Thrombosis (1969)

Pages
775

Size
5 x 8

ISBN
0309340004

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THROMBOSIS

DIVISION OF MEDICAL SCIENCES
NATIONAL RESEARCH COUNCIL

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National Academy of Sciences
WASHINGTON, D.C.
1969

Publication 1706

Available from

Printing and Publishing Office
National Academy of Sciences
2101 Constitution Avenue
Washington, D.C. 20418

Library of Congress Catalog Card Number 70-600517

Preface

Thromboembolic disease is a leading cause of morbidity and mortality in the United States, but its importance as a national medical problem is inadequately appreciated. This lack of appreciation of thrombosis as a major health hazard stems from at least two major considerations: first, thrombosis occurs often as a complicating event and—although it may be directly responsible for precipitating acute vascular obstruction, necrosis, infarction, and gangrene—the morbidity and mortality associated with it are usually related to some pre-existing disease, surgery, or trauma; second, it is current medical practice to identify diseases epidemiologically and clinically, with particular reference to organ systems (heart, brain, kidney, lungs, etc.), even though diseases in different organs may arise from identical pathologic events. Thus, acute thromboembolism is common to a number of major clinical problems, but the causative pathologic event is submerged in descriptions of effects of diseases in particular organs. In this respect, thrombosis contrasts sharply with cancer: whereas cancer has always been viewed as a disease that affects many organs, thrombosis has been viewed as only a finding associated with disease in many organs.

A somewhat analogous difficulty in appreciating the importance of thrombosis exists at the more fundamental levels of investigation. Here the structure of science tends to fragment the approach to this important health problem. The typical modern scientist, a skilled specialist, communicates regularly only with other workers in his own discipline. As a result, his studies concern highly selected aspects of a problem, generally without consideration of the broader biologic implications of his investigation. The anatomist, biochemist, biophysicist, physiologist, rheologist, and other specialists carry out their investigations, secure in their identification with their particular discipline, but encounter increasing

difficulty in communicating their concern over a phenomenon that interrelates their various scientific endeavors. Unfortunately, the clinical investigator, who might well bridge this gap, has involved himself almost exclusively with the elucidation of the coagulation defects that underlie some hemorrhagic phenomena, rather than with the factors that may be responsible for the more important clinical problem—thrombosis.

If we are to appreciate the importance of thrombosis in clinical medicine and to accelerate progress in understanding and controlling it, we must encourage a multidisciplinary effort to focus on the problem of thrombosis as a holistic entity. This book is intended to provide some impetus for such an effort; it presents the thrombosis problem as an entity and reviews and documents the current knowledge of its various facets. It is hoped that the information it contains will serve as a base for future development and reviews.

The publication is an outgrowth of one of the activities of the Task Force on Thrombosis of the Division of Medical Sciences of the National Research Council; the members of the Task Force served as the editors. The Task Force, in pursuit of its goal—to survey the thrombosis problem and to prepare a critique of the field—assembled a Conference on Thrombosis in Washington, D.C., in late November 1967. The immediate objective of the Conference was to provide a forum suitable for an interdisciplinary approach to the study of thrombosis. The invited participants were also asked to prepare scholarly reviews of the subject matter that they were to discuss briefly at the meeting. Their manuscripts form the contents of this book.

Acknowledgments

Financial support for the planning of the Conference on Thrombosis and for a major part of its conduct was provided by the Office of the Surgeon General, Department of the Army, and the Office of the Surgeon General, Department of the Navy (contract DA-49-193-MD-2077), the American Heart Association, and the National Institutes of Health (contract PH 43-64-44, task order 3). The Task Force on Thrombosis of the Division of Medical Sciences, National Research Council, was the scientific sponsor. The conference was held in association with the International Committee on Haemostasis and Thrombosis and constituted the principal scientific session of that Committee's 1967 annual meeting.

The Conference Program Planning Committee consisted of the members of the Task Force on Thrombosis—Drs. Sol Sherry (Chairman), Kenneth M. Brinkhous, Edward Genton, and James M. Stengle—and the following persons: Drs. Paul M. Aggeler, A. B. Chandler, Shirley A. Johnson, Abraham M. Lilienfeld, J. Fraser Mustard, George D. Penick, Oscar D. Ratnoff, Theodore H. Spaet, Stanford Wessler, and Irving S. Wright.

The Task Force wishes to express special indebtedness to Mr. Norman Grossblatt, staff editor in the Division of Medical Sciences of the National Research Council, for his extraordinarily perceptive and diligent work on the preparation of this volume for publication; to Mrs. Mary Freeman, who coordinated the typing and proofreading of all 50-odd manuscripts; and to Mrs. Joan Semasinghe, who verified the more than 2300 bibliographic references included in the manuscripts.

Under the auspices of the International Committee on Haemostasis and Thrombosis, financial support for some of the activities attending the conference and for a subvention that made publication of this book

possible was provided by contributions from the following laboratories:

Abbott Laboratories, Scientific Divisions
Armour Pharmaceutical Company
Astra Pharmaceutical Products, Inc.
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Smith Kline & French Laboratories
The Upjohn Company
Warner-Lambert Research Institute

The generous contributions of wisdom, time, effort, and money that made possible the successful completion of this important undertaking are gratefully acknowledged.

Contents

I
THE CLINICAL SPECTRUM OF THROMBOSIS

Introduction

IRVING S. WRIGHT

The presence of clots during disease and after death has been commented on by physicians for centuries, but serious study of the composition and possible significance of clots began only about a century ago. The progress was halting and confined largely to studies of gross pathology. The last 20 years have seen a renaissance. Clinicians have become increasingly aware of the diversity and subtlety of signs and symptoms produced by thrombi and the potential involvement of every organ of the body. Pathologists began to note the differences in the cellular constituency of thrombi. They also began to observe variations in the attachment to and the involvement of blood-vessel walls. Finally, mechanisms of formation, both orderly and disorderly, became apparent. Today, much information in these areas has been developed, and other disciplines are deeply involved.

Our understanding of the clinical spectrum of thrombosis should be broadened by the review and analysis of the disease states in which thrombosis and embolism are known to play leading, and in some cases dominant, roles. The examples that will be discussed in these papers have been selected because there is sufficient knowledge about them to warrant their consideration. However, the occurrence and the devastating effects of thromboembolism are far greater in magnitude and scope, in terms of numbers of patients, organ system involvement, and the nature and variety of pathologic states with which it is associated. Thrombosis and embolism are the most frequent terminal events in the occlusion of vessels, whether the process is associated with inflammation of the vessel walls, atherosclerosis, extensive pressure, or local injury or with processes of a generalized or distant pathologic nature, such as cancer, polycythemia, infection, dehydration, and inactivity and probably even blast injury in those who survive the initial effects. Indeed, it may

be safely stated that few serious diseases and fewer deaths occur in which some thrombosing or thrombolytic mechanism is not involved.

Each of the next 18 papers deals with a single aspect of the clinical spectrum of thrombosis: nine on venous and nine on arterial thrombosis. They are followed by a brief assessment of the clinical problems.

Venous Thromboembolic Disease in Medical and Malignant States

DAVID G. FREIMAN

It has been known for many years that numerous episodes of pulmonary embolism are unrecognized clinically, but the magnitude of this discrepancy has only recently become apparent. Earlier estimates of frequency based on necropsy data have usually ranged from 5% to 15%,[5,16,29,54,74] and have generally included only emboli that were readily recognized at the necropsy table. It is not surprising, therefore, that a high percentage of these emboli were considered to have contributed to death or that infarction was noted in 45–60%, or even more, of such patients.[11,16,49,54,70] More recently, incidence figures for pulmonary embolism ranging from 40% to 60% are being reported with increasing frequency.[2,27,57] Although some of this increase may well represent an absolute rise in pulmonary embolic disease, most of it is attributable to the inclusion of formerly unrecognized residua of emboli that failed to produce infarction or overt clinical manifestations and that were eventually reduced to traces by the two processes by which thrombotic obstructions are normally cleared from the circulation: fibrinolysis and organization.

The lung is protected from the effects of embolism by a number of local factors, including its dual blood supply and an airway that, when unobstructed, can provide oxygen directly to the parenchyma. These help it to function as a filter for the systemic circulation, just as the liver, which also has a dual blood supply, functions as a filter for the portal circulation. In addition, most thromboemboli, such as those deriving from sites of venous injury, are probably minor and are able to reach the smaller pulmonary arterial branches, where their effects are minimized, unless the lung is severely congested or otherwise abnormal. Even much larger thromboemboli may be tolerated, however, and there is now abundant evidence, both experimental [21,36,72] and clinical,[3,24,63]

that they may undergo rapid diminution in size, presumably as a result of the intrinsic fibrinolytic mechanism. As a result, occluded channels may be reopened or emboli, reduced in size, may be carried into smaller branches, permitting re-establishment of normal circulation if no irreversible damage has been done. The lytic process is probably assisted by the fragmentation of the thromboembolus and the resulting increase in surface area that may occur in transit from the vein of origin.[72]

The thrombus appears to be most susceptible to fibrinolysis during the first few days after formation [9,26,28,37,72]; during this period, an unknown number of thrombi and thromboemboli, especially smaller ones, probably disappear without recognizable trace. The rate and effectiveness of fibrinolysis are undoubtedly influenced by a variety of factors, however, including the amount of thrombus present, the changes in the fibrin substrate that occur with time either before or after embolization, the accessibility of the circulating blood to the thrombus, and the general activity of the fibrinolytic mechanism. As a result, variable amounts of thrombotic material often persist until organization begins—a matter of 1–5 days after thrombus formation or embolization, depending on the prior state of the vessel wall and the amount of local cellular activity. Lysis may well continue beyond this point, probably at a rapidly diminishing rate, and certainly continues to play an active role in helping to remove freshly formed thrombi that may develop *in situ* at embolic sites. It is through the process of organization, however, that the ultimate disposal of the residua is accomplished; many of them are reduced to organized nubbins, mural plaques, and fibrous webs and bands [25,28,35,36,43,50] in weeks to months, depending on the volume of unlysed thrombotic material and the local factors that affect the rate of organization. The phenomenon usually called "recanalization" is, in fact, only another manifestation of shrinkage of the thrombus as a result of these processes, with endothelialization of clefts and channels that may or may not traverse the obstruction.[72] If fibrinolysis has been relatively ineffective, the occluded vessel may remain partially or completely obstructed by organized thrombus, and organized emboli occluding multiple small pulmonary branches occasionally result in pulmonary hypertension and chronic cor pulmonale.[30,53]

The frequency with which these embolic traces are missed is clearly evident in the doubled incidence of pulmonary embolism observed at necropsy at the Beth Israel Hospital, Boston, after 1958, compared with 1951–1954, when no special effort was made to include such residua (Table 1). In spite of increased care and more rigid criteria, however, some emboli are still being overlooked, and several careful prosectors with an awareness of the problem have been able to detect evidence of pulmonary embolism in more than 60% of necropsied cases (Table 2).[27]

TABLE 1 Incidence of Pulmonary Embolism and Infarction, Beth Israel Hospital, 1951–1965

Years	No. Consecutive Adult Necropsies	Pulmonary Emboli Present		Infarcts Present		Emboli Associated with Infarction, %
		No.	%	No.	%	
1951–1954	581	125	22	58	10	46
1955–1957	516	145	28	42	8	29
1958–1960	491	217	44	46	9	21
1960–1962	531	214	40	54	10	25
1963–1965	500	226	45	73	15	32

Even this high figure probably represents an underestimation, inasmuch as it does not include emboli that may have undergone complete lysis or have been reduced by organization to nonspecific intimal thickenings. These unavoidable omissions, together with the complications of a terminal illness, point up the serious limitations of a necropsy series in estimating either frequency or predisposing factors.

Unfortunately, however, clinical criteria have thus far proved even less adequate. Most pulmonary embolic episodes, as well as those of peripheral vein thrombosis, are clinically silent or, in the former case, are often attributed to other causes, even when recent and massive. In a series of 500 consecutive adult necropsies performed between January 1963 and March 1965, only 10 (16%) of the 64 cases in which emboli were found in the major pulmonary arterial branches were diagnosed clinically (Table 3). Evidence of previously unrecognized embolic episodes in the form of fibrous webs and other organized residua was also present in nearly half the cases, occasionally resulting in the entrapment

TABLE 2. Incidence of Pulmonary Embolism in Unselected Adult Necropsy Samples, 1959–1964

Prosector	No. Cases Examined	Pulmonary Emboli Found	
		No.	%
J.S.	61[a]	39	64
P.B.	40	26	65
C.K.	28	17	61
C.F.	31	19	61
D.Y.	61	35	57
TOTAL	221	136	62

[a] Consecutive case series, previously reported.[27]

TABLE 3 Analysis of Cases with Pulmonary Embolism Involving Major Arteries in a Series of 500 Consecutive Adult Necropsies Performed between January 1963 and March 1965

			Clinically Recognized		Evidence of Previous Embolic Episodes		Associated with Infarction	
		No. Cases	No.	%	No.	%	No.	%
Thromboemboli found in one or both main pulmonary arteries:	Recent emboli present	18	6	33	13	72	11	61
	Emboli exclusively old	0	—	—	—	—	—	—
Thromboemboli found in lobar or segmental arteries:	Recent emboli present	38	4	11	18	47	24	63
	Emboli exclusively old	8	0	0	—	—	2	25
TOTAL BOTH AREAS		64[a]	10	16	31	48	37	58

[a] Represents 12.8% of the total series of 500 cases.

of a recent thromboembolus in a partially obstructed arterial branch (Figure 1).[43] Different stages of organization in recent thromboemboli also attested to the frequency of such multiple episodes during the terminal illness.[11] Of particular interest is the fact that 58% of the 64 cases (which represented 12.8% of the entire series of 500) were associated with infarction; this is remarkably close to incidence figures often quoted in the literature for major pulmonary embolism.[32,54] In contrast, the over-all incidence for this same group when emboli in subsegmental branches and organized embolic remnants are included is 45%, with only 32% of the emboli being associated with infarction (Table 1).

There is now considerable evidence that most clinically significant pulmonary emboli (if not most pulmonary emboli) derive from the veins of the lower extremities and pelvis, where stasis is common and frequently severe.[11,17,84] Estimates of the incidence of venous thrombosis tend to be even more unreliable than those of pulmonary embolism, in

FIGURE 1 Recent thromboembolus (arrow) arrested in a main branch of the pulmonary artery at the site of organized fibrous webs.

part for similar reasons, but also because, as a result of restrictions, the peripheral veins are rarely examined adequately at necropsy. In a few careful postmortem studies, however, peripheral venous thrombosis has been demonstrated in one fourth to one half of unselected necropsies on hospital patients,[29,39,48] and a considerably higher incidence has been recorded in selected groups.[64] It has also been shown that most of these, in addition to being clinically silent, are multiple and bilateral, and that they tend to develop in areas of stasis, particularly in valve pockets, where stasis can be demonstrated *in vivo* [47] and where organization tends to be more advanced than elsewhere.[48] The frequent presence in the pulmonary arteries of large emboli that could have come only from the leg veins or that show the imprints of venous valves constitutes additional evidence of origin from these sources.

Although other sites, such as the veins of the upper extremities (where stasis is less likely to be severe) and the chambers of the right side of the heart and particularly the right atrial appendage, may also contribute,[15,23] these sources are probably much less important. The role of the right side of the heart in particular as a source of significant embolism has probably been exaggerated; not only are such emboli likely to be small, but patients with atrial fibrillation or other cardiac abnormalities predisposing to atrial thrombi are also prone to develop peripheral venous thrombi and, consequently, larger and more serious emboli. Even the absence of peripheral venous thrombi in the major leg veins at the time of necropsy is not reliable evidence, in that little trace may be apparent after embolization unless a careful search is made. Thrombi appearing to arise in the inferior vena cava, where there are no valves, are more likely to be extensions from below or may represent emboli that originated more distally and were delayed en route to the lung.[73] The latter may also be true of the right side of the heart, and organization of such emboli trapped in the chordae of the tricuspid valve can occasionally be demonstrated both experimentally [72] and at necropsy (Figure 2). The occasional webs and bands found in the great veins probably represent the end stages of similar processes.

All these findings suggest that venous thrombosis and pulmonary embolism must be comparatively common, recurrent phenomena of little clinical significance, unless the normal mechanisms whereby thrombi are formed and cleared from the circulation are grossly disturbed. Allison [2] has come to similar conclusions. Excluding postoperative, posttraumatic, and puerperal states,* the most common medical conditions that appear capable of disturbing the homeostatic balance sufficiently to produce sig-

* Discussed elsewhere in this volume.

FIGURE 2 Organizing thromboembolus caught in the chordae of the tricuspid valve. Recent and organizing thromboemboli were also present in the pulmonary arteries and in the right atrium at the site of the network of Chiari.

nificant venous thromboembolic disease are cardiovascular disease (particularly cardiac failure), malignant neoplasms, and infection; many other conditions, including obesity, dehydration, hemiplegia, gastrointestinal hemorrhage, and various blood dyscrasias, have also been mentioned.[1,12,13,19,20,49] Because most studies are based on necropsy populations in which it is almost impossible to find a patient who did not have at least one of the above conditions at the time of death, correlations are certain to be high, but are not necessarily meaningful. Peripheral venous stasis, particularly in patients with chronic terminal disease, is undoubtedly an important common feature in many of them and may also help to explain the repeatedly noted increased frequency of embolism with advancing age. This is apparent in our series as well, although the relatively constant or diminishing incidence of infarcts indicates that many emboli in these age groups are small or represent organized residua of previous episodes (Table 4).

TABLE 4 Age Incidence of Pulmonary Embolism and Infarction in 500 Consecutive Adult Necropsies

Age, years	Total Cases	Emboli		Infarcts		% Emboli Associated with Infarct
		No.	%	No.	%	
Under 50	58	20	34	8	14	40
50–59	78	27	35	11	14	41
60–69	121	51	42	23	19	45
70–79	160	84	53	22	14	26
80 and over	83	44	53	9	11	20
TOTAL	500	226	45	73	15	32

The incidence of pulmonary embolism in patients with malignant, infectious, and cardiovascular disease (which often coexist in the same patient), who make up the bulk of the necropsy population at the Beth Israel Hospital, is not appreciably different from that of the group as a whole, as might be expected (Table 5). The incidence in postoperative states, however, appears significantly higher, but it is often impossible

TABLE 5 Incidence of Pulmonary Embolism in Various Disease Processes in 500 Consecutive Adult Necropsies

Disease	Total Cases [a]	Pulmonary Emboli Found	
		No.[a]	%
Malignancy, all types	152	76	50
Cardiovascular disease, all types	279	117	42
Infections, all types	121	56	46
Postoperative states (death within 21 days of operation)	84	51	61
Trauma	14	6	43
Specific conditions			
Cirrhosis of liver	10	2	20
Gastrointestinal hemorrhage	9	8	89
Severe respiratory disease	6	2	33
Cerebrovascular accident	31	10	32
Factor XI deficiency	1	0	—
Polycythemia vera	1	0	—
Miscellaneous (glomerulonephritis, etc.)	12	2	17
TOTAL CASES	500	226	45

[a] Cases showing multiple diseases are recorded more than once.

to separate the contribution made by the postoperative state from the neoplasm or other condition that led to the operation or to the subsequent infection or cardiovascular complication that may have resulted in death. Although subject to the same type of objection, and in spite of the small sample, the unusually high incidence of thromboembolism in association with gastrointestinal hemorrhage is noteworthy, as is the low incidence in cirrhosis.[65] Abnormal hematologic states, such as thrombocytosis and polycythemia vera,[52,56] and chronic respiratory disease, such as emphysema,[62] have also been associated with an increased incidence of thrombosis but are inadequately represented in this series. In the latter connection, however, it is important to distinguish between the frequency of embolization and the frequency with which symptoms are evoked. Embolism to the pulmonary arteries of patients with emphysema or other chronic respiratory disease may be very poorly tolerated and give the impression of increased frequency or severity, in spite of an over-all incidence no different from that of the control group.[10,42]

Although the importance of stasis as a critical factor in the genesis of venous thrombosis is now well established, both experimental and clinical evidence suggests that stasis alone may not be enough and that some additional triggering mechanism may be required.[71] Anoxic injury to the endothelium after prolonged stasis or minor traumatic injury to such a vessel may result in thrombosis, presumably by exposing the underlying connective tissues,[7,67] and the degree of trauma necessary to produce such changes can be shown experimentally to be very minor.[7,60] Whether such a thrombus becomes significant, however, probably depends on other factors as well, one of the most important undoubtedly being the activity of the fibrinolytic system.[8,18] This has not only been reported to increase in the presence of stasis [14,38] but may also help to explain why thrombosis is comparatively rare in very small vessels.[6] It is possible, therefore, that the high incidence of venous thrombosis and subsequent pulmonary embolism in association with cardiac failure or in elderly bedridden patients with chronic disease may often require no explanation beyond the occurrence of prolonged stasis associated with anoxic injury to the vessel wall [68] or multiple minor traumatic injuries in patients in whom the normal balance between fibrin formation and lysis has also been disturbed by local or general anoxia.

Whether the reported effects of infection, malignancy, and other conditions can be attributed entirely to such a process seems doubtful. In the small Beth Israel series, only carcinoma of the pancreas and lung showed an appreciable increase over the mean for the group (Table 6) and were thought to have thrombogenic potential.[22,46,55] In contrast with many reports in the literature,[41,44,66] no distinction was evident

TABLE 6 Incidence of Pulmonary Embolism in Association with Various Malignant Tumors in 500 Consecutive Adult Necropsies

Neoplasm	No. Cases	Emboli Found in	
		No.	%
Pancreas, total	12	9	75
head	9	7	
body and tail	3	2	
Lung	19	12	63
Stomach	17	9	53
Colon and rectum	26	11	42
Breast	17	7	41
Lymphoma and leukemia	17	7	41
Prostate	9	3	
Kidney	5	3	
Glioma	5	2	
Myeloma	4	2	
Bladder and ureter	4	2	
Uterus and tube	3	1	
Melanoma	2	2	
Thyroid	2	1	
Ovary	2	1	
Ampulla of Vater	2	0	
Mesothelioma	1	1	
Gallbladder	1	1	
Mouth	1	1	
Esophagus	1	0	
Hepatoma	1	0	
Undetermined	1	1	
TOTAL MISCELLANEOUS	44	21	48
TOTAL TUMORS	152	76	50

between carcinomas of the head of the pancreas and of the body or tail. Numerous explanations for this increased incidence have been offered, including release into the circulation of trypsin or active thromboplastic substances from necrotic tissue,[31,51,66] mucin production or other histologic characteristics of the tumor,[40,44,61] and acceleration of the coagulation process due to abnormalities in plasma coagulation factors or platelets,[4,45,58] but none of these has thus far been confirmed. Mucin production, at least, appears to bear no relation to thrombogenesis in our cases, nor does the presence of massive necrosis in tumors or in myocardial infarcts correlate well with the occurrence of pulmonary embolism. The coagulation mechanism can be activated and the level of

fibrinolysis altered [33,59] in many ways, however, and it seems reasonable to assume that factors released or activated by disease processes, including neoplasms elsewhere in the body, or even by bacterial endotoxin,[69] may at times disturb the balance sufficiently to produce significant thrombosis in areas of stasis. Because venous stasis is rarely complete, platelet accumulation and propagation, with the alteration of the vessel wall that must inevitably follow, would quickly convert such a thrombus, however it was induced, into the mixed red thrombus so characteristically seen in the veins or in the pulmonary arteries at necropsy.

The work reported here was supported by Public Health Service research grant HE 09342 and program project grant HE 06316 from the National Heart Institute.

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Lethal Pulmonary Embolism

RICHARD E. HOROWITZ AND DOROTHY TATTER

Pulmonary embolism is unique in that it is almost invariably a complication of another disease and is not diagnosed often or early enough to prevent death. This presentation will describe the pathology of lethal pulmonary embolism and will categorize the types and relative frequencies of the diseases that cause, or are associated with, pulmonary embolism.

METHODS

Necropsies performed at Los Angeles County General Hospital from 1961 to 1965, totaling 10,991, were reviewed. In 316, pulmonary embolism was considered the cause of death or a major contributory cause. The necropsies of an age- and sex-matched group of patients dying without pulmonary embolism were reviewed for comparison. Diagnosis of pulmonary embolism as the cause of death was determined only after confirmation of the gross and microscopic findings by one of the senior staff pathologists.

INCIDENCE

Pulmonary emboli were found in one third of all the necropsies. However, the incidence of lethal pulmonary embolism was 3%. The ratio of females to males was 6:4, and the average age of persons who died of pulmonary embolism was 69 years—the youngest was 18 and the oldest, 97. Lethal pulmonary embolism was more frequent in Caucasians in general and less frequent in patients of Mexican origin.

CLINICAL IMPRESSION

Of primary interest was the correlation between the clinical impression of the cause of death (Table 1) and that determined by necropsy. In only 11.5% of patients who died of pulmonary embolism was the diagnosis made clinically; 21.8% of patients were diagnosed as having died from myocardial infarction, 14.9% cerebral vascular accidents, 14.2% pneumonia, and 13.2% heart disease other than myocardial infarction.

There may be several reasons for this low level of diagnostic accuracy. First, the person who performed the necropsy may attribute the death to pulmonary embolism unjustly. Pulmonary emboli are dramatic and difficult to dismiss as the main cause of death, but their role in death is difficult to evaluate, particularly if other potentially lethal lesions are found. Second, it may be (and this happened) that a patient who died of pulmonary embolism actually had a myocardial infarct or pneumonia and that the clinical features of pulmonary embolism did not make a striking impression on the clinician. Third, in pulmonary embolism, there may not be a good correlation between morphologic alteration and clinical manifestation.[4,6] Fourth, the only clinical manifestation of pulmonary embolism may be the sudden death itself.[1,3]

TABLE 1 Clinical Cause of Death in 303 Patients with Lethal Pulmonary Embolism

Clinical Cause	Number	%
Myocardial infarct	66	21.8
Cerebral vascular accident	45	14.9
Pneumonia	43	14.2
Other heart disease	40	13.2
Pulmonary embolism	35	11.5
Malignant disease	24	7.9
Uremia	12	4.0
Chronic pulmonary disease	7	2.3
Sepsis	5	1.6
Malnutrition	3	1.0
Gangrene	2	0.7
Gastrointestinal hemorrhage	2	0.7
Pancreatitis	2	0.7
Parotitis	2	0.7
Miscellaneous	15	4.9
TOTAL	303	

PATHOLOGY

Table 2 lists the sources of the pulmonary emboli in the 316 patients. At Los Angeles County General Hospital, limitations of necropsy dissection prevented direct examination of the deep calf veins. On the basis of other studies,[2,9,10] it may be assumed that, when the source of the pulmonary emboli is not determined, the deep leg veins are implicated. On this basis, over 80% of lethal pulmonary emboli probably had their source in the lower extremities. The source of 12.3% (in 40 patients, all with cardiac arrhythmias) was mural thrombi in the right atrium. Lethal pulmonary emboli originating in the pelvic veins were considered rare. Two patients had major tumor emboli, one from a renal carcinoma, the other from a bladder carcinoma. In eight patients, there was more than one possible source for the pulmonary emboli.

Table 3 lists the location of the emboli within the pulmonary vasculature. In the 316 patients, a total of 532 separate emboli were identified. A solitary embolus was found in only 83 patients (about 25%). Location of a pulmonary embolus is determined mainly by the size of the clot, which in turn depends on several factors, including the size of the vessel in which the clot originally forms, the degree of stasis in that vessel, the rapidity of clot formation, and fragmentation of the preformed clot as it passes through the right side of the heart.[11] The most common locations of the pulmonary emboli were the right and left pulmonary arteries; next most common were the peripheral vessels. The massive embolus in a large vessel was single or associated with other, smaller emboli distally. The small peripheral emboli were invariably multiple.

Table 4 shows the unequal distribution of pulmonary infarcts relative to the locations of the pulmonary emboli. Of the 316 patients with lethal pulmonary emboli, 98, approximately one third, had pulmonary

TABLE 2 Sources of Lethal Pulmonary Emboli

Source	Number	%
Leg veins	139	42.9
Heart	40	12.3
Pelvic veins	19	5.9
Tumor	2	0.6
Undetermined	124	38.3
TOTAL	324[a]	

[a] Emboli had two sources in each of eight patients.

TABLE 3 Locations of 532 Lethal Pulmonary Emboli in 316 Patients

Location	Number	%
Main pulmonary artery	38	7.1
Right pulmonary artery	138	25.9
Left pulmonary artery	103	19.4
Right upper lobe artery	19	3.6
Right middle lobe artery	21	3.9
Right lower lobe artery	53	10.0
Left upper lobe artery	10	1.9
Left lower lobe artery	47	8.8
Peripheral arteries	103	19.4
TOTAL	532	

infarcts. These 98 patients had a total of 191 separately identifiable infarcts. Over 70% of all the pulmonary infarcts were in the lower lobes, even though most emboli were either in the hilum or equally distributed throughout the small peripheral vessels.

UNDERLYING DISEASE

Table 5 lists the major associated or underlying diseases in the 316 patients with lethal pulmonary embolism. There were a total of 507 separate diagnoses; many of the patients had multiple diseases all of which contributed significantly to death. Table 6 compares the 11 leading medical causes of death in patients without pulmonary embolism with the 11 most common underlying or associated diseases in patients with lethal pulmonary embolism. It should be noted that the most frequent cause of death in patients without pulmonary embolism during the period 1961–1965 was malignant disease. These were 103 patients with malignancies

TABLE 4 Locations of 191 Pulmonary Infarcts in 98 of the 316 Patients (Who Had 532 Pulmonary Emboli)

Location	Number	%
Right upper lobe	23	12.0
Right middle lobe	21	11.0
Right lower lobe	69	36.1
Left upper lobe	13	6.8
Left lower lobe	65	34.1
TOTAL	191	

TABLE 5 Major Associated or Underlying Diseases in 316 Patients with Lethal Pulmonary Embolism

Disease	Number	%
Coronary arteriosclerosis	89	17.6
Malignant disease	79	15.6
Cerebral arteriosclerosis	78	15.4
Postoperative state	28	5.5
Diabetes mellitus	27	5.3
Chronic renal disease	20	3.9
Chronic pulmonary disease	18	3.6
Hypertensive heart disease	18	3.6
Peptic ulcer	18	3.6
Cardiac arrhythmia	15	3.0
Cholecystitis	12	2.4
Peripheral arteriosclerosis	12	2.4
Pickwickian syndrome	10	2.0
Rheumatic heart disease	10	2.0
Decubitus ulcer	8	1.6
Pancreatitis	8	1.6
Pneumonia	6	1.2
Tuberculosis	6	1.2
Cirrhosis	5	1.0
Diverticulosis coli	4	0.8
No associated disease	4	0.8
Senility	4	0.8
Sepsis	4	0.8
Enterocolitis	3	0.6
Puerperal pelvic infection	3	0.6
Syphilis	3	0.6
Aortic aneurysm	2	0.4
Endocarditis	2	0.4
Islet cell adenoma	2	0.4
Sickle cell disease	2	0.4
Small bowel obstruction	2	0.4
Miscellaneous	5	1.0
TOTAL	507	

in the control group, compared with 79 in the pulmonary embolism group. The list of underlying or associated diseases excludes cases that came under the jurisdiction of the county medical examiner; thus, traumatic deaths and many postoperative deaths were underestimated in the tables.

The diseases most commonly associated with lethal pulmonary embolism were those in which hypercoagulability has been postulated, namely, coronary and cerebral arteriosclerosis, malignant disease, and

TABLE 6 Comparative Ranking of Medical Diseases in Patients with and without Lethal Pulmonary Embolism

Rank	With Pulmonary Embolism	Without Pulmonary Embolism
1	Coronary arteriosclerosis	Cerebral arteriosclerosis
2	Cerebral arteriosclerosis	Coronary arteriosclerosis
3	Postoperative state	Cirrhosis
4	Diabetes mellitus	Chronic renal disease
5	Chronic renal disease	Chronic lung disease
6	Chronic lung disease	Pneumonia
7	Hypertensive heart disease	Diabetes mellitus
8	Peptic ulcer	Peptic ulcer
9	Cardiac arrhythmia	Hypertensive heart disease
10	Cholecystitis	Peripheral arteriosclerosis
11	Peripheral arteriosclerosis	Rheumatic heart disease

the postoperative state.[5,7,8] The diabetic, with concomitant severe arteriosclerosis, frequently succumbed to pulmonary embolism. With the exception of the postoperative state, however, these conditions are also common causes of death in patients without pulmonary embolism. Cardiac arrhythmias, often associated with electrolyte disturbances, were seen in frequent association with lethal pulmonary embolism but were not diagnosed in the control group. The Pickwickian syndrome also appeared to be regularly associated with pulmonary embolism and possibly was related to the inactivity and right heart failure, with resultant venous stasis, in such patients.

Striking by its absence from the list of diseases associated with pulmonary embolism was alcoholic liver disease. After cancer, heart disease, and stroke, cirrhosis of the liver is the leading cause of death in our hospital population. In patients with lethal pulmonary embolism, cirrhosis ranks 19th! The clotting defects in the patient with hepatic dysfunction conceivably "protected" the patient from thromboembolic disease.

Of the 316 patients who died of pulmonary embolism, 79 had an associated or underlying malignant disease. Microscopic or incidental tumors were excluded from the tabulation. Table 7 lists the associated malignant disease according to site of origin. Table 8 ranks the 10 most common tumors in patients with and without pulmonary embolism. Pancreatic carcinoma ranks third among tumors associated with pulmonary embolism and sixth among tumors in patients without pulmonary embolism. Three types of neoplasms, ranking third, fourth, and fifth in our control group, do not appear at all in the top 10 tumors associated with pulmonary embolism. Carcinoma of the breast ranks third in the

TABLE 7 Associated or Underlying Malignant Diseases in 79 Patients with Lethal Pulmonary Embolism

Site	Number	%
Colon	12	15.2
Lung	8	10.1
Pancreas	8	10.1
Prostate	8	10.1
Stomach	8	10.1
Ovary	7	8.9
Uterus	7	8.9
Brain	3	3.8
Bladder	2	2.5
Gallbladder	2	2.5
Kidney	2	2.5
Lymph node	2	2.5
Bone	1	1.3
Breast	1	1.3
Lip	1	1.3
Melanoma	1	1.3
Thyroid	1	1.3
Tongue	1	1.3
Site undetermined	4	5.1
TOTAL	79	

patients without pulmonary embolism and only 14th in patients with pulmonary embolism. The lymphoma–leukemia group was the fourth most common neoplasm in patients without pulmonary embolism, but there were no cases of lymphoma or leukemia among the patients with lethal pulmonary embolism (except for two patients with lymphosarcoma

TABLE 8 Comparative Ranking of Malignant Disease in Patients with and without Lethal Pulmonary Embolism

Rank	With Pulmonary Embolism	Without Pulmonary Embolism
1	Colon	Lung
2	Lung	Colon
3	Pancreas	Breast
4	Prostate	Lymphoma
5	Stomach	Esophagus
6	Ovary	Pancreas
7	Uterus	Prostate
8	Brain	Stomach
9	Bladder	Uterus
10	Gallbladder	Brain

limited to lymph nodes). These patients did not demonstrate the clotting defects that are so common in disseminated lymphoma and leukemia and that may have protected this group of patients, as the cirrhotic was protected. Carcinoma of the esophagus was also common in the control population, ranking fifth; yet, there were no esophageal carcinomas in the patients with pulmonary embolism. Thus, with the exception of lymphomas and carcinomas of the breast and esophagus, the frequencies of tumors were comparable in patients with and without pulmonary embolism.

CONCLUSIONS

Review of 10,991 necropsies disclosed 316 cases of lethal pulmonary embolism, from which conclusions may be drawn:

1. Pulmonary embolism was the major cause of approximately 3% of all adult deaths. Pulmonary emboli were relatively more common in Caucasians.
2. The diagnosis of pulmonary embolism was made premortem in only 11.5% of patients who succumbed to it. The presence of pulmonary infarcts increased the diagnostic accuracy by only 1%.
3. Over 80% of all pulmonary emboli originated in the veins of the lower extremities. Slightly more than 10% of pulmonary emboli originated in the heart, but only in patients with cardiac arrhythmias.
4. The most common locations for emboli were the right and left pulmonary arteries. The next most common site was the small peripheral vessels, in which emboli were always multiple. The large hilar emboli were most often associated with small peripheral emboli, but in 25% of cases a massive embolus was single.
5. Only one third of pulmonary emboli resulted in infarction, 70% of which were in the lower lobes. The location and occurrence of pulmonary infarction appeared to be related to passive congestion of the lung, in addition to the embolus. However, most emboli were either in the hilar vessels or equally distributed in the peripheral vessels of all lobes. The location of a pulmonary infarct does not necessarily give any clue as to the location of clinically significant pulmonary emboli.
6. The most prominent predisposing diseases are those in which hypercoagulability states have been postulated, over 40% of the patients having associated coronary, cerebral, aortic, or peripheral arteriosclerosis. Patients with malignant disease and postoperative patients also ranked high with respect to lethal pulmonary embolism, again presumably

related to hypercoagulability. Conversely, patients with cirrhosis of the liver rarely had pulmonary emboli, presumably because of the clotting defects which accompany hepatic dysfunction.

7. In 15% of patients with lethal pulmonary embolism, malignant disease was an associated or underlying process. This incidence was somewhat below that in the general adult necropsy population or the control group, in which approximately 20% of patients had malignant disease as a cause, or the major cause, of death. Pancreatic carcinoma was frequently associated with lethal pulmonary embolism. However, three very common malignancies were only rarely associated with pulmonary embolism: carcinomas of the breast and esophagus; and lymphoma–leukemia, which constituted the fourth most common malignant neoplastic disease in the control group of patients (without pulmonary embolism). Presumably, as in the cirrhotic, the complicating clotting defects of the lymphoma–leukemia patient may have protected the patient from thromboembolic disease.

Although this study has re-emphasized the relationship between hypercoagulable states and pulmonary embolism, and has shown the negative relationship of pulmonary embolism and diseases with clotting defects, the essential problem is the establishment of the premortem diagnosis, which ultimately depends on a high index of clinical suspicion.

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Venous Thrombosis in Injured Patients (with Some Observations on Pathogenesis)

S. SEVITT

Pulmonary embolism has been known for over a century and is increasingly recognized as a common cause of illness and death as a postoperative complication of general, orthopedic, and gynecologic surgery, after parturition, in various medical conditions, and after trauma. The frequency of embolism is greatly underestimated clinically. Major embolism was found in 20% of 468 patients who reached necropsy after a wide variety of injuries, which corresponded to a frequency of fatal embolism of about 1.0% among inpatients, excluding those admitted to short-stay wards.[16,18] Pulmonary embolism was the most frequent single cause of death in the elderly injured. This picture has been considerably changed by the introduction of routine oral anticoagulant prophylaxis for many groups of injured patients.[17,19] However, in a recent analysis, 19 of 250 subjects who reached necropsy after road accidents had major lung embolism (Table 1); in most of them death was too quick for prophylaxis or prophylaxis was contraindicated because of the fear of hemorrhage. Seventeen of the cases of embolism were among the 60 subjects over 45 years old who lived more than 4 days (28%). This confirms other evidence [7,11,18] that duration of bedrest and age over 40 or 45 years are the main predisposing etiologic factors in thrombosis and embolism.

Because the great majority of emboli arise from thrombi in the deep veins of the legs and pelvis, this paper is concerned mainly with clinicopathologic aspects of the underlying thrombosis. The pathologic data were obtained by routine systematic dissection at necropsy of the deep veins of the legs and pelvis by a technique previously reported.[18,19] Studies on coagulation and fibrinolysis in injured patients [10] and on microthrombosis [4] are also included, because they have a bearing on the vexed problems of hypercoagulability and pathogenesis.

TABLE 1 Major Pulmonary Embolism among 250 Patients Who Died after Road Traffic Accidents with a Wide Variety of Injuries, by Age and Survival Time (Bedrest)

Age, years	Survival Time, days: 0–4 No. Patients	0–4 No. with Embolism	5–14 No. Patients	5–14 No. with Embolism	>14 No. Patients	>14 No. with Embolism	Totals No. Patients	Totals No. with Embolism
<45	86	0	12	1	8	0	106	1 (0.9%)
45–60	30	0	9	3	7	3	46	6 (13%)
>60	54	1	19	4	25	7	98	12 (12%)
All ages	170	1 (0.6%)	40	8 (20%)	40	10 (25%)	250	19 (7.6%)

ORIGIN AND EXTENSION OF THROMBI

Recent thrombi are attached to the walls of veins only at valve cusps, bifurcations, or small saccules; otherwise, they float almost freely in the bloodstream. The minor attachments explain the relative ease of detachment and the risk of embolism. The thrombotic process is abacterial and histologically noninflammatory, and the distinction between so-called thrombophlebitis and phlebothrombosis (deep-vein thrombosis) is not justified. Older thrombi are often invaded by endothelial or monocytic fibroblasts and thereby become adherent to the venous walls.

Thrombi begin as small primary nidi in valve pockets (Figure 1), most prominently in the femoral veins, or at vein junctions or in small venous saccules, particularly in the deep calf veins. The places of origin are related to the development of stagnant local pools that form under conditions of venous stasis in the legs, as demonstrated by McLachlin *et al.*,[11] using serial phlebography when the limbs of the patients were horizontal, supine, and still. This and other evidence [7,18] strongly suggest that the common denominator in the etiology of venous thrombosis in traumatic, surgical, medical, and obstetric cases is venous stasis of the lower limbs; bedrest plays the major role and is more important than the reason for confinement to bed. The primary thrombi extend in two ways: (1) under conditions of venous stasis, by forward propagation in the direction of the venous stream through the deposition (from the blood) of further thrombus material on the growing end; and (2) in a retrograde direction through thrombosis of a column of blood distal to the propagating thrombus, presumably when that has begun to obstruct the venous flow significantly. These mechanisms are outlined diagram-

FIGURE 1 Primary valve-cusp thrombus with early propagation.

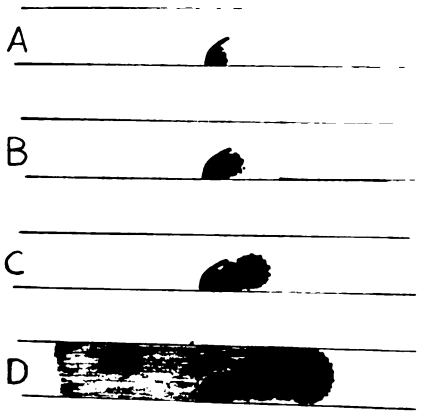

FIGURE 2 Diagrammatic representation of thrombus propagation and retrograde extension. A, formation of a primary thrombus in a valve cusp. B, C, and D, forward growth (propagation) in the direction of the venous flow through deposition of successive layers of thrombus material. Retrograde extension of the thrombus may occur with significant blockage of the venous flow, as in D.

matically in Figure 2. Small, independent primary thrombi arising in two, three, or more venous foci thereby often become long and confluent, and their sites of origin become hidden.

FREQUENCY AT NECROPSY: AGE AND BEDREST

Venous thrombi are common in injured subjects; venous dissection studies revealed an over-all incidence of 65% among 125 cases.[18] This was a low estimate, in that subjects with pulmonary emboli were deliberately excluded, and they all had thrombosis. Other estimates are relatively few, but indicate a high incidence of deep-vein thrombosis in medical, surgical, and other necropsies ranging from 36% to 60%.[6,7,9,12–14]

Age and survival period (which may be taken as equivalent to duration of bedrest) have a major influence on frequency. Consequently, the over-all incidence in a series will depend on the proportions of subjects of different ages and survival times. Table 2 shows that the frequency of thrombosis was 19% among 26 subjects who died within the first 3 days of injury, 44% among 18 subjects who died between 4 and 7 days, and between 79% and 88% among those who died during the second, third, and subsequent weeks. Fresh thrombi were almost never found among those who died within a day of injury. The absence of thrombi on the first day, their relative infrequency during the first 3 days, and the subsequent rapid rise in incidence indicate that the factors giving rise to the thrombi are not present or not sufficient in the early posttraumatic period, but develop or become sufficient later. With regard to age (Table 2), more than half of those over 45 years old and most (75%) of those over 60 years old died with thrombosis. But even in young per-

TABLE 2 Deep-Vein Thrombosis at Necropsy among 125 Injured Patients without Pulmonary Embolism by Age and Survival Time (Bedrest)

Age, years	Survival Time, days: 0–3 No. Patients	0–3 No. with Thrombosis	4–7 No. Patients	4–7 No. with Thrombosis	8–14 No. Patients	8–14 No. with Thrombosis	15–21 No. Patients	15–21 No. with Thrombosis	>21 No. Patients	>21 No. with Thrombosis	Totals No. Patients	Totals No. with Thrombosis
<15	2	0	4	0	—	—	2	0	5	4	13	4
16–45	4	1	4	0	6	6	2	2	5	3	21	12
46–60	8	3	4	2	—	—	—	—	4	4	16	9
>60	12	1	6	6	19	16	10	9	28	24	75	56
All ages	26	5 (19%)	18	8 (44%)	25	22 (88%)	14	11 (79%)	42	35 (83%)	125	81 (65%)

sons, thrombi were not infrequent; 31% of those under 15 years old had thrombosis. Advancing age was found associated with a rising frequency of thrombosis in the veins of both the thigh and calf, whereas prolonged bedrest (more than 28 days) was associated with a particularly high rate of thigh-vein thrombosis (73% of 30 cases). This is important in view of the large risk of serious embolism from these wide and often long thrombi. When age and survival period are taken together, it is clear that the risk of thrombosis is particularly large in middle-aged and elderly patients subjected to bedrest for longer than 3 days and much less common in those under 45 years old subjected to bedrest for less than a week before death. This broad separation was useful in the selection of high-risk patients for routine prophylaxis with oral anticoagulant drugs.[17,19,20]

Evidence was obtained that thrombogenesis may continue for weeks; many who died weeks or months after injury had older organizing, hemosiderin-stained thrombi and more recent, nonadherent thrombi in the same or different veins.

DISTRIBUTION AND PATTERNS OF THROMBOSIS

Thrombi were found most commonly in veins of the calf (74% of the 81 cases and 64% of the 162 limbs), next most commonly in pelvic and/or thigh veins (70% of cases, 49% of limbs), and less commonly in popliteal veins (37% of cases, 23% of limbs). The left was rather more often affected than the right limb. In the leg, thrombi were more common in intramuscular calf veins, usually the soleal plexus (67% of cases, 48% of limbs) than in the posterior tibial veins (54% of cases, 39% of limbs). In the thigh, thrombi in the common femoral vein (48% of cases, 27% of limbs) and the deep femoral vein were about equally frequent, and thrombosis of the superficial femoral and popliteal veins (each in about 33% of cases and 20% of limbs) were less common. In the pelvis, thrombi were relatively uncommon; but, when present, were usually in external iliac veins (26% of cases, 17% of limbs) and were less often found in common or internal iliac vessels. Thrombosis had extended into the inferior vena cava in 5% of the patients with thrombi.

The combinations of veins and vein groups affected by thrombi varied considerably in the different patients and often between the two limbs of the same patient (Figure 3). The most frequent combinations of veins with thrombi (Table 3) were calf, popliteal, and iliofemoral veins (18% of limbs) and calf and iliofemoral veins (13% of limbs). These

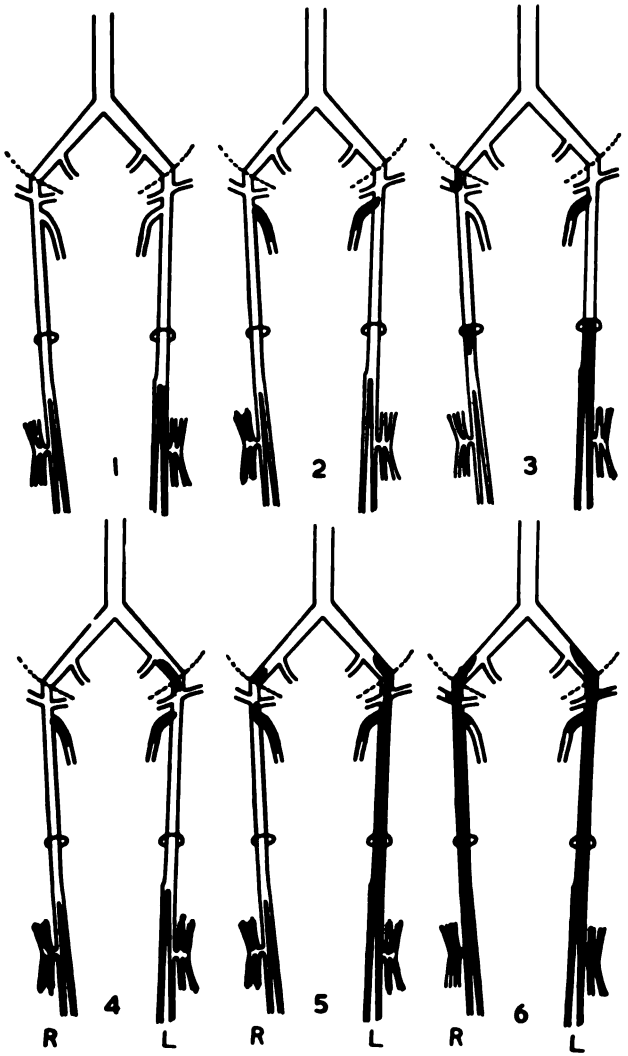

FIGURE 3 Diagrams of the lower venous tree, showing patterns of thrombosis found at necropsy in six injured patients. The examples were selected to show the independence of thrombosis in the main primary sites (see Figure 4) and the variety of patterns of thrombosis in different cases and often in the two limbs of the same subject.

TABLE 3 Thrombosis in Calf, Popliteal, and Iliofemoral Veins of 162 Limbs of 81 Patients with Thrombosis but No Embolism at Necropsy

Veins with Thrombi	Right Limbs, %	Left Limbs, %	Right and Left Limbs, %
Calf only	25	32	28
Iliofemoral only	11	23	17
Calf and iliofemoral	17	9	13
Calf and popliteal	4	4	4
Iliofemoral and popliteal	1	1	1
Calf, iliofemoral, and popliteal	16	20	18
TOTAL WITH THROMBOSIS	74	89	81

necropsy findings and those of Frykholm,[6] McLachlin and Paterson,[12] and Gibbs[7] are in general agreement.

PRIMARY SITES OF THROMBOSIS

The finding in various sites of small separate thrombi, often at valve cusps, indicated that thrombi can begin at one or more of a number of independent sites in the thigh and leg. This conclusion was supported by the variety of patterns of thrombosis that may be present and by the isolation of thrombi in various veins in different subjects. Six main primary sites were recognized (Figure 4): (1) the iliac vein, generally the external iliac just above the inguinal ligament; (2) the common femoral vein, including the mouths of the medial and lateral circumflex veins; (3) the deep femoral vein, often at or near its termination; (4) the popliteal vein near the adductor ring; (5) the posterior tibial veins; and (6) the intramuscular veins of the calf, particularly the soleal plexus. Primary thrombosis in the superficial femoral vein is uncommon; when this vein is involved, it is usually by retrograde extension following common femoral thrombosis. Propagation from popliteal thrombi is uncommon and from calf-vein thrombi, almost unknown. The commonly held view that thigh-vein thrombi arise from calf veins is therefore incorrect. The concept of multiple independent sites of thrombosis is supported by necropsy evidence from patients who had been given oral anticoagulant prophylaxis: when prophylaxis was delayed for a few days after injury, so that thrombi may have already begun to form but could not then extend, small thrombi were sometimes found at widely separate foci, especially in the soleal vein, the posterior tibial vein, the termination of the deep femoral vein, and the common femoral vein.

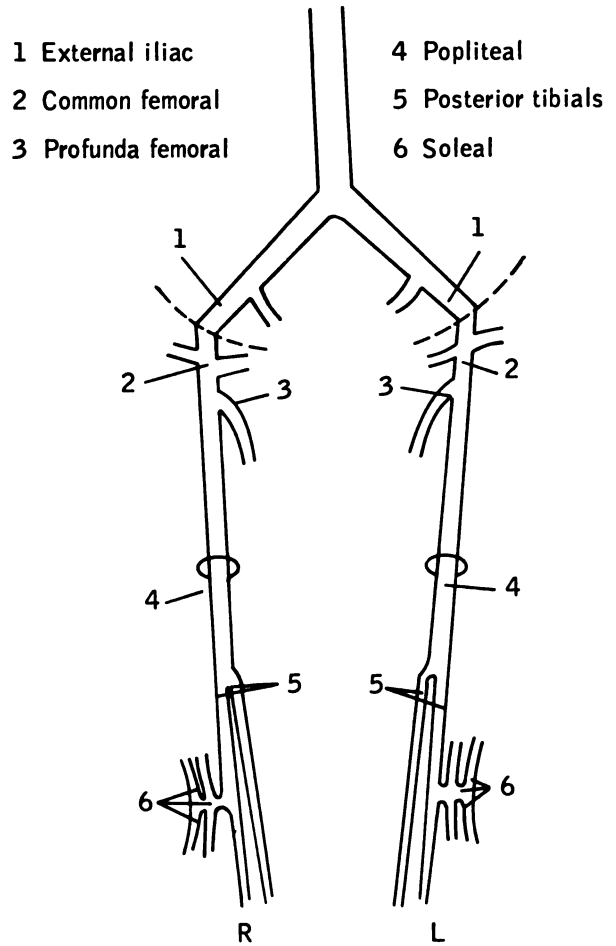

FIGURE 4 Diagram of the lower venous tree, showing the six main sites at which deep-vein thrombosis begins. These primary sites are independent of each other, although thrombosis is frequent in two or three of them (see Figure 3).

The anatomy of the lower venous tree and the dynamics of a slowed venous flow decide where thrombi begin to form when the body is in the horizontal position of bedrest. Emptying of veins depends on muscle contraction, and the blood will tend to become stagnant when this is lacking, especially in those lying supine in bed and inactive because of advancing years. Soleal veins form a number of intramuscular arcades, which increase in number and in lumen diameter with advancing age;

thrombosis in them is facilitated when they become distended with stagnant blood.[7] Thrombosis of the posterior tibial vein is probably encouraged by its relationship to the fibrous band of origin of the soleal muscle under which it passes. The popliteal vein passes through the adductor ring and may be compressed by it from the bulk of overlying adductor muscles, thus favoring thrombosis at its proximal end. The intramuscular location of the deep femoral vein and pressure from overlying muscles may help to slow its venous stream and promote thrombosis. The inguinal ligament appears to be a restraining band on the common femoral vein, and above it, the external iliac vein executes an acute weir-like change in direction, which may promote eddying and silting of formed elements from the blood when the flow is slowed. Eddying and silting of platelets from a stagnant flow of blood may be important at least for propagation; the resulting aggregation of platelets probably contributes to the postulated chain reaction involving the platelet-release phenomenon and thrombin formation. Eddying and silting are likely to occur at valves and mouths of vein junctions—for example, at the termination of the deep femoral, medial and lateral circumflex, and long saphenous veins, all tributaries of the common femoral vein. Thrombosis is more common in the left than the right limb, particularly in the thigh, and it is likely, as Aschoff[1] noted, that that is because the left common iliac vein is generally crossed by the origin of the right common iliac artery.

The anatomic findings support the old contention that venous stasis plays the major etiologic role in venous thrombosis and that the peculiarities of the venous anatomy in the legs determine the primary location of thrombi.

NATURE OF THE INJURY AND VENOUS THROMBOSIS

The frequency of thrombosis is only indirectly related to the injury, and there is no evidence that thromboplastin released from damaged tissue plays any substantial role. Although the main influences are age and bedrest, these are often related to the nature of the injury. For example, the incidences of thrombosis in the three groups of patients who died after head (or head and chest) injury, burns, and fractured neck of the femur were 37%, 60%, and 83%, respectively. The lowest figure was associated with a large proportion of patients with a short survival period, few of whom had thrombi; the very high rate was related to relatively long survival periods combined with advanced age; the intermediate rate (burned subjects) was associated with a relatively even mixture

of children, adults, and elderly subjects, of whom most survived longer than a week but some only a few days. Prolongation of survival may also be related to improvements in therapy, so that many injured and burned patients who previously would have died during the first 2 or 3 days with little or no thrombosis now survive weeks but remain in bed and then may die, often with venous thrombosis and sometimes from pulmonary embolism.

In those with a fracture or other injury to a leg, clinical evidence of thrombosis is usually in the same limb; therefore it would appear that the local trauma increases the likelihood of the thrombosis. However, venous dissection among 109 subjects who died after unilateral fracture of the femur or tibia did not show that. Thrombosis was generally bilateral (62% of the subjects). Unilateral thrombosis was much less common and was as frequent in the uninjured (14%) as in the injured limb (12%). Analysis of extent of thrombosis also failed to reveal any statistical difference between the injured and uninjured limbs.

The undoubted clinical association of ipsilateral limb trauma and thrombosis requires an explanation. Limb swelling often begins after the patient has sat out of bed, and then the precipitating cause is the dependent position of the limb, which is both immobile from injury (perhaps also by a plaster cast) and thrombosed. This explains at least part of the apparent paradox.

CLINICAL AND SILENT THROMBOSIS

Swelling of the limb, often restricted to the calf, is the most reliable sign of thrombosis, but it is often minor and equivocal. Other evidence—pain or tenderness in the calf, a definite Homan's sign, a sensation of tightness in the calf, or a difference in skin temperature in the two legs or feet—is of value when present but is often absent.

Those with symptoms are analogous to the visible part of the iceberg: clinicopathologic correlation showed that deep-vein thrombosis is symptom-free in the majority of cases (about two thirds) and in most of the limbs (about three fourths). In a special study on a series of subjects with fractured hips, the legs were examined for clinical thrombosis at frequent intervals from admission, and thrombi were searched for in those who died by dissection of the lower venous tree. Thrombosis was diagnosed clinically in 13 of 41 subjects who later died, but 32 of the 41 (including all 13 with limb swelling) had significant thrombosis at necropsy. Thus, 60% of whose who died with thrombi had been free of symptoms. A high frequency of silent thrombosis was probable in the

survivors, inasmuch as clinical thrombosis was as frequent in them as in those who died. This has since been confirmed by clinical venographic studies of the legs.[3,5]

Venous dissection also showed that bilateral thrombi are usual when thrombosis is silent in both limbs and are even more frequent when one limb is swollen. Thus, clinical thrombosis in one limb should be taken as evidence of bilateral thrombosis.

The factors influencing the onset or absence of leg swelling and other symptoms in subjects with deep-vein thrombosis are complex, and only some of them are understood. Of importance for swelling are the site and extent of thrombosis, the degree of vein blockage, thrombus retraction and contraction, adherence of the thrombus to the vein wall, the effects of gravity, and the efficiency of anastomotic channels.[15] Vein dissections at necropsy have shown that leg swelling is associated with confluent thrombosis of the main venous channels of the thigh and leg veins in the majority of cases or with extensive but nonconfluent thrombosis in a minority. However, extensive or even confluent thrombosis in the thigh and leg was also found in some without leg swelling, so that extensive thrombosis *per se* (especially in the iliofemoral vein) is necessary but not sufficient for swelling. Extensive thrombi are not necessarily occlusive; as already noted, they often lie in the middle of the vein attached only distally at one or two points. This, together with the natural retraction and contraction of thrombi and the presence of collateral channels, explains the lack of significant blockage of flow and of limb swelling in many cases. Retrograde thrombosis, which may follow growth by propagation, is probably important for swelling, because it often produces a long, continuous thrombosed segment that reduces drainage through anastomotic channels.

Dependence of the limbs and the effects of gravity on an already handicapped venous return are certainly influential. Leg swelling does not occur in many subjects during confinement to bed, but first appears when the patient sits out on a chair or begins to walk. Precipitation of swelling by the dependent position explains the relative infrequency of clinical thrombosis in elderly patients with fractured hips who undergo prolonged bedrest, compared with a much higher incidence in those with a period of bedrest that is shorter but sufficiently long to allow thrombi to form. Thrombogenesis seemed equal in both groups, in that deep-vein thrombi were equally frequent among those who reached necropsy.

There are probably mechanical and morphologic factors, as well. Partial venous obstruction is not likely to produce edema, unless and until the effect of gravity is added. Moreover, a recent thrombus may be able to elongate in the horizontal position and in the direction of blood flow, because its attachments are distal and small; thus, it would permit

the onward flow of blood around it (Figure 5). In the vertical position, the thrombus may become shorter and squatter, owing to gravity; then the venous flow is more likely to become obstructed. Of course, when thrombi seriously obstruct main venous channels, swelling occurs in the horizontal position of the limbs, but often worsens with the vertical position—hence, the value of bedrest for reducing leg edema.

Silent thrombosis explains the phenomenon of unheralded embolism, i.e., pulmonary embolism without clinical symptoms or signs referable to the limbs. Figures 6 and 7 illustrate such a case. A woman 72 years old died suddenly 17 days after admission to the hospital with a fracture of the odontoid. Death was due to a large thromboembolus in the right pulmonary artery. Dissection of the main veins of the legs (Figure 6) showed extensive thrombosis in one iliofemoral vein but no thrombi in the other. It had probably become dislodged and caused the fatal embolism. In spite of the extensive thrombosis, the legs and thighs showed no swelling (Figure 7).

At least half the cases of fatal embolism occur in patients without clinical thrombosis, and that is the basis of the superiority of anticoagulant prophylaxis over treatment.[17,19] Obviously, even effective therapy of every clinical case of thrombosis cannot prevent embolism in those without limb signs. Even in patients with clinical thrombosis in one limb, fatal emboli may arise from veins in the other silently thrombosed limb. Indeed, either limb is equally likely to provide the embolus; that is understandable in view of the finding that thrombosis is bilateral in about 80% of patients with major emboli at necropsy.

COAGULATION, PLATELETS, AND THROMBOSIS AFTER TRAUMA

The existence of a hypercoagulable state in the pathogenesis of venous thrombosis is controversial, and little is known of the special clotting

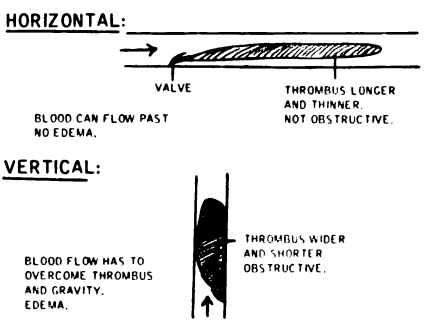

FIGURE 5 Postulated effect of the horizontal and vertical positions of a leg on the shape of a thrombus and on the venous flow.

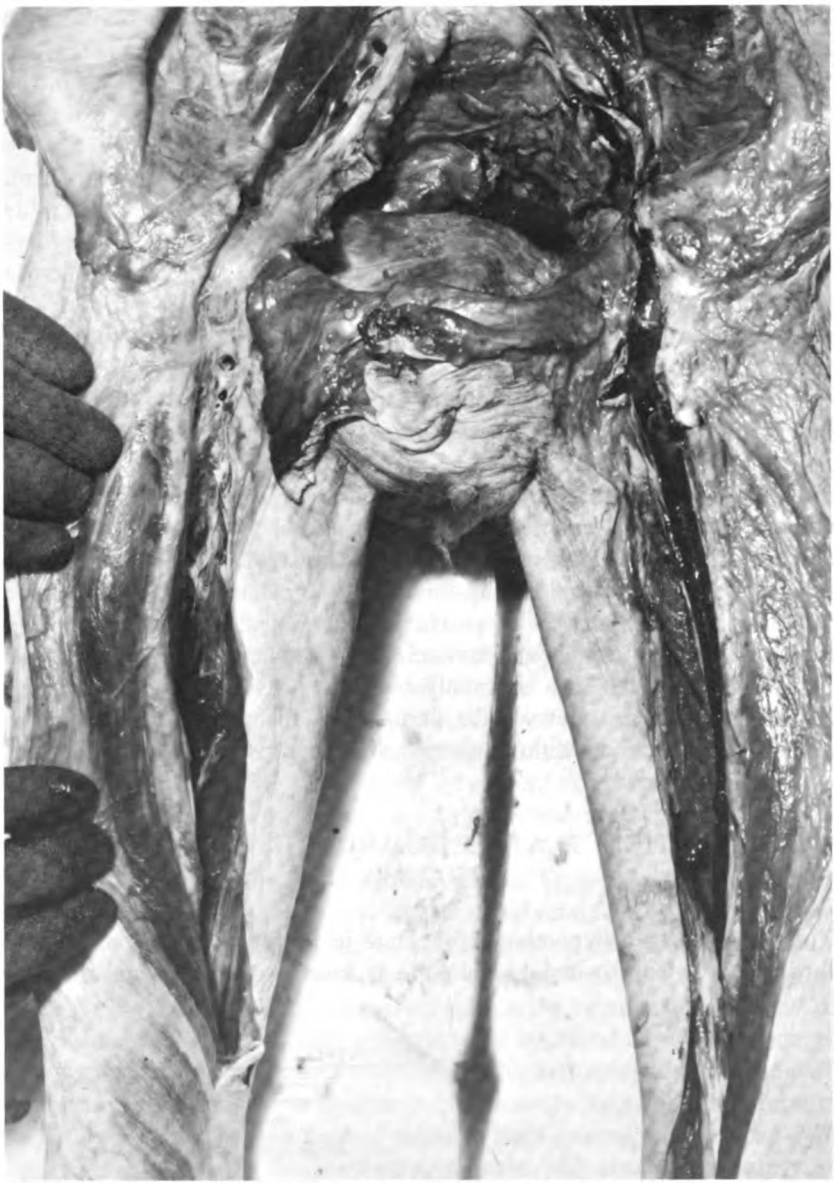

FIGURE 6 A case of silent thrombosis. Continuous iliofemoral thrombus of the left leg with none in the right leg (a fatal embolus came from the right leg).

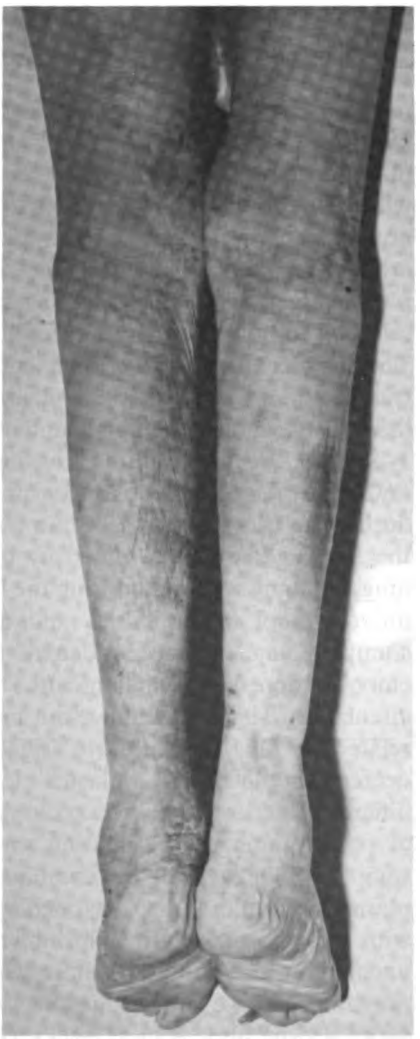

FIGURE 7 The lower legs of the patient with extensive venous thrombosis shown in Figure 6. Note the absence of calf or thigh swelling (silent thrombosis).

conditions on which venous thrombosis depends, if indeed such conditions exist. One or more phases of hypercoagulability might account for the initiation of thrombosis in the veins of particular groups of subjects, such as women taking contraceptive pills and susceptible ambulant subjects. These groups require special study. Gurewich and Thomas [8] considered that venous thrombosis occurs when stasis permits the local accumulation of normal intermediate products of blood coagulation and prevents rapid clearance of their activity by passage through the liver.

Hypercoagulability may have no relevance in most injured subjects with thrombosis, even though some, especially those with major trauma, develop an early transient hypercoagulable phase. This conclusion was indicated by serial *in vivo* studies on coagulation and fibrinolysis in animals [2] and injured persons [10] and correlation of the findings with microthrombi in the lungs and venous thrombi in the limbs of patients who died after trauma.[4] The first few hours after severe injury were dominated by a phase of acute hypercoagulability manifest as an acceleration of whole-blood clotting time, and that was associated with activated plasma fibrinolysis (Figure 8). These changes were followed by an abrupt rebound to normal or prolonged clotting time and inhibited fibrinolysis. The early changes were not detectable in those with moderate or minor trauma, even though they are eligible for venous thrombosis and embolism. The platelet count fell and the fall continued or accelerated for 1–3 days. Various plasma clotting factors also diminished, and the findings were consistent with consumption of platelets and clotting factors during an acute episode of intravascular thrombosis. Experimentally, these changes were prevented by prior administration of heparin.[2] The lungs of many who died during the first day or two of injury showed tiny microthrombi in capillaries and arterioles (Figure 9), composed of clumped platelets and condensed fibrin. These were probably microemboli trapped in the lung after microthrombogenesis in the venous circulation. Their appearance during the first day or two (Figure 10) was related in time to the early triggering of hypercoagulability and the decrease in platelets and plasma clotting factors. The later changes were different: clotting time was prolonged or normal, and the consumption of prothrombin during clotting was reduced (raised prothrombin-consumption index), with the combination indicating a defect in thromboplastin generation. This "hypocoagulable state" was usually associated with inhibition of plasma fibrinolytic activity. Tiny microthrombi were uncommon in the lungs of those who died days after injury, but arterial microthrombi began to appear, with the frequency increasing with survival time after 1 or 2 weeks (Figure 10). These had the structure of pulmonary thromboemboli and were often associated with macroscopic emboli and almost always with deep-vein thrombi in the lower venous tree. The separation of early tiny (capillary) microthrombi from later arterial microthrombi (pulmonary emboli) seems significant. The former appear related to early posttraumatic hypercoagulability, whereas pulmonary emboli, small or large, and the deep-vein thrombi from which they arise may develop in these subjects during the subsequent "hypocoagulable" period. The associated inhibited fibrinolysis may play a role, in that deep-vein thrombogenesis is not opposed by significant thrombolysis.

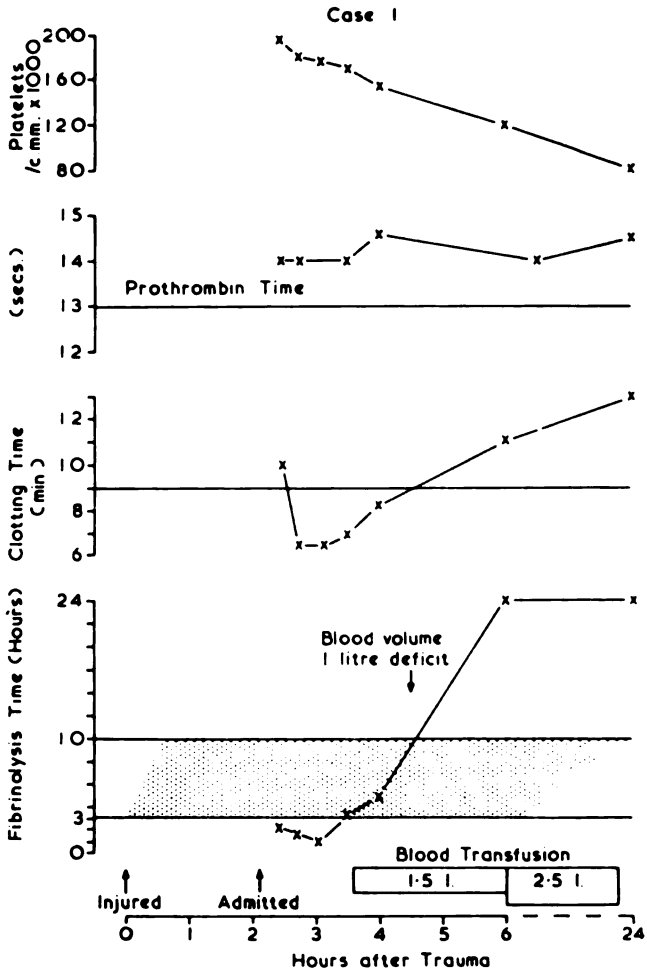

FIGURE 8 Serial changes in fibrinolysis time, clotting time (in plastic tubes), prothrombin time, and platelet count in a man 23 years old with fractures of both femurs and the pelvis. The horizontal lines represent normal limits for fibrinolysis time, the low normal limit for clotting time, and the upper normal limits for prothrombin time.

The blood platelet level rises 2 or 3 days after injury, and relatively high levels are often attained during the next week or two.[10] The thrombocytosis develops at a time when deep-vein thrombi are found (Table 2); although this is consistent with a relationship between thrombosis and platelets, the association is not necessarily causal.

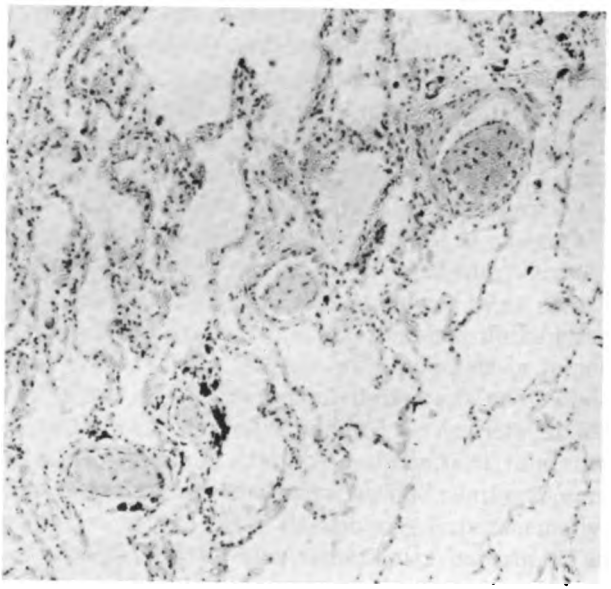

FIGURE 9 Pulmonary microthrombi in small arterioles and capillaries. Death came 36 hr after injury. Hematoxylin and eosin. (×75)

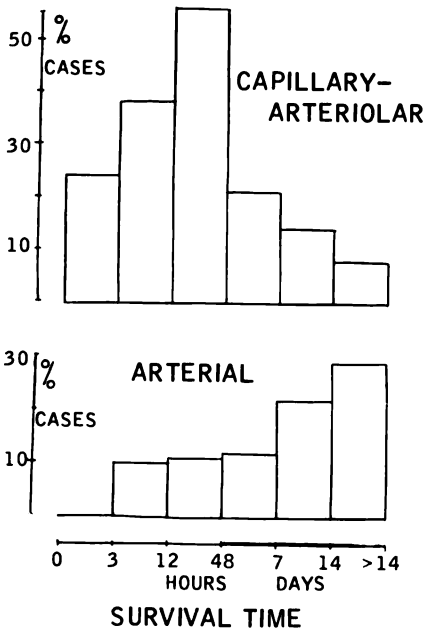

FIGURE 10 Frequency of capillary (capillary-arteriolar) and arterial microthrombosis in the lungs of injured subjects related to survival time. Note the high frequency of tiny capillary microthrombi during the first 2 days and the increased incidence of arterial microthrombi after 7 days.

The role of platelets in the initiation of deep-vein thrombi is unproved, but they are probably important at least for propagation: thromboplastin released from platelets aggregated on the surface of the thrombus would set in motion the blood-clotting process and release thrombin. Even in low concentration, the thrombin formed would aggregate more platelets and deposit them on the surface of the thrombus; fibrin would be formed when thrombin concentration was sufficient. The process would be repeated again and again, provided that the local venous flow was sufficiently slowed to prevent the rapid carrying-off of platelet thromboplastin and thrombin.

Surprisingly little is known of the structure of early tiny thrombi in valve cusps. Preliminary studies have shown that fibrin is usually present in the oldest parts of the thrombus, i.e., at its contact with the crypt of the valve pocket, but significant platelet collection may or may not be present. Other evidence in man points to the importance of fibrin in the formation or at least stabilization of thrombus-nidi. This was obtained through dissection of the lower venous tree in injured subjects who were known to be susceptible to thrombosis and were maintained on oral anticoagulant prophylaxis from soon after injury until death.[19] Thrombi, small or large, were absent at necropsy in the great majority, but a few subjects showed one or two valve-cusp or other small thrombi that could be attributed to the onset of thrombus formation before effective drug action cut the process short. The absence of small thrombi in most patients given anticoagulant prophylaxis suggests that platelet clumps are not essential for the initiation of thrombosis. However, the evidence is not conclusive, inasmuch as platelet aggregation can be a reversible process and requires fibrin for stabilization. Thus, small nidi of platelets may have formed and later dispersed, leaving no trace of thrombosis at necropsy.

SUMMARY

Venous thrombosis was investigated in injured subjects by dissection of the lower venous tree at necropsy. Thrombi were frequent, were related mainly to age and survival period (bedrest), and were particularly common in middle-aged and elderly subjects confined to bed for more than a few days. The nature of the injury had little direct influence. Thrombi were usually bilateral when present, even in cases with unilateral leg injury, and in most cases they were symptom-free. Thrombi occur independently in veins of the calf, thigh, and pelvis but most commonly in calf veins and in the iliofemoral channel. Thrombi begin to form in one or more of six independent sites, *viz.*, soleal veins, posterior tibial

veins, popliteal vein, deep femoral vein, common femoral vein, and external iliac vein. Venous stasis from recumbency, immobility, and age, together with the peculiarities of venous anatomy, determine when and where thrombi will begin to form. There is no evidence that posttraumatic hypercoagulability plays a role in producing deep-vein thrombi. Posttraumatic coagulation changes, including the early fall in platelets and reduction in plasma clotting factors, seem related to a different form of thrombosis manifest at necropsy as tiny microthrombi in the lungs. These appear within a day or so of severe trauma, unlike pulmonary emboli and deep-vein thrombi, which appear later and are unrelated to the severity of injury.

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Venous Thromboembolism in Surgical Patients

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The gravity of the problem of postoperative venous thromboembolism has become widely recognized since the original report of a pulmonary embolus after ovariotomy by Spencer Wells in 1866.[9] In 1944, Bauer [4] called attention to the frequency of venous thrombosis in surgical patients, especially after trauma to the legs. A more recent review of 748 cases of phlebitis at the Boston City Hospital [7] found the postoperative state to be the second most common predisposing factor, only heart disease being more commonly associated. Thrombotic complications were particularly frequent in surgical patients with malignant disease, with operations involving the pelvic viscera, or with trauma of the legs. The early literature on thromboembolism in surgical patients was reviewed in 1954 by De Bakey,[6] who emphasized the variability in reported incidence in different clinics and even in different years in the same clinic. Although De Bakey reported the same incidence of thromboembolism in operative as in nonoperative cases on the same surgical service, other authors have claimed a clear relationship between the operative incident and the subsequent development of a thrombotic complication. For example, Evoy [12] examined the time of occurrence of 111 fatal pulmonary emboli in postoperative patients and reported the maximum incidence on the 7th postoperative day; most of the episodes that occurred earlier were in debilitated patients who were confined to bed before operation. Eighty percent of fatal pulmonary emboli in this series occurred within 14 days of operation.

The development of intravascular thrombi is favored by many characteristics of the postoperative state. Direct trauma to vessels in a field of dissection is followed by changes in various constituents of the blood: increases in the number of platelets [8] and in platelet adhesiveness,[11,22] increases in plasma level of fibrinogen [21] and in the concentration of

other clotting factors,[1,10] and alterations in the fibrinolytic system.[14,16] The tendency toward thrombosis is increased in the legs by reduction in linear blood flow because of bedrest, abdominal binders, or abdominal distention, and sometimes by an increase in the viscosity of the blood secondary to dehydration.

The ability of prophylactic anticoagulation to prevent venous thrombosis and pulmonary embolism, which we have demonstrated in several categories of patients,[15,19,20] makes it important to identify within a surgical population the patients most likely to develop thrombotic complications. If some surgical patients have a greater risk of developing venous thrombosis than others, the recognition of predisposing causes might make it possible to anticoagulate only the patients who are particularly prone to thromboembolism, and so to spare the low-risk patients, if any, the hazards of anticoagulation.

Study of prophylactic anticoagulation in general surgical patients [20] has afforded the opportunity to examine the significance of various predisposing influences in such a patient group. In the course of 1 year, 1223 patients entered the participating surgical service and were considered for prophylactic anticoagulation. On the basis of previous reports, we attempted to predict which patients in this group were at high risk by virtue of manifesting conditions thought to predispose to thromboembolism, including prolonged bedrest, malignant disease, venous stasis, and local trauma (e.g., pelvic surgery).[2-7,9,13,15,17,18]

The composition of the predicted high-risk group in the study of general surgical patients is shown in Table 1. Patients in this group were given warfarin as a prophylactic anticoagulant unless they also had some contraindication to therapy, such as an active peptic ulcer or other predisposition to bleeding. The balance of the patients were tentatively considered as the low-risk group and did not receive anticoagulant prophylaxis, because they appeared to have no specific indication. The course of these patients is a measure of our success in predicting venous

TABLE 1 Indications for Prophylactic Anticoagulation (High-Risk Patients)

	Treated Group	Contraindicated Group	Total
Total no. patients	336	80	416
Bedrest	220	58	278
Obesity	70	13	83
Pelvic surgery	50	16	66
Visceral carcinoma	57	27	84
Previous thromboembolism	8	2	10

thromboembolism and the factors that predispose to it. Patients in the high-risk group who did not receive anticoagulants because of a contraindication to therapy had a 10% incidence of venous thromboembolism; the incidence in high-risk patients who were anticoagulated was 1.7%, and the incidence in the low-risk group was 1%. This experience supports the concept of a high-risk population.

Further insight is furnished by detailed consideration of patients in the so-called low-risk group who did in fact develop thromboembolism. There were eight such patients among a group of 807; three died with a pulmonary embolus (Table 2). Three of the eight patients had malignant disease, three were bedfast, and two were obese. They were not prophylactically anticoagulated because their indications were erroneously considered equivocal, or, in two cases, because they died while awaiting operation after a period of bedrest before entry into the hospital. Thus, a more strict enforcement of the prescribed indications for prophylactic anticoagulation would have resulted in assignment of all eight of these patients to the high-risk group and might have prevented all eight cases of thromboembolism in patients originally thought to be at low risk.

In summary, there is reason to believe that a predisposition to venous thromboembolism can be predicted with reasonable success in surgical patients, and that such predictions can be effectively used in the application of prophylactic anticoagulation and perhaps other preventive measures.

TABLE 2 Thromboembolism in Patients Assigned to Low-Risk Group

Diagnosis	Complication	Result
1. Carcinoma of stomach	Pulmonary embolus	Died
2. Carcinoma of stomach	Pulmonary embolus	Died (preoperatively)
3. Hiatus hernia, aspiration pneumonia (bedrest)	Pulmonary embolus	Died (preoperatively)
4. Ruptured appendix and pelvic abscess (bedrest)	Pulmonary embolus	Survived
5. Carcinomatosis	Pulmonary embolus	Survived
6. Obesity	Pulmonary embolus	Survived
7. Obesity	Pulmonary embolus	Survived
8. Aortic atherosclerosis (bedrest)	Thrombophlebitis	Survived

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Thrombophlebitis during Pregnancy and the Puerperium

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Thrombophlebitis is a serious problem when it occurs during the antepartum and postpartum periods, but the incidence of such occurrences has been difficult to determine. Findley [10] in 1912 gave one of the earliest accounts of the occurrence of thrombophlebitis associated with pregnancy. In 1945, Yahr *et al.*[37] reported the use of anticoagulant drugs during pregnancy. Ullery [32] in 1954 reviewed 135 published reports of cases of thromboembolism associated with pregnancy, including nine reported from his own experience. He recorded 15 deaths from pulmonary emboli among the 97 patients who did not receive anticoagulants, but noted that no pulmonary emboli had occurred in the 38 patients who were treated with anticoagulants. Finnerty and MacKay [11] in 1962 collected 208 reported instances of antepartum thrombophlebitis, including 57 from one institution.[23] By 1964, the number of reported cases had risen to approximately 300.[33] The reported incidence of thrombophlebitis associated with pregnancy has varied widely from 0.002 to 0.500.[38]

NEW YORK LYING-IN HOSPITAL SERIES

During the years 1945–1966, there were 76,473 live births at New York Lying-In Hospital. Of the mothers represented, 153 had a diagnosis of antepartum thrombophlebitis, and 815 developed postpartum thrombophlebitis. Thus, the incidence was 0.002 for the antepartum group and 0.0106 for the postpartum group. The data on 462 of these patients, who had 536 episodes of thrombophlebitis, were available for analysis and form the basis of this preliminary report.

The distribution by age and admission status is shown in Table 1. There were 64 instances of thrombophlebitis during pregnancy and 398

TABLE 1 Previous Pregnancies and Admission Status of 462 Patients with Thrombophlebitis Associated with Pregnancy (New York Lying-In Hospital, 1945–1966)

No. Previous Pregnancies	No. Patients			
	Antepartum		Postpartum	
	Age, years		Age, years	
	<25	⩾25	<25	⩾25
0	12	3	26	24
1	3	13	35	56
2	2	15	22	85
3	2	4	10	52
4	1	5	3	43
5	0	0	2	14
6	0	2	0	13
7	0	1	0	7
8	0	1	0	3
9 or more	0	0	0	3
TOTAL	20	44	98	300

after pregnancy. Thrombophlebitis appears to be more common in the older groups both during and after pregnancy.

The incidence of previous history of varicose veins is shown in Table 2. Unfortunately, the incidence of varicosities among the total admissions to this service is not known. In the antepartum group, only one of the 20 younger patients gave a history of having had a previous episode of

TABLE 2 Incidence of Varicose Veins in 462 Patients with Thrombophlebitis Associated with Pregnancy (New York Lying-In Hospital, 1945–1966)

Degree of Varicosity	No. Patients			
	Antepartum		Postpartum	
	Age, years		Age, years	
	<25	⩾25	<25	⩾25
Slight	2	11	23	58
Moderate	2	12	21	82
Severe	1	10	11	50
TOTAL	5	33	55	190
	(25%)	(75%)	(56%)	(63%)
No information	0	0	2	6

thrombophlebitis, but eight (18%) of the 44 older patients gave such a history. In the postpartum group, eight (8%) of the 98 younger patients had experienced a previous episode of thrombophlebitis, but 42 (14%) of the 300 older patients had had thrombophlebitis.

The locations of the thrombophlebitis are listed in Table 3. In some patients in each group (129 of the 462), more than one site was involved. Superficial unilateral thrombophlebitis was the most common in all groups; deep phlebitis, both unilateral and bilateral, occurred at a higher incidence among the younger antepartum patients. Thromboembolic complications among the 462 patients are summarized in Table 4. The statistical significance of associated diseases could not be evaluated, but it is interesting to note that iron-deficiency anemia was more common among the younger postpartum patients with thrombophlebitis than among the older patients. Obesity and diabetes were more prevalent in the older postpartum group. The complications of pregnancy—including hypertension, eclampsia, and pre-eclampsia—occurred most frequently among the older postpartum group.

Table 5 lists the known recurrences of thrombophlebitis. It was impossible to obtain full and accurate information, because 75 follow-up records were incomplete beyond 6 weeks, and no follow-up was available for 103 patients (Table 6). Therefore, the listed 74 recurrences in Table 5 were in 54 patients among the 284 who were followed for 1–15 years. This represents roughly a 20% incidence of recurrences, a figure which suggests that this is no minor problem.

At the last follow-up, which in 103 patients was the time of discharge, 140 of the 462 had no evidence of varicosities; 292 (63.2%) had superficial varicosities; two had vulval; nine had both vulval and superficial;

TABLE 3 Location of Thrombophlebitis Associated with Pregnancy in 462 Patients (New York Lying-In Hospital, 1945–1966)

	No. Patients			
	Antepartum		Postpartum	
	Age, years		Age, years	
Location	<25	≥25	<25	≥25
Superficial unilateral	11	30	77	233
Superficial bilateral	0	1	12	29
Deep unilateral	6	6	5	24
Deep bilateral	7	6	6	22
Lesser saphenous	0	7	5	10
Pelvic veins	0	2	1	3

TABLE 4 Thromboembolic Complications Associated with Pregnancy in 462 Patients (New York Lying-In Hospital, 1945–1966)

	No. Patients			
	Antepartum		Postpartum	
	Age, years		Age, years	
Complication	<25	⩾25	<25	⩾25
None	19	38	91	286
Extension of thrombophlebitis	0	3	6	9
Pulmonary emboli	1	3	1	5
Death	0	0	0	0

and 16 had evidence of postphlebitic syndrome, six of whom also had ulcers. Twelve (60%) of the younger antepartum patients had some form of phlebitic sequelae. Among the patients in the other three groups who could be followed, the total incidence of phlebitic sequelae was 61% for the older antepartum, 54% for the younger postpartum, and 63% for the older postpartum patients.

Husni *et al.*[16] reported that 90% of their patients (both antepartum and postpartum) with thrombophlebitis were between 20 and 40 years old, but they did not consider age as an etiologic factor. Of the patients reported by McElin *et al.*,[18] 50% were over 30, and only one was under 25. The majority of episodes occurred during the last trimester of pregnancy, or within 2 weeks following delivery. No seasonal influence

TABLE 5 Recurrence of Thrombophlebitis Associated with Pregnancy in 462 Patients during Follow-up (New York Lying-In Hospital, 1945–1966)

	No. Patients			
	Antepartum		Postpartum	
	Age, years		Age, years	
No. Recurrences	<25	⩾25	<25	⩾25
1	2	8	12	21
2	0	1	1	6
3	0	0	0	1
4	0	0	0	0
5	0	0	0	0
6	0	0	1	1
No history	13	28	66	206

TABLE 6 Duration of Follow-up of 462 Patients with Thrombophlebitis Associated with Pregnancy (New York Lying-In Hospital, 1945–1966)

Duration of Follow-up	No. Patients			
	Antepartum		Postpartum	
	Age, years		Age, years	
	<25	⩾25	<25	⩾25
6 weeks	2	5	20	48
1 year	2	6	16	49
2 years	1	2	11	20
3 years	3	6	16	37
5 years	3	4	7	26
7 years	0	4	5	22
10 years	3	4	3	21
15 years	0	2	4	7
No follow-up	6	11	16	70

was apparent. Although the majority of occurrences of thrombophlebitis associated with pregnancy involve veins of the legs (left more often than right),[22,38] it is obvious that veins of any area may be involved. Pelvic [27] and vulval and vaginal [9] veins are more commonly involved than is generally appreciated. Infrequently, cerebral,[12] visceral,[17] and ovarian [2,26] veins are involved.

Carroll *et al.*[6] have recently redirected attention to the occurrence of cerebral thrombophlebitis. They have collected 158 cases that occurred during pregnancy and the postpartum period. Thirteen patients had cerebral thrombophlebitis during the first trimester, and five during the second. Of the cases during the third trimester and the puerperium, 21 were before labor, and 119 were in the postpartum period. The mortality rate was 33% for this entire series, with the highest rate in the first trimester. Rath and Chandy [25] have reported a similar mortality rate in their series of 20 patients with primary intracranial cortical venous thrombosis during the postpartum period. Batson [3] demonstrated in 1940 that radiopaque fluid can move from pelvic veins through vertebral veins to the dural sinuses. McKay and colleagues [20] advanced the theory that thrombosis of the vessels that supply the pituitary may be responsible for hemorrhage and shock, pituitary necrosis, and the diabetes insipidus that sometimes accompanies anterior and posterior pituitary lobe insufficiency. This concept of initial thrombosis of cerebral vessels reverses the concept of Sheehan and Murdoch,[29] who postulated that hemorrhage initiated these complications.

The incidence of postpartum thrombophlebitis has been estimated by the Cincinnati General Hospital as 1165 cases per million (on the basis of 39,491 consecutive deliveries)[30] and by Dr. Kohl, Director of Obstetrical and Statistical Cooperative of New York, as 3512 per million (on the basis of 209,527 deliveries). These figures are derived from hospitalized pregnant women, but many patients develop superficial thrombophlebitis after discharge from the hospital and do not see a physician. Patients who develop deep thrombophlebitis may be admitted to another hospital or another service of the same hospital and thus may not be coded as "postpartum thrombophlebitis." Indeed, postpartum deaths from this cause may go unreported as such. Wright [36] cites three instances of maternal death en route from a hospital in a taxi and a fourth instance in a woman who died on her own doorstep, all from pulmonary emboli.

Data obtained from the Dominion Bureau of Statistics of Canada, for the Conference on Thromboembolic Phenomena in Women,[30] are presented in Table 7. Ochsner and his colleagues [22] analyzed thromboembolism found among 647,868 patients admitted to Charity Hospital in New Orleans from July 1938 through June 1950. The lowest incidence of thromboembolism was found on the obstetric service, 106 cases per 100,000 admissions. Zilliacus [38] in 1946 reported the incidence of deep venous thrombosis from three maternity hospitals in Sweden. The figures from two of them are pertinent to this discussion. At hospital A, in 1939–1945, there were 16,377 admissions. Deep venous thrombosis or sudden pulmonary embolism developed in 60 patients (0.37%). Pulmonary embolism occurred in 15 (25%) of the 60 patients; one was fatal. In hospital B, in 1940–1944, there were 24,882 admissions. There were 121 cases of deep thrombosis or sudden pulmonary embolism (0.49%). There were 22 pulmonary emboli, three of which were fatal.

Data regarding maternal mortality from pulmonary embolism obtainable from the Bureau of Vital Statistics of the United States are not separated into antepartum and postpartum periods. However, Finnerty and MacKay [11] have reported 208 antepartum patients who had 36 pulmonary emboli (Table 8). The 16 maternal deaths from this cause were all among the 135 patients who did not receive anticoagulants. Although there were four pulmonary emboli among the 73 patients who did receive anticoagulants, there were no maternal deaths among them, but there were 12 fetal deaths, eight of which were attributed to hemorrhage. Because fetal deaths from hemorrhage during anticoagulant therapy have been reported by several authors,[8,15,23,35] it should be remembered that coumarin compounds, by the nature of their low molecular weights, are able to pass the placental and milk barriers. Heparin, with a molecular weight of 20,000, cannot.

TABLE 7 Thromboembolic Phenomena in Women (Data from Dominion Bureau of Statistics of Canada)

	Age, years					
	15–19	20–24	25–29	30–34	35–39	40–44
Covered population (female)	35,628	27,767	26,398	27,997	28,725	27,466
No. pregnancies	2,462	7,312	6,263	4,372	2,642	855
Phlebitis, thrombophlebitis, legs (during pregnancy)		2	6	9	8	1
Rate [a]		27.4	95.8	205.8	302.8	117.0
Phlebitis, thrombophlebitis, other sites (during pregnancy)	1	3	2	1	3	2
Rate [a]	40.6	41.0	31.9	22.9	113.6	233.9
Total phlebitis: thrombophlebitis, pulmonary embolism (during pregnancy)	1	5	9	10	11	3
Rate [a]	40.6	68.4	143.7	228.7	416.4	350.9
Puerperal phlebitis and thrombophlebitis	1	—	3	2	2	2
Rate [a]	40.6	—	47.9	45.7	75.7	233.9

[a] Rate/100,000 pregnancies.

TABLE 8 Antepartum Thrombophlebitis [a]

	No. Patients	No. Patients with Pulmonary Emboli	No. Maternal Deaths	No. Fetal Deaths	No. Fetal Deaths Due to Anticoagulants
Not anticoagulated	135	32	16	18	0
Anticoagulated	73	4	0	12	8

[a] Derived from Finnerty and MacKay.[11]

Breckenridge and Ratnoff [5] have analyzed the necropsy records of the Coroner's Office of Cuyahoga County, Ohio, for the period January 1951 through August 1962. There were 26 cases of unexpected death from pulmonary embolism in otherwise normal persons. Among these 26 deaths, five were in women in the first trimester of pregnancy. The incidence of unexpected death due to pulmonary emboli was estimated to be 2.7 per million per year for males, 3.7 per million per year for females, and, based on the number of live births for 1959 (22,600 was the number of pregnancies), 18 per million pregnancies per year.

ETIOLOGIC FACTORS

The following list includes most etiologic factors contributing to thrombophlebitis during pregnancy and the puerperium:

1. Varicose veins
2. Increasing age and parity
3. Trauma (the injury may be minor, such as a twisted ankle, or caused by constricting garments, forceps delivery, or prolonged pressure in stirrups)
4. Some physiologic changes during pregnancy, including (a) increasing plasma volume, (b) increasing venous distensibility, (c) change in state of vessel walls, (d) decrease in laminar flow, (e) increasing fetal size, (f) coagulation factor changes (including X, VIII, VII, and fibrinogen) and postpartum increase in platelets, suppression of the fibrinolytic system, and release of tissue thromboplastin from the placenta
5. Anemia (actual iron-deficiency anemia, not only dilution)
6. Associated disease (such as diabetes, hypertension, toxemia of pregnancy, cardiac disease, and systemic lupus erythematosus)
7. Obesity
8. Suppression of lactation
9. Possible genetic factors

Most of these predisposing conditions need no further discussion. However, recent studies do merit attention. Goodrich and Wood [13,14] have shown that venous distensibility occurs in the veins of arms and legs before the increased pressure from the growing fetus can play a part. They have also demonstrated changes in laminar flow that contribute to stasis.

Changes in the coagulation factors [1,31] have been well documented in recent years, as have platelet increase in the postpartum period and sup-

pression of the fibrinolytic system during pregnancy.[8,28,34] Both Quick[24] and McKay[19] stress the importance of the state of the blood-vessel wall, perhaps some focal irritation, as a determinant of the occurrence and location of thrombosis. Puzos and others cited by Morrell [21] proposed in the eighteenth century that unconsumed mother's milk is directed to the legs to account for what was later called "milk leg" and is now known as thrombophlebitis. It remained for Daniel *et al.*[7] in 1967 to report statistics that suggest that suppression of lactation significantly increases the risk of thromboembolism. They studied the incidence of thromboembolism in Cardiff, Wales, in all mothers delivered of a single child in 1956–1966. Among the nonlactating mothers—i.e., those receiving diethylstilbestrol to suppress lactation—there were four times as many thromboembolic episodes as among lactating mothers (5.1 versus 1.2 per 1000).

The work reported here was supported by Public Health Service grant 5-RO1 HE 10530 from the National Heart Institute and grants from the Wallace Fund, the Albert and Mary Lasker Foundation, the Stella and Charles Guttman Foundation, and the Cornell Medical College Vascular Research Fund.

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Thromboembolic Complications Following Gynecologic Operations: Role of Prophylactic Anticoagulant Therapy

ELLEN MCDEVITT

The incidence of postoperative thromboembolic complications following gynecologic operations has been repeatedly emphasized. Ochsner *et al.*[4] reviewed their experience on the Gynecological Service of Charity Hospital. The total admissions to the Gynecological Service between 1938 and 1950 were 348 per 100,000 admissions. The incidence rate for all pulmonary emboli was 152 and for fatal emboli, 94 per 100,000 cases on that service. For 40% of fatal emboli, the authors were unable to find any clinical evidence of antecedent thrombus.

In a series reported by Zilliacus [5] in Sweden covering the years 1940–1944, among 14,204 patients, 60 (0.42%) developed thrombosis, and of these 16 (26.7%) had pulmonary emboli. Two of the 16 died.

Allen *et al.*,[1] in the first edition (1946) of their classic monograph on peripheral vascular diseases, reviewed their experience at the Mayo Clinic in successive gynecologic operations. This review is summarized in Table 1. Among the 5730 patients who underwent abdominal hysterectomies, 230 (4.01%) developed either thrombophlebitis or embolism. Eighty-seven of the 230 had one or more pulmonary emboli, and 42 died. In 163 of the 230 patients a clinical diagnosis of thrombophlebitis was made. Among 1192 who underwent vaginal hysterectomies, 30 (2.52%) had some type of thromboembolism: 14 of the 30 (1.17%) had pulmonary emboli, and six died; 22 of the 30 had clinical evidence of thrombophlebitis. Among 141 patients who underwent cesarean section, four developed evidence of thromboembolism. One pulmonary embolus resulted in death. Among 4048 patients who underwent other gynecologic operations, 75 (1.85%) developed thromboemboli. Pulmonary emboli occurred in 28, and 10 died. Thus, it is clear in this series that, when a form of thromboembolism occurred, the mortality

TABLE 1 Thromboembolic Complications of Gynecologic Operations

Type of Operation	Total Patients Having Operations	Complications			
		Thrombophlebitis or Embolism [a]	Pulmonary Embolism	Fatal Pulmonary Embolism	Thrombophlebitis
Abdominal hysterectomy	5730	230 (4.01%)	87 (1.52%)	42 (0.73%)	163 (2.84%)
Vaginal hysterectomy	1192	30 (2.52%)	14 (1.17%)	6 (0.50%)	22 (1.85%)
Cesarean section	141	4 (2.84%)	1 (0.71%)	1 (0.71%)	3 (2.13%)
Other gynecologic operations	4048	75 (1.85%)	28 (0.69%)	10 (0.25%)	56 (1.38%)

[a] Derived from Allen *et al.*[1]

TABLE 2 Results of Prophylactic Use of Dicumarol in 832 Cases of Abdominal Hysterectomy [a]

	No. Cases Expected	No. Actual Cases
Venous thrombosis	33	3 [b]
Fatal pulmonary emboli	6	0

[a] Derived from Allen *et al.*[2]
[b] Minor.

rate was high, approaching 50% once a pulmonary embolism had occurred.

Subsequently, Allen *et al.*,[2] reported the result of the prophylactic use of oral anticoagulants (dicumarol) in 832 cases of abdominal hysterectomy. On the basis of their previous experience at the Mayo Clinic, 33 episodes of thromboembolism were expected. Actually, three did occur, and they were minor (Table 2). Six fatal pulmonary emboli were expected; actually, there were none.

At the Dijkzigt Conference on the Prevention of Thromboembolism in Surgery (1961), Chalmers [3] listed his criteria for the prophylactic use of anticoagulants: (1) major gynecologic operation in a patient over 40, (2) cesarean section, (3) operative procedure for malignant disease of the genital tract, or (4) gynecologic or obstetric history of previous thrombosis. A summary of his experience is shown in Table 3.

Among a total of 4574 patients who underwent gynecologic operations in 1949–1953, there were nine fatal emboli, an incidence of 1.8 per

TABLE 3 Thromboembolism Following Gynecologic Operations with and without Prophylactic Anticoagulants [a]

1949–1953 (prophylactic anticoagulants not used):	
No. patients	4574
Fatal thromboembolism	9
number/year	1.8
number/100 patients	0.2
1953–1960 (prophylactic anticoagulants used):	
No. patients	7233
Fatal thromboembolism	2
number/year	0.28
number/100 patients	0.028

[a] Derived from Chalmers.[3]

year, or 0.2 per 100 patients. In 1953–1960, anticoagulants were used prophylactically. Among 7233 patients who underwent gynecologic operations, there were two fatalities from emboli, an incidence of 0.28 per year, or 0.028 per 100 patients.

The role of anticoagulants in the suppression of thromboembolism in gynecologic operative procedures has been established for almost 20 years. It hardly seems necessary to stress the point, but the mortality figures continue to reflect the neglect of this prophylactic measure.

The work reported here was supported by Public Health Service grant HE 10530 from the National Heart Institute and grants from the Wallace Fund, the Albert and Mary Lasker Foundation, the Stella and Charles Guttman Foundation, and the Cornell Medical College Vascular Research Fund.

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Fibrination–Fibrinolysis: Toxemia of Pregnancy, "Obstetric Shock," and Exsanguinating Diatheses

CHARLES L. SCHNEIDER

Delicacy of attachment of the placenta to the uterus predisposes to fibrination (during abruptio placentae) and foreign debris microembolism (during amniotic embolism). These are peculiar to human pregnancy. In the extreme (once in some thousands of live births), each may cause death directly by its own kind of widespread intravascular obstruction, notably at the level of the pulmonary arterial tree—the one by fibrin, the other by squamae; the one transient, the other persistent. Thus, each may cause a syndrome of clinically unheralded "obstetric shock" by obstruction of the lesser circulation in the sense of Steiner and Lushbaugh,[18] and placental separation of abruptio may cause hypovolemic shock by its "concealed accidental hemorrhage" or other obstetric hemorrhage. When prolonged, either obstetric shock or hypovolemic shock can probably cause focal ischemic degenerations, sufficient to cause histologically visible "incubated" lesions, and stasis anaerobic metabolic disturbances in these foci may have already caused peripheral thrombosis–fibrinolysis. From such loci of coagulation–fibrinolysis, a feedback exchange into the circulation of small pools of "defibrinated blood" (erythrocytes in serum), which contains fibrinopeptides from fibrinolysis and inhibits coagulation, may cause total incoagulabilities of the blood.

FIBRINATION OF ABRUPTIO

The retroplacental hematoma of premature separation transects the maternal arterial supply to the placenta. Like any arterial hematoma, this expands with the force of a hydraulic pump. Unlike other hematomas, when it ruptures its restraining capsule (Figure 1) by laceration of the

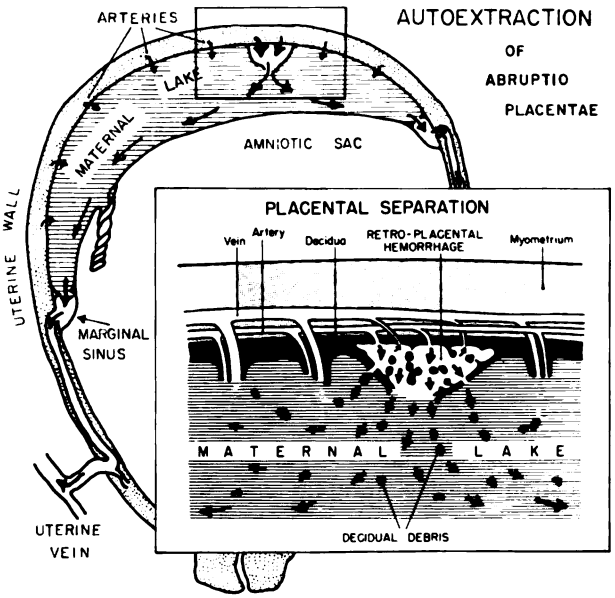

FIGURE 1 Autoextraction pathways during abruptio placentae. Feedback of materials, including tissue thromboplastin from the decidua. Gross autoextraction: via laceration into the maternal lake. Microautoextraction: via arteriovenous shunts (via the hematoma). (Reprinted with permission from Schneider.[14])

decidual (basal) plate of the placenta (Figure 2), small volumes of escaping blood may feed back into the maternal intervillous lake. Decidual materials, including tissue thromboplastin, can be swept with the blood into the maternal circulation (Figure 1) and initiate fibrination.

A congealed anatomic model of this autoextraction (Figure 2) is provided by coagulation of the hematoma. This brings the autoextraction to a halt; additional feedback lacerations of the same kind are opened during retroplacental expansions of the intradecidual hematoma and can be demonstrated in the same way in the delivered placenta of abruptio.[13]

Only in rare cases do the maternal intra-arterial fibrin deposits from fibrination become as dense and complete as suggested in Figure 4, and as persistent as the actual occlusions of Figure 5, which caused circulatory failure and death. Usually, fibrinogenopenia is the only evidence of the fibrination; the degree of defibrination provides an estimate of the

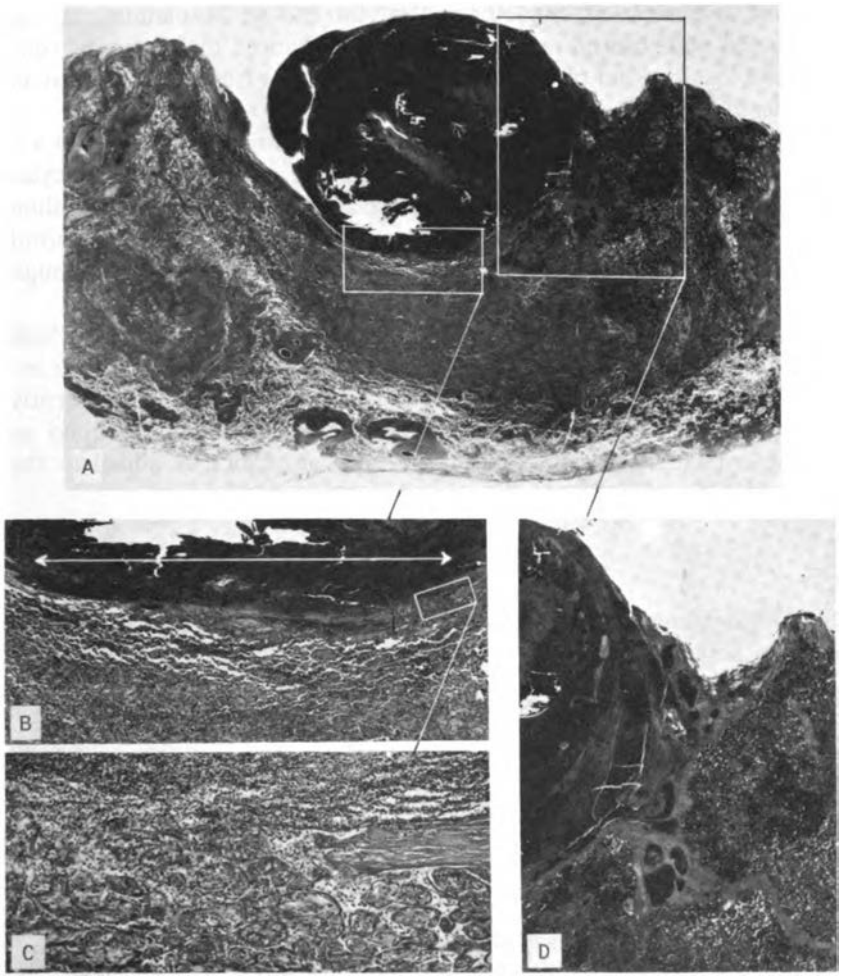

FIGURE 2 Histologic relationships of the hematoma to the delivered placenta of abruptio (cf. Figure 1). (A) Coagulated, *in situ* model of the hydraulic pump, autoextraction. The basal plate of the placenta is distended over the expanding hematoma. The subtending placental tissue is compressed and infarcted. The serum is expressed by retraction of the clot. (Uterine wall, above, not present.) (B) Low magnification. The expansion of the hydraulic (arterial) pump has lacerated the basal plate. (C) Higher magnification. Lacerated margin of basal plate. The hematoma, above, communicated freely with the maternal intervillous lake, below (until the hematoma coagulated). (D) Intradecidual position of the hematoma. The decidua extends over both the uterine (upper) and the placental (lower) surfaces. (Reprinted with permission from Schneider.[18])

amount of fibrination. Sometimes abruptio and its fibrination may be associated with central-nervous-system disturbances that produce convulsions [10] (one kind of eclampsia), and even less frequently with coma and hemiplegia.

During fibrination and its "whipping out" of fibrin (Figures 3 and 4), of course, the resulting "whipped blood" (suspension of erythrocytes in serum) remains fluid; it tends to become mixed into and to dilute any remainder of the circulating blood not so affected. The partial "defibrination" causes an over-all fibrinogenopenia, much as though defibrinated bank blood were transfused into the circulation.

However, unlike a serum or whipped-blood transfusion from which the fibrin is discarded, products of lysis of the fibrin deposits are returned to the circulating blood as inhibitors of coagulation. Consistently with this interpretation, during spontaneous fibrination of abruptio or during animal fibrination experiments, anticoagulation is added to the pancoagulation deficiency.[5-17]

OBSTETRIC SHOCK

Simulated amniotic meconium embolism produced by infusion of meconium suspension [18] leads to the interpretation that the hyperacutely

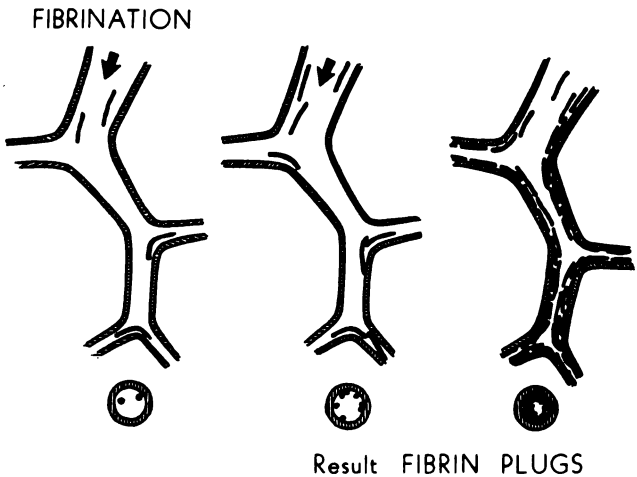

FIGURE 3 Intra-arterial fibrin deposition by fibrination. Fibrin filaments straddle bifurcations; more threads add on to the pioneer deposits until a cast is built up within the arterial tree. Platelet contribution is incidental. Unlike platelet thrombi, which resist lysis, the fibrin of this deposit spontaneously lyses away. (Reprinted with permission from Schneider.[11])

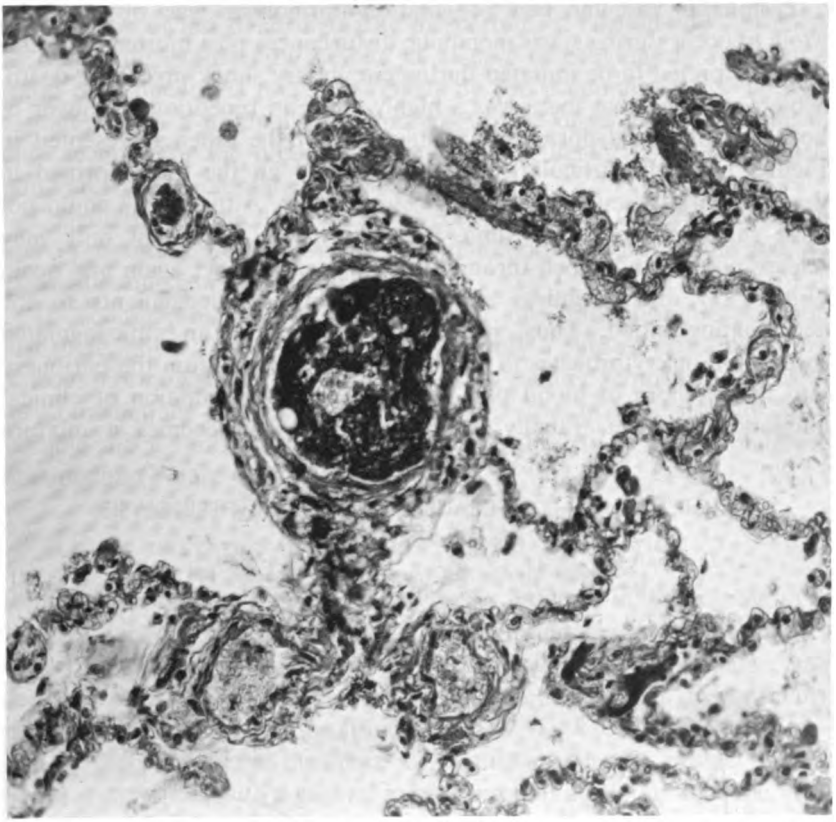

FIGURE 4 Intra-arterial fibrin obstructions from a case of abruptio with fatal fibrination. (From a case of Bartholomew *et al.*[1]) There is no lamination, as in a classic thrombus. Low magnification, lung. Fibrin occlusion in a small pulmonary artery, in cross section. The filaments are seen end-on, and parallel the lines of flow; they form a hollow cast as diagrammed in Figure 3. Smaller arterial fibrin obstructions are sectioned transversely (upper left) and longitudinally (lower right). (Reprinted with permission from Schneider.[10])

acquired pulmonary circulatory obstruction that causes severe intractable circulatory shock (as in maternal amniotic embolism) can cause an anticoagulant to be released and cause incoagulability.[5] Thus there is evidence for the production of defibrination and profound inhibition of coagulation during the severe and characteristic obstetric shock of amniotic embolism.

There is no evidence of initiation of fibrination in amniotic embolism comparable with that activated in abruptio placentae.[5,7]

Peripheral vascular bed coagulation–fibrinolysis was observed by Mole to occur during stasis metabolic disturbances post mortem, and this was interpreted to be initiated during circulatory shock preceding death. Mole concluded that there was a highly efficient, repetitious turnover of both thrombin and fibrinolysin (suggestive of the schema presented in Figure 5)[7] with particularly effective lysis of all the fibrin formed in the peripheral vascular bed during severe shock. Although he could not have suggested so at the time, the lysis of the fibrin not only may release pools of adsorbed thrombin and plasmin, to act again and again (Figure 5), but doubtless adds fibrinopeptide anticoagulants to the defibrinating blood. Thus, incremental boluses of an anticoagulated suspension of erythrocytes in serum may be released from the periphery into the plethora of blood pooled in the venous circulation of clinical amniotic embolism. Finally, the mother's blood becomes a mixture

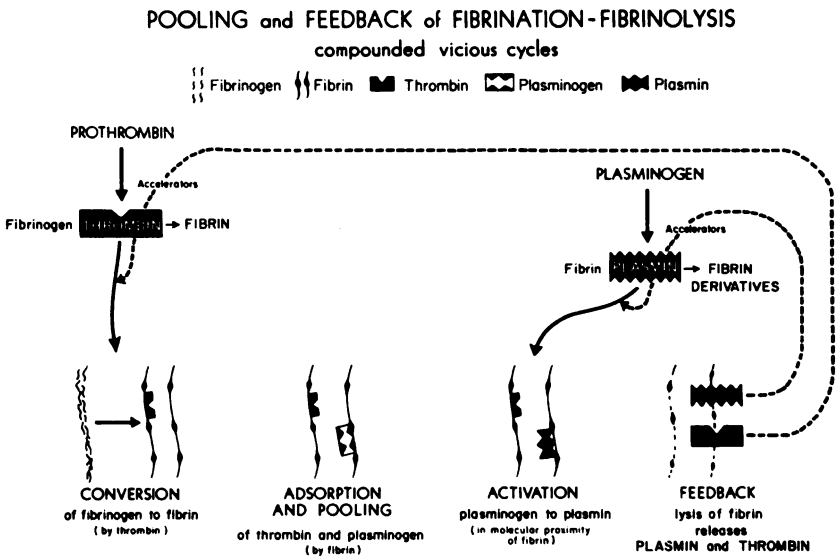

FIGURE 5 Cyclic fibrination–fibrinolysis. Pooling (by adsorption) and feedback (by fibrinolysis) of thrombin and of plasmin. During abruptio placentae, thrombin and plasmin may both act again, within the confines of the local fibrin deposit; but probably both are destroyed when liquefaction permits return into the circulation. Within loci of peripheral stasis, coagulation–fibrinolysis (in the sense of Mole) seems to generate inhibitory fibrinopeptides effectively. By gradual "exchange perfusion" during prolonged obstetric shock of amniotic embolism, it may cause total incoagulability (with fibrinogenopenia); but, during hypovolemic shock (e.g., from the concealed accidental hemorrhage of abruptio, or from any severe hemorrhage), may progress toward afibrinogenemia. Redrawn from Schneider.[7]

that is more or less defibrinated by the peripheral coagulation–fibrinolysis, but more importantly, despite its reserve of coagulation and hemostatic components, is totally inhibited by the accumulation of fibrinopeptides. Such a mechanism, secondary to the disseminated embolism, may account for the observed exsanguinating hemorrhages at parturition in relation to amniotic embolism.

Coagulation–fibrinolysis with efficient turnover of the enzymes (Figure 5) is interpreted to cause total inhibition of coagulation and an exsanguinating hemorrhagic diathesis.

The comparable obstetric shock from the fibrination of abruptio placentae, however real transiently,[10] is rarely a clinical problem. Spontaneous fibrinolysis and other mechanisms usually remove the circulatory obstructions of fibrin before they cause foci of necrosis and "irreversible shock" and before they cause death directly by total circulatory obstruction (Figure 4).

GESTATIONAL DURATION VERSUS FIBRINATION

Remarkably, although fibrination can be induced in the rabbit in a kind of laboratory abruptio (traumatic placental separation), it becomes increasingly difficult to induce this autoextraction as labor and delivery are approached (at 31 days); the maximum susceptibility occurs at 25 days of gestation [Figure 6(a)].

The gestational age incidence of fibrination in the rabbit [Figure 6(a)] is consistent with a general impression that abruptios with the most severe complications in the human occur before the cervix uteri is ready for effacement and dilatation for delivery [Figure 6(b)]. In contrast, the vast majority of abruptios occur near term [Figure 6(c)] and terminate (or are terminated obstetrically) promptly, often before development of serious complications to the mother or to the fetus. The relatively few cases in which abruptio occurs many weeks before term or in the primigravida provide a disproportionate incidence of fibrination and its complications or hemorrhage and the complications of hemorrhagic shock (including coagulation–fibrinolysis).

Figure 7 shows fibrinogen levels in a series of cases of abruptio placentae, severe in the sense that the placental separation was extensive enough to cause death *in utero*. In this series, there was a high incidence of fibrinopenia, but the fibrinogen levels ranged from the high normals of late pregnancy to afibrinogenemia. Figure 7 confirms that the predominant decrease of the fibrinogen level occurred early in the course of the abruptio placentae. In a few cases, the fibrinogen level tended to decrease further during the next few hours of retained abruptio placentae.

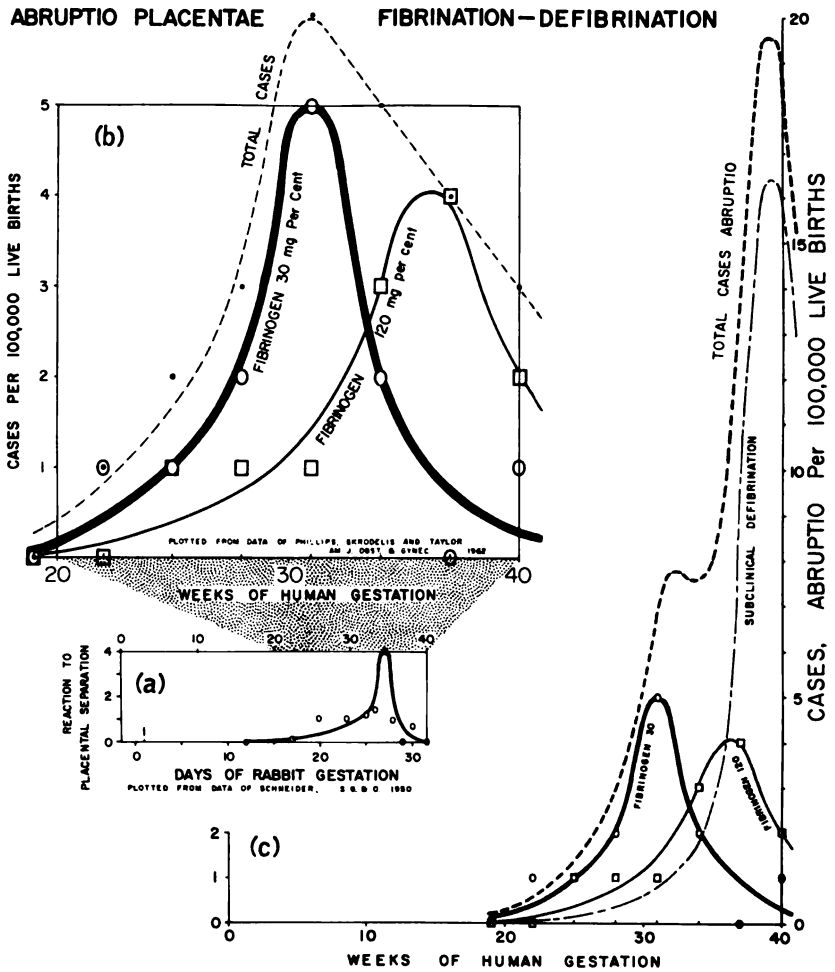

FIGURE 6 Comparison of the gestational age incidence of severe human abruptio (b) with that of experimental rabbit abruptio (a) and with the general incidence of abruptio (c). (a) Severity of fibrination complications following traumatic abruptio in the rabbit at increasing gestational ages. (Plotted from the data of Schneider.[6]) (b) Gestational age incidence of abruptio placentae, "afibrinogenemia" versus fibrinogenopenia (fibrinogen, 30 versus 120 mg%). The separation between the gestational age groups, 31 and 35 weeks, is not statistically significant.[3] It is not excluded that the prematurity corresponds to a reluctance on the part of obstetricians to carry out prompt obstetric delivery (e.g., by cesarean section) in the absence of a viable fetus, unless ultimately forced to do so by deterioration of the mother's condition during abruptio "retained *in utero*." (Plotted from the data of 24 cases of Phillips *et al.*[3]) (c) Gestational age incidences, abruptio. The incidences of severe abruptios (b) are superimposed. (Reprinted with permission from Schneider.[15])

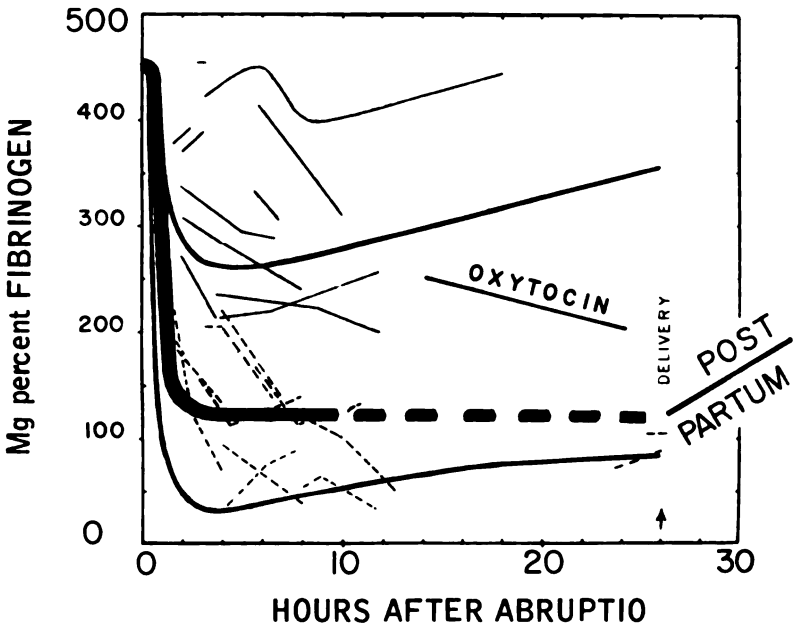

FIGURE 7 "Retained *in utero*" abruptio placentae. Some courses of fibrinogenopenia from 67 measured cases of "severe" abruptio (fetal death). Heavy curve: fibrinogen level from an intermediate group (18%) fell promptly to 120 mg% after onset of the abruptio. Two medium-heavy curves: average fibrinogen levels, respectively, for mothers whose levels remained above 200 and fell below 100 mg% (some to afibrinogenemia). (Spontaneous recovery, 3 mg%/hr.) Fine curves: courses in some individual cases. "Oxytocin," fibrinogen decrease of 5 mg%/hr during uterine stimulation. "Postpartum," fibrinogen increase of 10 mg%/hr. Deficit during oxytocin stimulation: 8–15 mg%/hr (0.3–0.6 g/hr). Plotted from data of Pritchard and Brekken.[4]

That the rate of spontaneous restoration of fibrinogen was depressed as long as the abruptio was retained *in utero* becomes more apparent when that rate is compared with the improved rate after delivery (Figure 7). Moreover, there was an actual decrease of the fibrinogen level during the stimulation of labor with oxytocics (Figure 7). Thus, the impression is supported that, as long as abruptio is retained *in utero,* there may be some degree or kind of continuing fibrination (manifested by relative depletion of fibrinogen) and that this is aggravated by stimulation of uterine contraction.[10]

A neglected direct observation consistent with active coagulation in relation to abruptio placentae and in relation to severe toxemia of pregnancy is hypercoagulability of the circulating blood manifested by a

decrease in Lee-White coagulation time, sometimes to the degree that it is difficult to withdraw blood for coagulation studies because the blood coagulates in the syringe while it is being filled.

Eventually, in the course of abruptio with fibrination, the circulating blood may become less coagulable, in terms of the Lee-White coagulation time, but the drawn blood still exhibits a persistent tendency to coagulate. Threads of retracting fibrin form within the whole blood or its plasma even upon storage in a refrigerator. Because no comparable fibrin formation develops in blood or plasma samples from normally pregnant women, or from the same abruptio patients, after delivery, it follows that there has been coagulation in the circulating blood during the abruptio.

SUBCHORIONIC THROMBOSIS OF THE PLACENTA

In approximately one of every 10 placentas, true thrombi, with a diameter of 1 cm or greater, develop on the intervillous lake aspect of the chorionic plate of the placenta. The hemoglobin within all but the most recent laminae (adjacent to the subchorionic space) is leached out. It is remarkable that these thrombi are merely incidental findings at examination of the placenta.

DISCUSSION

The data in the literature on persistent shock in amniotic embolism, based on a small number of cases, are consistent with experimental data leading to the interpretation that metabolic changes caused by prolonged stasis in the peripheral vascular bed may initiate a potent focal coagulation–fibrinolysis. Accumulation of thrombin and of fibrinolysin by absorption onto fibrin and their release by fibrinolysis (Figure 5) may efficiently reinforce coagulation–fibrinolysis locally in a biochemical cycle leading to local afibrinogenemia. The final result may be not only release of successive small pools of defibrinated blood, devoid of fibrinogen and of other hemostatic components, but simultaneous addition within those pools of fibrinopeptide inhibitors of coagulation, derived from the lysis of the fibrin. With admixture of a succession of such afibrinogenemic and anticoagulated boluses into the circulation, a gradually increasing "exchange perfusion" of anticoagulation and coagulopenia of the entire circulation may develop. This provides a mechanism for production of the observed total incoagulability of the circulating blood during prolonged obstetric shock from amniotic embolism,[5,9,15] but with

preservation of considerable and variable levels of fibrinogen and other agents of coagulation. It is unnecessary to invoke exogenous initiation of fibrination or fibrinogenolysis. Turnover cycles of initiation in peripheral pools, of coagulation–fibrinolysis, potent enough to lyse all the fibrin that is formed locally, as it is formed, render the process highly efficient (as proposed by Mole for circulatory shock).

The same mechanism does not seem to develop to the same degree during the fibrination phase, alone, of abruptio placentae. During fibrination, the fibrin is physically whipped out of the circulation, and intravascular fibrin deposits build up, leaving the defibrinated blood to return to the circulation. If this occurs before or without lysis of the fibrin (Figure 4), there will be little or no generation of fibrinopeptide inhibitors to be returned to the circulation at that time. During subsequent lysis of the fibrin deposits, there may be simultaneous fibrinolysis and production of accompanying anticoagulation. This does not exclude development of stasis thrombosis–fibrinolysis, later, if hypovolemic shock develops.

Flow diagrams of the development of fibrinopenias are shown in parallel in Figure 8, for the fibrination of abruptio and for the thrombosis–fibrinolysis of hemorrhagic shock (or, as described above, for obstetric shock of amniotic embolism). Of course, the more severe thrombosis–fibrinolysis from circulatory shock can follow fibrination, just as the concealed accidental hemorrhage or other hemorrhage and its hypovolemic shock can follow autoextraction and fibrination of abruptio placentae.

From the data accumulated in the literature, and in particular from the severe abruptios of Phillips *et al.*,[3] it seems probable that a limiting level of fibrinogenopenia is approached at approximately 100 mg%.[15] If so, then the rare cases of further degradation of fibrinogen toward afibrinogenemia in the report of Phillips *et al.* and the few cases of Pritchard and Brekken[4] (Figure 7) may have resulted from some secondary process attendant upon hypovolemic shock. Stated in the positive sense, fibrination of abruptio can progress efficiently until it has caused a depletion of circulating fibrinogen to approximately 100 mg per 100 ml of plasma, beyond which, especially in the presence of spontaneous release of coagulation inhibitors by concurrent fibrinolysis, even large pulse doses of tissue thromboplastin would have little or no capacity to exacerbate the fibrination further.

Severe defibrinations in the literature that tend to penetrate through the 100–120 mg% level of fibrinogen at once, or to progress stepwise toward afibrinogenemia (Figure 7), may be the result of intercurrent hypovolemic shock. Thus, the alternatives are here suggested in abruptio

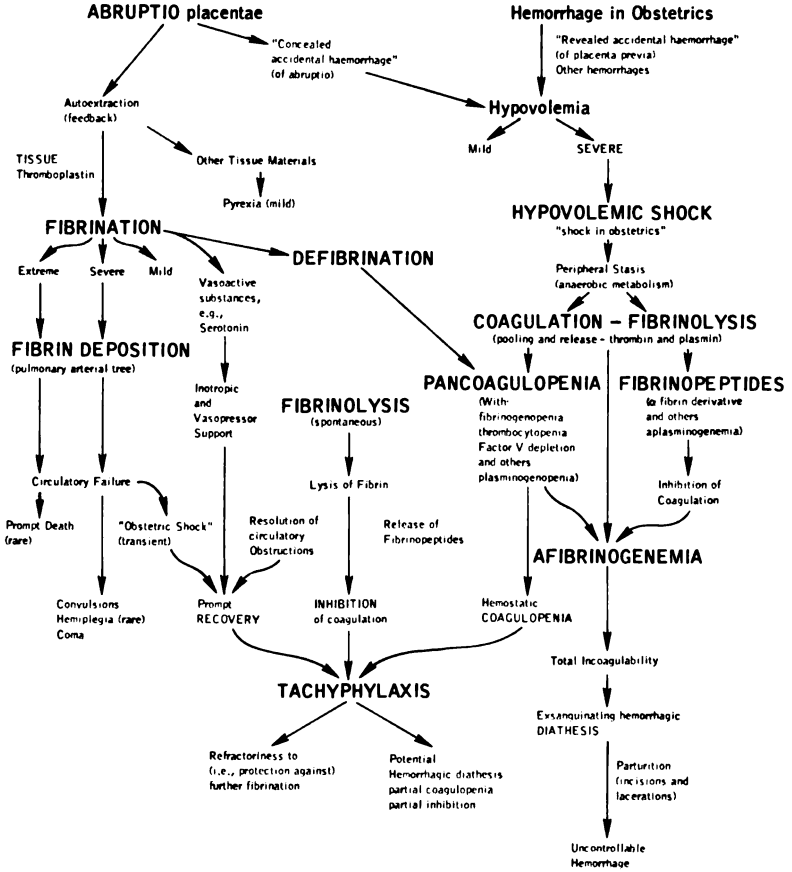

FIGURE 8 Flow diagrams of fibrination and thrombosis fibrinolysis, abruptio placentae versus circulatory shock. Abruptio placentae may cause depletions and anticoagulation of hemostatic mechanisms by two pathologic mechanisms: (1) fibrination and (2) hypovolemic shock. The fibrination is mediated by an autoextraction peculiar to the arterial hematoma of abruptio placentae. The hypovolemic shock may be caused by the "concealed accidental haemorrhage" and/or other hemorrhage, whether or not associated with abruptio or with pregnancy. Fibrination may cause pulmonary arterial fibrin deposition and circulatory failure (rarely lethal, and more often relieved by spontaneous plasminogenolysis of the fibrin). Anticoagulant fibrinopeptides tend to reinforce a tachyphylaxis that tends to prevent further fibrination and thereby to limit depletion of fibrinogen to a level of around 100 mg%. By contrast, the coagulation–fibrinolysis of hypovolemic shock can "exchange perfuse" small pools of afibrinogenemic and aplasminogenemic erythrocytes in serum (analogous to the defibrination of Mole) into the hypovolemic circulation, to the degree of total incoagulability. The two processes may be interrelated as in the diagram, and either may develop alone.

placentae (Figure 8), when there is prompt progression of afibrinogenemia, (1) that peripheral stasis coagulation may have superimposed itself secondarily during the severe fibrination, and adequately to cause circulatory failure, but of a duration less than required to cause death (rare), or (2) by circulatory failure from hypovolemia, or (3) that hypovolemic shock may have caused all or nearly all the coagulation failure directly, by itself.

It is consistent with the latter possibility that hemorrhagic shock from placenta previa, postpartum hemorrhage, other obstetric hemorrhage,[2] and nonpregnancy hemorrhage is known to have occurred with total incoagulability when no mechanism for initiation of hemostatic disturbance of fibrination comparable with that of abruptio was evident before the hemorrhage. In such cases, it follows that the shock of severe hemorrhage itself begets further exsanguinating hemorrhagic diatheses—i.e., hemorrhage begets hemorrhage.

The work reported here was supported in part by Public Health Service research grants HE 08397 from the National Heart Institute and HD 03538 and HD 34976 from the National Institute of Child Health and Human Development and by grants from the State of Michigan to Wayne County General Hospital.

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Natural History of Pulmonary Embolism

LEWIS DEXTER

As one of its functions, the lung is a filter. It is an efficient filter, protecting the systemic circulation, particularly that to the highly sensitive brain and heart, from being exposed to microobstruction or macroobstruction of the arterial blood supply by embolism.

The normal human lung can be deprived of over half of its vascular volume without creating any important cardiovascular or pulmonary disturbance. This has been demonstrated by excision of one lung [1] and by balloon occlusion of either the left or right main branch of the pulmonary artery.[5] Under these circumstances, the entire cardiac output passes through the remaining or unobstructed lung, so that its blood flow doubles. That this does not create circulatory compromise is not surprising, inasmuch as pulmonary blood flow with exercise trebles before there is any rise in pulmonary arterial pressure.[9]

Minor degrees of thromboembolism probably take place frequently in cardiac patients, in patients with fractures of the lower extremities, and in postoperative patients. There is evidence that no circulatory or respiratory symptoms occur in the normal until the cross-sectional area of the pulmonary vasculature has been reduced by over 50%.[16] In the lung compromised by disease, e.g., congestive heart failure, emphysema, or prior embolism, embolism of lesser magnitude can probably produce disability. Tachypnea, hyperventilation, and bronchoconstriction are the earliest respiratory changes, and a rise in pulmonary arterial pressure is the first circulatory change, as shown in Figure 1. The number of emboli required to produce a rise in pulmonary arterial pressure varies with the number of pulmonary vessels of the same size as the emboli (Figure 2).[10] Thus, it takes only seven emboli of lobar-vessel size to produce pulmonary and circulatory compromise, whereas it takes 1600 emboli to a third-order-size vessel and 90,000 if of major-artery size.

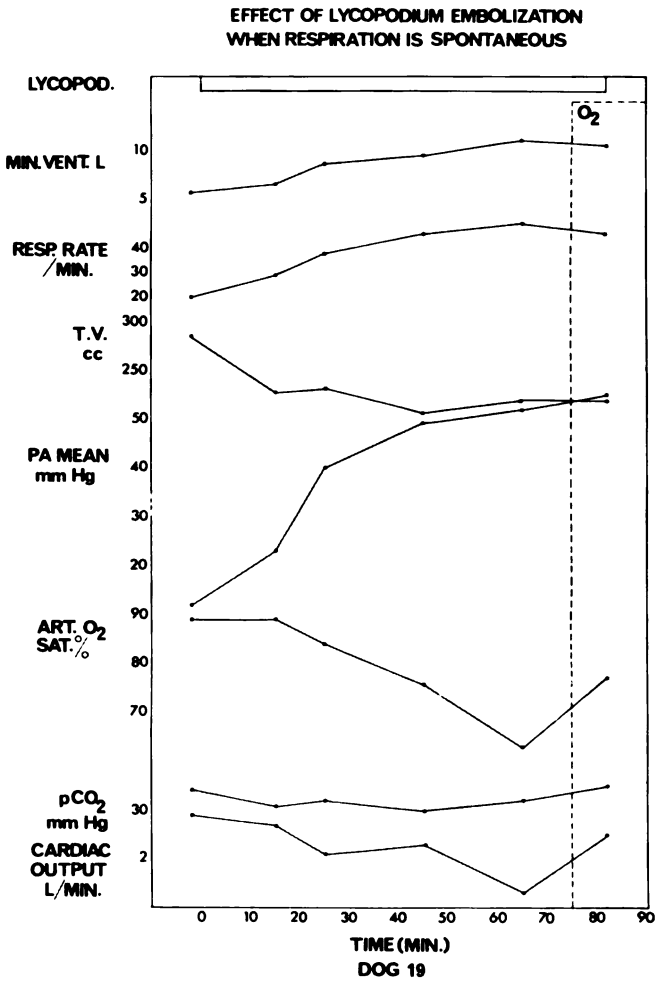

FIGURE 1 Respiratory and circulatory responses to experimental pulmonary microembolism. Hyperventilation, tachypnea, reduced tidal volume, pulmonary hypertension, hypoxemia, and fall of cardiac output are consistent sequelae.

Until these numbers of emboli are injected, there are no symptoms, signs, or measurable circulatory or respiratory changes in the experimental animal or, presumably, in man.

The circulatory and respiratory changes that occur are transient; they begin to disappear almost as soon as the infusion of embolic material

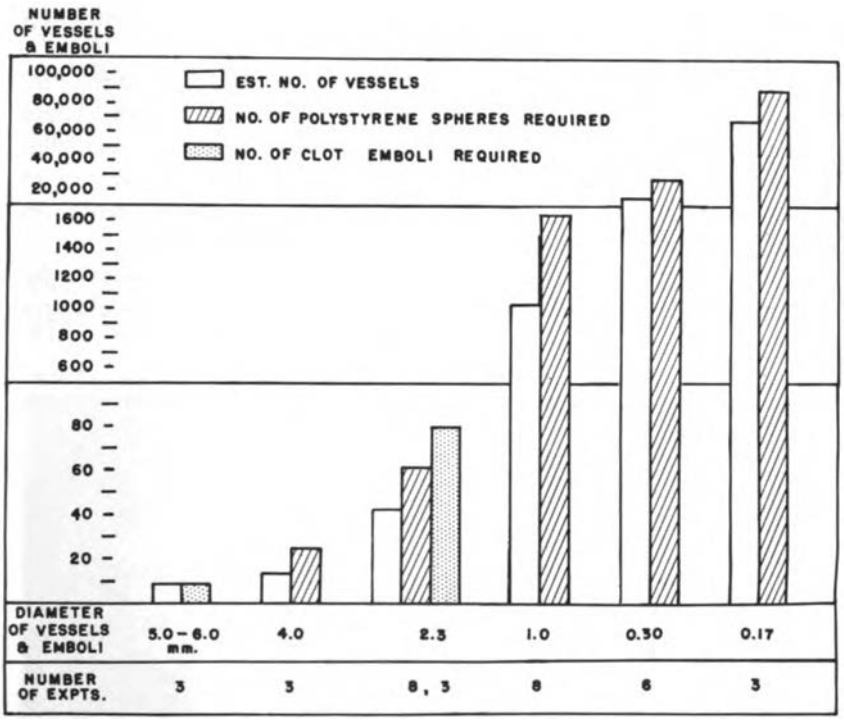

FIGURE 2 The number of emboli of a given size required to produce an incipient rise in pulmonary arterial pressure is related to the number of pulmonary vessels of the same size. Note that the required number of emboli is small when the emboli are large and becomes progressively larger as the emboli become smaller.

is stopped in the experimental animal. The same sequence occurs in patients, i.e., sudden and perhaps catastrophic appearance of disability, followed rapidly by improvement. The nature of this sequence has given rise to much speculation over the last three decades or so.

Three explanations have been put forward. The first is widespread reflex vasoconstriction. There is some evidence that this may occur when pulmonary arterioles smaller than 180 μ in diameter are occluded,[11] but there is little evidence that embolism of vessels larger than 180 μ produces reflex vasoconstriction.[11] Thromboemboli, as opposed to some other types of emboli, characteristically occlude arterial vessels greater than 180 μ; the involvement of arterioles is sparse, according to the studies of Dexter and Smith.[10] Furthermore, in persons who die of thromboembolism, we have found the clot in the lung to be uniformly

massive.[32] Attractive and logical as the concept of reflex vasoconstriction may be, it is difficult to support it with facts.

The second explanation is the possibility of a release of serotonin, which is the most powerful pulmonary vasoconstrictor known. There is evidence that bronchoconstriction by pulmonary embolism is mediated by this agent,[34] but there is no good evidence that it plays any role in producing pulmonary vasoconstriction. The most definitive work on this has been carried out by Sanders *et al.*[27] and by Marshall.[20]

The third explanation is that total occlusion of pulmonary arteries by thromboemboli is only transitory (Figure 3). Total occlusion changes

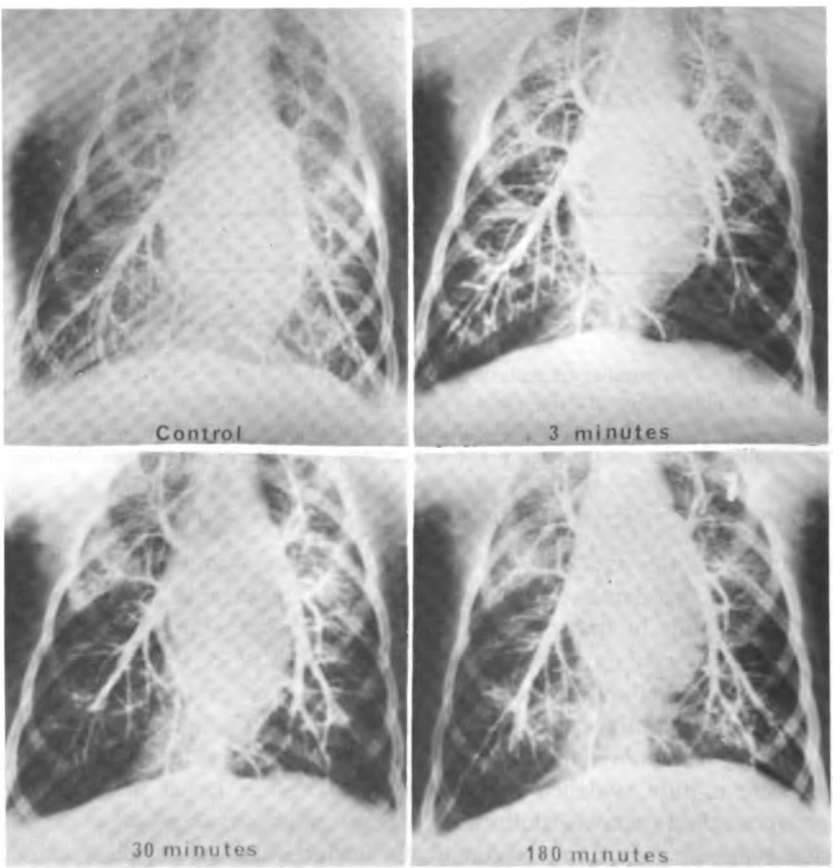

FIGURE 3 Pulmonary angiograms before and 3, 30, and 180 min after embolization with autologous blood clot. Note progressive vascularization of the left lower lobe with time.

within minutes to partial occlusion. Avascularity of segments of the lung gives way to partial perfusion of these areas; as this occurs, hemodynamic improvement follows (Figure 4). This has been clearly demonstrated in the experimental animal [8]; it is only by analogy that it can be presumed to occur in man.

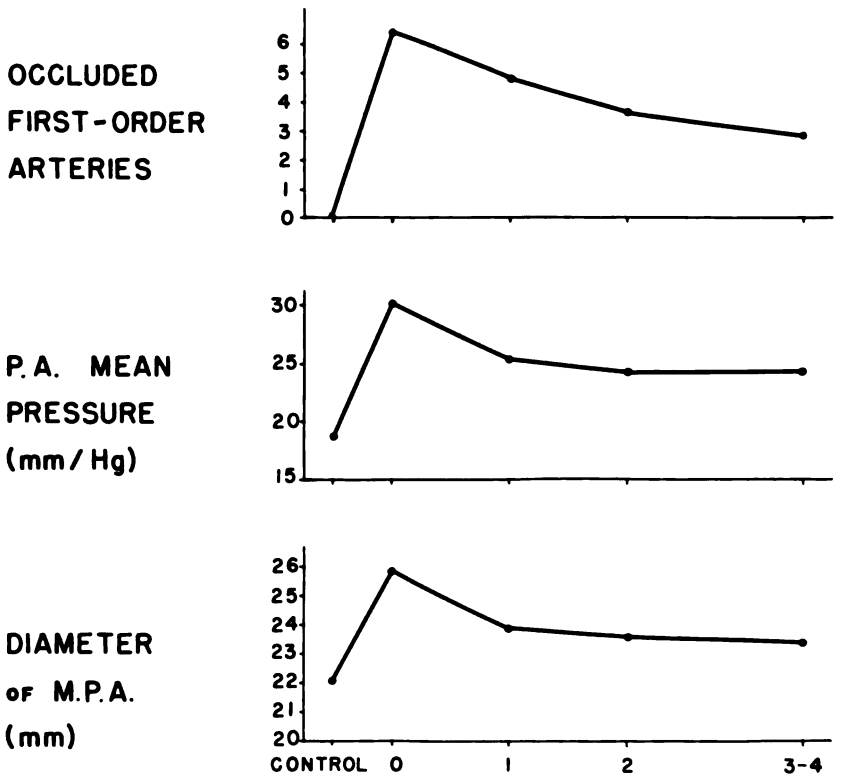

FIGURE 4 The time course after embolism in a dog that received 5 ml of fresh autologous blood clot. There is a striking parallelism between pulmonary arterial mean pressure, diameter of the main pulmonary artery, and number of occluded first-order arteries, as determined by pulmonary angiography. The progressive improvement that occurs is related to the rapid process of pulmonary revascularization.

The foregoing discussion serves as a background in interpreting the clinical course of naturally occurring human thromboembolism. A minimal volume of embolus must lodge in the lung before any manifestations of disability appear. Current evidence indicates that it must be massive. Anything short of massive will go unsuspected by both patient and physician. When, however, it is large enough to produce symptoms, the manifestations will vary from transient breathlessness, faintness, or cardiac arrhythmia, on the one hand, to catastrophe and death on the other. The more severe the symptoms, the easier it is to recognize the disorder; the less severe they are, the harder it is to identify them as due to embolism. That is why all clinical statistics on incidence are subject to criticism: they grossly underestimate the incidence of the disease.

When embolism does produce manifestations, it is abrupt in onset and evanescent. If death occurs, it occurs promptly. As shown in Table 1, about half of the patients who die of pulmonary embolism die within ½ hr of the onset, two thirds within 1 hr, and about three fourths within 2 hr. The remaining 25% die within the next few hours or days. It is difficult to obtain figures from the literature regarding the mortality of pulmonary embolism. The oft-quoted figure of 24.4% of Barker and associates [2] in 1940 is undoubtedly high, inasmuch as the true prevalence of embolism, on which death rate has to be based, is higher than most reports recognize.

When pulmonary embolism occurs in patients with normal lungs, the manifestations are very much the same as in the experimental animal and as described in textbooks. When it occurs, as it so frequently does, in those with underlying pulmonary disease and heart disease, the manifestations are often not those of embolism, but rather those of deterioration of pulmonary or cardiac function. This does not need documentation here, because it is emphasized in almost every article on the subject. It is included here for completeness and to point out that this

TABLE 1 Survival Time after Massive Fatal Pulmonary Embolism

Time, hr	Mortality,%						
½	56	68	50	—	60	67	49
1	61	—	—	34	—	75	58
2	—	—	—	—	—	78	—
6	—	—	—	51	—	83	—
No. cases	223	81	606	97	26	271	90
Reference	17	29	7	14	22	12	23

response to embolism poses one of the main difficulties in clinical recognition. As the embolic episode subsides in these patients, pulmonary and cardiac function improves.

If the patient survives and measures are taken to prevent further embolism, improvement in health occurs promptly. There is progressive re-establishment of the pulmonary circulation, due at first probably to the prompt conversion from total to partial occlusion of the vessel by the clot, with some return of circulation, followed by progressive lysis of the clot. The latter has been shown in experimental animals, in which there was almost complete resolution in 2–4 weeks.[4,19,21,28,30,33,36,37] In man, the fate of emboli in the lungs has only recently been studied by means of pulmonary angiography and by lung scans. Angiography is usually limited to one postembolic study, whereas scans can be performed repeatedly without discomfort to the patient. It has been demonstrated by pulmonary angiography that complete or almost complete resolution of large emboli has occurred in from 1 week to 6 months,[6,15,31,35] but it has been pointed out by Chait *et al.*[6] that resolution of the clot has often been poor in patients with cardiopulmonary disease. Perhaps the most definitive study to date is that of Tow and Wagner,[35] who used repeated scans of the lung to measure regional pulmonary blood flow. Within 4 months of pulmonary embolism, many of the 69 patients had complete or nearly complete return of pulmonary blood flow: of 27 patients with minimal involvement, 67% had complete return of blood flow and 75% had improvement; of 31 patients with intermediate involvement, 38% had complete return and 51% improvement; and of nine patients with severe involvement, 20% had complete return and 70% had improvement. Another interpretation of their data is that 25% of their patients with minimal involvement, 49% with intermediate involvement, and 30% with severe involvement showed no improvement. By calculation, in 37% of their patients, there was no improvement in pulmonary blood flow in 4 months.

If measures are taken to prevent further embolization, the patient usually returns to good health, although evidence of pulmonary vascular impairment persists. Occasionally, chronic cor pulmonale is a sequela of embolism. This is not common, but probably occurs more frequently than the literature indicates.[18] At last count in 1963, there were 250 cases of this reported in the literature.[18] We have 12 unpublished cases in our own files. Billings [3] in 1921 reported a necropsy incidence of 0.2–0.7%. Ring and Bakke [25] in 1955 reported an incidence of about 0.2%. Zollinger and Hensler [38] in 1958 reported an incidence of 1.7% in 4000 necropsies. Phear [24] reported that two of 71 survivors of pulmonary embolism developed chronic cor pulmonale, and eight others had severe

persisting disability. The difficulty of differentiating these from primary pulmonary hypertension has been emphasized.[26]

The implication of the studies of Phear,[24] Chait *et al.*,[6] and Tow and Wagner [35] is that emboli may not resolve as completely or as frequently as heretofore suspected, and that there may be more postembolic disability than the older literature indicated. Because heightened interest in a given disease leads to an increased recognition of its occurrence, it can be anticipated that the incidence of reported chronic postembolic pulmonary vascular obstruction by embolism will become higher than it is now.

The occurrence of chronic cor pulmonale after embolism has usually been attributed to multiple embolization, rather than to persistence of a single massive embolus.[13] Although this is undoubtedly true, chronic cor pulmonale may also turn out to be related to incomplete resolution of emboli.

In conclusion, pulmonary embolism is of acute onset. Depending on the volume of embolic material that lodges in the lung, there may be no discernible manifestations, there may be minor symptoms of short duration, there may be a life-threatening catastrophe, or death may occur within minutes of onset. In those who survive, recovery is relatively rapid if no further embolism occurs. Although it appears that progressive resolution of the embolic material in the lung is the rule, complete resolution does not always occur, and chronic cor pulmonale sometimes remains as a sequela.

The work reported here was supported by Public Health Service grants 5-RO1 HE 00450-18 and 5-TO1 HE 05234-10 from the National Heart Institute and Public Health Service fund 5-SO1 FR 05489-05.

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Pathophysiology and Management of Postphlebitic Venous Insufficiency

J. ALEX HALLER, JR.

The most serious aftereffect of deep thrombophlebitis is chronic venous stasis, which follows destruction of the venous valves. In the normal leg, the resting venous pressure in the foot when a person stands erect is about 100 mm Hg. With one or two contractions of the powerful muscle pumps of the calf, this pressure falls immediately to 0–5 mm Hg.

This remarkable hemodynamic change depends on the directional effect of competent venous valves. When the majority of valves in the deep system are rendered incompetent by the processes of repair and recanalization, muscular contraction produces a chaotic, nondirectional swing in flow and pressure. This inefficient pumping process creates a static venous pool and a continuous venous pressure of nearly 100 mm Hg in the dependent calf and foot.

The sequelae of stasis in venous insufficiency that result from unrelieved pressure have not received their due emphasis, probably because they are typically insidious. The hallmarks of this condition are pain, edema, hyperpigmentation, and ulceration. Basically, they all result from chronic, uncontrolled venous hypertension and secondary interstitial edema.

How many patients with serious postphlebitic limbs have a history of deep thrombophlebitis? Positive histories of deep phlebitis in patients with serious stasis sequelae range from 10% to 87%.[3] The wide discrepancy is probably due largely to differences in criteria for previous thrombophlebitis. In our experience, it seems likely that, if not all, at least the vast majority of cases of significant deep venous incompetence have resulted from phlegmasia alba dolens or from smaller recurrent epi-

Portions of this paper are incorporated in a monograph, *Deep Thrombophlebitis: Pathophysiology and Treatment*, by J. Alex Haller, Jr. Philadelphia: W. B. Saunders Co., 1967, 130 pp.

sodes of deep thrombophlebitis, which eventually involve the same deep venous system.

How many patients with extensive deep thrombophlebitis, phlegmasia alba dolens, will develop serious postphlebitic venous incompetence if healing is allowed to take place naturally, without operative intervention? This is an equally pertinent question, but much more difficult to answer. Accumulation of sufficient experience by a single person is difficult, and collected clinical data are almost wholly retrospective. If the natural pathogenesis of iliofemoral thrombophlebitis runs toward resolution with a low incidence of venous incompetence, then questions of comparative therapy are almost academic. There can be little question but that a clotted vein regularly becomes recanalized, and its valves are predictably and demonstrably incompetent.[6] It is our impression that only in few patients with phlegmasia alba dolens will deep thrombosis be naturally resolved without significant venous incompetence. Those lucky few in whom resolution does take place probably have a higher potential for clot lysis than for organization and recanalization, and also a larger collateral venous bed with undamaged valves.

In any event, the personal significance of the late sequelae depends largely on the activity demands of each patient. If every patient with a postphlebitic limb could keep his feet raised, there would be no serious medical or economic problems. But most patients with stasis sequelae are young or middle-aged adults who must work for a living, and their crippled limb is a heavy cross to bear. A young mother with an aching, edematous leg may find it intolerable to wash dishes, to cook, and to care for her young children. The farmer cannot walk or plow his fields; the laborer cannot stand for the necessary long hours on his job. Most occupations involve leg usage and foot dependency. Much can be done to control symptoms and prevent further organic damage in these patients, but they are all seriously handicapped by their postphlebitic venous incompetence.

ACUTE PATHOPHYSIOLOGY

Within a few days after the occurrence of acute thrombosis, long before the subsidence of swelling or symptoms, repair begins. The clinical findings in the acute stage are shown in Figure 1. A white thrombus is adherent to the intimal surface, but the red propagation thrombus either lies against the vein wall or is free in the venous stream. In all venous thrombi, the process of organization begins in the vein wall and progresses centrally.

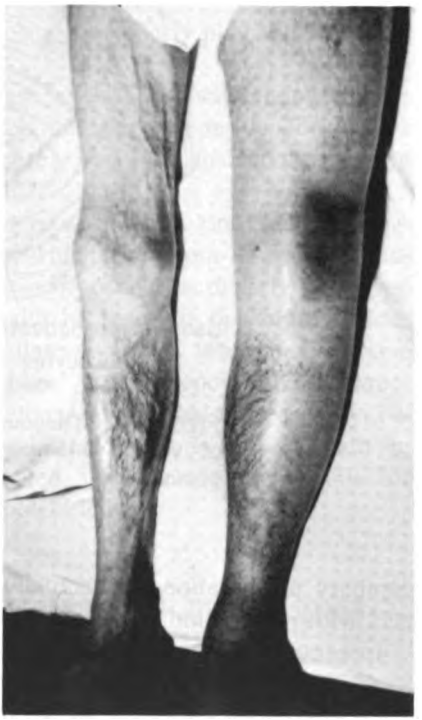

FIGURE 1 Diffusely edematous leg, which is characteristic of the acute phase of massive deep thrombophlebitis.

Organization of thrombus material begins with a migration of fibroblasts, which grow in from the vein wall.[1] As early as 5 days after thrombosis, they can be identified in the periphery of some clots. Inflammatory cells of all kinds participate in the cellular reaction. Large numbers of polymorphonuclear leukocytes and lymphocytes invade the thrombus.

Especially in large red propagation thrombi, which are seen in extensive deep thrombophlebitis, involution occurs by a process of partial fibrosis and partial liquefaction. Fibroblasts are thought to be responsible for laying down the fibrous matrix that results in peripheral organization. The polymorphonuclear leukocytes digest cell fragments and fibrin within the clot and are thereby responsible for the central liquefaction. In most red thrombi, the center is liquefied and the periphery becomes a dense fibrous ring (Figure 2). Intrinsic capillaries in the vein and valve walls seem to play a leading role in recanalization. This results largely from a process of dilatation and extension through the thrombus material. Edwards and Edwards[6] suggested that this early

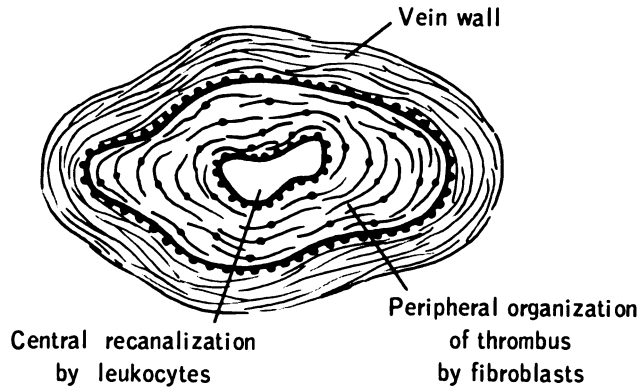

FIGURE 2 Diagram of two simultaneous processes of repair of venous thrombosis: central liquefaction and peripheral organization.

capillary proliferation may actually bridge the thrombus and thus connect with patent lumina on each side.

In some thrombosed veins, a spotty liquefaction occurs, with fibrous bands criss-crossing the old lumen and dividing it into many small lumina and crypts. The best-recognized example of this is in portal vein thrombosis, in which the multiple recanalized channels are technically unsatisfactory for a portocaval shunt, even though they may be adequate for flow into the liver.

Whether by a single lumen or by multiple lumina, the ultimate result of recanalization is some restoration of function to the vein. Its lumen is always partially obstructed, its wall is densely fibrotic and irregular, and the once-delicate valves are grossly crippled or destroyed.

It is important to recognize that normally the deep venous valves are not completely competent all the time. Changes in position and sudden alterations in intravenous pressure may permit a significant reflux of blood through normal valves. This observation was first made by Luke [9] in a series of retrograde venography studies in normal legs. This degree of insufficiency is both minimal and transient and, therefore, not hemodynamically significant.

The process of valve destruction in thrombophlebitis has been elucidated by Edwards and Edwards [6] in both animal and human material. They have shown that in many cases the collagen of the valve cusps is fragmented as the fibroblastic organization of the thrombus proceeds. Some fragments of the elastic tissue may remain, but they are the only

remnants of valve tissue. Capillaries and fibroblasts play the key roles in this destructive process.

When the phlebitic reaction is less severe, the valve may not be acutely destroyed. In such cases, it is incorporated in the fibroblastic organization. The valve cusps ultimately contract against the scarred vein wall as recanalization takes place.

The most significant effect of the recanalization process is the reestablishment of central luminal flow, which is without directional control because the valves have become incorporated in densely organized fibrous tissue. Function would actually be better if the entire venous return took place through dilated collateral vessels whose valves have not been destroyed by thrombophlebitis. It is patency without directional flow that initiates the vicious effects of venous stasis and hydrostatic pressure. These grossly altered hemodynamic forces result in dependent edema, which, if uncontrolled, is ultimately responsible for all the distressing sequelae of the postphlebitic limb.

CHRONIC SEQUELAE OF THE POSTPHLEBITIC LIMB

Pain, edema, hyperpigmentation, and ulceration are the hallmarks of postphlebitic venous incompetence. Serious crippling of the leg results from extensive destruction of the deep venous system, but even well-localized incompetence of the superficial femoral vein and popliteal vein can produce unattractive and uncomfortable edema of the ankle and foot.

The most significant factor in the development of postphlebitic sequelae is the hydrostatic pressure of venous incompetence. In the presence of recanalization and destroyed valves, a main-line pressure head from right atrium to toes is brought to bear on the lower leg every time the patient stands up (Figure 3). This may result in a hydrostatic pressure of 150–175 cm H_2O at the ankle, or more than 10 times the normal peripheral venous pressure. Inasmuch as the normal mean arterial pressure is around 100 mm Hg (135 cm H_2O), there can be little wonder that a progressive transudation from intravascular to interstitial spaces occurs when incompetent legs are dependent.

The initial discomfort of venous incompetence is comparable with the pain that results from varicose saphenous veins. It is an uncomfortable sensation of dependent fullness, which may have a throbbing quality. Like the pain of saphenous incompetence, it is dramatically relieved by raising the feet higher than the rest of the body. Unlike the pain of superficial varicosities, which is often made worse by walking, the pain

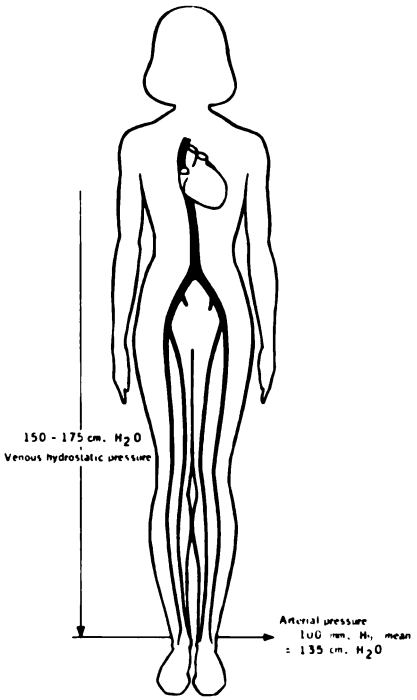

FIGURE 3 Relative pressure relationships in ankle vessels with patient upright and with no effective muscle pumps or venous valves.

of pure deep venous incompetence is usually decreased by the milking action of muscular activity, such as that involved in walking and flexing the ankles and toes. If there is combined incompetence of the superficial and deep systems, elastic-stocking support will often decrease pain by making the muscle more effective as a pump in evacuating pooled blood from the leg. If the superficial system is competent, pain is made worse by elastic-stocking compression, and most patients will not tolerate it.

The secondary or late pain of chronic incompetence is a burning, stinging sensation, which seems to result from interstitial edema and the inflammation that this extravascular fluid excites. Nerve endings are both stimulated by the inflammation and compressed by the increased tissue pressure. This discomfort is relieved much more slowly by elevation. If the edema and inflammatory reaction are permitted to become chronic, an extensive interstitial fibrosis results, which may compress muscle and nerve in a brawny induration. This chronic discomfort may defy intensive analgesic therapy.

Finally, a tertiary discomfort results from tissue necrosis and ulceration with superimposed cellulitis. This is characteristically a throbbing pain, with varying degrees of burning and stinging. Many patients with

this late stage of the disease plead for amputation to relieve them of the constant anguish in their ulcerated, infected leg.

Edema is due to hydrostatic transudation, which is stopped by raising the feet but recurs on dependency. If secondary fibrosis and induration are allowed to occur, then inflammatory edema may be added to the transudate, and simple elevation becomes less effective. Local lymphatic obstruction may result from the proliferation of fibrous tissue. This produces secondary lymphedema, which leads to permanent enlargement of the leg.

The single and catastrophic effect of venous insufficiency is interstitial edema from the hydrostatic load. Chronic accumulation of uncontrolled edema is responsible for all the late complications of a postphlebitic limb. To the extent that treatment is successfully directed toward control of edema, an incompetent deep system will remain a nuisance but will not lead to crippling disease.

Hyperpigmentation of the skin and recurrent eczematoid reactions are manifestations of long-standing edema. This is caused mainly by hemosiderin deposits, resulting from both capillary hemorrhages and prolonged capillary stasis. If chronic irritation of the skin persists, melanin deposits will be added to the pigmentation. Interstitial fibrosis further reduces the available blood supply to the skin and subcutaneous tissues by compressing the capillary bed. Venous stasis compounds the circulatory insufficiency by lowering the oxygen content of the venous blood. Thus, a state of chronic hypoxia accelerates a vicious circle of cell breakdown and scar formation.

Local ischemia, interstitial fibrosis, and brawny edema set the stage for ulceration from the slightest mechanical injury to the skin. A trivial blow, usually unrecognized by the patient, may initiate extensive ischemic necrosis.

Another significant factor in the local breakdown of tissues is incompetence of the perforating veins that connect the deep and superficial venous systems. This mechanical effect has been well described by Cockett and Jones, as follows [4]: The main venous damage of the tissues where varicose ulcers occur is done by perforating veins (especially the lower two) directly into the deep veins. Incompetence of one or more of these perforations throws a great stress on the delicate venous mesh of the subcutaneous tissues that they drain. In fact, incompetence of one or more of these perforators is probably the main local factor in the causation of ulcer in this region. Once the skin is opened, previously trapped edema fluid pours forth as a rich medium for bacterial growth. Infection is instantly added to damaged cells, and cellulitis is the predictable result. Depending on the organisms producing the infection,

further necrosis may be superimposed and there may be an alarming dissolution of tissue (Figure 4). Such stasis ulcers are most common in the region of the internal malleolus and very rare in the calf. It is almost impossible to heal a stasis ulcer until both the cellulitis and the dependent edema are controlled. Practically, this means constant elevation of the affected foot and the use of both topical and systemic antibiotics.

Two major types of venous insufficiency result from the processes of repair in thrombophlebitis: saphenous and deep venous incompetence. An incompetent deep venous system is actually composed of deep varicose veins; but, because they are not visible, they are seldom thought

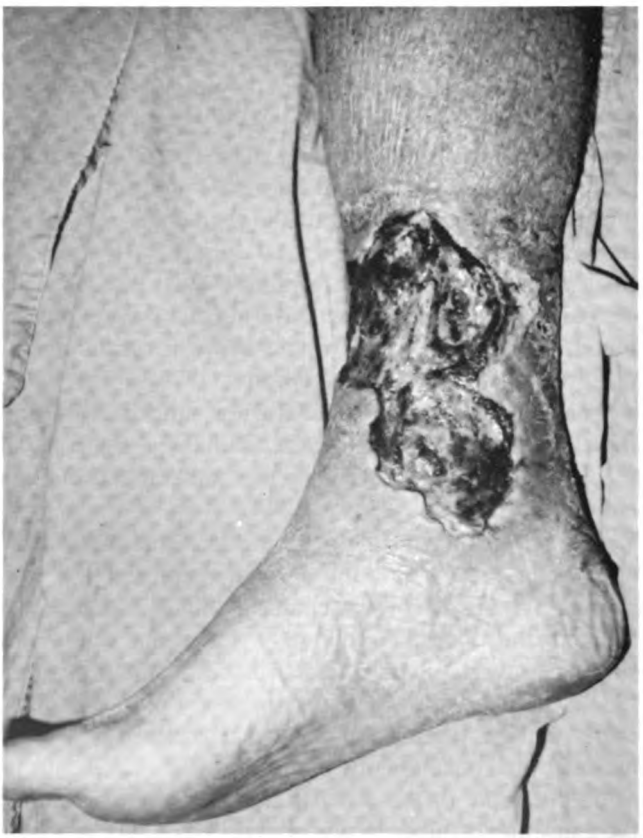

FIGURE 4 Postphlebitic stasis ulceration with superimposed infection and extensive tissue necrosis.

of in this way. A large vein is not necessarily a varicose vein, but an incompetent vein is. Thus, some of the symptoms of deep venous incompetence are present with a less extensive process of saphenous varicosities. Stasis and absence of directional flow in a varicose saphenous system will result in some edema after long dependency and will also produce the well-known aching pain of varicose veins.

An incompetent or varicose saphenous system may result from extensive thrombophlebitis in the saphenous vein, but this is rarely the etiology.[8] More commonly, incompetence results from congenitally faulty valves or from overdistention of the superficial veins. This overdistention may result from an associated deep thrombophlebitis that drives venous return into the saphenous veins as a preferential collateral channel. In other cases, the connecting or perforating veins between the deep and superficial systems become incompetent. The muscle pump then squeezes increasing volumes of blood into the already distended saphenous system. The valve cusps can no longer coapt themselves, and incompetence results.

If the clinical picture is due primarily to saphenous incompetence, the signs and symptoms will disappear when this system is obliterated. This can be tested by applying a snug elastic bandage up to the knee. If the complaints attributable to stasis disappear, then one can be almost certain that operative removal of the varicose saphenous veins will cure the patient. If the symptoms persist, one must look elsewhere for their etiology.

Significant incompetence in the deep system is a result of extensive deep venous thrombophlebitis. It can usually be diagnosed on the basis of an accurate history and the typical features of dependent venous stasis.

MANAGEMENT OF VENOUS INSUFFICIENCY

Theoretically, the ideal treatment would be reconstruction of the faulty valves, but the destructive nature of thrombophlebitis almost eliminates this possibility. Attempts to replace destroyed venous valves in experimental animals with autologous and even homologous valves have recently been reported,[10] but there has been a high failure rate, because of thrombosis. A few preliminary reports of autologous human-vein valve grafts have appeared, but the data are too scanty to evaluate properly.

In 1955, Bauer [2] popularized an earlier approach to deep venous hypertension by attempting to interrupt the column of static blood.

He divided the superficial femoral vein at several levels and ligated the popliteal vein behind the knee. He reported dramatic results,[3] but generally the effect of interruption was short-lived, because collateral channels that bypassed the two divided ends quickly enlarged and the same hydrostatic pressure head was soon re-established.

Several bypassing procedures have been suggested, including an ingenious bypass across the pubic symphysis.[5] Unfortunately, the indications for use of these procedures are rare, and there is a high incidence of thrombosis. Surgeons will be the first to agree that there is no good operative treatment for extensive deep venous incompetence. The best approach is to prevent destruction of the valves by early complete removal of the clot by venous thrombectomy.

Many French surgeons (such as Leriche and Fontaine and associates) believe that sympathetic overactivity and other altered neurovascular reactions are as important as stasis and local venous pressure increases in producing postthrombotic ulceration.[7] They have therefore recommended sympathetic blocks and even sympathectomy in the treatment of advanced venous stasis. Although sympathetic denervation increases blood flow in skin and subcutaneous tissues, most experienced observers feel that sympathetic overactivity plays only a secondary role in stasis ulceration and that the primary factors are interstitial edema and fibrosis. For that reason, sympathectomy has not been widely used in the treatment of the postphlebitic limb.

All this is not to say, however, that no satisfactory treatment is available for the postphlebitic limb. All effective palliative treatment of deep venous incompetence is directed toward prevention of interstitial edema. This can be accomplished by excising incompetent superficial veins (varicose veins), by establishing adequate elastic-stocking support, by avoiding prolonged dependency, and by emptying the venous overload frequently with short periods of elevation of the affected limb. If edema is thereby controlled, none of the more serious late complications—fibrosis, induration, ulceration, and cellulitis—will occur. It can be a difficult and frustrating task to convince a young postphlebitic patient of this lifelong commitment. But these rules must be followed religiously if he is to avoid the otherwise inexorable curse of chronic uncontrolled edema.

The typical postphlebitic syndrome of severe throbbing pain associated with brawny dependent edema and cellulitic stasis ulcers can be effectively prevented by strict avoidance of chronic edema. All the miserable sequelae of deep venous incompetence are secondary to interstitial edema. All successful preventive measures must, therefore, be predicated on this principle: Control the edema!

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Acute Myocardial Infarction: Magnitude of the Problem

M. A. IBRAHIM, D. L. SACKETT, AND W. WINKELSTEIN, JR.

With increasing interest in coronary-heart-disease prevention programs and in the establishment of coronary-care units, it has become mandatory to estimate the magnitude of the problem of acute myocardial infarction in the U.S. population. However, the development of this estimate proves to be extremely difficult, in spite of extensive research efforts.

A simplified working model that uses age–sex-specific incidence rates, as well as short-term survival rates, has been applied to permit an approximation of the prevalence of this disorder in the U.S. white population aged 30 years and over for 1970.

METHOD

Acute myocardial infarction has been operationally defined on the basis of the demonstration of one or all of the following: definite history, typical electrocardiographic changes, and a characteristic rise and fall in enzyme levels.[4] The Framingham Heart Disease Epidemiology Study has accumulated considerable data bearing on this problem. Estimates have been derived from this source and combined and supplemented with those of similar longitudinal studies of free-living population groups. Estimated average annual age–sex-specific incidence rates per 1000 white population for acute myocardial infarction (exclusive of sudden death)[4,10] are shown in Table 1. Beyond age 60, the rates were obtained by extrapolation on the basis of the exponential relationship between age and incidence noted among younger groups (Figure 1). Thus, incidence rates for acute myocardial infarction (exclusive of sudden death) were estimated as 10 per 1000 males and 5 per 1000 females aged 30 and over.

TABLE 1 Estimated Average Annual Incidence Rate of Acute Myocardial Infarction per 1000 Population (White Persons, Aged 30 and Over)

Age, years	Male	Female
	(EXCLUDING SUDDEN DEATHS DUE TO MYOCARDIAL INFARCTION)	
30–39	1.4	0.1
40–49	3.4	0.5
50–59	7.5	1.6
60–69 [a]	17	6
70 and over [a]	40	24
30 and over	10	5
	(SUDDEN DEATHS DUE TO MYOCARDIAL INFARCTION)	
30 and over	4	1
Total, 30 and over	14	6

[a] Rates extrapolated.

Sudden death (death within 1 hr) was estimated from the frequency of sudden deaths attributed to myocardial infarction in the Baltimore population,[10] as well as several prospective studies.[7,8,14,17] Accordingly, for the white population, rates of sudden death attributable to myocardial infarction were estimated to be in the neighborhood of 4 per 1000 males and 1 per 1000 females aged 30 and over (Table 1).

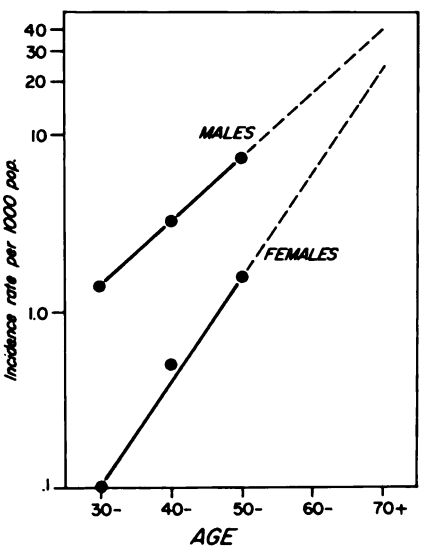

FIGURE 1 Estimated average annual incidence rate of acute myocardial infarction per 1000 population.

TABLE 2 Sudden Deaths among First Myocardial Infarctions

Criterion	Study Population	Population at Risk	Sudden Deaths, %
"Sudden unexpected death documented to have occurred within a matter of minutes" [8]	Residents of Framingham, Mass., aged 30–59, free of CHD at entry	124	20.2
Death "within three hours of onset of acute coronary episode" [3]	Adult white male civil servants in Los Angeles, free of CHD at entry	100	22
Death within 24 hr of onset of fatal event [10]	A sample of "sudden unexpected non-traumatic deaths" among Baltimore residents who were functioning in the community at large before onset of fatal event	estimated	22
Death within 24 hr of onset of fatal event [14]	Employees of E. I. du Pont de Nemours and Company, free of prior myocardial infarction	1331	25.2

Death within 24 hr of onset of fatal event [22]	Adult males enrolled in the Health Insurance Plan of New York, free of prior myocardial infarction	881	30.8
Death within 48 hr of onset of fatal event [17]	Approximately 110,000 adults aged 25–64 enrolled in the Health Insurance Plan of New York for 2 years or more	332	26.8
"Sudden death"—not further specified [5]	1913 male employees of New York State aged 39–55, free of prior myocardial infarction	36	19.4
"Sudden death" among subjects apparently in good health—not further specified [11]	North Americans who originally met physical requirements for military or civilian pilot duty	87	19.5
Unattended "sudden deaths"—not further specified [6]	White males aged 35–64 in Middlesex County, Conn., reported by physicians and/or vital records	74	24.3
"Sudden deaths"—not further specified [26]	First myocardial infarctions among 1886 males aged 35 or over, in a probability sample of dwelling units in six North Dakota counties	98	26.5

The incidence of death due to acute myocardial infarction at various intervals within a 1-year follow-up was derived from estimates of case-fatality rates. Studies concerned with the frequency of sudden death among first myocardial infarctions have been reviewed (Table 2). In these studies, the definitions of sudden death have varied from deaths "within a matter of minutes" to deaths within 48 hr. Sudden deaths occurred with frequencies of 20–30%, and, for the purpose of this paper, an average case-fatality rate of 24% has been chosen as the estimate for deaths within 1 hr of onset of the terminal event.

First-month case-fatality rates among first myocardial infarctions are presented in Table 3. Rates derived from observations based on free-living populations have ranged from 30% to 38%. Therefore, a first-month case-fatality rate of 35% does not seem unreasonable for our purpose here.

The first-year case-fatality rates for first myocardial infarctions are presented in Table 4. In this case, a rate of 40% would seem consistent with the estimates previously selected for first-hour and first-month case-fatality rates. The rates based on clinical and hospital studies [1,15] presented in Tables 3 and 4 have not been applied because of shortcomings to be discussed later.

The natural histories of "other coronary disease" (primarily angina pectoris) and "silent" myocardial infarction are relatively unknown; therefore, their contributions to the estimate of prevalence will not be considered.

TABLE 3 First-Month Case-Fatality Rates among First Myocardial Infarctions

Study Population	Population at Risk	First-Month C.F.R., %
First myocardial infarctions seen by a group of internists in Ohio [1]	156	13.5
Admissions to a Hartford, Conn., hospital diagnosed as having first myocardial infarctions [15]	204	18.6
Employees of the Du Pont Company with first myocardial infarctions [14]	1331	30
Adult males in H.I.P. with first myocardial infarctions [22]	881	36
35- to 64-year-old male subscribers to the Medical Sickness Annuity and Life Insurance Society, Ltd., with first myocardial or cardiac infarctions [13]	192	38

TABLE 4 First-Year Case-Fatality Rates among First Myocardial Infarctions

Study Population	Population at Risk	First-Year C.F.R., %
First myocardial infarctions seen by a group of internists in Ohio [1]	156	27
Employees of the Du Pont Company with first myocardial infarctions [14]	1331	36
Adult males in H.I.P. with first myocardial infarctions [23]	881	39.8
35- to 64-year-old male subscribers to the Medical Sickness Annuity and Life Insurance Society, Ltd., with first myocardial or cardiac infarctions [13]	192	40

ESTIMATES OF PREVALENCE

The average annual age–sex-specific incidence rates for acute myocardial infarction and sudden death (due to myocardial infarction) have been applied to the U.S. white population projected to 1970.[19] It can be seen from Table 5 that approximately three fourths of a million persons will suffer from first acute myocardial infarction (excluding sudden death) in 1970. If sudden deaths are added, one would expect that about a million persons will suffer from this disorder.

Figure 2 presents estimates of death or survival at various periods

TABLE 5 Estimated Annual Number of New Cases of Acute Myocardial Infarction in the United States, 1970 (White Persons, aged 30 and Over)

Age, years	Male	Female	Total
	(EXCLUDING SUDDEN DEATHS DUE TO MYOCARDIAL INFARCTION)		
30–39	16,000	1,000	17,000
40–49	39,000	6,000	45,000
50–59	68,000	15,000	83,000
60–69	113,000	49,000	162,000
70 and over	227,000	187,000	414,000
30 and over	463,000	258,000	721,000
	(SUDDEN DEATHS DUE TO MYOCARDIAL INFARCTION)		
30 and over	177,000	50,000	227,000
Total, 30 and over	640,000	308,000	948,000

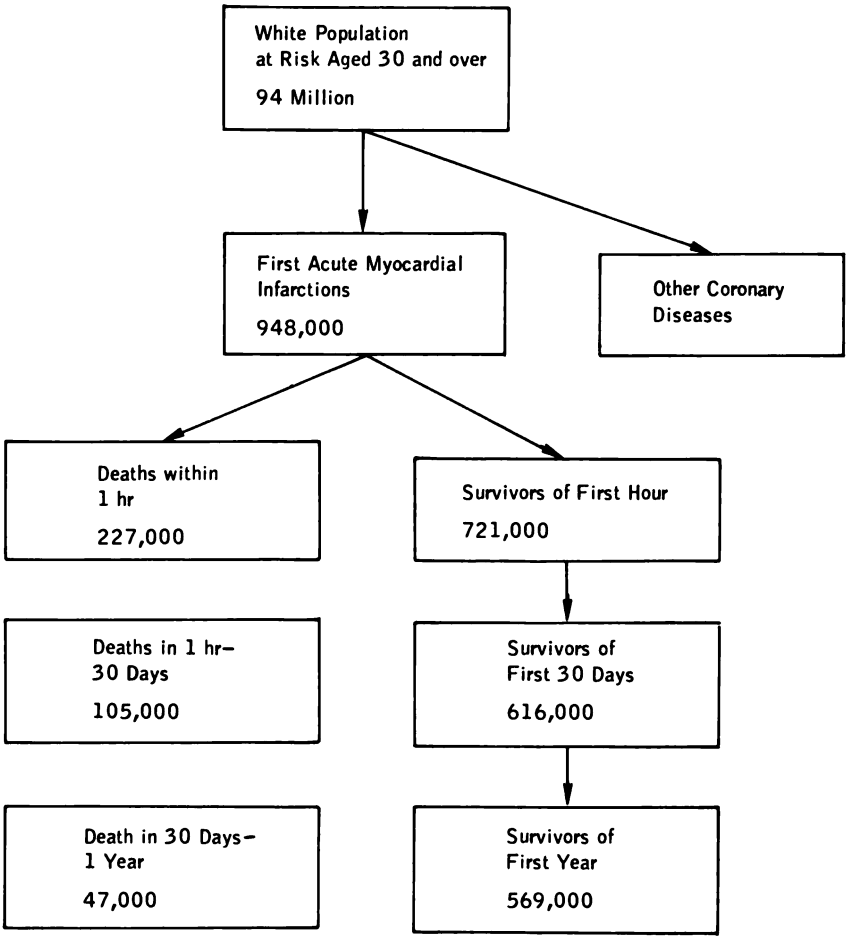

FIGURE 2 Estimates of death or survival at various periods following an acute myocardial infarction (1970).

following an acute myocardial infarction. The cumulative first-hour, first-month, and first-year case-fatality rates among first myocardial infarctions are 24%, 35%, and 40%, respectively. Accordingly, slightly more than one-half million victims of first myocardial infarctions will survive the first year.

The gaps in our current knowledge, particularly those relating to the contribution of "other coronary disease" and "silent myocardial infarctions" to this picture, render these figures minimum estimates (it should also be pointed out that cases in existence on January 1, 1970, are not

included). It may be expected that an additional million persons will develop "other coronary disease," mainly angina pectoris or coronary insufficiency, in 1970. Although the prevalence of "silent" myocardial infarction has been estimated as one fifth that of all infarctions,[18] its natural history is relatively unknown, thus precluding the estimation of its contribution to the total picture here.

DISCUSSION

It has become clear that hospital records may not provide accurate estimates of the prevalence of acute myocardial infarction in the community. In addition to their inherent selective biases in reflecting the "true" prevalence,[24] hospital records and clinical studies will exclude the majority of "silent" myocardial infarctions and "sudden" deaths.[9,16,25] Figures derived from this source would therefore be underestimates and would lack a proper population base.

Although mortality data are often useful in the determination of the prevalence of many diseases, they are of only limited value for acute myocardial infarction for several reasons. The major and most pertinent problem is the fact that acute myocardial infarction is not coded as such, but is included in the general International Statistical Classification (ISC) category of arteriosclerotic heart disease, including coronary. Second, the sensitivity and specificity for this cause of death are generally low.[2,12] Finally, mortality measures for this disorder cannot reflect the magnitude of its yearly incidence, because they are confounded by the contribution of cases already in existence from previous years, as well as by the exclusion of persons who survive the year following onset.

Nevertheless, an examination of mortality statistics may help in the evaluation of the estimates proposed in this report. Deaths attributed to the ISC category in the U.S. white population aged 30 years and over in 1964 numbered 505,000, for a death rate of 6 per 1000 population.[21] The number of deaths attributed to acute myocardial infarctions reported here is 379,000 (Figure 2), for a death rate of 4 per 1000 population. The difference between the two figures could be attributed to the contributions to mortality from "other coronary disease," "silent" myocardial infarction, and "leftovers" from previous years.

Morbidity data gathered by the National Health Examination Survey, in addition to being estimates of prevalence, are not specific for acute myocardial infarction. Among white persons aged 18–79 years, the prevalence of definite coronary heart disease was calculated as 2.7 million.[20] Considering these limitations, our figure of about three fourths of

a million new cases of acute myocardial infarction (excluding sudden death) seems reasonable.

An additional problem in the already complex picture is the lack of comparable definitions and criteria among epidemiologic and clinical studies of this disorder.[23] These limitations should be borne in mind before the estimates proposed here are accepted. Standard errors for the incidence rates presented in Table 1 were not calculated, simply because other errors included in these estimates exceed sampling variability.

The estimates given here should be adequate, at present, for the determination of the magnitude of the problem of acute myocardial infarction in the U.S. white population in 1970. The actual prevalence could be determined, of course, by a costly prospective study, using a randomly selected sample of the U.S. population. For practical purposes of administering medical care and planning health services, these estimates based on existing knowledge could be profitably used.

SUMMARY

Available estimates of the annual incidence of acute myocardial infarction have been applied to the U.S. white population, aged 30 years and over, projected to the year 1970. With the aid of a simplified working model of the natural history of this disorder, its prevalence at various periods of time after onset is approximated. It is expected that about a million persons will experience a first acute myocardial infarction in 1970 and that 250,000 of these would cause sudden death. It is anticipated that slightly more than one-half million would survive the end of the first year.

The work reported here was supported in part by Public Health Service research grant CD 00135 from the National Center for Chronic Disease Control.

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The Role of Thrombosis in Myocardial Infarction

J. R. A. MITCHELL

The reported prevalence of thrombotic coronary occlusion in patients dying with myocardial infarct has varied from 0% to 96% (Table 1). Two inescapable conclusions arise: (1) although one cannot decide at first sight whether 0% or 96% is more realistic, they cannot both be correct; and (2) if we are to use the word "infarct" in a pathologically meaningful way, to imply an area of necrosis due to impairment of blood supply, the acceptance of results that indicate no impairment of supply should lead us to rename the syndrome. The problem is not merely one of semantics; we urgently need effective measures to prevent and treat the syndrome that we know as "myocardial infarction," and we cannot develop such measures unless we understand the pathologic nature of the syndrome.

A fundamental scientific principle is that one should compare like with like by acceptable methods. This involves a clear initial definition of the material to be studied and a recognition of important variables within the material. Failure to apply this discipline may result in considerable confusion. To resolve the confusion, we need to concentrate on three points: (1) the selection of material for study, (2) the development of suitable techniques, and (3) the recognition that occlusion is a dynamic process and that a time scale must be incorporated into study results.

SELECTION OF MATERIAL FOR STUDY

In some studies, the prevalence of coronary occlusion in patients dying with a clinical diagnosis of cardiac infarct has been determined. There is abundant evidence [1,19] that clinical diagnosis is a fallible guide to necropsy findings. Moreover, death certificates fulfill the purpose for

TABLE 1 Prevalence of Thrombotic Coronary Artery Occlusion in Groups of Patients Stated to Have Died of Myocardial Infarct

Source	Prevalence of Thrombotic Occlusion, %
Master *et al.*[13]	0
Gross and Sternberg [8]	7
Spain and Bradess [24]	16–54
Branwood and Montgomery [4]	21
Snow *et al.*[23]	59
Davenport [5]	60
Barnes and Ball [2]	69
Blumgart *et al.*[3]	90
Harland and Holburn [9]	91
Harrison and Wood [10]	93
Muri [18]	94
Master *et al.*[14]	96

which they were intended (to reassure the community that death was due to a natural disease) and are not necessarily valid scientific documents. They therefore give only an approximate idea of which of many possible natural events has been responsible for death. If clinical diagnosis is to play a satisfactory part in the selection of patients for study, it must take advantage of all available ancillary aids and we must recognize that many of the groups in Table 1 were collected before the era of 12-lead electrocardiography and serum-enzyme studies.

Other groups have accepted sudden death as being synonymous with cardiac infarct. Everyone would agree that cardiac infarct can cause sudden death, but not everyone would agree that the class of sudden deaths is homogeneous. To obtain information on this point, we studied the hearts of 19 patients who had died suddenly and whose necropsies had failed to reveal the cause of death.[16] In six patients we found massive cardiac necrosis of recent origin; all these patients had occluding thrombi. The hearts of seven patients had fibrotic areas that indicated previous massive necrosis, but no histologic evidence of recent change could be found; all these patients had recanalizing thrombi or occlusion by plaques. The remaining six patients were the most interesting, for four of them had evidence of focal inflammatory disease either in the coronary arteries (one patient) or in the myocardium (three patients); in the other two patients, the heart was entirely unremarkable. Undoubtedly, the use of electron microscopy (if we can overcome the problems

of autolysis) and enzyme histochemistry will be helpful in studies of this kind. My purpose is not to present these as definitive results, but simply to show that "cardiac sudden death" is not synonymous with "cardiac infarct," inasmuch as one third of the sudden-death group may have conditions of an entirely different nature. This small study serves to reinforce the view of many pathologists [7] that it is simple to record the diseases that a patient died *with,* but not simple to decide which a patient died *of.*

Another way in which groups of patients with "cardiac infarct" have been collected is at necropsy. Some workers have considered that all areas of necrosis or scarring represent infarct,[11] but Schwartz and I challenged that view.[17] In an unselected necropsy series, we measured the length of each area of necrosis or scarring, in the long axis of the heart, by assessing the number of serial 4-mm transverse slices on which it appeared. The scarring and necrosis did not show a continuous distribution pattern but segregated into two groups: small and large lesions. The differential age and sex pattern of the two types of lesion is shown in Table 2, and the lack of correlation between the two groups is shown in Table 3. Large lesions occurred in patients with coronary stenosis and with occlusions; the presence of small lesions did not correlate in any way with coronary disease, and there is therefore no evidence that they form part of "ischemic heart disease" as currently defined.

In summary, if one attempts to assess the prevalence of occlusion in groups of patients selected on clinical grounds, in patients who have died suddenly, or in patients whose necropsies reveal various sorts of cardiac necrosis or scarring, one must expect to get low and variable values, for the group that one wishes to study is being diluted with unrelated and irrelevant material.

TABLE 2 Age and Sex Prevalence of Small and Large Cardiac Lesions

Age, years	Prevalence, %			
	Men		Women	
	Large	Small	Large	Small
35–44	7	13	0	9
55–64	13	29	5	11
65–74	25	40	8	31
75	13	38	11	32

TABLE 3 Prevalence of Small Cardiac Lesions in Men and Women with Large Lesions, Compared with Prevalence in Unselected Series

Selection Group	Prevalence of Small Lesions, %
Unselected sample, men ($n = 75$)	31
Men with large lesions ($n = 57$)	28
Unselected sample, women ($n = 62$)	21
Women with large lesions ($n = 22$)	18

TECHNIQUES USED FOR STUDYING THE CORONARY TREE

Branwood and Montgomery [4] suggested that "the incidence of coronary thrombotic occlusion is a direct function of the care and technique employed in the examination." In an analogous situation, Saphir [21,22] showed that myocarditis, considered by many to be rare, increased in apparent prevalence in a general necropsy survey according to the number of blocks examined, eventually reaching a stable value that approximated to its true frequency. In the study of coronary occlusion, there is no substitute for serial transverse sectioning of the major epicardial arteries. Injection of a radiopaque mass and subsequent radiography provide an invaluable guide to the anatomy of the coronary tree and a useful pointer to the areas that merit especially detailed study, but radiographs cannot be used as the sole arbiter of stenosis or occlusion.

Having developed an acceptable technique, one must then define "occlusion," because not all workers have used it in the same sense. The figures that appear in Table 4 were based on the following criteria:

1. An occluded artery is one in which there is no injection mass in a segment, as judged under a 40-power dissecting microscope, although the injection mass is present on both sides of the segment.
2. A stenosed artery is one in which at least one channel containing injection mass is present.
3. A probably occluded artery is one in which a fleck of pale material can be seen under a 40-power dissecting microscope but cannot be identified as a channel containing injection mass. If it is a channel, it is there-

TABLE 4 Occlusion Status and Nature of Occlusion in Men and Women with Large Lesions of Various Histologic Ages

Histologic Age of Lesion	No. Patients				Nature of Occlusions or Probable Occlusions, as Number of Arteries		
	Total	Occluded	Probably Occluded	Stenosis Only	Thrombus	Recanalizing Thrombus	Plaque
<2 days	10	7	3	0	15	0	1
2–13 days	11	7	3	1	15	1	1
2–4 weeks	2	2	0	0	1	3	0
1–4 months	2	2	0	0	0	1	3
>4 months	21	10	4	7	0	24	6
All ages	46	28	10	8	31	29	11

fore less than 100 μ in diameter. (On subsequent histologic examination, most of these areas proved to be zones of calcification in plaques.)

One also needs to define the nature of the arterial lesions seen and one can recognize three types of occluding mass:

1. A thrombus is a mass consisting of blood constituents but differing from a clot in having a nonhomogeneous, often laminar, structure due to a selective deposition of platelets, white cells, and fibrin.
2. A recanalizing thrombus is a mass containing multiple small channels.
3. An arterial plaque is a mass not recognizable as either of the preceding types and consisting of fibrous tissue, cellular areas, lipid deposits, and calcium deposits in varying proportions. As Duguid [6] showed, the distinction between plaques and recanalizing thrombi is difficult and probably artificial.

IMPORTANCE OF THE TIME FACTOR

Patients die at various intervals after the onset of cardiac infarction, and, if occlusive arterial disease is a dynamic process, the time factor must be included in any investigation of the infarction–thrombosis relationship. It is possible to assess the age of an area of cardiac infarction with reasonable accuracy.[12] Schwartz and I [17] applied these criteria and the techniques and definitions already outlined to a group of 46 patients who were found at necropsy to have a single massive area of cardiac necrosis or scarring and to another 33 patients with more than one large lesion. It is the first group, with single lesions, that I want to discuss in detail (Table 4).

Of the 46 patients with single lesions, 25 died within 4 months of their initial episode. In one of the 25, the most severe lesion found was a simple stenosis, with a lumen recognizable under the dissecting microscope. Eighteen of the 25 (72%) had occlusions, and six (24%) had lesions in which, if a lumen was present, it was too small to be recognized under the dissecting microscope or differentiated from plaque constituents (i.e., less than 100 μ). A very different pattern emerged in the 21 patients who died 4 months or more after the episode; in this group, only 10 (48%) had occlusions, four (19%) had probable occlusions, and seven (33%) had simple narrowing as their most severe lesion. The prevalence of occlusions is clearly related to the time elapsed since the initial episode (4% of the patients who died within 4 months of the ini-

tial episode had simple narrowing, compared with 33% of the patients who died 4 months or more after the episode).

The nature of the occluding masses is also totally dissimilar: of the patients with recent lesions, 76% had recognizable thrombi, 12% had recanalizing thrombi, and only 12% had plaques; of the patients with older lesions, none had fresh thrombi, 80% had recanalizing thrombi, and 20% had plaques.

One must always remember that the composition of a necropsy series is the result of multiple selection factors, and that any findings may be related to the lethality of a condition, rather than to its initial causation. Nevertheless, the evidence is strong that the development of a large area of cardiac necrosis is due to the occlusion of a coronary artery with a platelet–leukocyte–fibrin thrombus.

NATURE OF THE OCCLUSION

Although the great nineteenth-century pathologists, especially of the German school, made a clear distinction between a clot and an arterial thrombus, the distinction was lost sight of in the first half of this century.[20] The belief has therefore arisen that the two are synonymous, with the important and progress-preventing corollaries that if one wants to study thrombosis one must first study coagulation, and if one wishes to prevent or treat thrombosis one must use anticoagulant drugs.

When blood clots in a stationary system, it forms a soft, homogeneous, dark red mass in which one finds all the blood cells entrapped in a fine fibrin mesh in their expected proportions (5 million red cells, 250,000 platelets, and a few thousand white cells per unit volume). An arterial thrombus is a firm, pale, friable nonhomogeneous mass with zones in which various cell types predominate (the lines of Zahn).[25] It consists mainly of platelets, with each zone of platelets fringed by polymorphonuclear leukocytes, and the whole mass held together by coarse fibrin strands. Although the basic components of a clot and of a thrombus are the same, the way in which they are arranged and their frequency of occurrence are markedly dissimilar. An analogy can perhaps be drawn between two languages, such as French and English, that use the same alphabet but different letter patterns to produce very dissimilar words.

Morphologic studies lead one to the inescapable conclusion that thrombus-building, and not coagulation, is the key process to study. We need to know how the platelets cohere as they do, what role the leukocytes play, and how the investing fibrin strands develop. We need to

know whether these processes are themselves abnormal in thrombotic disease or merely indicate some underlying change in other components of the circulating blood.[15] Only then can we develop rational and effective measures for the prevention and therapy of thrombotic disease of which myocardial infarction is such an important example.

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The Pathogenesis of Myocardial Infarct and Coronary Thrombosis

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It seems remarkable that there is still uncertainty about the sequence of events that leads to myocardial infarct. Part of the uncertainty is due to imprecise definitions of such terms as "coronary heart disease," "ischemic heart disease," and "myocardial infarction." For example, many persons who die suddenly and unexpectedly have neither clinical nor anatomic features of infarct, but the diagnosis of infarct is commonly made on the basis of circumstantial evidence. Although it seems probable that such persons died of ischemic heart disease, it is impossible to say, even on the basis of special techniques, whether an infarct was present. I therefore propose to confine my discussion to those cases in which infarct—in other words, an area of ischemic necrosis—has been definitely demonstrated.

Uncertainty about the pathogenesis of myocardial infarct has arisen also because different techniques have been used to examine the coronary arteries. Techniques have varied from a simple opening of the arteries longitudinally to transverse sectioning with or without injection of radiopaque material and with partial or complete microscopic examination. It must be accepted that anything short of detailed histologic examination will yield incomplete information regarding the state of the coronary arteries and the incidence of thrombosis.

I shall demonstrate that most, although not all, myocardial infarcts are due to thrombotic occlusion of a coronary artery, and that in most cases occlusive thrombosis has resulted from rupture of an atheromatous plaque.

MATERIALS AND METHODS

The material used has been previously described.[10] In brief, 64 hearts were examined, 53 with recent infarcts (sometimes superimposed on old

infarcts) and 11 with old infarcts only. The hearts were fixed in formalin, the coronary arteries were sectioned transversely at 3-mm intervals, and each block was examined microscopically. The incidence and locations of thrombi were tabulated.

Later, to learn something about the pathogenesis of the thrombi, blocks found to contain thrombus were examined by serial section. In all, 52 thrombi were examined. They were sectioned at 7 μ, and every tenth section was stained and examined.

RECENT INFARCTS

Coronary thrombosis was present in 48 (90.6%) of the 53 cases of recent myocardial infarct. Multiple thrombi were present in 15 (28%); in six of these, two separate thrombi were found in the same artery, and it might be postulated that the distal thrombus was an embolus. However, in eight cases, two different arteries were thrombosed, and in one case, four separate thrombi were encountered, each major artery being involved. In five cases, no thrombus could be found: three men, aged 19, 64, and 65, and two women, aged 69 and 71.

Of the total of 65 thrombi, 51% were in the left anterior descending coronary artery, 18.5% were in the left circumflex coronary artery, and 31% were in the right coronary artery.

Infarct was of the multicentric variety in seven cases, including two in which thrombus was not demonstrated. In all seven cases, there was severe generalized atheromatous narrowing of the coronary tree, with at least one previous fibrous occlusion of the lumen. Previous localized infarct was present in four cases.

In 26 of the 53 cases with thrombosis, death had been caused by the first localized infarct. In all these, a thrombus was found to be occluding the vessel supplying the damaged area. In 22, recent infarct was superimposed on old infarct. In the remaining cases, fibrous occlusion was found in the artery leading to the area of infarct, and thrombus was present in another vessel.

HEALED INFARCTS

Thirty-three hearts with healed myocardial infarct were examined. In 22, there was a superimposed recent infarct. No thrombus was found in the remaining 11 cases, which had an old infarct only. In all 33, the artery supplying the area of fibrosis had an old fibrous occlusion. In seven instances, fibrous occlusions were found that were not associated with fibrosis of muscle. Thus, occlusion need not result in infarction.

SERIAL SECTIONS OF THROMBI

Blocks from 52 thrombi were examined. Some of these involved only a single 3-mm block, whereas others involved up to 20 cm of artery. In 45 (86.5%), the thrombus was found over a soft, atheromatous plaque, and in 37 (71.1%), the underlying plaque could be seen to have ruptured. Atheromatous material was often found trapped in the overlying thrombus. A constant feature of plaques that had ruptured was the presence of large numbers of lipid-filled macrophages in the wall of the so-called atheromatous abscess.

Another important feature of coronary thrombosis that appeared in the serial sections was the different ages of different parts of some thrombi. A portion near the site of rupture might show beginning organization, while a segment either distal or proximal had thrombus of more recent appearance. As many as three stages were apparent in one occluding thrombus.

As expected, serial sections proved especially useful in the cases with multiple thrombi. Material was available in 11 such cases. In three, embolism to a distal segment was thought to account for the second thrombotic occlusion. In the remaining eight, the individual thrombi represented separate pathologic events. In any one case, the thrombi were associated with ruptured plaques and were always of different stages of development.

DISCUSSION

THE INCIDENCE AND SIGNIFICANCE OF CORONARY THROMBOSIS

The reported incidence of coronary thrombosis in hearts with infarcts varies from 21% [3] to 100%.[15] It is generally agreed that the most important factor explaining these differences is the thoroughness of the search. The results of the present study indicate that thrombus was present in nine of 10 cases. Furthermore, the thrombus was usually found blocking the artery supplying the damaged area. Old fibrous occlusions, thought to represent organized thrombi, were demonstrated in association with healed infarcts.

Spain and Bradess [16] found an increasing incidence of coronary thrombosis with survival time and concluded that thrombosis was the result, rather than the cause, of infarction. This view had previously been suggested by Branwood and Montgomery [3] and has since been supported by Baroldi [1] and Meadows.[13] However, this study has demonstrated that the majority of thrombi are found in association with rup-

tured plaques, and confirms the earlier work of Leary,[11] Chapman,[4] Constantinides,[5] and Friedman and Van den Bovenkamp.[9] To accept the hypothesis that the infarct causes the thrombosis, one would have to propose a mechanism by which the infarct (of unknown cause) caused not only the thrombus in the artery supplying the damaged area but also rupture of the underlying atheromatous plaque. It seems more reasonable to accept the traditional view that thrombosis causes infarction.

LOCATION OF THROMBI

More than half the thrombi in our series occurred in the left anterior descending coronary artery. This does not necessarily indicate that thrombosis is more prevalent in that branch. Forty-six percent of left anterior descending thrombi were in the first centimeter, where occlusion would most probably prove fatal. More distal occlusions are more likely to have a satisfactory clinical course. Postmortem findings probably differ from clinical experience in this respect.

OCCURRENCE OF MULTIPLE THROMBI IN FATAL MYOCARDIAL INFARCTS

In a very careful study of 315 cases of coronary heart disease, Foord [8] found 23 cases (7.3%) that had recent thrombi in two coronary arteries and two cases (0.6%) in which all three arteries were occluded by thrombi. In the present study, 28% of recent infarcts showed multiple coronary thrombi. Careful examination revealed that these usually represented separate thrombotic episodes. It would therefore appear that fatal myocardial infarct sometimes results from a series of coronary thromboses, each due to rupture of a soft atheromatous plaque. Such a view is in keeping with the conclusions of Blumgart *et al.*,[2] who demonstrated that coronary thrombosis does not necessarily produce any characteristic clinical manifestation, a finding confirmed by Muri.[14]

TYPE OF INFARCT

The common type of infarct is a localized area of necrosis involving the entire thickness of the myocardium. Less commonly, necrosis is multicentric, being largely subendocardial and patchy and sometimes extending around the entire left ventricle. In our series, there were seven multicentric infarcts. In two, there was no thrombus. This tends to confirm previous reports of a low incidence of thrombosis in this type of infarct.[6,12] Infarct of this type is perhaps related to the arcade of anastomosing arteries that supplies the subendocardial tissues.[7]

AGE

Yater *et al.*[18] reported a higher incidence of recent thrombotic occlusion in patients under 40 years old than in older patients. Taken together with some experimental studies, this led to the suggestion that myocardial infarction in the young or middle-aged man is a separate disease.[17] Our observations lend no support to this hypothesis. Thrombosis was equally common in all age groups. Furthermore, the youngest patient in our series, a man of 19, died of a multicentric infarct without thrombosis.

CONCLUSIONS

It is clear that coronary thrombosis is the decisive factor in the natural history of coronary atheroma. Myocardial infarction results from one or more coronary thromboses in the large majority of cases. In the majority of cases, too, thrombosis results from rupture of atheromatous plaques. Thrombi frequently propagate, so that thrombi may extend or occlusions develop over some period. It follows that, to prevent coronary thrombosis, future research must be directed at the questions of why atheromatous plaques change from firm fibrous structures into soft, cellular, mushy lesions, why they then rupture into the arterial lumen, and how the extension of existing thrombi can be prevented.

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Initiation of Thrombosis in Human Coronary and Cerebral Arteries

P. CONSTANTINIDES

Seven years ago, we produced advanced fibrous atherosclerosis of the human type in rabbits.[8] We then found that we could induce thrombi in the aorta and coronaries of such animals if we exposed them to a combination of pressor amines (e.g., epinephrine, norepinephrine, angiotensin, and serotonin) and agents that promoted coagulation and attacked the endothelium, such as Russell's viper venom.[9,11]

The most remarkable thing about the thrombi obtained in this manner was that they reproduced precisely the anatomic characteristics of human coronary thrombosis: they could be induced only in animals whose arteries displayed fibrous atherosclerosis or other wall damage (e.g., calciferol damage)—but never in rabbits with normal vessels—and they were intimately associated with plaque hemorrhages. Histologic study showed that most hemorrhages developed by the entry of blood into the arterial wall through breaks in plaque surfaces, and that the thrombi therefore represented physiologic seals over these cracks.[3,10] It appeared that our experimental procedure had cracked the rigid collagenic wall of the atherosclerotic vessels, and that the resulting breaks were sealed by thrombi after allowing some blood to penetrate into the wall. It also seemed that the reason such breaks could not be elicited in normal arteries was that the normal walls were sufficiently elastic to stretch without cracking.

To find out whether human coronary and cerebral artery thrombosis was likewise initiated by cracks in atheroma surfaces, we then made for the first time a complete serial-section study (every 10 μ) of 20 thrombosed and 16 control coronary arteries and of 10 thrombosed cerebral arteries from persons necropsied at the Barnes Hospital in St. Louis and the Vancouver General Hospital, respectively.

After examining more than 55,000 sections, we discovered that all

thrombi were associated with breaks (of various sizes and shapes) in the atherosclerotic or fibrosed arterial wall, and that almost all hemorrhages could be traced to entry of blood through the same breaks.[2,4-7] Most of the cracks were narrow, short, and hard to identify without connective-tissue stains (which explains why breaks and ulcerations were found responsible for thrombosis in only a minority of all the cases previously studied). If it is considered that most of the histologic studies of the past were done on just a few random or low-frequency step-serial H&E sections, it will be realized that under those circumstances only the biggest breaks would be detected; all the small ones would be missed.

Similar findings, with various degrees of documentation, were recently reported by Sinapius,[14] Chapman,[1] and Friedman and Van den Bovenkamp,[12] who studied thrombosed coronaries with step-serial sections. (W. A. Harland, author of the preceding paper, has also made step-serial sections of a large number of thrombosed coronaries and has found breaks in the wall in most cases.)

We have concluded that thrombosis in the major arteries of the human heart and brain is initiated in most cases by breaks in the atherosclerotic or fibrosed walls of these vessels, although the rate of growth, the ultimate size, and the fate of the resulting thrombi may well depend on systemic chemical and local rheologic factors.

We are convinced that the subthrombic breaks do not materialize *after* the establishment of thrombosis but *precede* and *cause* the thrombi, because we could demonstrate:

1. That most breaks were overlaid and filled by the platelet nuclei of the thrombi;
2. That blood had penetrated into the wall through subthrombic fractures of sometimes thick collagenic sheets (this would not be conceivable if the wall had broken *after* the thrombi formed, because it would entail the passage of blood through solid thrombi); and
3. That fragments of the atherosclerotic wall, including shreds of collagen, were buried within several thrombi.

Our findings also show that the exposure of fatty atheroma gruel to the bloodstream is by no means indispensable for the development of thrombi over wall fractures. Many of the fractured plaques were of the predominantly fibrous or collagen-rich type, without fatty gruel contents, and in a few cases of cerebral artery thrombosis the fissured vessels were merely fibrosed, with no atheromata in the region of the thrombi.

The next important problem is, of course, to find out what causes these breaks.

We feel that future research should explore the following possibilities:

1. That the breaks are caused by the action of chemicals that attack the endothelium and inner wall and "unzip" interendothelial junctions (e.g., enzymes, vasoactive amines, and antigen–antibody complexes);
2. That the breaks are caused by mechanical forces acting on the brittle vessels from within the lumen (e.g., pressure explosions) or from outside (e.g., the rhythmic back-and-forth bending of the coronary arteries with every heartbeat); and
3. That the breaks are due to changes in the plaque itself, leading to increased fragility or spontaneous disintegration of the plaque surface (e.g., cell depopulation, molecular collagen changes, and continuing destructive atherogenic processes).

I might add that ruptures of plaque capillaries cannot be responsible for the thrombi, as theorized by Paterson,[13] inasmuch as we found no evidence of capillary rupture in the great majority of the cases we studied by the complete serial-section technique.

Further progress toward the solution of this problem is likely to result from the increased use of *in vivo* and *in vitro* experimental models combined with the continued analysis of human material.

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Strokes: An Estimate of the Magnitude of the Problem in the United States, 1965

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No direct measurement has ever been made of the magnitude of the problem of cerebral infarction or any other kind of cerebrovascular accident in the United States. Indeed, the fact that nothing on this subject was published in connection with the National Health Interview Survey can be taken as an indication of its complexity. Therefore, if we are to attempt to assess this problem, not only must we depend on estimates, but we must base the estimates on inadequate data. Two types of data are available: rates derived from local population studies and rates derived from national mortality figures. Although the local studies may have been carried out well, all of them are small and unrepresentative of the nation as a whole. Mortality data have long been known to provide little more than a general index of the epidemiologic characteristics of the more commonly fatal and easily diagnosed diseases. But there is a growing literature that examines their validity in general and their validity as it relates to cerebrovascular disease in particular. Therefore, although we know that these data are erroneous, we are learning where the errors lie and can sometimes make adjustments for them.

Because of the poor quality of the fundamental information, it has been necessary to make many assumptions, but these have always been kept as simple as possible. For instance, in estimating prevalence, which requires consideration of data for several years, it has been assumed that the incidence has not changed during the period 1953–1965. In fact, there is good reason for believing that the incidence changed over that period, but, because we have little idea of the magnitude of the change, the possible gain due to guessing must be weighed against the loss in simplicity of the calculations.

All the estimates in this presentation are for 1965, the most recent year for which mortality data are available (I. M. Moriyama and

R. Israels, personal communication) for the population of the continental United States. In all the tabulations, data are given for cerebral hemorrhage and for all cerebrovascular accidents, as well as for cerebral infarcts. This is done because, in addition to diagnostic problems in differentiating between infarct and hemorrhage, there are statistical and procedural reasons, to be outlined below, why the two may be confused. Thus, the most accurate estimates that can be made are unquestionably those for rubrics 330–334 combined—that is, for all "vascular lesions affecting central nervous system."

MORTALITY

Published tables of mortality from cerebral infarct provide a gross underestimate of the importance of this disease, for two main reasons: (1) the annual reports of the National Center for Health Statistics are concerned only with a single entry on each death certificate, the underlying (or principal) cause of death, and (2) internationally accepted coding conventions require that such death-certificate entries as "cerebrovascular accident" and "stroke," which do not explicitly state whether a lesion is hemorrhagic or an infarct, must be coded under ISC rubric 331, "cerebral hemorrhage." Attempts will be made below to adjust the 1965 national mortality data for both these sources of error.

A third problem is to distinguish between cerebral thrombosis and cerebral embolism, which, in the usual publications of mortality data, are grouped under the single ISC rubric 332, "cerebral embolism and thrombosis." Partly because any correction factor designed to distinguish between the two would be a gross approximation, and partly because current clinical research indicates that emboli play a larger role in the pathogenesis of cerebral infarct than was previously supposed, no attempt will be made here to subdivide "cerebral infarct." Other difficulties for which no allowance can be made in the present study are discussed elsewhere.[1]

The last year for which information is available concerning death-certificate entries related to contributory (or secondary) causes of death is 1955. A recently published supplement to the mortality statistics for that year presents an extensive analysis of all the entries appearing on a stratified sample of death certificates registered in the continental United States.[11] Among the tabulations is one that presents sex-, age-, and race-specific estimates of the total number of death certificates bearing an entry coded under rubrics 331, 332, and 330–334. These data were age grouped, by 10-year intervals from 35 through 84, with

the sexes and two principal color groups (white and nonwhite) kept separate. Those below and above these age limits were taken as two pooled groups. Equivalent data from the regular mortality report for 1955,[10] which concerned only underlying causes of death, were then grouped similarly, and each sex-, age-, and race-specific subtotal was divided into the equivalent subtotal of contributory and underlying deaths combined. The resulting ratios provide a measure of the extent to which use of underlying causes alone underestimates mortality from cerebrovascular disease and can be used as multipliers to correct the figures on deaths from underlying causes for other years. Such corrections for the 1965 data are shown in Table 1 *; age-, sex-, and race-specific rates estimated from them are shown in Table 2.

Next, we attempted to correct for the regulation that requires that imprecise entries be coded and tabulated as "cerebral hemorrhage." The correction factors for this purpose were derived from an ongoing study in New Haven, Connecticut,[3] in which all available clinical, pathologic, and other information concerning the city residents certified as having died with a cerebrovascular accident between 1962 and 1964 has been collected and analyzed.[4] This has permitted us to estimate the proportion of deaths coded as "cerebral hemorrhage" that were actually associated with cerebral infarct. Because the study is relatively small, the ages had to be grouped on a broader basis than in Table 1, but sexes and color groups were kept separate. Appropriate proportions were subtracted from the total national deaths reported under "cerebral hemorrhage," and added to those reported under "cerebral infarct," and *vice versa*. The results of these adjustments, with rates calculated from them, are given in Table 3. It should be noted that there are no grounds for assuming that New Haven is typical of the nation.

INCIDENCE

With a single exception, to which we shall return, incidence studies of cerebrovascular disease in general, and of cerebral infarction in particular, fall into two broad groups: (1) those which extend surveys originally designed to study coronary heart disease (such as those in Framingham and Los Angeles) and (2) newer surveys that are directly

* The assumption here, as in the estimate of prevalence referred to above, is that there has been no secular change over the period 1955–1965, except that in this instance changes in age-specific mortality rates would be immaterial, provided that the ratio of underlying-cause entries to all entries remains constant. We have no way of knowing whether that is the case.

TABLE 1 Estimated Gross Numbers of Persons Dying with Specified Cerebrovascular Diseases, U.S. Population, 1965, by Age and Sex *

	Cerebral Hemorrhage (331)				Cerebral Infarct (332)				All Cerebrovascular Accidents (330–334)			
	White		Nonwhite		White		Nonwhite		White		Nonwhite	
Age, years	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Less than 35	2,218	949	561	322	294	325	60	39	3,959	2,445	940	741
35–	1,224	893	769	640	499	412	122	75	2,779	2,299	1,262	1,141
45–	3,304	2,629	1,993	1,502	1,598	1,014	516	381	6,870	5,647	2,638	2,418
55–	8,374	6,018	2,740	2,597	4,896	3,297	1,115	940	17,803	12,076	4,770	4,222
65–	18,016	16,744	3,380	3,599	11,776	10,783	1,705	1,683	38,887	35,420	6,378	6,192
75–	22,363	28,462	2,069	2,234	16,099	19,842	1,189	1,612	51,789	64,738	4,096	4,578
85 and over	14,084	15,258	694	942	6,468	10,764	325	550	20,776	36,322	1,275	1,831
Unstated	5	13	17	6	16	25	4	6	29	47	25	12
TOTAL	69,588	70,966	12,223	11,842	41,646	46,462	5,036	5,286	142,892	158,994	21,384	21,135

* 331, 332, and 330–334 in the headings of this and the ensuing tables are based on the 1957 edition of International Statistical Classification of Diseases.

TABLE 2 Estimated Rates per 1000 of Persons Dying with Specified Cerebrovascular Diseases, U.S. Population, 1965, by Age, Race, and Sex

	Cerebral Hemorrhage (331)				Cerebral Infarct (332)				All Cerebrovascular Accidents (330–334)			
	White		Nonwhite		White		Nonwhite		White		Nonwhite	
Age, years	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Less than 35	0.04	0.02	0.07	0.04	0.006	0.006	0.008	0.005	0.08	0.05	0.1	0.1
35–	0.11	0.08	0.63	0.45	0.04	0.04	0.10	0.05	0.3	0.2	1.0	0.8
45–	0.34	0.26	1.9	1.3	0.16	0.10	0.50	0.33	0.7	0.5	2.6	2.1
55–	1.1	0.75	3.7	3.3	0.66	0.41	1.50	1.2	2.4	1.5	6.4	5.3
65–	3.8	2.9	8.2	7.5	2.4	1.8	4.1	3.5	8.2	6.0	15.5	12.9
75–	10.3	9.5	10.9	9.8	7.4	6.6	6.3	7.1	23.8	21.5	21.7	20.2
85 and over	38.0	25.1	16.9	17.4	17.4	17.7	7.9	10.2	56.0	60.0	31.1	33.9
Crude rate	0.83	0.82	1.09	1.00	0.50	0.53	0.45	0.44	1.7	1.8	1.9	1.8

TABLE 3 Estimated Rates per 1000 of Persons Dying with Specified Cerebrovascular Diseases, U.S. Population, 1965, by Age, Race, and Sex, with and without Adjustment for Coding Regulations

Age, years	Cerebral Hemorrhage (331)				Cerebral Infarct (332)			
	White		Nonwhite		White		Nonwhite	
	Male	Female	Male	Female	Male	Female	Male	Female
NOT ADJUSTED								
35–64	4.6	3.3	16.3	14.1	0.8	0.5	1.1	0.9
65+	74.8	71.4	95.8	85.2	3.1	4.2	2.4	2.8
ADJUSTED								
35–64	3.1	3.0	13.6	10.3	2.2	0.8	3.9	9.4
65+	54.9	53.1	75.3	50.1	11.8	23.6	22.9	47.3

concerned with cerebrovascular disease (such as those in Seal Beach, California; Chicago; and Missouri). In both groups, the numbers of new cases have been small—in the first, because the study population is just moving into the age groups in which stroke is a serious risk, and in the second, because the studies have not on the whole been under way long enough to produce a satisfactory yield of publishable information. The exception is a survey conducted by the Connecticut State Department of Health in Middlesex County, Connecticut. Records were kept of every person who suffered a cerebrovascular accident between April 1, 1957, and March 31, 1959; each was identified and followed for 5 years. A person's inclusion in the survey did not depend on whether the cerebrovascular accident was the first one the person had had. In all, there were 191 such persons, 91 of whom had an infarct, in a population of about 83,500.

In this paper, estimates of incidence and prevalence of cerebral hemorrhage and of all cerebrovascular accidents are based on only the Middlesex data; for cerebral infarcts, the Framingham cases are added.[2,5] In the Seal Beach population, although by the end of 1965 there were 84 cases of cerebral thrombosis among 132 new cases of cerebrovascular accident, the rates differ considerably from those in any other study (W. B. Kannel, personal communication), probably because of the highly selected nature of the population. We have therefore omitted them.

The age- and sex-specific incidence data for the three diagnostic categories under consideration were plotted on semilogarithmic paper, with both Framingham and Middlesex data being used in the case of cerebral

infarct. With the single exception of cerebral hemorrhage in males, for which a curve was fitted by eye, the points came very close to being rectilinear (Figures 1 and 2). Therefore, to smooth the other five curves, the following regression was fitted to the published incidence data:

$$\log y = a + bx,$$

where y is the incidence and x is age. The five regression formulas are given in Table 4, and the sex- and age-specific incidence rates derived from them, with the age-specific incidence rates for cerebral hemorrhage in males, are given in Table 5. by relating these to the estimates

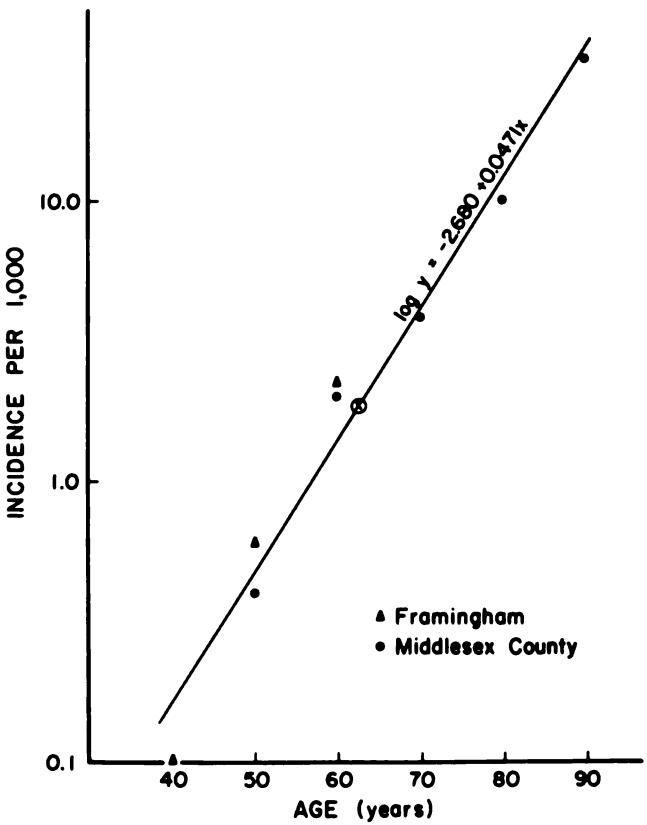

FIGURE 1 Regression between logarithm of incidence of cerebral infarct in females and age in Middlesex County, Connecticut, and Framingham, Massachusetts. Note how well the regression fits the data. (Similar plot for males is shown in Figure 4.)

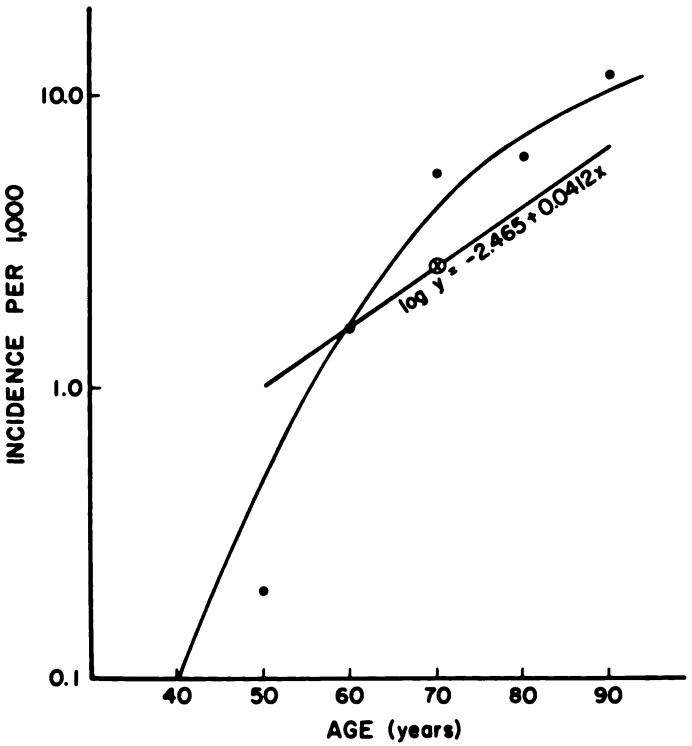

FIGURE 2 Regression between logarithm of incidence of cerebral hemorrhage in males and age in Middlesex County, Connecticut. The regression does not fit the data, so a curve that was fitted by eye was used as a basis for estimates.

TABLE 4 Regression Formulas for the U.S. Logarithms of the Incidences of Specified Cerebrovascular Disease, White Persons Only

Sex	Cerebral Hemorrhage (331)	Cerebral Infarct (332)	All Cerebrovascular Accidents (330–334)
Male		$0.0445X - 2.491$	$0.348X - 1.486$
Female	$0.0411X - 2.406$	$0.0471X - 2.688$	$0.0474X - 2.453$

TABLE 5 Estimated Incidences in U.S. White Persons of Specified Cerebrovascular Disease, per 1000 Population

	Cerebral Hemorrhage (331)				Cerebral Infarct (332)				All Cerebrovascular Accidents (330–334)			
	Males		Females		Males		Females		Males		Females	
Age, years	Rate	S.E.	Rate	S.E.	Rate	S.E.	Rate	S.E.	Rate	S.E.	Rate	S.E.
Less than 35	—	—	—	—	—	—	—	—	—	—	0.1	0.0
35–	0.1	0.10	0.18	0.17	0.24	0.17	0.17	0.17	0.75	0.33	0.27	0.2
45–	0.2	0.19	0.46	0.30	0.64	0.33	0.48	0.30	1.7	0.57	0.80	0.3
55–	1.6	0.64	1.2	0.54	1.7	0.66	1.4	0.58	3.9	1.00	2.4	0.7
65–	5.4	1.40	3.0	0.94	4.4	1.26	4.1	1.09	8.9	1.79	7.3	1.4
75–	6.1	2.25	7.5	2.16	12.5	3.21	12.0	2.73	20.0	4.02	23.0	3.7
85 and over	11.8	6.51	10.9	4.71	30.0	10.29	35.0	8.39	45.0	12.5	64.0	11.1

of the U.S. population in 1965,[8] it was possible to estimate the total number of new cases in white persons in that year (see Table 6).* Table 1 indicated that about 14% of the total mortality for cerebrovascular accidents was in Negroes; if one assumes that the proportion is the same for new cases, then it may be estimated that the total numbers of new cases in the nation in 1965 were about 210,000 for infarct and 462,000 for all cerebrovascular accidents.†

PREVALENCE

The direct estimation of prevalence requires an approach to a total population, or a probability sample of that population, by mail, interview, or medical examination. Theoretically, the interview and examination phases of the National Health Survey have been in a position to do this; presumably, the reasons that no publications have been forthcoming have been (1) that many persons who have suffered a stroke no longer live in their own homes and are therefore not available to the survey, and (2) that the retrospective self-diagnosis of stroke among survey respondents is inaccurate. Therefore, for our purposes, there is no choice but to use an indirect approach and, provided survival information is available, this can be done from incidence or mortality data.

* The standard errors were estimated as follows:

$$S_{(i)} = \sqrt{\frac{P(1-P)}{n}},$$

where $S_{(i)}$ is the standard error of a given age-specific incidence, P is that given incidence rate, and n is the relevant population at risk.[8] The standard error s_t for the whole population can then be calculated as follows:

$$S_t = \sqrt{\sum_{i=1}^{n} S_{(i)}^2}.$$

The 95% confidence limits of the age-specific estimates of the numbers of new cases in the nation will be:

$$1.96S_t \text{ (national population at risk)}.$$

It should be stressed, however, that, because P is derived from a smoothed curve and not directly from a population, these standard errors and confidence limits are at best gross approximations.

† This method of estimating the statistics for the nonwhite population is, like so many others in this paper, an approximation. It does, however, allow for the fact that all available data indicate that incidences are higher among Negroes than among white persons.

TABLE 6 Estimated Numbers of New Cases of Specified Cerebrovascular Diseases, U.S. White Population, 1965

Age, years	Cerebral Hemorrhage (331)		Cerebral Infarct (332)		All Cerebrovascular Accidents (330–334)	
	Males	Females	Males	Females	Males	Females
Less than 35	—	—	—	—	—	4,811
35–	1,063	1,988	2,551	1,939	7,973	3,079
45–	1,937	1,870	6,200	4,878	16,468	8,130
55–	11,810	9,648	12,548	11,256	28,686	67,536
65–	25,612	17,562	20,869	24,001	42,213	42,734
75–	13,267	22,522	27,187	36,036	43,500	59,069
85 and over	4,378	6,627	11,130	21,280	16,695	38,912
TOTAL	58,067	60,217	80,485	99,390	163,257	234,271
95% confidence limit	±19,610	±20,760	±22,875	±25,443	±32,009	±34,431

Because even the adjusted mortality data given in this paper almost certainly fall short of the true figures, we estimate prevalence from the incidences shown in Table 5 and the survival data derived from the survey of Middlesex County.

Prevalence was calculated in two ways. For both, it was necessary to estimate the probable maximum survival of a stroke victim, because the Middlesex follow-up lasted for only 5 years, by which time between 26% and 42% of the study population were still alive, depending on sex and the kind of stroke involved. This step, common to the two methods, was done by plotting the published survival data on arithmetic graph paper and then extrapolating linearly to identify the point at which the curve intersected the abscissa (Figure 3). Mean survival times were then calculated and multiplied by the number of new cases. The resulting estimates of gross prevalence and of prevalence rates in the population aged 35 and over on June 30, 1965, are given in Tables 7 and 8 under heading "A."

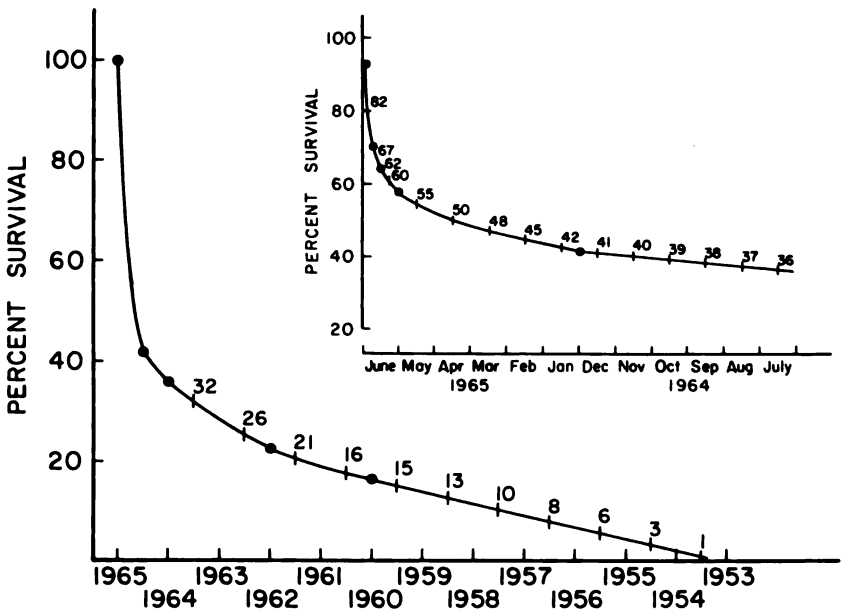

FIGURE 3 Survival curves for cerebral infarct for the sexes combined for Middlesex County, Connecticut, assuming that they were the basis for estimates of prevalence in mid-1965. The inset shows detailed data for cases occurring over the immediately previous 12-month period. The larger graph shows a curve for the total period; survival estimates for the years 1953–1960 have been made by linear extrapolation.

TABLE 7 Age- and Sex-Specific Estimates of the Prevalence of all Cerebrovascular Accidents in Persons Aged 35 and Over as of June 30, 1965, U.S. White Persons Only

Age, years	Males						Females					
	New Cases	Average Survival	Cases		Prevalence/1000		New Cases	Average Survival	Cases		Prevalence/1000	
			Estimate A	Estimate B	A	B			Estimate A	Estimate B	A	B
35–64	53,127	4.52	240,134	222,567	8.67	8.03	78,745	2.23	175,601	181,052	6.00	4.69
65–74	42,213	1.31	55,299	57,914	11.66	12.21	42,734	1.69	72,220	96,836	12.34	16.54
75 and over	60,195	0.72	43,340	43,213	17.02	16.98	107,981	1.12	120,939	118,939	33.49	32.94
All persons *	155,535	1.83	284,629	323,694	8.13	9.25	229,460	1.97	452,036	396,827	11.68	10.25
95% confidence limits	±33,477						±34,431					

* Prevalence estimate A for all persons = new cases × average survival. Prevalence estimate B for all persons = sum of age-specific estimates.

TABLE 8 Estimates of the Prevalence of Specified Cerebrovascular Diseases in the Total U.S. White Population Aged 35 Years and Over as of June 30, 1965

Disease	New Cases	Average Survival, years	Cases		Rate	
			Estimate A	Estimate B	per 1000	Population at Risk
Cerebral hemorrhage	117,221 ± 28,626	0.78	91,432	112,764	1.24	1.53
Cerebral infarct	179,875 ± 34,213	1.96	352,555	347,066	4.78	4.71
All cerebrovascular accidents	384,995 ± 47,052	1.89	736,665	720,521	9.87	9.78

The second method involved the initial assumption that there were 100 new cases from July 1, 1964, through June 30, 1965, distributed equally throughout that year, and that there had been 100 new cases in every previous 12-month period from which there were survivors. Then the numbers of survivors from each of the years 1954–1964, each of the months July 1964–May 1965, and each of the weeks in June 1965 were summed separately, and each separate total was corrected to the national level, taking into account the period under consideration. The statistics used in the computation of prevalence of cerebral infarct are shown in Figure 3, and the estimated prevalence figures are given as "estimate B" in Tables 7 and 8. In the absence of information about survival after a cerebrovascular accident in nonwhite persons, it is not possible to attempt any estimates of prevalence for them. It is probably fair to assume, however, that their inclusion would raise the number of cases of all kinds of stroke in the nation in mid-1965 to around 1 million. On a similar basis, one can conclude that over 400,000, in both color groups, had suffered a cerebral infarct.

By relating the statistics for white persons only to the national white population aged 35 and over,[8] we can determine, on the basis of the present data, that the gross prevalence of all cerebrovascular accidents in mid-1965 was around 1%, and that for cerebral infarct it was about 0.5% (Table 8); age- and sex-specific prevalence data for all cerebrovascular accidents in the white population are given in Table 7. The available data did not permit detailed calculations of this kind for the separate types of cerebrovascular accident.

DISCUSSION

In Figure 4, age-specific incidence rates are compared with age-specific mortality rates, with and without correction for cases in which the cerebrovascular accident was considered to be only contributory to death. It can be seen that, although the inclusion of contributory causes of death increases the estimates considerably, the mortality rates still fall far short of the incidences. Inasmuch as the vast majority of strokes are irreversible, it is fair to assume that persons who have suffered them die with them and that there should therefore be a fairly close correspondence between the two kinds of rates. I have stated that one important reason for the consistent discrepancy shown in this analysis is that the numbers of deaths in Table 1, and therefore the rates in Tables 2 and 3, fall short of the true figures. The amount of information given on a death certificate varies considerably from one state to another, and the propor-

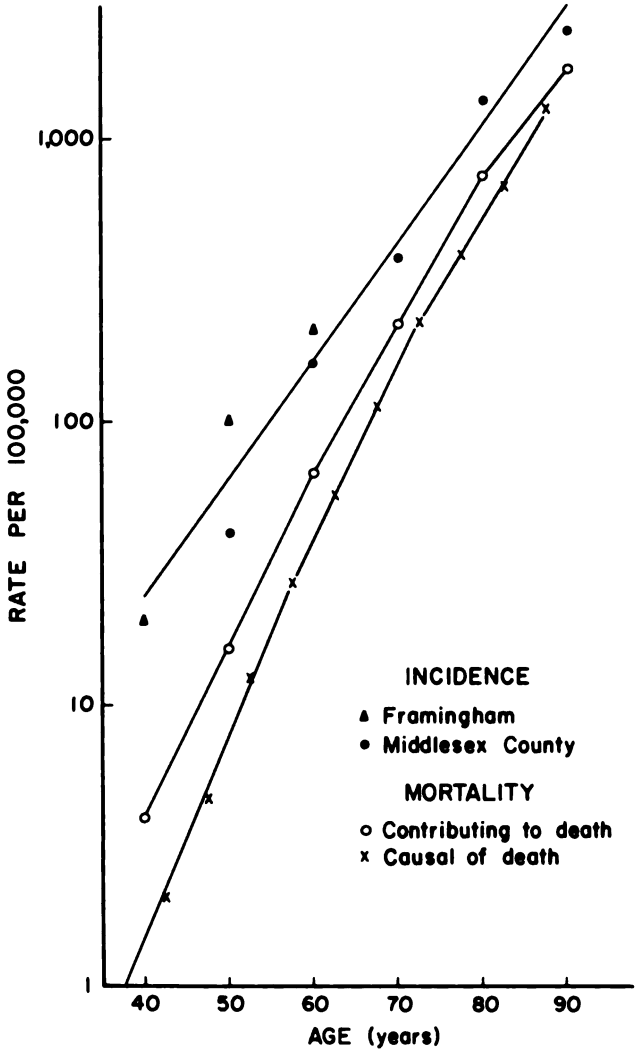

FIGURE 4 A comparison for 1965 between (1) reported mortality ascribed to cerebral infarct, (2) estimates of mortality rates for all deaths in which cerebral infarct was a contributory as well as an underlying cause, and (3) age-specific incidences. (Semilogarithmic plot for white males only.)

tion of certificates listing more than one cause ranges from about 62% in New England and the Mountain States to about 45% in Texas and parts of the deep South. Thus, it can be assumed that, in many instances in the southern states, a cerebrovascular accident was a contributory cause of death but was not mentioned.

It is more difficult to evaluate the incidence and prevalence data given in Tables 5 through 8. With the exception of cerebral infarct, in the consideration of which the Framingham Survey was taken into account, these data depend entirely on a 5-year study of some 85,000 people living in a semirural area. The addition of the Framingham data increases the size of the sample, but Middlesex County, Connecticut, and Framingham, Massachusetts, are less than 100 miles apart, so that not by any stretch of the imagination can data drawn from them be taken as representative of the nation.

Apart from the comparison with mortality data that has already been made, the only available statistic is in the frequently quoted statement [2,6] that in the late 1950's there were 2 million persons living in the United States who had suffered a stroke. This statement was first made by the staff of the Heart Control Program, United States Public Health Service. It later appeared in the 1964 report to the President by the Commission on Heart Disease, Cancer and Stroke,[7] with the addition that 80% of persons who suffer a cerebrovascular accident "survive the acute initial phase of the disease." The technical committee responsible for preparing that report re-evaluated the existing estimate in the light of subsequently published data on series of hospital cases and such information as was available from the National Health Survey (N. Borhani, personal communication). Even with the inclusion of cases among the nonwhite population, an estimate based on the present data cannot possibly approach 2 million, although it may be close to 1 million.

Aside from the parochial nature of the present data—the age-adjusted death rates for cerebrovascular accidents are lower in Connecticut and Massachusetts than in many other states [1]—an important discrepancy between them and the data cited in the report to the President lies in the 1-month survival rate. In Middlesex, this rate ranged between 18% and 65%, according to age, sex, and type of cerebrovascular accident. These rates cannot be ascribed to the fact that the Middlesex investigators included subsequent as well as primary strokes in any person, because the 1-month survival rate for all 154 primary attacks was only 45%. In our own New Haven study,[4] only 58% of those who died with a stroke survived for 2 days, and 16% survived for 1 month. The figures were very similar, whether the stroke was a primary or subsequent one. Thus, the question arises as to whether the 80% survival rate of the re-

port of the President's Commission is a serious overestimate that arose from bias in the selection of the hospital series on which it was based. A significant proportion of those who die of stroke within a few hours probably never reach a hospital, and the hospitals concerned with the rehabilitation of stroke patients (presumably those most likely to study and report survival) will almost certainly reserve beds for those with a favorable prognosis, in preference to those about to die. Therefore, the figure 2 million—which would mean a crude prevalence of 2.5% for the total population over the age of 35—may well be an exaggeration, not only for 1958 but also for 1965. But, with the rapidly increasing size of the population, and the slow but continuing increase in the expectation of life, it will not be an exaggeration for long.

Finally, the question of particular relevance in this volume is the estimate of 400,000 cases of cerebral infarct. If the numbers given for white persons in Table 8 are added to a hypothetical 50,000 for the nonwhite population, and the estimates for cerebral hemorrhage and cerebral infarct are then summed, the total falls more than 200,000 short of the total for all cerebrovascular accidents. This discrepancy can be accounted for by the fact that, first, 15% of the cases in Middlesex County were unclassified, and, second, 57% of those survived over 1 month. It is reasonable to assume that many of the survivors suffered a cerebral infarct, and that there were probably close to 500,000 cerebral infarct cases in the nation in 1965, giving a crude prevalence of over 0.5% among persons aged 35 and over.

In conclusion, these extensive tabulations provide little better than an educated guess as to the magnitude of the problem of cerebral infarct in particular or cerebrovascular disease in general in the United States. It is sobering that, 2 years after the Regional Program was launched, we know so little about the demands raised by one of the disease groups with which the Program is principally concerned.

I am grateful to Miss Jennifer Kelsey for her help in the preparation of Tables 4, 7, and 8. The research for this paper was supported in part by contributions from the United Fund of Old Saybrook, Connecticut.

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Incidence and Significance of Thromboemboli in Acute Cerebrovascular Occlusion

CLARK H. MILLIKAN

Cerebrovascular occlusion can be observed at autopsy examination without evidence of brain damage and without a history of any defect in neural function. In such instances, it cannot be stated whether the occlusion occurred suddenly or over a considerable period. "Acute cerebrovascular occlusion" is commonly defined in clinical terms as the swift onset of a focal neurologic deficit in a diagnostic setting in which evidence of trauma, neoplasm, abscess, or intracranial bleeding is absent and some evidence of concurrent cardiovascular disease is present —e.g., atrial fibrillation, myocardial infarction, carotid bruits, or demonstrated stenosis or occlusion in cerebral angiograms. If this liberal clinical definition is not permitted, we are restricted to the study of instances in which acute occlusion and appropriate brain infarction are revealed at autopsy. A reasonable approach is to analyze evidence from clinical and autopsy material, inasmuch as the latter provides limited insight into mechanisms that may change from minute to minute.

When the term "thromboemboli" is used in discussing cerebrosvacular disease, it may mean various things:

1. A thrombus that produces a fragment, which by definition is an embolus; if so, the most frequent site of thrombus formation—lung, heart, aorta, carotid artery, vertebral artery, basilar artery, etc.—is a matter of importance, because the neurologic community has clung to the idea that an embolus must come from a cardiac source;
2. A thrombus that is formed after an embolus narrows or blocks an artery;
3. Emboli that are not visibly related to thrombosis, e.g., cholesterol emboli observed in the retina;
4. Thrombosis without any evidence of embolism; and
5. Emboli of air, fat, amniotic fluid, etc.

If "thromboemboli" includes all these, it clearly is the important mechanism, in addition to atherosclerosis, in over 80% of strokes. Thromboembolic phenomena then are involved in virtually all cases of cerebral infarction. I choose to interpret "acute cerebrovascular occlusion" as referring to a pathogenic process that produces focal cerebral ischemia through some mechanism of thrombosis that may or may not involve embolus production and is sometimes of sufficient duration to cause infarction. This interpretation includes the idea of temporal change, to explain the sudden onset and often limited duration of the focal neurologic defect. The interpretation has not been proved; only bits and pieces appear as fact. Information about incidence is not available; therefore, these comments are of only relative significance.

To establish the significance of the thromboembolism as the causative mechanism in most transient focal cerebral ischemic attacks and in progressing cerebral infarction, it is necessary to review the evidence for the theory—evidence that applies to either the carotid or the vertebral basilar arterial system.

As early as 1875, Gowers [2] described a case of unilateral blindness associated with contralateral hemiplegia; autopsy revealed mitral stenosis and clots in the atrial appendixes, an embolus in the left middle cerebral artery, and several small emboli in the central artery of the retina. The potential importance of primary occlusion of the internal carotid artery in the neck received no significant attention until 1914, when Hunt [3] wrote about carotid thrombosis associated with wounds of the neck. By 1951, Johnson and Walker [4] had found 101 angiographically proved cases of carotid occlusion in the literature and added six of their own. Since then, dozens of articles have appeared concerning internal carotid artery thrombosis, with many descriptions of the "clinical picture." There is some variation in these descriptions, but most include impaired motor and sensory function on the contralateral side of the body, some disorder of speech and language if the carotid occlusion is on the dominant side, and at times a history of episodes of defective vision in the ipsilateral eye. All this is commonplace. The amazing thing is how little attention is paid to the fact that carotid occlusion and stenosis commonly occur without any signs of brain dysfunction! This was clearly demonstrated in studies by Martin *et al.*,[5] Stein *et al.*,[9] and Schwartz and Mitchell.[8] Fisher [1] noted that one third of patients with occlusion of one internal carotid artery were asymptomatic, although the emphasis, in discussion, was characteristically placed on the two thirds who were symptomatic.

These observations point to something that should be self-evident: there are multiple main routes by which local areas of brain receive

blood, and there must be some peripheral or distal arterial lesion to produce well-demarcated focal ischemia. The technical difficulties in studying this portion of the arterial tree at autopsy are well known to neuropathologists. Because of the difficulties, observations are frequently limited, as in the study by Fisher,[1] to the circle of Willis (including middle cerebral stems, anterior cerebral stems, communicating vessels, posterior cerebral stenosis, and basilar and vertebral arteries). Rarely is the time taken for a careful search for an appropriate distal arterial occlusion. Moossy [7] conducted such a search, also studying the extracranial circulation; in brains with old infarctions, most of the "thrombi" were in the extracranial circulation, but, in brains with recent infarcts, six of eight "thrombi" were in the intracranial arteries, distal to the circle of Willis. Study of such matters is particularly difficult because of inability to be certain whether some lesions are thrombi, emboli, atherosclerosis, or combinations of them. It is apparent that investigation of autopsy material can give only limited data concerning the complex spectrum of events that produce focal cerebral ischemia with or without infarction.

By 1955, our description and observation of transient ischemic attacks led us to write [6]:

> Our present concept of the pathogenesis of attacks is as follows: A thrombus begins to form on an area of diseased endothelium. This soft material may reach a size sufficient to produce enough alteration in blood flow to cause symptoms, break from its source, fragment and be carried away. More likely, however, appears the possibility that the newly formed clot becomes dislodged before symptoms occur, travels to a place where the vessels branch, lodges for a few minutes (symptoms produced) and then fragments and is carried away.

We were led to develop this hypothesis because of bits of positive evidence interrelated to items of negative evidence. The positive evidence was:

1. Individual attacks of transient ischemia are of swift onset. Patients note that each attack reaches its maximal degree in a matter of seconds, often within 10 sec. The attack arrives in all-or-nothing fashion; i.e., there is no gradual spread or worsening of the phenomena—they are suddenly present, e.g., as a hemiparesis.
2. Most attacks are of short duration, often lasting less than 20 min. Then circulation is obviously restored. This implies that the process is transitory.
3. The neurologic content of an individual attack, particularly in the carotid system, is remarkably similar from one attack to another in an individual patient.

4. It is curious that an individual attack is seldom maximal—e.g., hemiplegia, aphasia, hemianesthesia, and hemianopsia—but is more often a monoparesis or slight weakness of the right hand associated with defective speech, and so forth. Blindness in one eye and contralateral hemiplegia almost never occur simultaneously. Amaurosis is frequently separate, in time, from cortical ischemia. Similarly, patients who have had amaurosis fugax and hemiparesis, in attacks, when a completed stroke develops later do *not* get permanent loss of vision in the one eye. This implies that, although the source of the trouble may be in a proximal large artery, the occlusive event that causes the focal neurologic sign is far out distally in the system.

5. Emboli in the retinal circulation have been observed on many occasions. They differ in type, in some instances being made up of cholesterol and in others of platelets mixed with red blood cells and fibrin. The behavior of these emboli (how they move and/or fragment) would be characteristic of the theoretical phenomena, in the brain circulation, that could account for the transient focal ischemic attack.

6. It has been noted that the highest incidence of focal neurologic complications of angiography occurs when the dye is introduced into the carotid artery close to the takeoff of the internal carotid. Embolization to the retina has been noted in some such instances. This implies that manipulation of the vessel is responsible for embolization to smaller vessels and the latter produces a focal neurologic event.

7. Manipulation (palpation, massage, or compression) of the carotid bulb may be associated with the immediate onset of a focal neurologic deficit, the brain lesion and the manipulated carotid being ipsilateral. This implies that one or more embolic fragments have been dislodged by the manipulation.

The negative evidence that supported the theory was:

1. The attacks were not associated with systemic arterial hypotension. Fortuitously, the blood pressure was recorded at the beginning of several attacks in a small number of patients; there was no change in blood pressure in those patients. In addition, patients with transient ischemic attacks commonly do not have attacks when the blood pressure is significantly lowered by maintenance in the upright position on a tilt table or administration of a hypotensive drug. A significant observation concerns some patients having orthostatic hypotension and transient ischemic attacks. The orthostatic reaction was accompanied by syncope, not by a transient ischemic attack. The transient ischemic attacks occurred without the hypotension. It is also significant that transient

ischemic attacks are only infrequently noted in large numbers of patients receiving active antihypertension treatment in special clinics, although a precipitate, short-duration decrease in blood pressure is common in such patients.

2. Electrocardiographic tracings taken at the onset of attacks show no evidence of a change in cardiac rhythm, a change which might imply either a decrease in cardiac output or production of minute emboli.

3. Arterial shunts do not seem to be a likely pathogenetic explanation for attacks. When there is occlusion of the proximal portion of a subclavian artery with reversal of blood flow in the appropriate vertebral artery (which is fairly common), transient ischemic attacks are not produced. The symptomatic manifestations of this phenomenon are nondescript, and even exercise of the appropriate arm does not induce focal neurologic disability.

4. The inhalation of carbon dioxide, a potent vasodilator, even during the first few seconds of an attack, does not alter the usual temporal pattern of that attack in the patient.

5. When the carotid artery is occluded surgically, as in the treatment of intracranial aneurysm, the focal neurologic signs (when they do appear) frequently begin many minutes or even hours after the surgical manipulation. These procedures are most commonly performed in young patients, and it appears likely that some buildup of thrombus, with distal embolization, must be the explanation for the late-onset focal deficits.

6. Rarely, turning of the head or extension or flexion of the neck is associated with the onset of an attack. When a kinking or external compression mechanism has been pathogenically related to previous attacks, the phenomenon (head-turning producing an attack) can be verified by having the patient perform the maneuver while being examined. Such verification is very rare.

This series of positive and negative items leads inescapably to the conclusion that embolic phenomena are a most likely explanation for transient ischemic attacks. To this must be added the concept of thrombotic occlusion—not, as is commonly supposed, thrombotic occlusion of a cervical cerebral vessel but thrombotic occlusion of or other interference with flow in an intracranial cerebral artery. It is important to recall that the small branches of the basilar artery supply an area that subtends a massive amount of neurologic function. If a thrombus were to begin to form near the orifice of one of the paramedian branches of the basilar artery, it is possible that there would be some interference with flow into that artery, creating ischemia in the

distribution of that particular vessel. If the beginning thrombus were dissolved by the natural lytic action in the blood, flow could be restored and function returned to normal. Another possible explanation for termination of an attack would be the separation of this fragile thrombus from the wall of the artery, with its subsequent fragmentation at a distal, perhaps unimportant site. This would then immediately restore circulation in the artery originally involved. If the thrombus did not dissolve or separate from the wall, symptoms would continue as an expression of ongoing brain stem ischemia; continuation of sufficient ischemia, of course, would produce infarction. This appears to be a logical sequence of events, in that, in many instances of basilar artery thrombosis that have been subjected to autopsy examination, there is definite lamination or stratification of the occluding material, which implies that layers of clot were formed at different times. The basilar artery is simply used as an example of a vessel in which this type of dynamic pathophysiologic and pathomorphologic change occurs; it is presumed that the same phenomenon occurs in other large arteries or in smaller arteries.

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Ocular Manifestations of Ischemic Disease

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Vascular occlusions in the eye may result from hemodynamic disturbances, from arterial compression, and from inflammatory vessel disease. Before the appearance of the occlusion, the patient sometimes has ocular symptoms, which are usually referable to ischemia and may consist of visual blurring, described by patients in various ways, such as a veil, curtain, or haze, or even of a blackout. If the symptoms are unilateral, one may think of carotid artery disease; if bilateral, of basilar or vertebral vessel involvement.

With ocular ischemia, the characteristic visual loss is brief. If it persists longer than about ½ hr, the patient probably is suffering from migraine or optic nerve disease of other origin than ischemia, or it may represent the onset of an actual occlusion of the central retinal vessels.

When the patient also complains of diplopia or vertigo associated with the visual haze, this tends to indicate primarily basilar or vertebral, rather than carotid, artery insufficiency.

RETINAL ARTERY OCCLUSION

Retinal artery occlusive disease may be classified as embolic, arteriosclerotic, ischemic, and inflammatory.

Embolic phenomena, usually unheralded, may cause sudden complete blindness. The funduscopic examination is characteristic and reveals a diffuse retinal cloudiness secondary to swelling of the ganglion cells and a contrasting cherry-red spot at the macula. Blood may be segmented in the arterioles in a so-called boxcar fashion. One hundred years ago, it was felt that central retinal artery occlusions were never the result of emboli. Subsequent studies have shown embolism to occur only

rarely. Indeed, in a series of 84 retinal artery occlusions reported by Liversedge and Smith,[14] only two were thought due to emboli, the remainder resulting from thrombosis associated with generalized arteriosclerosis. Reports, however, have begun to appear in the literature showing pathologic proof of embolization.[1,17,34] Zimmerman [34] feels that the entire question of central retinal obstruction (i.e., thrombosis versus embolization) should be restudied.

The following criteria suggest the diagnosis of embolism:

1. The presence of a disease capable of producing emboli, such as mitral stenosis, subacute bacterial endocarditis, or endocardial myxoma
2. Evidence of embolization to other organs
3. Absence in the vessel in question of a disease capable of producing thrombosis

Hollenhorst [9] reported a 20% incidence of retinal emboli after carotid endarterectomy.

Histopathologically, the emboli have varied. Zimmerman's case [34] revealed fibrin with enmeshed red cells and leukocytes. Ball [1] reported cholesterol crystals, red blood cells, fatty debris, and mononuclear cells. In the case of McBrien *et al.*,[17] there was an aggregate of platelets, some leukocytes, and a little fat. In a younger patient, one should suspect cardiac or pulmonary sources of the emboli, whereas in the older patient, atheromata of the carotid system may be indicated.

Of the arteriosclerotic phenomena, atheromatosis probably accounts for a large number of retinal arterial occlusions. The occlusive phenomenon is often preceded by one or more attacks of amaurosis fugax. Transient signs of central-nervous-system vascular embarrassment may also be present. In Liversedge and Smith's report [14] of 84 central retinal occlusions, right and left eyes were equally affected. The group consisted of 53 males and 31 females; 75% of the patients were over 60 years old. It was interesting that 18 of 33 male cases studied had diastolic blood pressures of less than 100 mm Hg, causing the authors to speculate on the possibility that hypertension protected the ocular circulation through narrowed sclerotic vessels. In 33 of 63 cases studied, arteriosclerotic vascular disease in the form of angina, claudication, and vascular accidents affecting other areas of the body was present. Neurologically, of 63 cases, only 13 had symptoms at the time of occlusion, and three more developed symptoms after the occlusion.

The general category of ischemic phenomena includes all processes that reduce blood flow to the eye sufficiently to cause blackout or blindness. This process may be seen not only in arteriosclerotic vascular

disease, but also in severe hemorrhage in pilots making a "tight" turn, and in other conditions. According to Hollenhorst,[9] 40% of all cerebral vascular problems are due to occlusive disease within the extracranial arteries. Furthermore, about 80–90% of these patients have ocular manifestations. Because the location of these arteries makes them accessible to surgical intervention, accurate early diagnosis is essential. Examination of such patients by arteriography carries a definite risk of permanent complication (blindness or stroke). The average rate of complication is about 2%; however, this figure may be even higher among patients with atheromatous disease. In spite of this, complete vascular evaluation is necessary. It has been stated [9] that 50% of patients with intermittent insufficiency in either the carotid or the vertebral basilar system would have a serious stroke within an average of 3 years (25% will have no stroke but will continue with intermittent symptoms, and 25% will recover fully).

In Hollenhorst's series [9] of 235 patients with symptomatic involvement of the carotid system, 206 (88%) had ocular signs or symptoms. In Rob's series [23] of 181 patients, 41 had visual disturbance. The commonest symptom in both series was amaurosis fugax (38% in Hollenhorst's series and 19% in Rob's series). Twenty-three patients in Hollenhorst's series had retinopathy consisting of one to 10 cotton-wool patches in the eye on the same side as the carotid occlusion.

Microaneurysms, small round hemorrhages, new vessel formation, and irregularly dilated veins have been described in association with lowered retinal arterial pressure.[12] Bright plaques (possibly cholesterol crystals) are seen in approximately 10% of persons with carotid occlusive disease. Furthermore, in 21 hypertensive patients, asymmetric retinal vascular changes were noted. The eye on the side with the carotid insufficiency exhibited little hypertensive change, whereas the eye on the other side had typical hypertensive retinopathy.[9,12] Visual loss was encountered in the following forms: (1) partial (altitudinal) or complete monocular loss, due to central retinal artery or branch arteriole occlusion; (2) partial or complete monocular loss, with a temporal cut in the contralateral eye from involvement of the central retinal artery and middle cerebral artery; and (3) incongruous homonymous hemianopia due to middle cerebral artery thrombosis.

When carotid occlusion becomes complete or almost complete, further ocular signs include ipsilateral proptosis, Horner's syndrome, dilatation of and decreased flow rate in conjunctival vessels, optic atrophy, and ocular hypotension.[12]

In summary, then, ocular symptoms of carotid insufficiency include amaurosis fugax, altitudinal hemianopia, and other field defects. Signs

include retinopathy and asymmetrical hypertensive changes and bright plaques in the retinal arterioles.

Mechanisms leading to arterial occlusion include [23] (1) narrowing of the lumen by buildup of plaques, (2) complete occlusion developing over a primary stenosis, (3) hemorrhage behind an atheromatous plaque, (4) emboli from an area of stenosis, (5) spasm of the artery, and (6) change in the general status of the patient, leading to decreased vascular flow, pressure, or both.

Although it is not a cause of central retinal artery occlusion, one must consider arteriosclerotic vascular disease that affects the vertebral basilar system as a possible cause of the patient's visual disturbance. Symptoms in the presence of insufficiency of these vessels are variable and complex owing to the multiple structures involved. The hallmark of this condition is intermittent insufficiency, as described by Millikan and Siekert.[19] Blurred vision, diplopia, transient homonymous hemianopia, scintillating scotomata, and ptosis constitute the ocular symptoms and signs.

Of Kearns and Hollenhorst's cases,[12] 79% had ocular symptoms, 40% had experienced intermittent blurring, and 27% had experienced diplopia; 14% had bilateral homonymous hemianopia, and 12% had unilateral homonymous hemianopia. Macular sparing was frequent. Third- and fourth-nerve palsies were rare, whereas sixth-nerve palsies were noted in two cases. Ten patients of 183 had paresis of conjugate gaze, 15 intranuclear ophthalmoplegia, 18 nystagmus, and two vertical diplopia or skew deviation.

Westby and Dietrichson [32] reported 12 of their 13 patients to have ocular symptoms (blurred vision, diplopia, and field defects). All 13 had ocular signs, including muscle palsy, field defects, nystagmus, and cortical blindness. (Cortical blindness including Anton's symptom has been reported in this condition.) These patients are in the older group and exhibit other manifestations of atheromatous disease (hypertension and intermittent claudication). The narrowing commonly occurs in the vertebral vessels at their takeoff from the subclavian arteries.

The aortic arch syndromes represent disease processes whose pathophysiology is based on chronic circulatory insufficiency. Clinically, they are characterized by the absence of detectable pulses and by inability to measure blood pressure in the arms or the neck. Pathologically, there is obstruction of the major vessels arising from the aorta. Abnormalities of this general type have been called "Takayasu-Ohnishi's disease," [27] inverse or "reversed coarctation" of the aorta,[30] and "chronic subclavian carotid obstruction syndrome." The inclusive term, "aortic arch

syndrome," was suggested by Frøvig [7] and propagated by Ross and McKusick [25] in their extensive review of the subject.

There appear to be two main types. Briefly, the first is inflammatory and found predominantly, but not exclusively, in young Japanese women. Occlusion of the vessels is caused by a diffuse nonspecific arteritis. An affected vessel reveals medial thinning, with disorganization of elastic membrane and endothelial thickening. The serology is negative. The patient may have fever, leukocytosis, increased sedimentation rate, and often a positive PPD (indeed, tuberculosis has been suggested as a cause, but no proof has ever been offered). The second type is much less homogeneous and may or may not be associated with inflammation. It occurs in both males and females 40–60 years old. Occlusions may occur anywhere along the great vessels but favor their points of origin.[5]

Ross and McKusick [25] analyzed over 100 of these cases and found syphilitic aortitis, with or without aneurysm, to be implicated as the most common cause. Atheromatosis was not common. Other authors, however, feel that this should receive greater recognition as a cause.[8] Other causes included congenital abnormalities; some types of trauma to the chest, neck, and axilla; and nonsyphilitic arteritis.

The ophthalmologic picture presented in these patients includes chronic hypotension and hypoxia, which may have superimposed on it acute circulatory embarrassment precipitated by sudden thrombosis of one of the great vessels. Central-nervous-system manifestations abound (seizures, cranial nerve palsy, syncope, headache, and deafness).[5] The ocular symptoms include flashes of light and amaurosis fugax, particularly on tilting the head upward. Clinical signs include rubeosis and atrophy of the iris. The lens may develop a cataract prematurely. The retinal vessels reveal venous dilatation, peripheral arteriovenous communications, microaneurysms, vascular occlusive phenomena, ischemic exudates, and optic atrophy. Less commonly, retinal detachment, retinitis proliferans, and vitreous hemorrhages may be seen.[8] In the arteriosclerotic form of pulseless disease, the retinal findings are mainly microaneurysms, whereas, in the inflammatory form of arteritis, exudates appear to be more common.

Of special note for the ophthalmologist is temporal arteritis. This is a systemic disease of older persons, characterized by fever, elevated sedimentation rate, and giant-cell arteritis. Pathologic changes in this condition also center about the elastic membrane of the arteries, and, according to Cogan,[4] affected patients show a fall in elastase inhibitor substance. This disease process may cause blindness with no symptoms

referable to the temporal artery; 50% of patients will have visual symptoms.[29] Sedimentation rate is always elevated and may be used as a guide to the efficacy of treatment. The disease sometimes responds to steroids. Visual symptoms generally occur in 3–4 weeks after the onset of other systemic signs, which include malaise, elevated temperature, and signs and symptoms of arterial disease. Visual loss is usually permanent. There may be swelling of the optic nerve head, anomalous anastomoses of artery and veins, proliferation of vessels, and microaneurysms.

Hemorrhagic glaucoma, a frequent complication of central vein thrombosis, is considered rare in occlusion of the central retinal artery. Few, if any, ocular complications follow arterial occlusion. Discussion of this entity is pertinent because more and more cases are being reported in the literature. Imperceptibly and gradually, the anterior chamber becomes filled with vascular and connective tissue associated with the formation of peripheral anterior synechiae and marked tension elevation. The iris, too, is involved in this process. The majority of these eyes are lost because of severe pain. Rigorous medical and surgical treatment is indicated and may save the eye and indeed may even salvage some vision.[31] The unaffected eye has never been found to have elevated tension.[10,15,31,33]

RETINAL VEIN OCCLUSION

Many names have been ascribed to central retinal vein occlusion. In 1854, Liebreich coined the term "apoplexia retinae" to describe obstruction of the central retinal vein with papilledema and hemorrhages. "Retinitis hemorrhagicae" was used by Leber [13] in 1877 and implies an inflammatory process. In 1878, von Michel [18] called this pathologic process "thrombosis of the central retinal vein without diagnosis." Today, the term "central retinal vein occlusion" connotes the clinical picture of papilledema, dilated veins, and multiple hemorrhages, without attempting to designate a specific disease entity.

Retinal vein occlusion occurs most commonly in the middle or later years of life, about 50–70 years of age. Males and females appear to be affected equally.[21,22] However, the process occurs about one decade earlier in men.

The most common site for obstruction of the central retinal veins is at or just behind the lamina cribrosa. Branch vein occlusion usually occurs at arteriovenous crossings or where a vein branches. At all these sites, an anatomic constriction is placed on the vessel. Two factors

appear to play a role: (1) anatomic predisposition to obstruction and (2) an underlying disease state.

In the series reported by Paton *et al.*,[21] 56% of the males and 70% of the females had diastolic pressures greater than 100 mm Hg. Atheromata (manifested by a history of intermittent claudication, ischemic heart disease, or cerebral vascular disease) were present in 53% of the males and 25% of the females. In 81% of this British series of 118 patients with central retinal vein occlusion, hypertension and/or atheromata were present. The authors of the article draw no conclusions. But they do state that there appears to be a high incidence of arterial disease associated with retinal vein thrombosis.

Fluorescein studies of these patients revealed prompt passage of the dye bolus through the so-called thrombosed veins in the acute stages. In fact, arteriolar occlusions and constrictions were demonstrated. Furthermore, in the later stages (the period was highly variable), arteriolar changes were noted in an increasing number of patients. These changes included narrowing, unevenness of diameter, whitish deposits in the vessels, and fading of the artery. Field changes, consistent not with the hemorrhages, but rather with the arterial segment involved, were seen in 64% of cases. Ophthalmodynamometry in these patients revealed that 22 of 73 tested had decreased systolic pressures. Experimental venous occlusions performed in monkeys did not mimic the classical picture of central retinal vein thrombosis. However, when the central retinal artery was accidentally occluded in attempting to occlude the vein, the fundus picture of vein occlusion was produced. This adds further support for the theory that venous occlusion is basically an arterial disease. Kearns and Hollenhorst [12] presented 22 cases of retinopathy consisting of microaneurysms and hemorrhages, with dilatation of the retinal veins, most marked in the midperiphery of the retina and associated with occlusive disease of the ipsilateral retinal artery. (Evidence of arterial disease was not found with as great a frequency in the 400 cases of central and branch vein occlusion reported from Scandinavia.[22])

The picture of central retinal vein occlusion may be seen in lymphoma, dysproteinemia, leukemia, and polycythemia and after common carotid ligation. The common factor in all these may be hypoxia, which leads to venous atony and then to leakage of blood through the dilated vessels. It has been suggested that sudden rapid arterial occlusion leads to pallor, arteriolar narrowing, etc., whereas a slower blockage leads to hypoxia and venous occlusion.

Primary glaucoma has been reported to precede central retinal vein occlusion in 23–43% of patients.[2,28] The low value represents frank

pressure elevation; the high value represents tonographic evidence of decreased facility of outflow. Raitta [22] reported an incidence of 28% of primary glaucoma. Inclusion of glaucoma suspects brought this percentage to 35. In summary, then, glaucoma is present in approximately 20–30% of patients before central retinal vein occlusion, and possibly more if glaucoma suspects are included.

A complication of central retinal vein occlusion is the development of hemorrhagic glaucoma. Most series report the incidence as between 10% and 30%.[3,22,28] In a patient who does develop hemorrhagic glaucoma, it is important to evaluate the other eye. No hemorrhagic glaucoma has been reported after branch vein occlusion.

BLOOD DYSCRASIAS

A retinopathy consistent with the clinical picture of central retinal vein occlusion and exhibiting all or some of its features has been reported in the following: severe anemia (including the anemia that follows acute blood loss), hemoglobinopathies (sickle-cell and sickle-cell hemoglobin C disease), neoplastic disorders of the reticuloendothelial system and hematopoietic tissue, and the hyperglobulinemias (macroglobulinemia, cryoglobulinemia, and multiple myeloma). Blood viscosity has been shown to be elevated in all these conditions.[6] Martin and Bayrd [16] in 1954 showed blood viscosity to be increased to 5–8 times normal in diseases associated with increased blood elements (e.g., leukemia and polycythemia). In the hemoglobinopathies (particularly SS and SC diseases), low oxygen tension leads to increased sickling. This leads to increased viscosity, which in turn leads to decreased flow and lower oxygen tensions. A toxic cycle begins that is very difficult to reverse. Increased serum proteins (as in macroglobulinemia, multiple myeloma, and cryoglobulinemia), some cases of hemolytic anemia, and occasional cases of lymphocytic leukemia and lymphosarcoma have associated increased blood viscosity.

In Foulds's article [6] on blood diseases with ocular manifestations, he states that there may be changes in the intracranial pressure with any of the above-mentioned conditions leading to papilledema. There may be changes in the systemic venous pressure (e.g., polycythemia secondary to chronic lung disease).

Two factors, then, may give rise to the observed clinical picture: increased viscosity and decreased flow rate.

It has been postulated that the stasis induced by the hyperviscosity of the serum is so extreme that it even overcomes anticoagulant tendencies that may be present in some of these disease states.[26]

Digital pressure to the eye in conditions with increased viscosity produces sludging of blood in the veins. This appears as a breaking up of the column of blood into aggregates, separated by clear plasma. The exact mechanism is not clear, but it probably involves a combination of abnormalities within the blood, as well as decreased flow.[11]

Although venous damage is the *sine qua non* of this group, symptoms referable to the arterial tree have been described. Specifically, intermittent insufficiency of the carotid and basilar vertebral systems associated with polycythemia has been reported.[20]

Another common cause of central retinal vein occlusion is diabetes.[24] Less common are inflammatory conditions, particularly those involving the paranasal sinuses.

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Acute Aortic and Peripheral Arterial Occlusion: Incidence and Significance of Thromboemboli

JOHN A. SPITTELL, JR.

Consideration of the incidence and significance of thromboemboli in acute occlusion of the aorta and its major branches involves primarily the natural history of atherosclerotic disease of the peripheral arteries and the heart.[2,9,50] In addition, nonatherosclerotic cardiac disease is a significant source of emboli that may suddenly occlude peripheral arteries.[3,24] Acute "thrombotic" arterial occlusion may also result from trauma and complicate the arteritides, infectious diseases, hematologic disorders, and debilitating diseases[1,2,46]; on occasion, acute arterial occlusion occurs in the absence of identifiable cardiac, arterial, or systemic disease (so-called primary arterial thrombosis[17]).

Data on the incidence of acute arterial occlusion are of several types but, singly or in combination, probably do not give a true picture of the frequency. There are several reasons for the inadequacy of available data: (1) the clinical manifestations of acute arterial occlusion are variable, so not all instances come to the attention of physicians; (2) the incidence of amputation gives an incomplete picture, because even without treatment many arterial occlusions do not result in gangrene, and indeed such processes as infection and trauma may be the principal cause of limb loss; (3) the clinical differentiation between embolic and thrombotic arterial occlusion is usually inferential and based on the identification of the source of emboli or the presence of arterial disease or precipitating factors; (4) distinction between embolic and thrombotic arterial occlusion by pathologic examination of the artery alone is often not possible; and (5) acute peripheral arterial occlusion does not contribute as heavily as coronary and cerebral thromboemboli to mortality, so less attention has been focused on the problem. Recognition of these shortcomings is important in reviewing the available information, because the incidence of acute occlusion of the aorta and

its major branches is undoubtedly greater than most of the studies indicate.

Thrombogenesis has been implicated in all aspects of atherosclerosis,[20] which is the commonest underlying cause of acute occlusion of the aorta and its major branches. Not only has mural thrombosis been proposed as a factor in the formation of the atheromatous lesion,[11,12,32] but thrombosis is the final event in the total occlusion of the atherosclerotic artery,[47] as illustrated in Figure 1. Indeed, Barker [4] reported that, although there was considerable variation in the relative extent of atheroma formation and thrombosis in different cases, thrombosis was found to be responsible for at least part of the occluding process in the arteries of all limbs amputated because of advanced arteriosclerosis obliterans. Alteration of the surface of the arterial lumen by atheromas producing a change in electrical charge,[39] more subtle endothelial alterations [34] or disturbances in local flow, and changes in the coagulation of the blood,[31,47,49] perhaps due to its content of lipid or coagulation factors,[6,7,33] have been proposed to explain the tendency to thrombosis

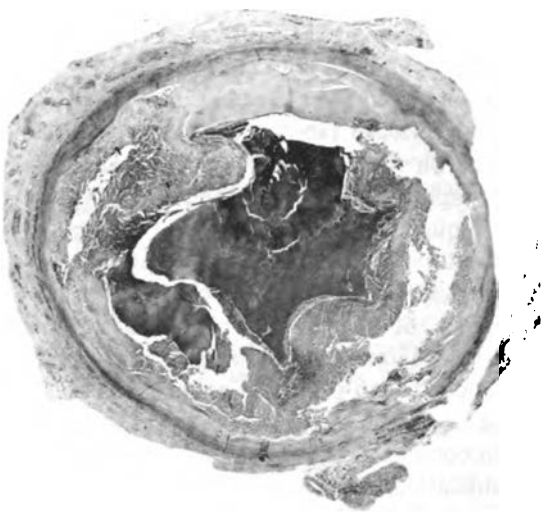

FIGURE 1 Cross section of abdominal aorta of a 54-year-old man whose clinical picture was compatible with acute aortic occlusion 2½ weeks before. Atheromatous changes and complete occlusion of lumen by organizing thrombus are evident. Hematoxylin and eosin. (×5) (Reprinted with permission from Spittell *et al.*[47])

in the atherosclerotic vessel and will receive ample attention later in this volume.

What is the frequency of acute thrombotic occlusion in the atherosclerotic aorta and its major branches? One can begin to answer this question by reviewing the several available studies [5,27,40–42] of the natural history of the disease (Table 1). Most reports are confined to the incidence of amputation, gangrene, or both. Data such as those presented in Table 1, although they give a perspective of the morbidity associated with the disease, fall short of a true picture of the incidence of acute thrombotic occlusion in peripheral arteriosclerosis because of variation in the clinical course, as mentioned previously. In a more recent study of the natural history of arteriosclerosis obliterans, deWolfe and colleagues [10] directed their attention to the occurrence of acute arterial occlusion as one facet of the problem. Of 513 limbs affected by aortoiliac disease, 29 (6%) presented with an acute arterial occlusion, and in another 50 limbs (10%) an acute arterial occlusion developed later; of 473 limbs with combined aortoiliac and femoropopliteal arteriosclerosis, 40 (8%) presented with an acute occlusion, and in another 115 limbs (24%) an acute occlusion developed; and of 864 limbs with only femoropopliteal disease, 229 (27%) presented with an acute occlusion as the first symptom, and in another 188 limbs (22%) an acute occlusion developed later. From their data, deWolfe and colleagues concluded that acute arterial occlusion is much more common than previously reported; that the more distal the disease process, the higher the incidence; and that no available criteria make it possible to predict which limb will be the site of an acute arterial occlusion.

The over-all incidence of acute arterial occlusion reported by deWolfe and colleagues [10] is probably lower than incidence of thrombotic occlusion. The basis for that statement is the excellent study of acute arterial occlusion by Wessler and coauthors.[50] Of 98 lower limbs amputated for gangrene or unremitting ischemic pain, 47 (48%) were shown by injection and dissection techniques to have one or more fresh thromboembolic occlusions. Of the 47 limbs, 39 (83%) had multiple lesions. It is significant that in fewer than half the limbs with fresh arterial occlusions the diagnosis had been entertained clinically. In general, there was a correlation between length of occluded portion and clinical recognition: when the occluded portion was more than 10 cm long, the diagnosis was made in 94% of the cases, but when it was less than 5 cm long, the diagnosis was made in only 10% of the cases. Thus, many acute arterial occlusions are asymptomatic because of their size, but a small occlusion may significantly impair collateral circulation or serve as a nidus for the deposition of further clot.

TABLE 1 Prognosis in Arteriosclerosis Obliterans

Authors, Year	Site of Occlusion	No. Patients	Follow-up, years	Major Amputations, %	
				Non-diabetic	Diabetic
Silbert and Zazeela, 1958 [41]	Unselected	799	3–30	8	34
Juergens *et al.*, 1960 [27]	Femoral	271	5	6	—
Juergens *et al.*, 1960 [27]	Aortoiliac	194	5	4	—
Schadt *et al.*, 1961 [40]	Femoral	362	9–18	7	27
Begg and Richards, 1962 [5]	Unselected	198	5–12	7	—

The main source of arterial emboli (Table 2) is the heart—most frequently in coronary heart disease and rheumatic heart disease,[22] but emboli may arise from a heart that is failing from any cause.[2,3,24] Although the cardiac aspects of emboli are covered in detail by Dr. Genton elsewhere in this volume, it should be noted at this point that the incidence of arterial embolism is not accurately known. Only emboli that occlude major arteries are detected clinically, and necropsy data, although they yield a higher incidence, vary according to the interest and completeness with which examination is carried out. A smaller number of arterial emboli arise from thrombi within arterial aneurysms,[45] from atheromatous plaques, and from the rare phenomenon of paradoxic embolism associated with patency of the atrial or ventricular septum.[2,8] (It should be mentioned in passing that emboli usually lodge at bifurcations, for these are the points at which the caliber of the artery is abruptly reduced.)

The arteries to the extremities, including the aorta, are occluded by emboli from the heart less frequently than are the arteries to the brain, according to clinical data (Table 3). In a study by Daley and colleagues,[8] almost half of 393 emboli in 194 patients with rheumatic heart disease lodged in arteries to the brain, whereas 28% occluded arteries to the lower extremities, including the aorta at its bifurcation, about 14% lodged in arteries supplying the abdominal viscera, and about 10% occluded arteries to the upper extremities. Similar distribution has been noted by others.[3] Such percentages are probably more nearly accurate, although undoubtedly low, in the case of cerebral and extremity arteries than in the case of visceral arteries, where only a small proportion of emboli are recognized clinically. Hoxie and Coggin [25] and Miller and colleagues [30] found that fewer than 3% of the

TABLE 2 Sources of Arterial Emboli

Cardiac disease:
Rheumatic heart disease
Coronary heart disease
Valvular prostheses
Failing heart—any cause
Subacute bacterial endocarditis
Arterial disease:
Atherosclerotic plaques
Aneurysms
Paradoxic embolism

TABLE 3 Distribution of 393 Arterial Emboli in 194 Patients with Rheumatic Heart Disease [a]

Site of Lodgment	No.	% of Total
Arteries to brain	188	47.8
Aortic bifurcation	23	5.9
Arteries to lower extremities	87	22.1
Arteries to upper extremities	39	9.9
Arteries to abdominal viscera	56	14.2
TOTAL	393	—

[a] Modified from Daley *et al.*[8]

cases of renal infarct and embolism of the kidney and spleen, respectively, were recognized clinically.

The frequency of acute arterial occlusion from emboli arising in arterial aneurysms is unknown, but clinical experience indicates that such emboli are much less common than emboli arising from the heart. However, aneurysms of some arteries produce thromboembolic complications more frequently than others.[45] Emboli arising from aneurysms of the thoracic or abdominal aorta must be rare, in that this complication is scarcely mentioned in the numerous large series of cases recorded in the literature.[14,26,44,51]

As one proceeds distally in the arterial tree, the incidence of thromboembolic complications of aneurysms increases. Thus, in 26% of 89 patients with femoral artery aneurysms, Pappas and colleagues [35] reported thromboembolic complications, of which half were acute with evidence of recent thrombosis or embolization. In a series of 100 popliteal aneurysms, Gifford and co-workers [18] reported thromboembolic complications as the most frequent; in 45 of the aneurysms, thrombosis, distal embolization, or both occurred, and most of the 20 amputations were necessitated by gangrene. Just why the incidence of thromboemboli increases both in atherosclerotic narrowing and in aneurysm with the more distal location of the disease is as intriguing a problem as is the predilection of the atherosclerotic disease for particular locations in the arterial tree.[37,38,48]

The distinction between embolic and thrombotic arterial occlusion has been stressed for a number of reasons, including understanding of pathogenesis, physiologic mechanisms, prognosis, and therapy. As mentioned, in some cases (for example, rheumatic valvular heart disease), the conclusion that an acute arterial occlusion is embolic is almost

always correct, as is the conclusion that an occlusion is thrombotic in arterial trauma or polycythemia vera. In atherosclerotic disease, however, unless the patient has an acute myocardial infarct, the distinction between thrombotic and embolic occlusion is difficult, although most observers at the bedside, in my experience, decide that thrombotic occlusion is present. Distal embolization from mural thrombi on proximal atherosclerotic plaques (Figure 2) may be considered, but with no way to prove it, this concept usually remains conjectural. The possibility that distal embolization from mural thrombi is much more

FIGURE 2 Extensive arteriosclerosis obliterans of abdominal aorta and iliac arteries. (Reprinted with permission from Allen *et al.*[3])

frequent than we can document seems likely, however, particularly in light of the phenomenon of cholesterol embolization,[36] which had received sporadic attention for the better part of a century until 1945, when the work of Flory [16] aroused interest in the subject. Necropsy data [19] indicate that the kidneys, pancreas, and spleen are involved most frequently, but Flory's statement that "gangrene of a toe or some other portion of a lower extremity, occurring in an old person with advanced arteriosclerosis, may occasionally be caused by cholesterol crystal emboli" [16] has received increasing confirmation in recent years.[15,43] Most often, the manifestations are digital infarcts and livedo reticularis (Figure 3), first reported by Fisher and colleagues.[15] Resemblance to periarteritis nodosa with multisystem involvement has also been stressed.[13,21] Biopsy of muscle in the affected limb may show occlusion of a small artery by embolic cholesterol material (Figure 4). Although these emboli occlude chiefly smaller arteries, the occurrence

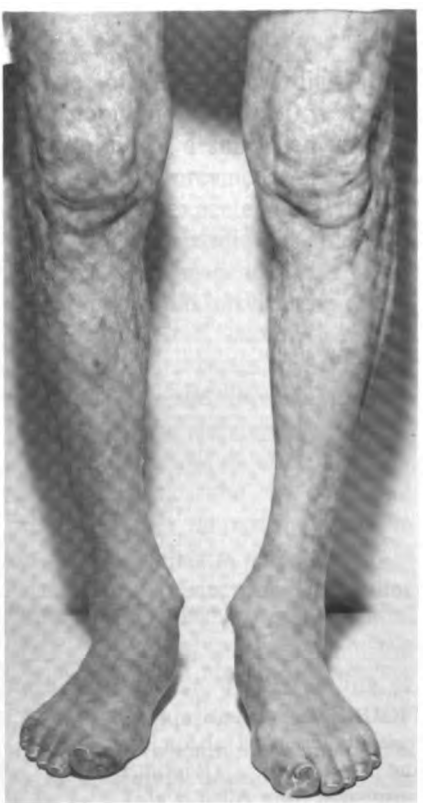

FIGURE 3 Digital changes and livedo reticularis of lower extremities. (Reprinted with permission from Kazmier *et al.*[28])

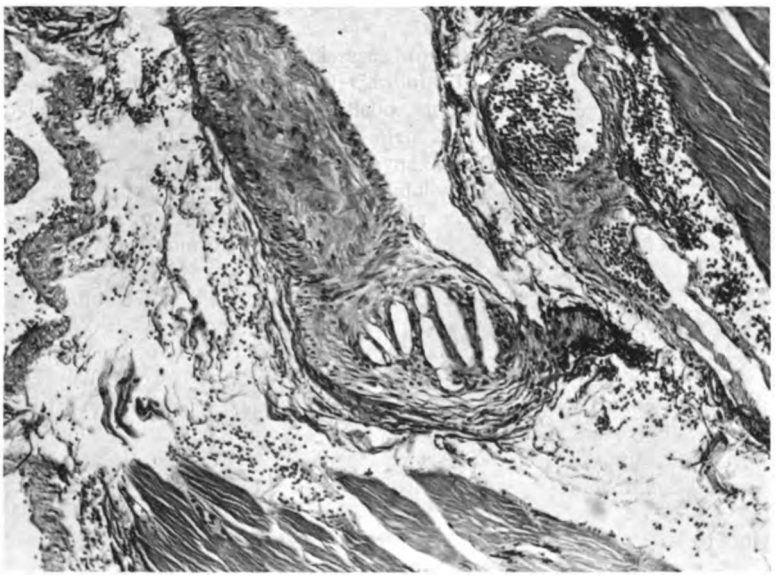

FIGURE 4 Biopsy of right gastrocnemius muscle, showing small artery occluded by embolic cholesterol material. Hematoxylin and eosin. (×120) (Reprinted with permission from Kazmier *et al.*[28])

of this phenomenon with greater frequency than we suspected lends credence to the concept of distal embolization from mural thrombi; and it raises the question, at least in my mind, of whether many of the presumed thrombotic occlusions of atherosclerotic vessels are really embolic, with subsequent propagated clot.

Although it is decidedly less frequent than arterial occlusion associated with heart disease and arteriosclerosis, acute arterial occlusion that complicates other diseases can be equally catastrophic (Figure 5). A 57-year-old woman with chronic ulcerative colitis sustained an acute occlusion of her right brachial artery, which led to gangrene and loss of her arm. Pathologic examination of the amputated arm revealed no underlying disease of the occluded artery to account for what was presumed to be thrombotic arterial occlusion.

The consequences of acute peripheral arterial occlusion can vary from no disability to all degrees of ischemia and even to loss of limb and life. Factors influencing the outcome include the size of the occluded artery, the adequacy of the collateral circulation, the extent of the occluding lesion, and the age and associated diseases of the patient. In general, the older the patient and the larger the artery occluded, the

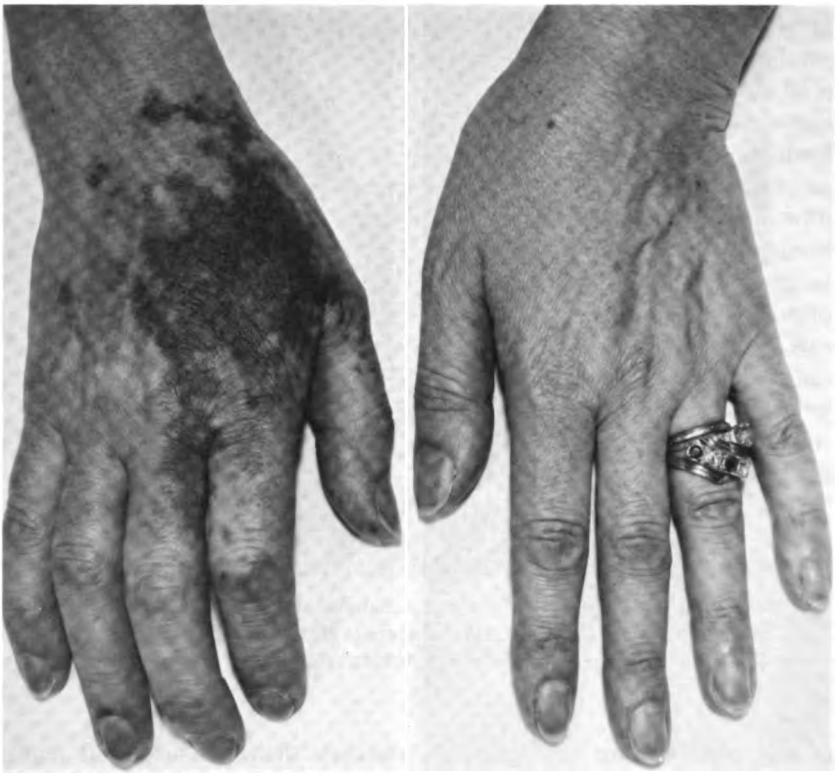

FIGURE 5 Pregangrenous changes of the right hand in a 57-year-old woman with thrombosis of the right brachial artery complicating chronic ulcerative colitis.

greater the mortality and the greater the loss of limb. Survival of the limb is more common when arteries of the upper limb are involved than when arteries of the lower limb are involved.[2]

The significance of thromboembolism in acute occlusion of the aorta and its major branches lies not only in the morbidity and mortality it produces but also in its intimate relationship to cardiac disease, particularly atherosclerosis. An acute aortic or peripheral arterial occlusion can be the initial manifestation of otherwise unknown significant heart disease.[23,29] It is also important that recurrences both of embolic arterial occlusion in the patient with heart disease and of thrombotic occlusion in the patient with peripheral arterial disease are distressingly common.[3,50]

Although less important than coronary or cerebral disease as a cause

of death, peripheral arterial disease affords greater accessibility for study; underlying factors important in thromboembolism are undoubtedly similar in the various parts of the arterial tree. Better understanding of these factors and the recognition and prediction of thrombosis should lead to more effective therapy and prevention.

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Cardiac Embolic Disease

EDWARD GENTON

Thrombosis is usually considered to be a peripheral disease that results from the deposition of fibrin in arteries or veins. However, the heart is not uncommonly the primary site of thrombus formation; when this occurs, it is almost always in association with pre-existing cardiac pathology. Some of the conditions frequently complicated by the development of cardiac thrombosis are shown in Table 1. The list includes lesions of the endocardium and myocardium, and valve structures. In recent years, the insertion of foreign material into the heart has accounted for many such events.

Brief consideration of the role played by thromboembolism in the natural history of the lesions listed will demonstrate the current incidence and significance of "cardiac thromboembolism."

ENDOCARDITIS

Inflammation of cardiac valves, whether it results from infection or from sterile processes, is regularly associated with deposits of thrombotic material.

In bacterial endocarditis, colonies of organisms on or beneath the affected valves are accompanied by deposits of platelet masses and fibrin. Generally, these deposits do not become large, but they may impinge on valve sinuses or subvalvular areas and interfere with their function. Occasional aortic lesions are so located as to narrow coronary ostia and alter coronary blood flow.

Of much greater significance are the effects produced by emboli that arise from fibrin and bacteria deposits on the affected valves. These emboli occur in virtually every case of bacterial endocarditis and pro-

TABLE 1 Conditions Predisposing to Cardiac Thromboembolism

Endocarditis
Bacterial
Nonbacterial
Myocardiopathy
Myocardial infarction
Rheumatic heart disease
Valve prosthesis

duce many of the physical signs that alert the physician to suspect the diagnosis.

Small, usually septic, emboli shower the systemic circulation, producing microinfarcts or abscesses in widespread areas. Such microemboli produce major complications when they involve vital organs and may cause death from organ failure, even if infection is eradicated.[12,33,35]

Major emboli are reported to occur in 15–30% of patients with subacute bacterial endocarditis,[33,34,40] and account for a significant proportion of deaths from this disease. The significance of this complication has increased since the beginning of the antibiotic era, as uncontrolled infection has become a less frequent cause of death (Table 2).

Valve vegetation in the absence of bacterial infection is being encountered with increasing frequency.[2] This condition is recorded in the literature under a variety of names, including "terminal," marantic, Libman-Sacks, and nonbacterial thrombotic endocarditis or endocardiosis. It is seldom found in the absence of systemic disease, and the systemic diseases most frequently predisposing to marantic endocarditis are uremia, cirrhosis, collagen diseases, and carcinomas with metastasis, especially of the mucinous variety.[1,2,31] The thrombotic masses are usually small and arranged in rows on the free or closing margins of the mitral, aortic, or tricuspid valve, in that order of frequency. Occasion-

TABLE 2 Cause of Death in 52 Cases of Subacute Bacterial Endocarditis [a]

Cause of Death	%
Persistent infection	44
Thromboembolism	31
Congestive heart failure	13
Arrhythmias	4
Noncardiac	4
Unknown	4

[a] Derived from Rabinovich *et al.*[34]

ally, they are large and pyramidal or massive sessile lesions (Figure 1). The thrombi consist of masses of agglutinated platelets capped with fibrin. Histologically, valve tissue beneath the thrombi reveals swelling of collagen fibers, eosinophilic or fibrinoid degeneration, and often tufts of proliferating capillaries, without significant inflammatory reaction.[6,32]

The etiology of these vegetations is unknown. Some workers suggest that they result from damage of valvular ground substance due to immunologic phenomena [1,2,27]; others have thought that they represent a local expression of a systemic state resulting from circulating thromboplastic material.[29] Although the vegetations are usually only an incidental finding at necropsy, the process may lead to embolic episodes in 10–20% of cases.[1,6,31] Such emboli are at times large enough to produce infarction of organs and cause death. In one series, 8% of patients suffered embolization to coronary arteries.[2]

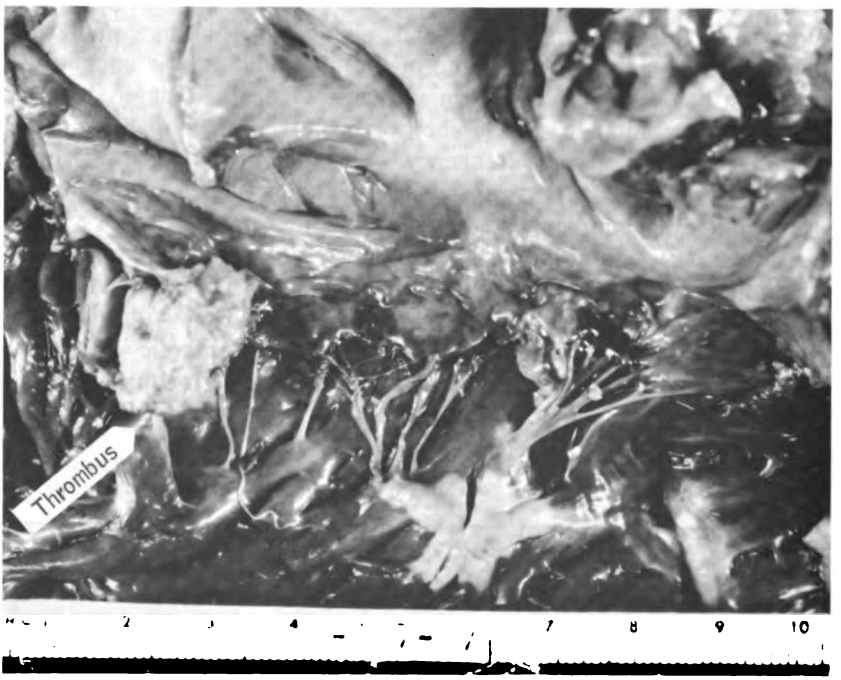

FIGURE 1 Large, friable, pyramidal thrombus involving chordae tendineae and free edge of anterior leaflet of tricuspid valve. Patient was a 46-year-old man with mucinous carcinoma of lung who had multiple pulmonary emboli thought to have arisen from marantic lesion.

MYOCARDIAL DEGENERATION

Pathologic processes that result in degeneration of myocardium are frequently associated with the development of thromboembolism. Patients with primary myocardial disease (i.e., myocarditis or myocardosis), regardless of etiology, are found at necropsy to have mural thrombi within one or both ventricles in approximately half the cases.[10,11,20] Embolic episodes involving pulmonary or systemic circulation occur in more than one third of these patients in most series. Occasionally, such an episode is what first brings the patient to the attention of the physician; more frequently, it is responsible for death.[10,17,20,37]

In such hearts, areas of endocardial damage underlie thrombi and presumably represent the primary focus for platelet aggregation and fibrin deposition, which may be initiated by exposure of collagen to the circulating elements at the site where endocardial integrity is lost.

Subjacent to a myocardial infarct resulting from coronary artery disease, a mural thrombus often develops. The lesions are most frequently seen in the left ventricle, which is invariably involved, but may occur in the right ventricle following septal infarction. In necropsy studies, the reported incidence of mural thrombi after infarction has ranged from 15% to 65% and has usually approximated 45%.[7,23–25,30]

The thrombi vary in size and consist of masses of fibrin enmeshed in ventricular trabeculations that overlie infarcted muscle. They may appear as rough irregular cauliflower masses or as more smooth, often laminated deposits within the cavity of an aneurysm (Figures 2 and 3). Either type may give rise to emboli of all sizes that enter the systemic or pulmonary circulation, depending on which ventricle is involved. Pulmonary emboli occur in about 75% of patients with right ventricular thrombi; they may produce infarction, but they seldom cause death.[7] Massive pulmonary embolism is much more likely to arise in the deep venous system of a lower extremity.

Systemic emboli originating in the left ventricle enter vessels to the spleen, kidney, and brain most often and vessels to mesentery and extremities less often. Evidence of emboli may be found at necropsy in approximately one third of patients with mural thrombus.[7,24] Clinically, systemic embolization complicates the course of 5–10% of patients with myocardial infarct, contributing significantly to the morbidity and mortality of the lesion.[19,30] Embolism occurs most frequently in the second and third weeks following infarction but may develop within the first few days.[24,41] It is uncommon for emboli to occur a month or more after an acute infarct, presumably because deposition of fresh fibrin

FIGURE 2 Large, irregular, mural thrombus enmeshed in trabeculations over entire lateral wall of infarcted left ventricle.

ceases as the mural thrombus becomes organized. Some patients with aneurysms that contain thrombi will have recurring embolization for months or years.

RHEUMATIC HEART DISEASE

Systemic thromboembolism constitutes a major complication of chronic rheumatic heart disease, occurring in 5–10% of cases. Patients with mitral disease, especially mitral stenosis, have the highest risk. Embolization develops in 10–20% and produces approximately 20% of the deaths in patients with mitral stenosis.[13,18,26] This is second only to congestive heart failure as a cause of death with valve disease [3,6] (Table 3). Nearly 60% of emboli enter the cerebral circulation; 30% involve

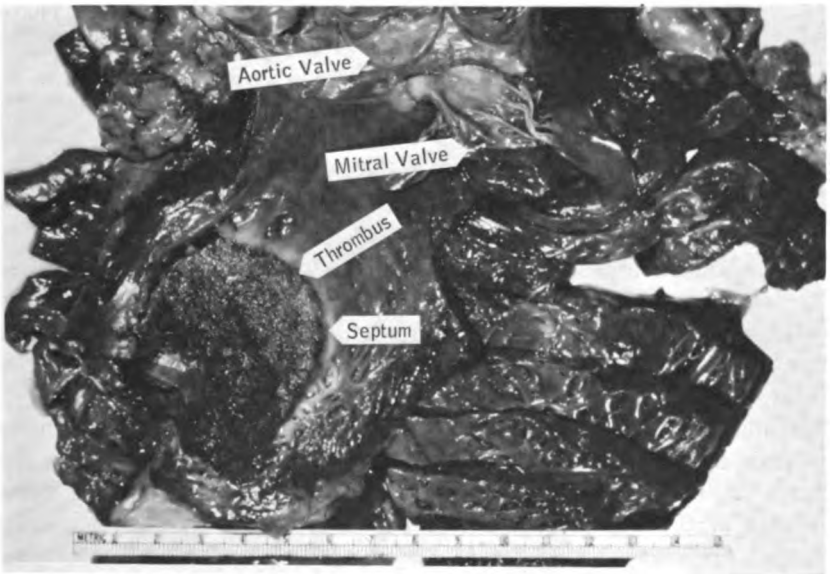

FIGURE 3 Smooth-surfaced thrombus filling aneurysmal cavity of infarcted septal wall of left ventricle.

the peripheral and 10% the visceral vessels. Initial emboli prove fatal in 15–20% of patients, and survivors have varying degrees of residual damage to organs or tissues. If they go untreated, a high risk of recurrence remains; more than half the survivors suffer repeat embolization, in 40% of the cases within 1 month and in 66% of the cases within 1 year of the initial attack.[36,39] Nearly one third of patients have more than two episodes.

Essentially, all emboli in mitral stenosis arise from within the left atrium, where mural thrombi are found at surgery or autopsy in

TABLE 3 Cause of Death in Cases of Mitral Stenosis [a]

Cause of Death	%
Congestive heart failure	61
Systemic emboli	19
Pulmonary embolism	9
Subacute bacterial endocarditis	5
Unrelated	6

[a] Derived from Rowe *et al.*[30]

15–55% of the cases, depending on clinical circumstances. These lesions may develop within the left atrial cavity, in the auricular appendage, or both [15,22] (Figures 4 and 5).

Stasis of blood due to valve stenosis has been considered the most important pathogenic factor in the formation of thrombi within the left atrium. Other factors are clearly important, inasmuch as correlative analysis fails to show that stasis alone is responsible.

Of other contributing factors, age is one of the most important (Table 4). Embolization before the fourth decade is unusual, but thereafter the incidence increases rapidly, until more than two thirds of patients with mitral stenosis are in atrial fibrillation at the time embolization occurs. Presumably, this reflects the effects produced by loss of atrial contractions, resulting in decreased turbulence and greater stasis.[13,39] Maximum risk is present soon after onset of fibrillation, with many patients developing emboli within 1–3 months after the arrhythmia appears.[8,39] The risk of embolization has been estimated during sinus rhythm at 0.7%/patient-year, increasing to 5.0%/patient-year when

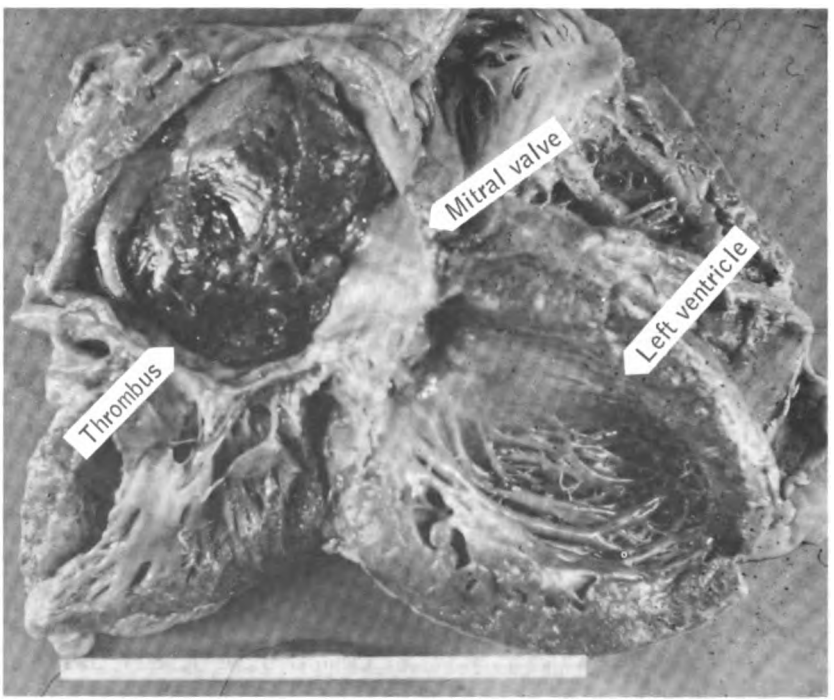

FIGURE 4 Large sessile thrombus filling almost entire cavity of left atrium in patient with mitral valve disease and history of recurrent systemic emboli.

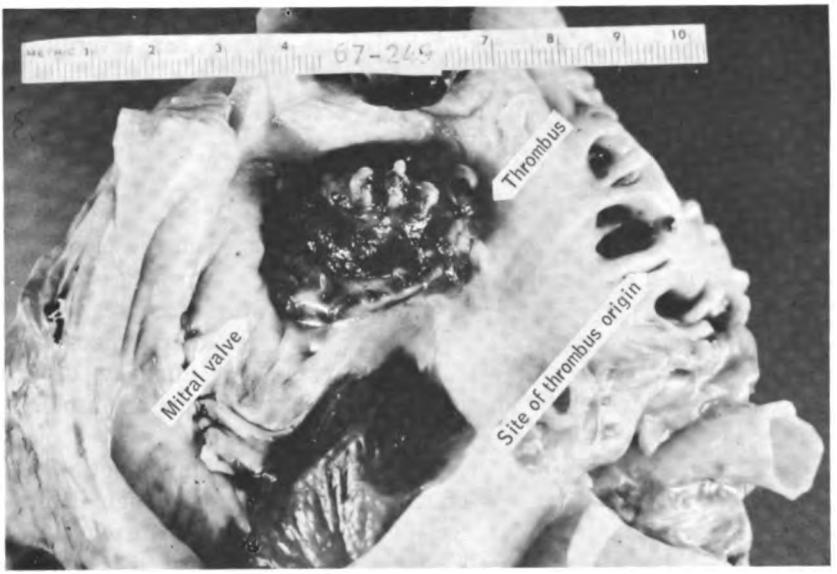

FIGURE 5 Opened left atrium in 36-year-old female with mitral stenosis who died suddenly. Thrombus completely obstructs mitral orifice. Projections on surface of thrombus correspond to trabeculations in atrial sidewall, suggesting site of thrombus formation.

TABLE 4 Factors Contributing to Cardiac Thromboembolism in Mitral Stenosis [a]

	Decade	% Incidence of Embolism
SIGNIFICANT CORRELATION		
I. Age	3rd	1
	4th	6
	5th	15
	6th	17
	7th	38
II. Cardiac rhythm: Sinus rhythm, 0.7%/patient-year		
Atrial fibrillation, 5.0%/patient-year		
III. Cardiac output		
INSIGNIFICANT CORRELATION		
Sex		
Severity of stenosis		
Functional classification		

[a] Derived from Casella *et al.*[13]

atrial fibrillation is present.[13] Reduced cardiac output has also been found to correlate with increased embolization, whereas sex, severity of stenosis, and functional classification have not.[13]

CARDIAC VALVE REPLACEMENT

During recent years, amazing progress has been made in the technical aspects of cardiac valve replacement, owing to improvement in surgical techniques, postoperative management, and pump procedures. Currently, the operative mortality, even in severely ill patients, does not exceed 20%.[14,38] Thromboembolic phenomena unfortunately occur with alarming frequency in survivors and constitute the major unsolved problem in valve surgery.

The Teflon cusps used initially were associated with only about a 5% incidence of embolization but were abandoned because of high failure rates.[16] Recently, ball or disk valves have been used almost exclusively because of their hemodynamic advantages. Placement of these prostheses, particularly in the mitral area, is frequently associated with thrombus formation on the valve.

The amount and location of the thrombotic material vary greatly, as do the consequences. Occasionally, the thrombus itself leads to alterations in hemodynamics by creating stenosis or insufficiency (Figures 6 and 7). For example, thrombus on an aortic prosthesis may interfere with coronary blood flow by impinging on coronary ostia and may produce an anginal syndrome or perhaps arrhythmia and sudden death.

The major consequences of valve thrombus are associated with fragments that embolize into the systemic circulation (Figure 8).

Early embolization, within a month of surgery, is clinically recognized in approximately 10% of patients. Survival is usual, but there may be permanent sequelae.[38] Late embolization presents an even greater problem and produces nearly half the deaths.[38] In one large series [16] (Table 5), during a 12- to 29-month follow-up, embolization occurred in 31% of patients with aortic valve prostheses, 33% with mitral prostheses, and 23% with multiple prostheses. These figures correspond to those reported by other authors.[21,38] After an embolic episode, the incidence of recurrence is high, especially in patients with mitral prostheses, and approximates 25%.[16]

Risk of embolization continues to be high, as shown in Figure 9, with a cumulative incidence as high as 40% by the end of the second year following surgery. A plateau then appears to occur, for reasons that are not known.

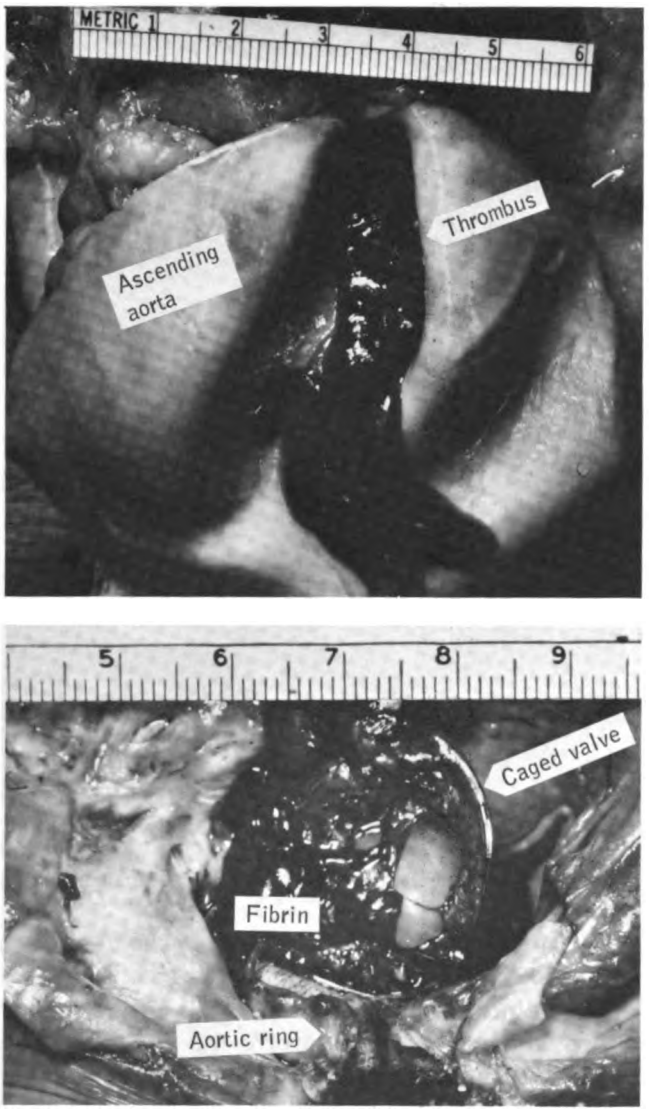

FIGURE 6 Specimen from patient who died suddenly 9 months after insertion of caged-ball valve for aortic stenosis. Severe angina developed several weeks before death. No embolic episodes were recognized. Top, view of opened ascending aorta revealing long, free-floating thrombus, whose proximal portion appeared to impinge on ostium of right coronary artery. Bottom, base of aorta opened. Valve is well seated, but almost completely covered by fibrin, which gave rise to thrombus.

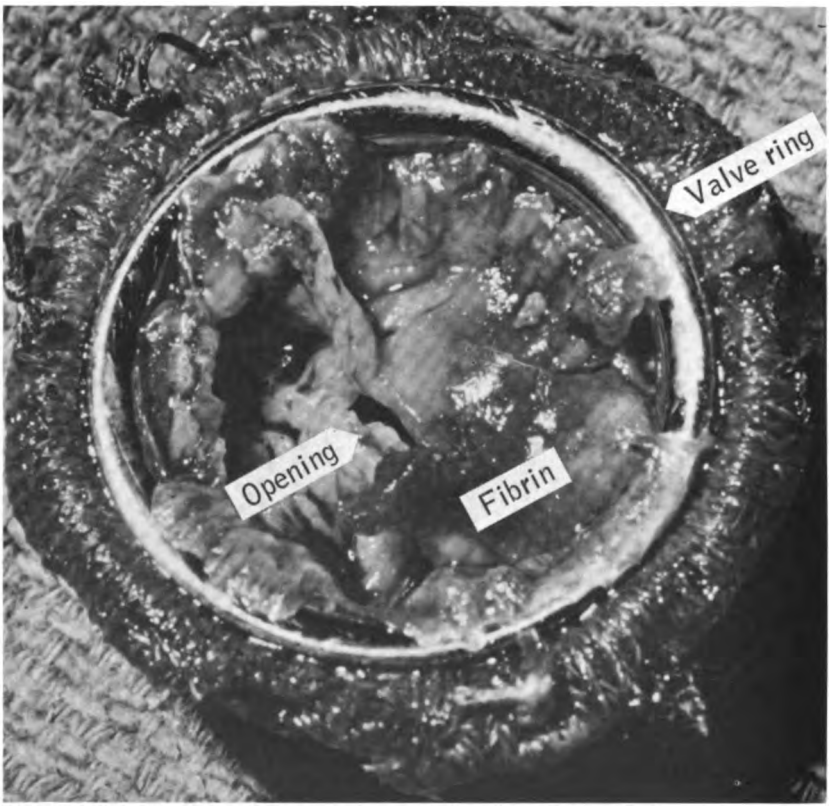

FIGURE 7 Disk valve removed from patient who developed recurrence of mitral stenosis symptoms 6 months after successful surgery. Orifice viewed from atrial side is almost covered with fibrin, with only a small opening in the center permitting blood flow.

The distribution of systemic emboli arising from prosthetic valves is listed in Table 6. Most involve the cerebral circulation, resulting in significant mortality and even greater morbidity. Coronary artery embolization is prone to develop, particularly in the multiple-valve replacement group, and is associated with a high mortality.

Numerous factors conceivably contribute to the development of thrombi on prosthetic valves. Hydraulic and hemic factors, including stasis, turbulence, viscosity and reactivity of platelets, and clotting factors, are all of potential etiologic significance.

Probably of greater importance are the electrical charge of the foreign surface and its ability to support tissue growth.

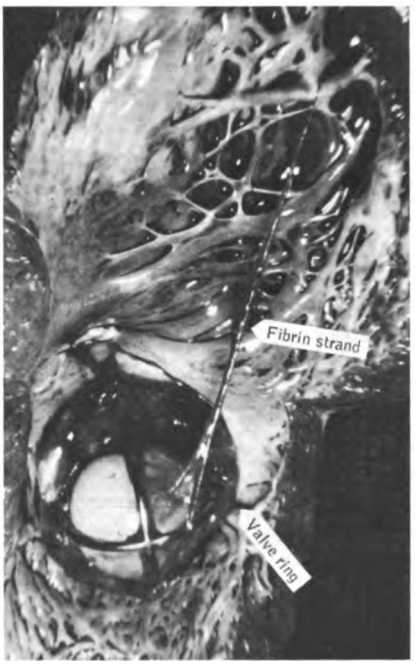

FIGURE 8 Ventricular view of caged mitral valve in patient who died following cerebral embolization. Metal ring and struts of the valve are covered with fibrin, which gave rise to long strands attached to ventricular trabeculations.

Much effort went into developing a material of sufficient durability that was nonthrombogenic. No such material was found, and attention turned to coating the valve surface with substances that retarded thrombus formation, such as a mixture of graphite, benzalkonium, and heparin. This coating decreased the rate of early thrombus development, but the substances were eluted after several weeks and benefit was lost.[9,38]

Thrombus forms most frequently where metal is exposed, especially at the base of struts, and seldom at the junction of the prosthetic valve

TABLE 5 Incidence of Embolism after Insertion of Starr-Edwards Prosthesis (after 12–29 months)[a]

Valve	No. of Patients	Thromboembolism	
		No.	%
Aortic	221	69	31
Mitral	166	54	33
Multiple	75	17	23

[a] Derived from Duvoisin *et al.*[10]

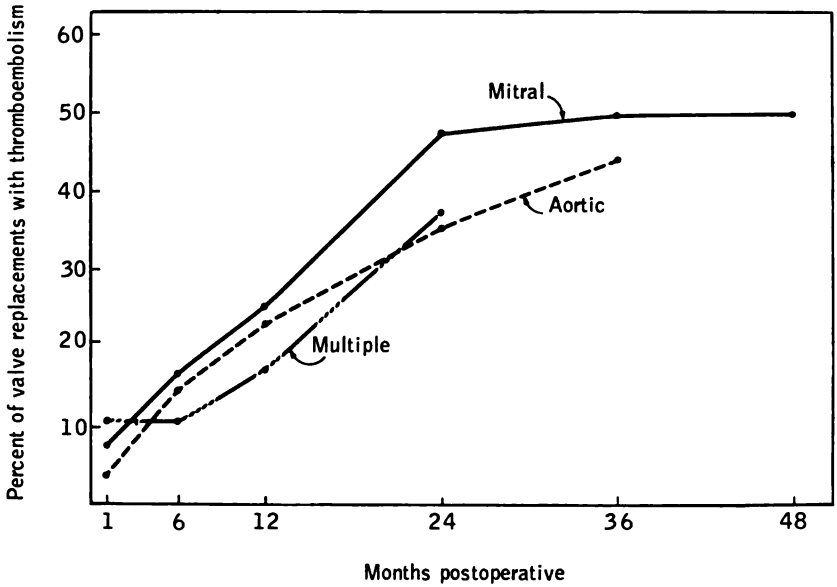

FIGURE 9 Actuarial curves demonstrating time of occurrence of thromboembolism following insertion of caged-ball valve.

ring and tissue. At that junction, a porous synthetic cloth, such as Dacron or Teflon, covers the metal to allow placement of sutures and undergoes endothelialization within several weeks of insertion. The discovery that endothelialization may protect from thrombus formation has resulted in the development of prostheses that are nearly totally covered with porous synthetic cloth. Currently, valves so constructed are being evaluated in animals and patients. Preliminary results are encouraging and suggest that the incidence of thromboembolism is greatly reduced.[9,38]

TABLE 6 Incidence of Embolism after Insertion of Cardiac Valve Prosthesis [a]

Location	% of Total	% Fatal
Cerebral	67	9
Coronary	15	35
Renal	5	3
Other	13	4
Recurrent	25	

[a] Derived from Duvoisin *et al.*[10]

If such results are confirmed, a major step in valve replacement will have been made.

Another approach to valve replacement has been to use homografts of aortic valves harvested from cadavers. In more than 200 patients in whom such valves have been placed, there have been no major embolic episodes. Unfortunately, structural changes frequently occur in the homografts, and further improvements in sterilization and preservation techniques appear necessary to allow acceptance of the method.[5]

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An Appraisal of the Clinical Problems

IRVING S. WRIGHT

From a clinical standpoint, the following unsolved problems and needs seem most important.

As in the case of many other disease states, we do not really know whether the spectrum and incidence of thrombosis are increasing or whether the new emphasis is merely a manifestation of our increased interest and acuity. However, it does appear that the spectrum is broadening as we are exposed to new drugs and other technical hazards of our civilization. In terms of the general population, we do not know the incidence of thrombosis. We have no valid information on its incidence in patients who do not see a physician, in those who do see a physician, or even in those who are hospitalized. Except in a few small population studies, such cases are not reported to any data-collecting agency. Even in small, detailed clinical studies, the figures are inevitably lower than the actual incidence, as shown by our autopsy findings and those of many others.[6] Assign a physician interested in this field to any medical, gynecologic, surgical, or fracture service, and the number of diagnosed cases of thromboembolism will immediately increase. This has happened in many hospitals.

Are we, as seems to be the case, encountering new coagulopathies as a result of longevity, with an increase in atherothrombosis, or of exposure to new industrial hazards and drugs? Does our diet influence this tendency? Currently, there is almost universal interest in what has been termed the thrombosing tendency, or the "hypercoagulable state." It is common knowledge that members of some families and many individuals thrombose more easily than most of their contemporaries, and more attention must be focused on this problem.[5] Does a genetic factor play a role in thrombosis, as with some hemorrhagic syndromes? We should certainly consider this possibility in such families; I reported a

remarkable example in the George Brown Lecture of the American Heart Association of 1951,[5] and we have seen many similar families since then.

Can we develop tests that will indicate more clearly the state of the platelets and other factors, to give us a better predictive capacity as to which patients will develop thrombosis? Even if this becomes possible in terms of blood taken from one vein, can we expect such tests to give us information regarding local thrombosing tendencies that exist at any distant plaque or area of injury of the vessel wall? Is there any difference between thromboses associated with different types of malignancy? If so, what is its significance?

What is the precise relation between atherosclerosis and thrombosis resulting in atherothrombosis? Is thrombosis an initial step in some atherosclerotic plaques, or does it follow after their formation or rupture?

Aside from the phlebothrombosis resulting from stasis, is there a true and significant difference between thrombosis as it occurs in the arteries and thrombosis in the veins? Problems regarding diagnosis of silent or small thromboemboli are uppermost in many clinicians' minds. Better detection of asymptomatic thrombi in the veins of the leg and thromboemboli in the lungs, the brain, and the kidneys would be a tremendous gain. A simple method, such as tagging the clots, should be developed. The high incidence of multiple thromboembolic involvement that is found when sought should change the attitude of the physician, whether he be an internist, a hematologist, a vascular surgeon, or a neurologist. The approach must certainly be holistic, rather than specialistic.

We do not have completely satisfactory methods for either prophylaxis or treatment. Methods for critical evaluation of anticoagulants, streptokinase, urokinase, clofibrate, estrogens, and other proposed therapeutic agents have proved elusive and difficult. There have been practically no serious attempts to evaluate surgical, as opposed to conservative, methods of handling these problems by use of random control series. Many of these questions are to be explored and in part answered in this volume, but others will remain unsolved and continue to challenge us.

To understand the mechanisms operating, we need more basic work in the field of physical chemistry, including the interplay of the electrical forces between the surfaces of cells and molecules.[1,2] In this regard, several interesting studies have been reported,[3-5] and this work should be extended. We need modern genetic studies of families who demonstrate striking thromboembolic tendencies. We need predictive tests of

validity. We need a systematic determination of the incidence of thromboembolic conditions. This will require a registry of all cases, however mild, that can be clinically diagnosed in a number of population groups, not only in the United States and Western Europe but also in such areas as Kampala, where, at Makerere Medical Center, coronary thrombosis in the natives is almost unknown. It will also require total autopsies of the vascular system, including the vessels of the extremities, neck, and brain. Experience has shown that these are hard to come by, but they should be a major objective. Otherwise, we can never satisfactorily evaluate the reported incidence of thromboemboli associated with possible etiologic factors, such as oral contraceptives, about which there is presently much estimate and guesstimate, but very little hard information. As is well recognized, we need both better anticoagulant and better thrombolytic agents. The status of these will be discussed later, at which time we hope that an effort will be made to differentiate clearly between the potential limitations and effectiveness of the drugs themselves, in contrast with the inadequacies of the protocols and techniques used in various studies that have been reported.

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II
THE EPIDEMIOLOGY OF THROMBOSIS

Epidemiology of Coronary Heart Disease: Risk Factors and the Role of Thrombosis

FREDERICK H. EPSTEIN

In recent years, epidemiologic studies have made it possible to predict with remarkable accuracy the chances that individuals and groups with various constellations of risk factors will develop clinically overt coronary heart disease. But epidemiology has contributed little, owing largely to methodologic problems, to the knowledge of the role of thrombosis in the development of atherosclerosis and its consequences. Morris's suggestion [22] that coronary stenotic occlusion, but not coronary atheroma, has become more common in recent decades made it plausible to think that the occlusive lesion is essentially thrombotic in origin, whereas the disseminated plaques have a different cause.[1] However, there is at least equally good reason to believe that there is only one type of atherosclerosis, which is characterized by a degree of pleomorphism. In either case, it is likely that thrombosis, perhaps in the form of platelet–leukocyte–fibrin aggregations, is involved, with other mechanisms, in the evolution of the atherosclerotic plaque, either as a primary or as a secondary phenomenon.[25] Apart from the chronic process, thrombosis is no doubt related to the acute event of a heart attack. The broad term, "heart attack," is used deliberately, in preference to "coronary thrombosis" or "myocardial infarction," in order to include the whole clinical spectrum from sudden death to myocardial infarction with survival. Unfortunately, neither the acute nor the chronic process is amenable to direct epidemiologic study, except on the level of so-called epidemiologic pathology, with all its shortcomings, because of the lack of clinical methods of detection.

Before proceeding, we should recall that there is a wide difference of opinion among pathologists regarding the frequency of thrombosis as the initiating event in heart attacks. On one end of the scale, it is stated that the infarct almost always precedes the thrombosis [29]; on the other,

it is believed that thrombosis is the primary event.[13] At any rate, thrombosis seems to be found more commonly among patients who die in the hospital than in patients who die suddenly.[5] Even among patients with infarcts, the frequency of thrombosis is variously reported as 21%,[2] 50%,[8] and around 90%.[21] Evidently, there is today no simple answer to the question of how often thrombosis is a cause of acute myocardial infarction. The differences in answers may be related to the thoroughness of the search, the kinds of patients studied, the length of survival, and the interpretation of the findings. In considering pathogenetic factors, the roles of intimal hemorrhage and plaque fissures [4,12] must also be kept in mind.

There is no doubt that many cases of sudden death bear no evidence of thrombosis at autopsy. In one study, only around 20% of patients who died suddenly had a fresh and recent thrombus [29]; in another, 27% of such patients had no demonstrable arterial occlusion.[5] Many persons who died suddenly without thrombosis may be presumed to have suffered a failure of the conduction mechanism of the myocardium. The problem of sudden death has recently become a matter of prime interest to epidemiologists [18]; in Baltimore, 60% of all deaths due to arteriosclerotic heart disease were sudden, and in only about half the cases was there a history of heart disease. According to the Framingham study, 24% of 229 men and women died within an hour or before reaching the hospital after experiencing their first heart attack [6]; this figure increases to 31% after exclusion of "silent" infarcts.

There is an urgent need to study the epidemiology of sudden death, separately for those who do and do not show thrombosis; the victims cannot possibly benefit from any therapeutic advances, such as intensive-care units. Little is known about factors that predispose to sudden death; only obesity [16] and physical inactivity [17] have so far been shown to carry excessive risk. Among persons who have died at any time within 3 or 4 weeks after the acute event, serum cholesterol level [15] and again physical inactivity [10] have been shown to be common.

In contrast with these areas of considerable ignorance and confusion, there is now much solid knowledge concerning the probability of developing coronary heart disease, considered, in its various manifestations, as a group. The accuracy of predictive tests in terms of multiple risk factors will be briefly reviewed, to serve as a background to the question of what proportion of new events of coronary heart disease remain "unexplained" in terms of current information. It is conceivable that additional predictive power might be obtained by including various measurements of "thrombotic tendency" among these risk factors.

What is the predictive power of available tests [9] for coronary heart

disease? Let it be assumed that there is a test, or a battery of tests, with almost complete ability to predict the disease. Such a test would concentrate, among a minority of the population at risk, a majority of the future cases. In Figure 1, the square represents 100 persons at risk. Twelve persons (12%), as shown in the rectangle on the left, have a positive test, and 10 of them (83% of the 12) will develop clinical disease within the next 10 years. Among the 88 persons with a negative test, only two (2.3%, rounded off to 2% in the figure) will develop the disease within the next 10 years. Thus, the nearly ideal test would concentrate five sixths of the future cases among a little more than one tenth of the population.

Moving from the ideal to the real, the predictive power of serum cholesterol level is shown in Figure 2, using 260 mg% or above as a positive test. Among middle-aged men, 16% show a positive test in these terms, and about one fifth of them will develop clinical disease within 10 years. By contrast, only 10% of the remaining 84 men at risk will become affected—a risk ratio of 2.1:1. Of the 12 men who become ill, only a little more than three men will have a positive test.

Predictive power is improved if men are chosen who show an increase in either serum cholesterol, blood pressure, or both (Figure 3). Now, 42 of the 100 men are put at preferential risk, and eight of them (almost 20%) will fall ill—almost three times as high a proportion as among

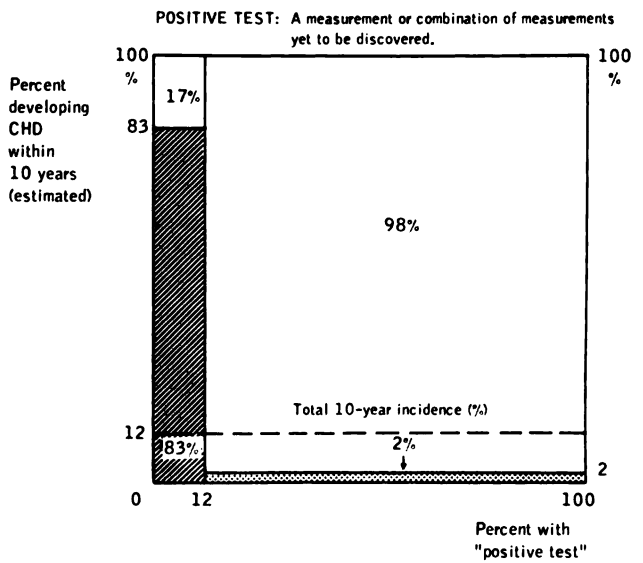

FIGURE 1 Predictive power of a hypothetical ideal test for coronary heart disease. Adapted from Epstein.[6]

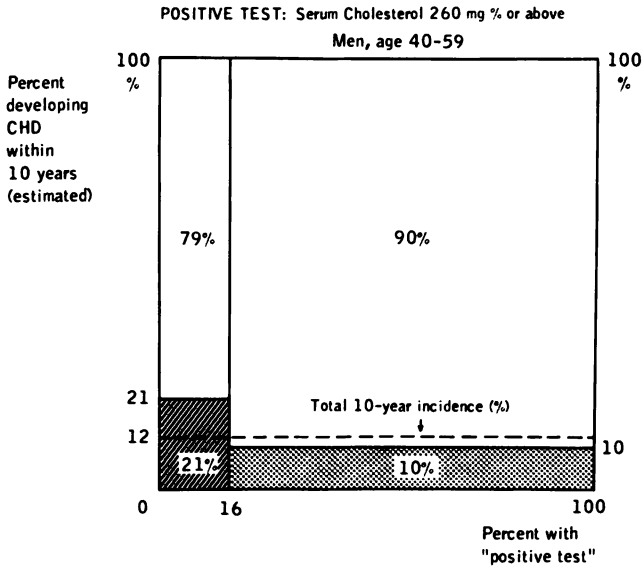

FIGURE 2 Predictive power of serum cholesterol level in coronary heart disease. Adapted from Epstein[9]; based on data from Kannel, W. B., T. R. Dawber, A. Kagan, N. Revotskie, and J. Stokes III. Factors of risk in the development of coronary heart disease—six year follow-up experience. The Framingham study. Ann. Intern. Med. 55:30–50, 1961.

those with a negative test (20% versus 7%). Thus, about eight of the 12 new events occur among those with a positive test, compared with three of 12 when serum cholesterol alone is used as a predictor. Data from another study, in Britain (Figure 4), are remarkably similar.

Thus, serum cholesterol and blood-pressure levels alone are good predictors. As would be anticipated, adding more risk factors will add predictive power among those with a positive test (Figure 5), but the price paid is that a smaller proportion of the population is put at preferential risk. In the example of Figure 5, taking four risk factors (serum cholesterol, blood pressure, smoking, and overweight), almost half the men (44%) of those high on all four will develop disease, but such men account for only 3% of the total population.

As a final example of this new, quantitative epidemiology, the predictive power of multivariate risk functions is presented (Figure 6). The seven risk factors shown are put into a multiple logistic function, and the values are divided into deciles of decreasing risk. A little over one third of the men in the highest 10% of the population will develop

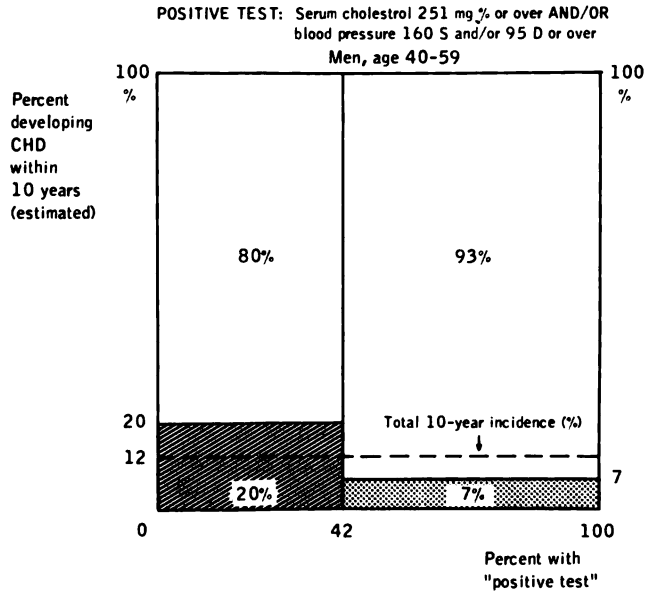

FIGURE 3 Predictive power of serum cholesterol level and blood pressure in coronary heart disease. Adapted from Epstein [9]; based on Framingham 8-year incidence data from T. R. Dawber (personal communication).

overt illness in the ensuing 12 years. It may be stated that 67 of the 88 men, or 75%, have risk function values above the median, so that 75% of the new cases arise from the 50% of the population that is at preferential risk.

The predictive power of currently available tests clearly falls very short of the ideal situation depicted earlier. Yet, there is no other disease that can now be predicted as well as coronary heart disease. If thrombotic tendency (for lack of a better term) is linked with coronary disease, any tests that would measure such a tendency should substantially increase predictive power. So far, only platelet adhesiveness seems to be a potentially practical characteristic to include in epidemiologic studies, but no published data are available. Needless to say, any such test would add predictive power only if its correlation with other known risk factors were relatively low, making it a risk factor in its own right. Using circumstantial evidence, however, one would suspect that a number of indices related to thrombosis and blood clotting might well be associated with the risk factors already mentioned, such as serum lipid

level and smoking, and others of likely importance, such as physical inactivity and, possibly, so-called "emotional stress."

Is there any relation between these risk factors and mechanisms related to thrombosis? I cannot review here the extensive (if controversial) literature on the effect of various dietary fats on coagulation. One excellent summary is contained in a report on dietary fat and coronary heart disease by a panel of experts appointed by the National Heart Foundation of Australia.[7] The report concludes that the evidence of increased coagulability among patients with atherosclerotic disease is conflicting, but that lipids play a significant part in the coagulation process, in that long-chain fatty acids probably accelerate platelet aggregation. It is therefore conceivable that serum lipid levels and platelet adhesiveness show some correlation. Smoking also releases fatty acids, as well as catecholamines, both of which have an effect on platelets.[23] Catecholamines are mobilized by some emotional stresses.[11] Catechola-

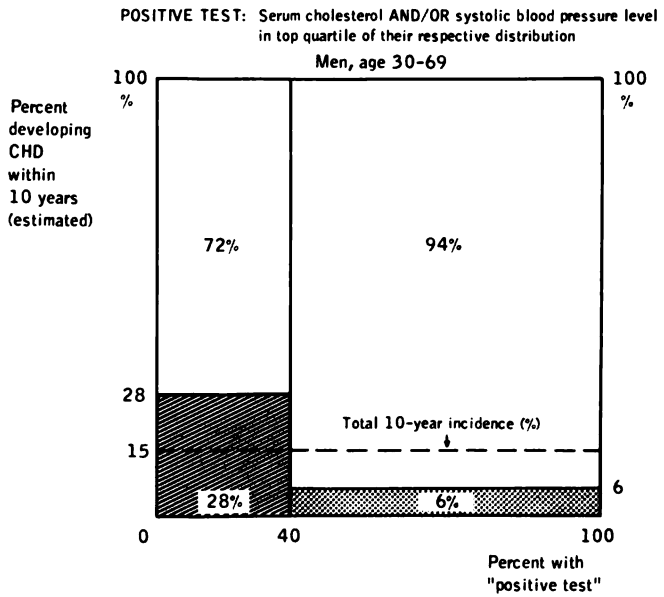

FIGURE 4 Predictive power of serum cholesterol level and blood pressure in coronary heart disease. Adapted from Epstein [9]; based on data from Morris, J. N., A. Kagan, D. C. Pattison, M. J. Gardner, and P. A. B. Raffle. Incidence and prediction of ischaemic heart-disease in London busmen. Lancet 2:553–559, 1966.

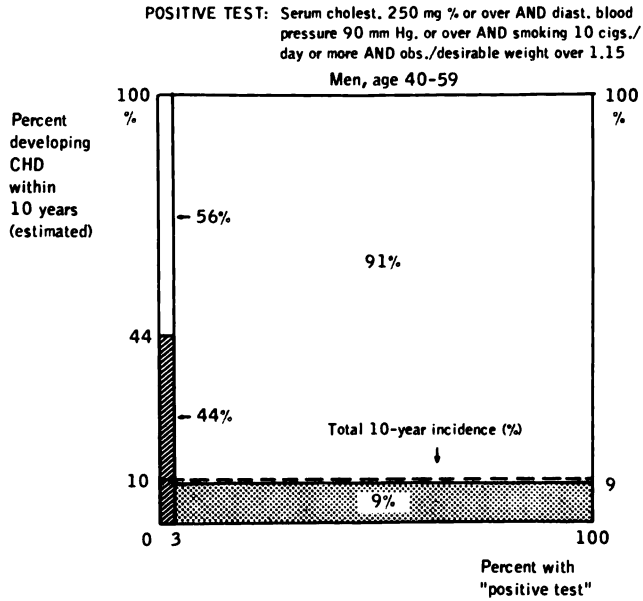

FIGURE 5 Predictive power of multiple factors in coronary heart disease. Adapted from Epstein[9]; based on data from Stamler, J. Atherosclerotic coronary heart disease. The major challenge to contemporary public health and preventive medicine. Conn. Med. 28:675–692, 1964.

mines, in turn, mobilize fatty acids.[24] Coagulability and fibrinolysis are both enhanced by exercise.[3,14] Clearly, there are many possible complex links between the thrombosis–coagulation–fibrinolysis system and atherosclerotic diseases, but it would be idle to speculate on them from the point of view of epidemiologic studies and the risk of coronary heart disease at this time. The need is for the development of methods that are sufficiently simple and accurate to measure these potential influences as part of epidemiologic surveys. In this context, the possible importance of familial-genetic factors must be kept in mind, with regard to both coagulation defects viewed as quantitative variables and anatomic variations that may predispose to thrombosis. Thus, small-caliber arteries have been shown to predispose to myocardial infarction,[27,30] perhaps in part because such vessels are more liable to thrombose.

Although differences in the frequency of thromboembolic phenomena over time and in different countries and their possible relation to coronary disease are difficult to interpret,[26] there is good evidence that

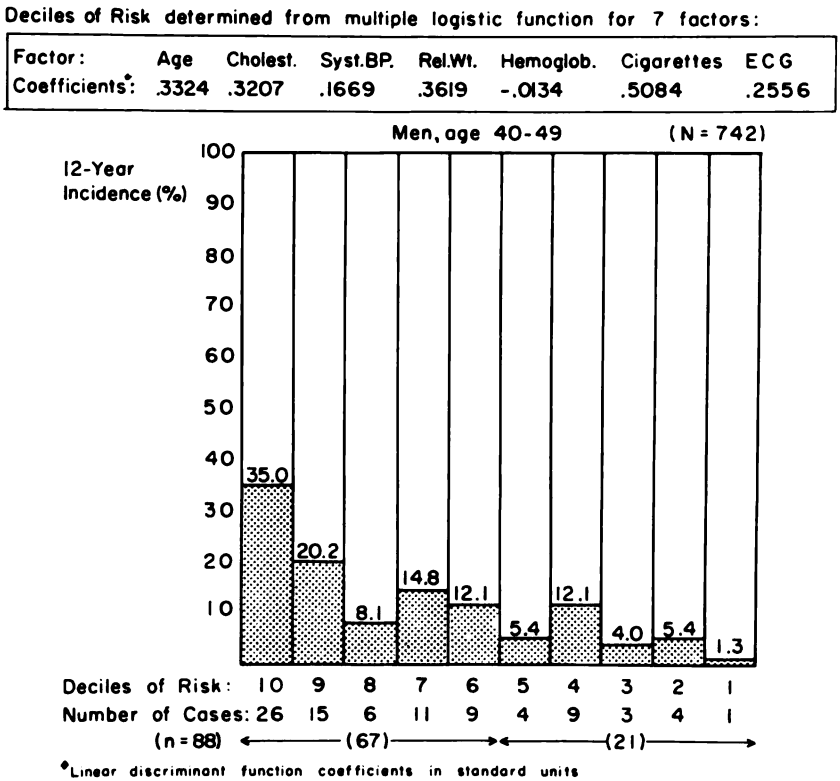

FIGURE 6 Predictive power of multiple factors in coronary heart disease. Based on data from Truett, J., J. Cornfield, and W. Kannel. A multivariate analysis of the risk of coronary heart disease in Framingham. J. Chronic Dis. 20:511–524, 1967.

coagulability is diminished and fibrinolysis enhanced among populations in which coronary disease is uncommon. Such data are available for the Bantu,[20] East Africans,[19,28] and Koreans.[19] But no cause-and-effect relationship can be inferred from these findings.

The work reported here was supported by Public Health Service research career award K6 HE 6748 from the National Heart Institute.

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The Epidemiology of Cerebrovascular Disease

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This discussion of the epidemiology of cerebrovascular disease is part of a volume on thrombosis, so I will attempt to concentrate on the aspects of the disease that have some potential relevance to the subject of thrombosis. The discussion must be tempered by the realization of the limitations inherent in any paper that attempts to deal with the factors affecting the distribution and occurrence of one of the components of the disease entity that has hitherto been covered by the blanket term, "cerebrovascular disease." We are truly limited in our ability to distinguish cerebral hemorrhage from cerebral thrombosis in most of the data that have been published thus far and on which the epidemiologist must rely in his initial investigations. I will attempt to discuss briefly the observations that have been made concerning the epidemiology of cerebral thrombosis, categorizing the different observations into those related to place, time, and person.

PLACE

Differences Between Countries

A number of studies have stressed the striking differences in mortality rates from the cerebrovascular diseases between different countries.[1,14,54,58] Unusually high mortality rates have been reported from Japan, Finland, Scotland, and Germany, whereas other so-called technologically advanced countries, including the United States, generally tend to group around a lower median. Marked differences have been noted in the portion of stroke cases attributed to the different kinds of cerebrovascular disease. In the English-speaking countries, about half

the total mortality associated with cerebrovascular disease is attributable to hemorrhage, and 25–30% to thrombosis and embolism. In contrast, hemorrhage was said to account for 85% of such cerebrovascular deaths in Japan.[57] Only in recent years has evidence begun to accumulate indicating that the much-discussed excess of cerebral hemorrhage in Japan may be an artifact of reporting. Several studies in the literature indicate that there may be gross overreporting of hemorrhage and underreporting of thrombosis in the Japanese population. In one study that attempted to correlate death-certificate with autopsy data, it was found that in only 22% of deaths attributed to stroke on the death certificate was there confirmation that the same type of cerebrovascular accident was directly related to death. Nearly one third of the fatal cases of cerebral thrombosis were mistakenly attributed to cerebral hemorrhage on the death certificates, whereas only 2% of the cases of cerebral hemorrhage were mistaken for thrombosis.[20] In addition, follow-up of examinees in the Atomic Bomb Casualty Commission program has led to the realization that, when one is able to look at incidence, rather than simply mortality, thrombosis is more frequent than hemorrhage. Among 132 definite cases of cerebrovascular accident diagnosed among the atomic-bomb survivors in Hiroshima, the ratio of hemorrhage to thrombosis as the known cause was 0.36 for males (14/39) and 0.44 for females (16/36).[20] These findings are similar to those reported from a total-population study in Hisayama, Japan: among 1621 persons over the age of 40, 38 stroke cases were observed over a 3-year period, and the ratio of hemorrhage-related cases to the total was 0.45.[25] It is becoming increasingly evident that cerebral thrombosis is being underreported in Japan, casting some doubt on the probability that large-scale international studies will be very fruitful in helping to elucidate the epidemiologic features of the cerebrovascular diseases.

The problems involved in carrying out such studies in relation to cerebral thrombosis are evident in a recent report of blood-coagulation studies carried out on stroke patients in Minnesota and Japan.[12] The authors were able to demonstrate abnormalities in fibrinogen in acute stroke patients in both Minnesota and Japan, and they felt that the values obtained indicated that some identifiable coagulation changes do follow nonhemorrhagic strokes in both populations. But some unexplained differences between the two populations could not be interpreted, owing to the many variables influencing the test values. The tests used have been found to vary significantly, depending on their time of performance. There is also marked variation in results with the age of the subjects, so that, in the absence of known "normal" values for different age segments of the particular population from which the

cases were derived, it is not possible to interpret the results of the study. In addition, the authors were unable to compare the populations directly, because of slight variations in technique, differences in test reagents, mineral content of the water, and technician variability.

Differences Within Countries

Several studies have reported on the variability of stroke mortality rates in Ireland, Japan, and the United States.[1,4,57,61,62] In each of these three countries, the difference between the highest and lowest subdivisions was the same (with the highest rates being 2½ times the lowest). For white males in the United States in 1959–1961, the highest death rates from cerebrovascular disease were in the Southeastern States, and the lowest in the Southwestern and Mountain States. The age-adjusted rates range from a low of 34.3 per 100,000 in Wyoming to 90.3 per 100,000 in South Carolina. The same pattern is seen in the data for U.S. white females. To determine the validity of these differences in rates, there is in progress a study of stroke mortality in nine states of varying economic character and of reputedly varying stroke mortality rate. A sample of all deaths occurring in these areas has been reviewed, and all available medical records have been reviewed independently to determine the presence of cerebrovascular disease. The preliminary findings, recently reported,[31] indicate that the observed differences cannot be explained by such factors as variability in method of completing death certificates or by differences in percentages of deaths that occurred in hospitals. It is of interest to note that the types of cerebrovascular disease listed on the certificates were similar in all nine areas. If these findings of real mortality differences can be supplemented by similar findings of differences in morbidity rates, it may be possible to elucidate the epidemiology of cerebrovascular disease more completely by studying the manner in which suspected environmental and host factors differ.

TIME

Long-Term Trends

The figures for trends in death rates from cerebrovascular disease for the United States during the period 1900–1962 show a moderate but steady decline in total cerebrovascular disease for white males, white females, and nonwhite females and very little change for nonwhite males during that period. Decreases in total rates have recently been noted in

the United States,[66] Canada,[15] and Japan.[25] Reports from England and Wales note little or no change in age-adjusted death rates from cerebrovascular disease during the period 1932–1961,[67] and one report indicates an increase in the Republic of Ireland.[1]

Because of the rapid increase of cerebrovascular disease death rates with age, the trends based on age-adjusted rates may be markedly affected by changes in the ratios of the numbers of persons in the oldest age groups, which usually are open-ended (e.g., "75 and over"). Recent studies of trends of cerebrovascular disease mortality in Baltimore and Memphis show that the total death rates have declined in the age groups under 75, and that the decline has been greatest in the younger age groups—ages 45–64. The rates among nonwhites in both cities actually increased slightly in the age group 65–74.[33]

Some interesting leads have developed from analyses of trends of the specific types of cerebrovascular disease listed on death certificates. Such analyses must always be viewed with caution, in that there may be gross inaccuracies in the categorization of disease types. Discrepancies between death-certificate diagnoses and autopsy findings were previously described for the Japanese data.[20] A recent study in Baltimore has shown poor agreement between diagnostic information on clinical records and the specific type of cerebrovascular disease listed on death certificates. Terminal hospital records were reviewed independently from the death certificates. It was found that for deaths certified as being related to cerebral thrombosis, the reviewer concurred with the diagnosis in 54% of the cases, and that for deaths certified as being related to cerebral hemorrhage, the reviewer agreed with the diagnosis in only 37% of the cases.[30]

The limitations of such studies must be recognized, but it is important to note that several different reports have now documented the finding that cerebral hemorrhage has been declining since 1930, and that there has been an increase in cerebrovascular occlusive disease (i.e., cerebral thrombosis and embolism combined). Yates found an approximately twofold increase in thrombosis and embolism for England and Wales for the period 1932–1961 and stated that the increase was not noticeable under the age of 60. He also reported a temporary interruption in the upward trend for thrombosis during World War II.[67] Data from Memphis and Baltimore,[28,33] as well as a recent report on trends in age-adjusted death rates in Japan,[25] present similar findings of rising death rates from cerebral thrombosis and embolism and a decline in the death rates from cerebral hemorrhage. Such studies as these emphasize the importance of considering the different components of cerebrovascular disease separately.

SEASON

Mortality data from Japan, the United States, England, and Wales have revealed a consistent pattern of lower rates in the summer months and higher death rates in the winter.[6,56,65] The seasonal variation is most marked in Japan. It has been suggested that this may be due to (1) the decrease in blood pressure that has been observed in the summer or (2) an increase in pneumonia and respiratory infections in the winter, which precipitates death of stroke patients. No data have been developed that deal specifically with the seasonal distribution of death from cerebral thrombosis or any of the other categories of cerebrovascular disease.

PERSON

AGE

Mortality data clearly demonstrate the geometric increase of death rates with age for the total group of cerebrovascular diseases. Mortality rates above age 75 are about 10 times as high as mortality rates for ages 45–54. Up to age 75, the nonwhite population has higher rates, whereas beyond that age the white rates are higher. Age-specific death rates presented by Bronte-Stewart for England, Scotland, and Wales showed that the proportion of total deaths due to stroke varied with age from just over 4% at age 35 to 20% at age 75.[6] However, among the 193,588 deaths from cerebrovascular diseases reported in 1960 in the United States, 19.4% occurred in persons under 65.[3]

There is good evidence from the few morbidity studies that have been reported that the geometric increase in death rates is paralleled by a geometric increase in the incidence of new cases of the various categories of cerebrovascular disease. In a study of first strokes in an entire county in Connecticut with a population of 835,000, incidence rates approximately doubled for each successive 10-year age group up to the age of 75, and for cerebral thrombosis there was an even greater increase above that age.[11] The study of the Du Pont Company labor force experience revealed that rates for both disabling and fatal strokes among male wage and salaried employees increased progressively in each 5-year age group from 45 to 64. The data reported from the Framingham study on the 12-year incidence of thrombotic brain infarction revealed similar geometric increases with age.[23] The incidence data reported from Hiroshima [20] were similar to the U.S. data in the increase of total cerebrovascular disease death rates with age, and the reported rates

were not very different from those reported in the Connecticut study. The rates for cerebral thrombosis in Hiroshima were also quite similar to those reported from Framingham.

The data reported recently from Memphis and Baltimore [33] illustrate a problem in interpreting trends in mortality data with age-adjusted rates. An earlier report from Memphis [28] suggested that there had been no change in the total cerebrovascular disease death rates when both contributory and underlying causes of death were taken into account and when adjustments were made for changes in diagnostic fashion. Actually, there has been a substantial decrease in the rates in the age groups from 45 to 74 and an increase in the rates in the oldest group (75 and above).

Among the possible explanations for the opposite trends in death rates in the younger and older groups are differences in medical care and diagnostic acumen; changes in accuracy of reporting ages on death certificates; and an alteration in the composition of the open-ended 75-and-above age category, with a higher percentage of the group attaining ages of 80 and above in the most recent period. Another possibility stems from the finding that the fall in total cerebrovascular disease death rates during this period has been due primarily to a decrease in cerebral hemorrhage death rates, whereas the rates of death associated with cerebral thrombosis and embolism have been increasing. The increasing stroke death rates in the oldest groups may be simply a reflection of the greater role of thromboembolism in older persons.

SEX

A number of studies based on mortality rates have shown male:female ratios close to unity for cerebrovascular disease, quite unlike the picture seen for arteriosclerotic heart disease.[48] Data from Baltimore show a slightly higher male:female ratio for white persons and a lower ratio for Negroes. When broken down by specific type of cerebrovascular disease, the mortality data from Baltimore showed that the excess Negro female rates were due to higher rates from thrombosis or embolism.[29] There is less consistency in reported data based on morbidity as well as mortality.

The incidence data reported from the Framingham study [23] show that the incidence rates in women of a given age were similar for both cerebral infarction and myocardial infarction, whereas in males the myocardial infarction rates were several times higher than the cerebral infarction rates. The male:female ratios were very high for myocardial infarction, but they were close to unity for cerebral infarction. The inci-

dence data for cerebral thrombosis and cerebral hemorrhage in Middlesex County revealed similar rates for males and females.[11] The data from Hiroshima suggest a greater difference between males and females than was observed in the U.S. experience, especially up to the age of 70.[20] The reports from the Du Pont Company labor force study also reported somewhat lower stroke rates for female than for male employees. However, it seems that the marked differences between males and females in both morbidity and mortality, noted repeatedly for arteriosclerotic heart disease, are not characteristic of the cerebrovascular diseases.

RACE AND ETHNIC GROUP

The only data available in regard to racial differences in the cerebrovascular diseases are related to mortality; the reported morbidity population studies have not dealt with the nonwhite in this country. Routine vital statistics for the United States show sizable differences between nonwhite and white mortality rates associated with cerebrovascular diseases.[43] These can be considered primarily a reflection of the differences between the Negro and the white populations. But, it is interesting to note that other groups showed differences from whites in death rates related to stroke. For 1950, the highest rates were in the American Negro, and those for Chinese-Americans and Japanese-Americans followed, in that order. Several studies have shown the same mortality pattern, i.e., highest cerebrovascular disease death rates for the nonwhite female, followed by those for the nonwhite male, then for the white male, and finally for the white female.[54] Mortality data for Charleston County, North Carolina, for 1955–1958 revealed a ratio for nonwhite:white males of 5:1 and for nonwhite:white females of 11:1.[43] Data from Chicago showed markedly higher rates of mortality attributable to stroke and hypertensive disease in the Negro than in the white population.[3] In a study of stroke deaths in Baltimore residents aged 20–64, cerebral hemorrhage rates were found to be four times higher in nonwhite males than in white males and five times higher in nonwhite females than in white females; and cerebral thrombosis and embolism rates were twice as high in nonwhite males as in white males and four times as high in nonwhite females as in white females.[29]

As mentioned previously, there has long been interest in the higher reported mortality rates from cerebrovascular disease among the Japanese. A number of reports in the literature dealing with proportionate mortality data have supported the view that the Japanese have a marked excess of cerebral hemorrhage, compared with both cerebral and myocardial infarction.[26,42,45,56,59] However, the most recent reports from

Hiroshima and Hisayama suggest strongly that these reports must be re-evaluated, and that some of the major reported differences between U.S. and Japanese rates, as well as between rates for Japanese persons in Japan, Hawaii, and the continental United States,[16] may be attributable to differences in diagnostic criteria, coding, and methods of reporting. A recent report of an extensive review of almost 30,000 autopsied cases in Japan, covering the period 1959–1962, revealed cerebral hemorrhage in 2.2% of the autopsies and cerebral thrombosis in 2.0%.[25] Regardless of the selective nature of an autopsy series, these figures seem incompatible with previous estimates that attributed over 35% of all deaths in Japan to cerebrovascular disease, with a marked preponderance of cerebral hemorrhage.[26] Recent studies indicate a greater predilection for atherosclerosis in the cerebral vessels than in the coronary vessels in the Japanese, and there appears to be relatively more cerebrovascular disease than coronary disease.[49]

PHYSIOLOGIC FACTORS

Serum Cholesterol The Framingham study demonstrated a relationship between serum cholesterol level and the later development of thrombotic strokes. In men and women who were under 50 at the time of the first examination at which serum cholesterol was determined, there was an upward trend in the incidence of stroke with higher serum cholesterol levels. The serum cholesterol level was not a valid prediction of risk of stroke if first determined over the age of 50.[23] The data from Hiroshima reveal an increased risk of development of cerebrovascular disease in persons with serum cholesterol levels above 220 mg%. The Japanese population is known to have lower serum cholesterol levels than the U.S. population, and there is a suggestion that the risk may be somewhat higher for a given level in the Japanese than in the U.S. population. The relationship was more marked for females in the Japanese study.[20] The Los Angeles prospective study failed to find any relationship between initially determined serum cholesterol levels and subsequent risk of stroke.[7]

In a study of 257 patients with cerebral thrombosis compared with 124 control patients (free of clinical evidence of atherosclerotic disease), the serum cholesterol levels were found to be higher (but not significantly) in all age groups and both sexes among persons with cerebral thrombosis. The ratio of beta-lipoproteins to alpha-lipoproteins was significantly higher in all age groups and both sexes among persons with cerebral thrombosis.[50] Another report found no significant difference in mean serum cholesterol levels between 110 patients with cere-

brovascular disease, 79 patients with nonvascular neurologic disease, and 22 healthy men; however, a significantly greater percentage of patients with cerebrovascular disease had high serum cholesterol levels.[41]

In a recent study of patients with cerebrovascular disease (all of whom had acute or rapidly progressive monoplegia or hemiplegia, or had had one or more attacks of short-lasting hemiplegia), carotid angiography was performed to differentiate cerebral thrombosis (with a complete block of the internal carotid or its two main branches) from cerebral circulatory insufficiency (in which intermittent partial obstruction of the arteries was found). Patients and controls were 50–70 years old. Plasma cholesterol levels and triglyceride levels were significantly higher in patients with thrombosis than in controls, whereas the plasma lipid values were about the same in patients with cerebral circulatory insufficiency without occlusion and in controls.[7]

Weight Insurance-company statistics indicate that there is a moderate increase in stroke mortality with increase in degree of overweight, the degree of excess mortality being somewhat greater in females than in males.[52,53] In the Los Angeles prospective study, there was a gradient of increasing risk of developing cerebral thrombosis with increasing weight, but no relationship between weight and the risk of developing cerebral hemorrhage was found.[7] A similar relationship between obesity in college students and increased risk of later developing cerebrovascular disease was reported in studies of students from Harvard and the University of Pennsylvania.[46] However, in the Framingham study, no relationship was found between the degree of obesity at time of entry into the study and the later development of thrombotic strokes. In this study, subjects who were 20% above the median weight for their height and sex were considered obese. The data presented for the first 12 years of observation did not take into account changes in weight that occurred during this period.[23]

Blood Pressure The relationship between blood-pressure level and stroke has been the subject of considerable speculation and a fair amount of data production. Insurance-company mortality studies[52] showed that the ratio of actual to expected mortality from vascular lesions of the central nervous system increased with the degree of elevation of blood pressure. In a study of death certificates collected in Chicago during a 1-month period in 1956, it was found, by querying physicians, that a history of hypertensive disease as either an underlying or an associated cause of death could be elicited for 69% of deceased persons whose certificates carried a diagnosis of cerebrovascular

disease.[55] The frequency of cerebral hemorrhage among Japanese has been related to the high prevalence rates of hypertension.[44,45,56]

Studies of the South African Bantu [63] show death rates from cerebrovascular disease that are about the same as those among white persons in South Africa, England, and the United States. This is in marked contrast with the very low death rates from coronary heart disease among the Bantu. It has been postulated that the relatively high rate of hypertension among the Bantu, compared with the Japanese, predisposes to a high death rate from cerebrovascular disease, and that it is likely that many or even most of these stroke fatalities represent cerebral hemorrhages secondary to hypertension and associated with nonatherosclerotic vascular lesions.[34]

The mortality data for the United States show that in the last decade there has been a reduction in the middle-age death rate from hypertensive disease, which has been accompanied by a smaller but significant decrease in mortality from strokes.[8,60] It is tempting to infer that the reduction in stroke mortality, shown to be due primarily to reduction in cerebral hemorrhage, has been the direct result of advances in antihypertensive therapy in recent years, and that conclusion has been drawn repeatedly in the literature.[2,36,51] Although there seems no doubt of the value of antihypertensive therapy in patients with malignant hypertension,[10,19] the value of such therapy in treating other, less severe hypertension has not been firmly established.[40] A 20-year follow-up study suggested that the treatment of hypertension does increase survival.[5] That implies that antihypertensive therapy has reduced death rates from both hypertension and its complications, including stroke. However, several reported studies comparing the development of strokes in relatively small numbers of treated and untreated hypertensive persons have produced equivocal results. In three studies, there was some evidence of a reduction in the number of strokes that occurred in the treated groups.[17,35,38] In another study with somewhat larger numbers of patients, a follow-up of almost 2 years yielded two strokes in the treated group and only one in the untreated.[64] It seems clear that further studies are needed to elucidate the relationships between hypertension and the various forms of cerebrovascular disease. The mortality data from Memphis and Baltimore indicate that cerebral hemorrhage death rates were falling even before the widespread use of the antihypertensive drugs.[33] And a recent report showed that, in both animals and humans, the mechanisms of autoregulation of cerebral blood flow, which keep cerebral perfusion relatively constant when the cerebrovascular system is intact, lose their effectiveness when there is stenosis or occlusion of vessels; the cerebral blood flow then becomes more sensitive to changes in levels of blood pressure. That has been said to indicate that induced

hypertension may have clinical usefulness.[27] In addition, it has recently been suggested that reduction of blood pressure in persons with moderate hypertension and cerebrovascular symptoms may result in a worsening of transient cerebrovascular symptoms.[40] The evidence seems to be mounting that hypertension is closely related to cerebral thrombosis, as well as to cerebral hemorrhage, although the mechanism of the two relationships may be very different.

The most convincing body of evidence has come from the prospective epidemiologic studies, which have uniformly been able to demonstrate a relationship between hypertension and the risk of later developing a stroke. The Framingham study used categories of "normotensive" (less than 140 mm Hg systolic and less than 90 mm Hg diastolic) and "hypertensive" (160 mm Hg or greater systolic and/or 95 mm Hg or greater diastolic). Hypertension increased the probability of developing a thrombotic brain infarction to a probability more than five times greater than that among normotensive persons.[23] The Los Angeles study, using the same categories of hypertension, demonstrated a fourfold increase in the risk of developing cerebral thrombosis for the hypertensive person, and over a tenfold increase in the risk of cerebral hemorrhage, compared with normotensive or borderline persons. The data showed a relationship between systolic pressure and cerebral hemorrhage, with a gradient of increasing risk with rising systolic pressure. No such relationship was seen for systolic pressure and cerebral thrombosis. A gradient was, however, observed for both cerebral hemorrhage and cerebral thrombosis with increasing diastolic pressure.[7] The Hiroshima data showed the same marked increase in risk of developing cerebrovascular disease in both males and females who were hypertensive when they entered the study. There was a definite gradient with both rising systolic and diastolic pressures, and the risk of later cerebrovascular disease was 10 times higher in the extreme hypertensive group (180+ mm Hg systolic and 110+ mm Hg diastolic) than in the normotensive group (less than 140 mm Hg systolic and less than 90 mm Hg diastolic). Unfortunately, these data were not presented separately for cerebral hemorrhage and cerebral thrombosis.[20]

In another type of study carried out retrospectively in Seal Beach, California, elderly persons with stroke (evident from either history or physical examination) were found to have higher mean systolic blood pressures than their nonstroke controls. This did not hold true for diastolic pressure.[8]

Stress The relationship of stroke to "emotional stress" has received some attention in the literature. A recent controlled study found suggestive differences in residence, occupational class, and nativity between

stroke victims and controls. An excess of foreign-born females was found that, it was suggested, may have some meaning in terms of separation from close relatives and adaptation to a new culture.[13] Along these same lines, a 1958 study reported a higher stroke mortality among new immigrants to Israel.[22] Other indications of differences in life situations between stroke victims and controls include greater marital stability among the stroke victims and greater job stability among the male stroke victims. There was also an excess of male stroke patients with histories indicating a long period of employment in the industrial categories of "transportation" and "heavy industry."[13] There was no attempt in these studies to distinguish between types of cerebrovascular disease.

ASSOCIATION WITH OTHER DISEASES

In addition to hypertension, previously discussed, there are a number of diseases whose relationships to stroke have been investigated or suggested in the literature. These include diabetes, rheumatic heart disease, and other forms of cardiovascular disease.

Diabetes Retrospective data have shown that diabetics have a higher mortality rate from stroke than do nondiabetics.[21,30] The only prospective data on the relationship between diabetes and subsequent stroke come from the Hiroshima study, in which the diabetic women had increased risk of developing stroke, especially during the fifth and sixth decades of life.[20]

Rheumatic Heart Disease The relationship between rheumatic heart disease and cerebral embolism is well documented, and the control of rheumatic heart disease (with early therapy of streptococcal infections, with the use of penicillin prophylaxis, and with the introduction of anticoagulants for prevention of embolic phenomena in patients with valvular heart disease) has been and will continue to be effective in reducing morbidity and mortality from this form of stroke.[3]

Other Cardiovascular Disease One of the most significant findings of the Framingham study was that thrombotic strokes "unheralded" by illness were uncommon. In three of every four subjects who developed a thrombotic stroke there were antecedent findings of a major cardiovascular disease—hypertensive cardiovascular disease, coronary disease, congestive heart failure, or intermittent claudication—for which most were receiving some form of treatment at the time of the stroke. About one third of those who developed a thrombotic brain infarction had

what might be called "prodromal symptoms," which might have led to some attempt to prevent a full-blown stroke, if suitable measures had been available. A striking relationship was found between cardiac impairment (as evidenced by enlargement and electrocardiographic abnormalities) and stroke. It was suggested that impairment of myocardial function contributes to the precipitation of stroke in a person who may be predisposed to such an event by a pre-existing hypertension and high serum cholesterol level. There was a marked increase in the incidence of cerebral thrombosis among those with pre-existing abnormal electrocardiograms. When findings of left ventricular hypertrophy, nonspecific ST-T alterations, or intraventricular block were present, the incidence of thrombotic infarction was six times as high as in persons without such abnormalities. When the risk of developing a thrombotic stroke was analyzed in relation to ECG, serum cholesterol, and blood pressure, those with two or three abnormalities were found to have eight times the risk of those with no abnormalities.[23]

The findings reported recently from Hiroshima largely corroborate the data from Framingham. When ECG evidence of left ventricular hypertrophy (high QRS voltage with ST-T changes) was present at the initial examination, the risk of later developing stroke was almost three times the average risk in men and over five times the average risk in women. This same excess risk was found if there was cardiomegaly, identified by x-ray.[20] Because the analysis of the Hiroshima data did not treat the cerebral thrombosis cases separately, it is not possible to contrast the level of risk with that of Framingham. It was pointed out in the text that patients who developed cerebral hemorrhage had higher blood pressures, a greater frequency of ECG evidence of left ventricular hypertrophy, and a greater frequency of cardiomegaly, as shown by chest x-ray, than did patients with cerebral thrombosis.[20] The Seal Beach, California, study of an elderly population also furnishes evidence of a relationship between cardiac impairment and stroke. Significantly higher proportions of male and female stroke patients had abnormal ECG's and cardiomegaly, shown by x-ray, than of their nonstroke controls.[8]

The relationship between the cerebrovascular diseases and ischemic heart disease is one of the most intriguing problems confronting the stroke investigator today. A number of different approaches have been used to attempt to show either the presence or the absence of an association between these conditions, and much time could be spent reviewing the statistics on this subject. But this relationship will probably remain in question until there are adequate studies that effectively categorize the cerebrovascular diseases.

The Framingham study gives strong indication that the same risk

factors that seem to predispose to ischemic heart disease predispose to thrombotic brain infarction.[23] This is the one study that has set up rather clear definitions for the differentiation of cerebral thrombosis from the other forms of cerebrovascular disease, and has done it on the basis of clinical examination. This gives added weight to the conclusion that there is a relationship between at least a segment of the stroke cases and atherosclerotic coronary heart disease.

The Hiroshima group investigated several variables that have not been previously reported. They found proteinuria to be related to an increased risk of later development of stroke, especially in women. They also reported that women with hemoglobin values exceeding 15 g% had an increased risk of developing stroke. Although this was based on relatively small groups, it does furnish an interesting lead concerning the relationship between the component elements of the blood and the mechanisms that lead to formation of thrombi.[20] A recent report on the Framingham study has also identified the blood hemoglobin content as one of the factors that predisposes to the development of thrombotic strokes. The Framingham group found a twofold increase in risk of thrombotic stroke in subjects whose hemoglobin levels at the time of initial examination had been moderately elevated (15 g% or higher in males, 14 g% or higher in females). The presence of both hypertension and elevated hemoglobin level increased the risk of later development of thrombotic strokes sevenfold. They also found a positive relationship between elevated hemoglobin levels and the prevalence of hypertension.[24]

FAMILIAL AGGREGATION

Inferences have long been drawn from both clinical observations and life-insurance risk studies that there is a familial aggregation of cases of stroke.[52,53] In a recent study of 126 stroke patients (80% of whom were diagnosed as having cerebral thrombosis) and a similar number of controls, data were obtained on 2265 relatives, over 1000 of whom were deceased. This study showed a statistically significant excess of deaths from cerebrovascular disease among parents and siblings of the stroke patients.[13]

SOCIAL CLASS

United Kingdom mortality statistics for 1950 were analyzed by social class for both ischemic heart disease and strokes in males aged 65 and over.[6] Ischemic heart disease was found to be almost twice as common among professionals and executives as among laborers, but no such trend was obvious with respect to strokes. In younger men, there was a

slight downward gradient for strokes from upper to lower social classes, but it was not nearly so marked as that for ischemic heart disease. The Los Angeles prospective study showed a definite gradient for cerebral thrombosis and total cerebrovascular disease, with higher rates in the higher SES groups, based on job classification. No gradient was present for cerebral hemorrhage.[7]

A case-control study in Baltimore showed an excess of stroke patients residing in the upper socioeconomic half of Baltimore, as indicated by census tract of last residence. The stroke patients were also characterized by fewer changes of occupation, less social mobility from first to last job, and longer duration of last job, compared with a control group.[13] However, a more recent study in Baltimore has reported higher death rates from stroke in the lowest social classes.[32]

CIGARETTE SMOKING

Several studies [9,18] have shown a moderate increase in mortality rates from the cerebrovascular diseases in cigarette smokers, compared with nonsmokers. This relationship was represented by an observed:expected mortality ratio of 1.3, which was less impressive than the association demonstrated with most of the other cardiovascular-renal diseases. This was analyzed only for the combined ISC category of vascular lesions affecting the central nervous system. The relationship with cerebral thrombosis has been investigated in the Framingham study,[23] and there is an apparent trend in men, with the incidence increasing with the number of cigarettes smoked daily. The number of cases reported thus far is small, and no statistically significant association could be established. However, it is quite likely that the accumulation of larger numbers of cases may prove this trend to be significant. The recently reported follow-up study of college students also showed an increasing risk of later stroke among smokers.[46]

EXPOSURE TO ENVIRONMENTAL HAZARDS

There have been several suggestions in the literature regarding possible associations between exposure factors in the environment and increased risk of developing stroke. A relationship between carotid and vertebral stenosis and overexposure to carbon monoxide fumes has been suggested.[67] This hypothesis has received some support from the Baltimore case-control study, which revealed an excess of stroke cases in males whose work was associated with the operation of motor vehicles.[13] It has also been suggested that there is an inverse relationship between the

death rates from stroke and the hardness of water servicing particular geographic areas.[37] Finally, a relationship has been postulated between an excessive salt intake and the high rates of cerebrovascular disease in the northeast districts of Japan.[56]

CONCLUDING REMARKS

The true importance of the thrombotic process in the cerebrovascular diseases has been somewhat obscured in recent years because of inherent artifacts in the kinds of data most generally available for epidemiologic study. The necessity of developing hypotheses based on analysis of mortality data has led to an overemphasis of the differences between the epidemiologic patterns observed in arteriosclerotic heart disease and those seen in the cerebrovascular diseases. The marked difference in case fatality rates between the hemorrhagic and the thrombotic forms of stroke have also tended to overemphasize the importance of cerebral hemorrhage and underestimate the importance of cerebral thrombosis. Patterns of reporting disease and the prevailing differences in customs of completing death certificates and generating national mortality statistics have tended to overemphasize the international differences in stroke mortality, with the implication that major differences in the natural history of cerebrovascular disease can best be elucidated by the development of international studies.

A review of the current state of knowledge of the epidemiology of cerebral thrombosis calls for a re-emphasis on the areas of similarity between cerebral and coronary thrombosis, as well as the recognition that the Negro–white differences are probably of more immediate importance than the Japanese–white differences, in terms of contributing definitive knowledge to the epidemiology of cerebral thrombosis. The data developed as a by-product of the prospective studies of ischemic heart disease demonstrate that many of the "risk factors" associated with increasing risk of developing coronary thrombosis and myocardial infarction are also associated with increasing risk of developing cerebral thrombosis; these include elevated serum cholesterol level, elevated blood pressure, evidence of cardiac impairment by x-ray or ECG, and pre-existing diabetes. There is the suggestion that obesity and cigarette smoking will also prove to be "high-risk factors" with respect to later development of cerebral thrombosis. Moderately elevated blood hemoglobin levels have also been shown to be associated with an increased risk of developing cerebral thrombosis.

It would appear that the climate is favorable for an attack on the

problem of thrombosis, which could use for investigation segments of the population that seem to have an increased risk of eventually encountering a major thrombotic episode. These would include especially Negroes, hypertensive persons, diabetic persons, and hypercholesterolemic persons. The need is great for the development of standardized, reliable, repeatable, and simple procedures for measuring blood coagulability and fibrinolytic activity that can be used in epidemiologic investigations—to be added to the now usual battery of blood studies included in such studies of cardiovascular and cerebrovascular disease.

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Information on Thrombosis as a Cause of Death, from Studies Promoted by the World Health Organization

AUBREY KAGAN

INTRODUCTION

Thrombus, or consequent embolism, causes death as a complication of other disease processes, and data from studies connected with the World Health Organization (WHO) will be examined here in an attempt to establish how often that happens. Thrombosis might also cause death as part of the pathogenesis of disease, such as atherosclerosis, but the available data do not permit an evaluation of that role; although it is an important problem, it will be discussed only briefly. There will be no attempt to discuss the relationship of thromboembolic phenomena (TEP) and morbidity.

Mortality statistics are of limited value [5,7]; I have therefore referred to other studies:

1. Most of the data I will use are derived from a population-related autopsy study, whose objective was to compare extent and type of atherosclerosis in the aorta and coronary arteries in several European communities, by age, sex, cause of death, and a number of pathologic and physiologic variables. The principal investigators in this study were N. H. Sternby and E. Lundberg of Malmö, Sweden; A. M. Vihert, V. Zdanov, and E. Matova of Moscow, USSR; R. Vaněček and Z. Zahor of Prague, CSSR; A. Livsic of Yalta, Ukrainian SSR; and K. Uemura and I of WHO. The purpose and protocols are set out elsewhere.[17] They did not include the study of thrombosis other than in the coronary arteries. Nevertheless, some useful information can be obtained on the frequency of some TEP and of various forms of death in which thrombus or embolism is thought to play a part. For this paper, there has been a special analysis of part of the data: namely, the pooled data from deaths

of men and women aged 40–59 years during the period January 1963 through December 1966 from three of the communities, *viz.*, Malmö, Sweden; Prague Area II, CSSR; and Yalta, Ukrainian SSR. The living populations among which the deaths occurred numbered roughly 130,000. Some 84% of all deaths were studied, and information was available on deaths that were not subjected to special autopsy. The high rates of information retrieval, together with reliability tests, make possible realistic estimates of frequency of some pathologic processes at death in these communities.

2. Katsuki and his colleagues [9] studied cerebrovascular disease in Hisayama, Japan. They examined 90.1% of all stroke patients, both male and female, aged 40 or above, in 1960–1961 and observed them through the sickness. From 1962 onwards, 90–100% of the deaths among that population were autopsied. Because of the high rate of data retrieval, an idea of frequency of events in the community can be obtained. Data from this study and the other population-related autopsy study have been used to check the reliability of pathologic and clinical diagnosis and of mortality statistics.

Data from several WHO-supported studies have been quoted to challenge or support some of the observations on coronary thrombosis arising from the "population-related" autopsy material:

1. Carlos Marigo has examined autopsy material from Brazilians of European and non-European origin who died in the hospital. He examined coronary arteries and hearts macroscopically and, when large "myocardial lesions" were found, made a detailed histologic search in serial sections. His preliminary results, which show "what can happen," rather than "how often it happens," are quoted.

2. D. E. Christian has studied platelet adhesiveness in a sample of rural Jamaicans and compared those who had symptoms or signs of myocardial ischemia with those who did not, and with postmyocardial-infarction patients and controls in England.

3. A. G. Shaper and his colleagues have studied platelet adhesiveness and fibrinolysis in Africans, Asians, and Europeans. Their findings and opinions are quoted.

RELIABILITY OF PATHOLOGIC AND CLINICAL DIAGNOSIS AND MORTALITY STATISTICS

When relevant factors are assessed erroneously, clues may be missed and false ideas given. The population-related autopsy studies afforded

opportunities to assess the autopsy and clinical diagnosis of some TEP and diseases thought to be related.[17] In those studies, a high proportion (80% or better) of all deaths of persons aged 10 or above in several demographically defined communities were studied according to a protocol, and some information was obtained about the deaths not autopsied. I shall present relevant findings from deaths of men and women aged 40–59 from three of the communities (Malmö, Sweden; Prague Area II, CSSR; and Yalta, Ukrainian SSR) concerning the reliability of some autopsy and clinical data. In some instances, data from older groups will also be quoted. Information from a follow-up study of subjects aged 40 and above in Hisayama, Japan,[8] adds to the data on clinical diagnosis of fresh cerebrovascular accidents and, with data from the autopsy study in Malmö and Prague, shows some of the limitations of national mortality statistics.

PATHOLOGIC DIAGNOSIS

Autopsy data were checked for consistency by making repeat observations on the same specimens. Data on fresh cerebral hemorrhages and fresh cerebral infarcts were tested in Malmö and Prague II and found to be reliable; data on old hemorrhages and infarcts were not reliable. Tests were not made on the Yalta material.

Data on fresh and old myocardial infarcts were tested in Malmö and Prague II. Data on fresh infarcts and myocardial scars greater than 0.5 cm were found to be repeatable, but data on small lesions (including diffuse myocardial fibrosis) were not. Tests were not made on the Yalta material.

Data on coronary thrombosis were tested in all laboratories. The test was to compare the routinely recorded findings with those made by a panel on a sample of the coronary arteries when the investigators had been separated from all contact with the patient. Material from 10,000 subjects from all laboratories was treated in this way. Here I will report only the findings in subjects aged 40–59 years. The ratio of routine findings to special-inquiry findings varied with clinical and autopsy diagnosis; Table 1 indicates that, when a clinical or autopsy diagnosis of coronary heart disease (CHD) was made, the pathologist reported 80–90% of the cases of thrombosis found in the special inquiry. When a diagnosis of CHD was not made, the pathologist reported only half the cases of thrombosis found in the special inquiry. An "alertness" factor, the ratio of routine:special findings, has therefore been taken into account in later analyses.

No repeatability tests were made on observation of pulmonary embolism; peripheral (leg) venous thrombosis; mesenteric, carotid, or cerebral

TABLE 1 Routine Assessment of Thrombosis in Extramyocardial Coronary Arteries, According to Clinical or Autopsy Diagnosis Compared with Special Inquiry When the Clinical and Autopsy Diagnoses Were Not Known (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta, Ryazan, Tallin)

Diagnosis	Number of Deaths	Number in Which Thrombus Found		Alertness Factor, Routine/ Special
		Routine	Special	
Clinically, coronary heart disease	200	32	35	0.91
Clinically, not coronary heart disease	2300	18	34	0.53
Autopsy, "myocardial infarct"	478	44	56	0.79
Autopsy, not "myocardial infarct"	2022	6	13	0.46

artery thrombosis; or atrial or ventricular thrombosis. Thrombus in the arteries mentioned was seldom reported. The impression was that peripheral venous thrombosis was reported with varied frequency by different observers, depending on their interest, and this TEP has not been analyzed.

Pulmonary embolism was of interest to all the observers, and they dissected to the third branch of the pulmonary artery. It was noted in 137 cases altogether and as a principal cause of death in 16 of them. Table 2, however, shows that pulmonary embolism was reported more frequently in Prague II and less frequently in Yalta than in Malmö. The proportion of such cases regarded as the principal cause of death was lower in Prague than in Malmö. There were also differences in rates according to sex, between Malmö and Prague. Inquiry shows that much of the variation is due to reporting differences. I cannot yet say on the basis of the evidence whether these are community or observer differences. In analyses referred to later, I make no attempt to compare between communities, and in pooling data I adjust for age, sex, and source where it seems necessary.

CLINICAL DIAGNOSIS AND MORTALITY RATES

Clinical diagnosis was compared with autopsy diagnosis, and autopsy diagnosis with mortality rates for the whole community or nation.

Fresh Cerebrovascular Lesions Table 3 refers to 1478 deaths in men and women aged 40–59 years from Malmö and Prague II. Taking all

TABLE 2 Pulmonary Embolism and Pulmonary Embolism as Principal Cause of Death Diagnosed at Autopsy in Three Communities (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta; 1963–1966)

	Malmö	Prague II	Yalta	All Sources
Number deaths	1012	466	362	1840
Number cases pulmonary embolism	64	66	7	137
Number cases pulmonary embolism, principal cause of death	10	6	0	16
Pulmonary embolism, % of all deaths	6	14	2	7
Pulmonary embolism, principal cause of death, as % of all deaths	1	1.3	0	1
Pulmonary embolism, principal cause of death, as % of all pulmonary embolism cases	16	9	0	12

fresh "cerebrovascular accidents" as the principal cause of death, the sensitivity of the clinical diagnosis was 0.66. That means that in 66% of these cases the cause of death was diagnosed clinically (71 of 107). When autopsy showed fresh "cerebrovascular accident" as an associated factor, the sensitivity of the clinical diagnosis was very low (0.30, or 13 of 44 cases). Some of this may be due to underreporting of known events, but inquiry suggests that only a small proportion can be accounted for in this way.

TABLE 3 Comparison of Clinical with Autopsy Diagnosis for Several Conditions [a] (Deaths of 1478 Men and Women Aged 40–59 Years; Malmö, Prague II [b]; 1963–1966)

Condition	Sensitivity, Principal Cause of Death	Sensitivity, Not Principal Cause of Death	Specificity
Cancer	0.92	0.17	0.99
Cerebrovascular disease	0.66	0.30	0.99
Coronary heart disease	0.51	0.09	0.98
Thromboembolic phenomena	0.38	0.06	0.99

[a] Derived from Kagan *et al.*[7]

[b] Note that deaths from Yalta are not included.

TABLE 4 Wrong Clinical Diagnosis in 20 Cases in Which Autopsy Showed Fresh Cerebral Hemorrhage, and 4 Cases in Which Autopsy Showed Fresh Cerebral Infarct to Be the Principal Cause of Death * (Men and Women Aged 40–59 Years; Malmö, Prague II; 1963–1966)

Autopsy Diagnosis of Fresh Cerebral Hemorrhage		Autopsy Diagnosis of Fresh Cerebral Infarct	
Clinical Diagnosis	No. Cases	Clinical Diagnosis	No. Cases
Unknown	7	Pulmonary embolus	2
Hypertension	6	Coronary heart disease	1
Coronary heart disease	2	Hypertension	1
Senility	2		
Cancer	1		
Pyelitis	1		
Other	1		

* Derived from Kagan *et al.*[7]

Of the 36 cases in which autopsy showed the principal cause of death to be fresh "cerebrovascular accident" but clinical diagnosis was different, 12 cases involved extracerebral hemorrhage, 20 cases cerebral hemorrhage, and four cases cerebral infarct. The clinical diagnoses in the cerebral hemorrhage and infarct cases are shown in Table 4.

Seven of the 20 cases of cerebral hemorrhage were clinically of "unknown" cause. These were mainly cases in which the physician had not been called before death.*

Table 5 shows how national mortality rates of CSSR, Japan, and Sweden compare with rates from *ad hoc* studies of small communities.† There is reasonably good correspondence for vascular lesions of the central nervous system taken all together. For studies of etiology, it is necessary to differentiate between cerebral hemorrhage and cerebral infarct. Clinicians often avoid the issue by diagnosing "cerebrovascular accident" or "apoplexy." Table 6 compares the ratios of mortality rates from fresh cerebral hemorrhage and fresh cerebral infarction drawn from autopsy data in *ad hoc* studies with those drawn from national mortality data. In every case, the ratio from *ad hoc* studies is lower than the corresponding figure from national statistics. There is clearly

* Many of these come into the category of "sudden death," defined as death occurring unexpectedly within 6 hr of the onset of symptoms in an apparently healthy subject or in a sick person whose condition was either steady or improving.

† The reasons for assuming that the small communities are not very different from the nations with regard to cerebrovascular accidents can be found in Kagan *et al.*[7]

TABLE 5 Comparison of Mortality Rates for Vascular Lesions Affecting the Nervous System Based on National Statistics with Those Based on Special Inquiry in Small Areas of Czechoslovakia, Japan, and Sweden [a] (Men and Women)

Country or Area	Source	Year	Annual mortality rate, %		
			⩾40 years	40–79 years	40–59 years
CSSR	WHO Statistics Annual, Vol. 1, Geneva 1966	1963	2.7	1.9	0.3
Prague II	Population-related autopsy study	1963–1966	—	2.2	0.7
Japan	WHO Statistics Annual, Vol. 1, Geneva 1966	1963	5.9	5.0	1.5
Hisayama	Follow-up study	1961–1966	5.4	4.5	1.3
Sweden	WHO Statistics Annual, Vol. 1, Geneva 1966	1963	2.8	1.8	0.3
Malmö	Population-related autopsy study	1963–1966	—	1.4	0.4

[a] Derived from Kagan *et al.*[7]

considerable misclassification when the diagnosis is not based on autopsy.

Coronary Heart Disease Table 3 shows that the sensitivity of clinical diagnosis is low in Malmö and Prague II when CHD is the principal cause (0.51, or 145 of 282 cases) and extremely low when it is an associated factor (0.09, or four of 47 cases).

There were 324 CHD deaths in the men and women aged 40–59 years from Malmö, Prague II, and Yalta. Of these, 297 could be categorized without doubt into the following three groups: 26 cases of coronary occlusion without fresh or old infarction; 176 cases of fresh infarction with or without scars; and 95 cases of myocardial scar without fresh infarction.

Table 7 shows that the sensitivity of clinical diagnosis in the first group (occlusion without infarction) was only 0.31; 16 of the 18 missed cases were clinically of "unknown" cause. The clinical sensitivity in the second group (fresh infarction) was better: 0.63; 48 of the 66 missed cases were clinically of "unknown" cause. In the third group (myocardial scar without fresh infarction), the clinical sensitivity was only 0.44; 43 of the 53 missed cases were of "unknown" cause. The "unknowns" account for a large proportion—nearly 80%—of the clinically missed cases. These involved mainly persons who died before medical attendance; most died suddenly.

TABLE 6 Ratio of Brain Hemorrhage to Brain Infarction Mortality Rates: Comparison of Data (Men and Women) from National Statistics [a] and *Ad Hoc* Studies [b]

	National, 45–54 years	*Ad hoc*, 50–59 years	National, 55–64 years	*Ad hoc*, 60–69 years	National, 65–74 years	*Ad hoc*, 70–79 years	National, 75+ years
CSSR	6	1.5	5	1.0	4	0.7	1
Japan	12	4.0	7	2.5	4	0.5	3
Sweden	9	2.0	5	1.0	2	0.5	2

[a] Epidemiological Vital Statistics Report 18:253–259, 1965.
[b] Derived from Kagan *et al.*[7]

TABLE 7 Clinical Diagnosis in 297 Cases in Which Principal Cause of Death (Autopsy) Was Coronary Heart Disease, According to Type of Coronary Heart Disease (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta; 1963–1966)

Autopsy Diagnosis	Number of Cases	Number not Diagnosed Clinically	Wrong Clinical Diagnosis
Coronary occlusion, no infarction	26	18	16 unknown 1 "cerebrovascular disease" 1 "other myocardial degeneration"
Fresh myocardial infarction, single or multiple; with or without myocardial scar	176	66	48 unknown 3 diabetes mellitus 3 "other specified" 2 cerebrovascular 2 cor pulmonale 2 hypertension 2 "other disease of the heart" 1 other myocardial degeneration 1 peptic ulcer 1 pyelitis 1 senility
Myocardial scar, multiple or single; no fresh infarction	95	53	43 unknown 4 "other disease of the heart" 2 cerebrovascular 2 accident 1 hypertension 1 "all other specified"

In the whole series of 324 CHD deaths in men and women 40–59 years old from Malmö, Prague II, and Yalta, 195 (60%) were sudden. It is in sudden deaths that the cause, without autopsy, is much in doubt. In some areas, autopsy is carried out in such cases; but that is by no means universal. Often, it is assumed that all sudden deaths are due to coronary heart disease. In this series, there were 394 sudden deaths, excluding accidents. Fewer than 50% of these (195) were due to coronary heart disease. The causes of sudden death are listed in Table 8.

Thromboembolic Phenomena No special attempt was made to assess these factors clinically, but most of the deaths referred to in Table 3 occurred in the hospital or in other circumstances of relatively high standards of diagnosis. The clinical sensitivity was extremely low. Again, a proportion (probably small) of this is due to underreporting.

TABLE 8 Principal Cause of Death in 394 Nonaccidental Sudden Deaths (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

Rank	Principal Cause of Death	Number of Cases
1	Coronary heart disease	195
2	Intracerebral hemorrhage	33
3	Cancer	28
4	Unknown	26
5	"Other" disease of heart and aorta	20
5	Chronic bronchitis, bronchiectasis, etc.	20
7	Rheumatic heart disease	19
8	Subarachnoid hemorrhage	10
9	Pulmonary embolism	9
10	Alcohol	5
10	Hypertensive heart disease	5
10	Pulmonary tuberculosis	5
13	Cirrhosis of liver	4
14	Cor pulmonale	3
15	Peptic ulcer	2
15	Pneumonia	2
15	Cerebral infarction	2
18	Other arterial thrombosis	1
18	Cholecystitis	1
18	Nephritis	1
18	Diabetes mellitus	1
18	Other myocardial degeneration	1
18	"All other diseases"	1
	TOTAL	394

Venous Thrombosis Records were not searched especially for evidence of venous varicosity and thrombosis of the peripheral veins (legs) in the population-related autopsy studies. Perovsky [12] has pointed out the need for establishing precision, bias, and accuracy of methods of assessing thrombosis in the leg veins and has carried out some work showing the correlation between serial phlebography, venous pressure measurement, clinical examination, and infrared and color photography in 120 patients and normal persons. The data are not yet available.

SUMMARY

In the population-related autopsy material referred to, the macroscopic autopsy assessments of fresh cerebral hemorrhage, fresh cerebral infarction, fresh myocardial infarction ($\geqslant 0.5$ cm) and myocardial scar ($\geqslant 0.5$ cm) were found to be repeatable and without bias. No attempt was made to compare macroscopic and microscopic findings.

Autopsy assessment of thrombosis in the extramyocardial coronary arteries varied with clinical and autopsy diagnosis. Pathologist "alertness" was greatest when CHD was diagnosed clinically; less, but reasonably high, when CHD was diagnosed at autopsy but not clinically; and least when CHD was not diagnosed at all. In using heterogeneous data, it is necessary to make a correction for "alertness."

No tests of precision, bias, or accuracy of autopsy observations on other TEP were made. In their absence, it is not possible to know whether differences found between communities are real or due to the observer. In pooling such data, adjustments should be made for age, sex, and source.

Sensitivity of clinical assessment of fresh "cerebrovascular lesion," CHD, and TEP was low. In the case of CHD, but not in "cerebrovascular accident," much of the lack of sensitivity was caused by the fact that death occurred before medical attendance.

Although a high proportion of CHD deaths were sudden, less than half the sudden deaths were due to CHD. Diagnosis, without autopsy, in sudden deaths is unreliable.

The differentiation of fresh cerebral hemorrhage and fresh cerebral infarction, as principal cause of death, is unreliable without autopsy.

Many cases of clinically unreported fresh cerebral hemorrhage, fresh cerebral infarction, and CHD were found at autopsy as associated factors. It is safe to assume that, both as principal causes of death and as associated factors, these conditions and most TEP are underdiagnosed.

These observations are based on studies in which the standards of clinical and autopsy diagnosis were higher than average. They cannot

be extrapolated to other studies, but untested data should not be accepted.

THROMBOEMBOLIC PHENOMENA AS THE CAUSE OF DEATH

The role of TEP in the development of underlying disease processes, such as atherosclerosis, is unsettled, and the available data do not permit any conclusions to be drawn. TEP contributed to death as a complicating factor, and reference to the population-related autopsy material gives some idea of how often this happens. Any conclusions about this will, of course, underestimate the importance of TEP as a cause of death insofar as they play a role in the development of underlying pathology.

The autopsy material has therefore been examined to try to determine how often TEP constituted the principal cause of death in the presence of various types of underlying disease. The criterion used was that the TEP were present and caused death that, if they had been absent, would not have occurred for months or years. In most cases of embolism, a decision was easy. If, for example, a patient with carcinoma and multiple complications suffered a pulmonary embolism while lying in a hospital bed *in extremis*, the embolism could not be regarded as the principal cause of death. The patient was dying anyhow, and the embolism was incidental. However, a death caused by pulmonary embolism in a patient who had operative carcinoma or in whom treatment was leading to remission could reasonably be assigned to the embolism because, but for the embolism, the patient would have recovered from the immediate threat of death for months or years.

It was possible to decide when fresh cerebral infarction was the principal cause of death but not the frequency with which it was associated with TEP in the carotid, vertebral, and cerebral arteries. Here, discussion turns on whether death is always, never, or sometimes due to TEP.

It was possible to decide when CHD was the principal cause of death, and also when extramyocardial coronary thrombosis was present. In those cases, it is not possible to say whether the thrombus was incidental to, or the cause of, death. In cerebral infarction and CHD deaths, we can say that the maximum number of times that TEP could have been the cause of death was the number of times that fresh cerebral infarction was the principal cause of death or the number of times that CHD with coronary thrombosis was the principal cause of death. Table 9 summarizes the findings.

TABLE 9 Thromboembolic Phenomena, Complicating Disease Processes, as the Principal Cause of Death (Deaths of 1840 Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

Thromboembolic Phenomenon	Number of Cases Diagnosed	Number of Cases Estimated	Number of Cases as Principal Cause of Death [a]	
			Minimum	Maximum
Pulmonary embolism	137	>137	16 (0.9)	28 (1.5)
Atrial thrombosis	20	—	6 (0.3)	13 (0.7)
Mesenteric artery thrombosis	2	2	2 (0.1)	2 (0.1)
Cerebral, carotid, or vertebral thrombosis	—	—	0	23 (1.3)
Extramyocardial coronary artery thrombosis	170	198	2 (0.1)	179 (9.7)

[a] Numbers in parentheses are percentages of all deaths.

VENOUS THROMBOSIS AND PULMONARY EMBOLISM

In the population-related autopsy study in the three communities, peripheral venous thrombosis was probably grossly underreported, but the pulmonary artery was dissected to its third branch. Pulmonary embolism was reported in 137 (7%) of 1840 men and women aged 40–59 years, at death. This is an underestimate, in that cases not contributing to death were often not reported. In 16 of the reported cases, the pulmonary embolism was regarded as the principal cause of death, i.e., about 1% of all deaths. There were a further 12 cases in which there was some doubt as to whether the embolism was the principal cause of death. A reasonable estimate of deaths that could have been prevented if pulmonary embolism had been prevented, in this material, is 1–2%. In half of these cases, even if the embolism had been prevented, the patient would still have been very ill.

It is likely, inasmuch as many minor cases of pulmonary embolism were not reported, that in more than 88% of cases pulmonary embolism, when it occurred, was not the principal cause of death.

ATRIAL THROMBUS

Atrial thrombus was found in 20 cases. In 12, it was found in association with the 64 cases in which rheumatic heart disease was the principal cause of death, and in each of these it led to embolism. In five of the 12, it followed operation on a valve; in all five, it can be regarded as the principal cause of death. In the other seven of the 12, the issue is not clear because congestive failure occurred before embolization. Even if embolization had not occurred, the patients would probably have died, and if they had recovered they would have remained very ill. In one case, a thrombus from the aortic valve (in a case of aortic stenosis, probably nonrheumatic) embolized into the coronary artery. Thus, embolization from atrial thrombus accounted for six to 13 of 1840 deaths (0.3–0.7%). Atrial thrombus without embolization may have been underreported in this material. In 13 of the 20 that were reported (63%), embolism contributed significantly to death.

MESENTERIC ARTERY THROMBOSIS

Two of the deaths studied were caused by mesenteric artery thrombosis.

CEREBRAL, CAROTID, OR VERTEBRAL ARTERY THROMBOSIS

There was no consistent attempt to report on these vessels in the population-related autopsy study, and it is impossible to say how often throm-

bosis or embolism occurred or how often it was the principal cause of death. There was no evidence that cerebral hemorrhage as principal cause of death was due to embolism from, or thrombosis in, these vessels. On the contrary, there was evidence that thrombus formation was uncommon in subjects who died from cerebral hemorrhage. Fresh cerebral infarction was found in 57 cases. In 23 of them, it was the principal cause of death. Some, all, or none of these may have been due to thrombosis in, or embolism from, cerebral, carotid, or vertebral arteries. Our estimate for death due to thrombosis in, or embolism from, cerebral, carotid, or vertebral arteries is, therefore, zero to 23 cases, or at most 1.3%.

CORONARY THROMBOSIS

Thrombi were found in the extramyocardial coronary arteries in 170 cases. In 153 of them, CHD was regarded as the principal cause of death. Table 1 shows that coronary thrombosis was probably underdiagnosed, if autopsy showed that CHD was the principal cause of death, by 9–21%. It is probable, therefore, that about 26 cases of thrombosis were missed in deaths due to CHD, making an estimated total of 179 cases of coronary thrombosis that might have caused death. There were two cases in which death was assigned to CHD and in which coronary thrombosis was found, but no infarction. In each of these cases, the interval between symptoms and death was known and was sufficiently long for infarction, if present, to be manifest at autopsy. In those two cases, death must be assigned to coronary thrombosis, and that is the minimum number of deaths due to such cause. The maximum is the estimated number of CHD deaths in which thrombosis occurred, 179. How often thrombosis is the cause of death or incidental to CHD is not clear. But it is such an important question that the next section is devoted to it.

SUMMARY

Conditions in which the issue is fairly clear—*viz.*, pulmonary embolism, atrial thrombosis, or mesenteric artery thrombosis—account for a relatively small proportion (1.3–2.3%) of all deaths. But when the role of thrombus is not clear, as in cerebral infarction and CHD, TEP might cause as much as 11% of all deaths. Pulmonary embolism occurs frequently without causing death. In the reported data, 88% of the cases were nonlethal. That is an underestimate, in that many cases of nonlethal pulmonary embolism were not reported and many more may have occurred in subjects who recovered. Atrial thrombosis and, more so, mesenteric artery thrombosis were infrequent findings at death but, when found,

were commonly thought to have caused death. A true assessment of the danger of these conditions requires data that are not available in this material, i.e., a knowledge of how many subjects have these conditions without dying.

In the cases of cerebral infarction and CHD, the argument turns on how often the thrombus is primary or secondary to the parenchymatous lesion. The frequent occurrence of the latter condition, even in this relatively young population, makes it highly desirable to answer this question.

No attempt has been made to determine the contribution of TEP to death through their role in the development of underlying disease processes. This may be the most important question of all.

CORONARY THROMBOSIS AND CORONARY HEART DISEASE

The population-related autopsy study gives us some idea of the frequency with which different kinds of CHD are found at death in three European communities, how often thrombosis occurs in the extramyocardial main coronary arteries, how this is associated with death from CHD, how subjects with and without such thrombosis compare at death, and the relationship of coronary thrombosis to atherosclerosis of the coronary circulation.

FREQUENCY OF CORONARY THROMBOSIS ACCORDING TO TYPE OF CHD

Table 10 shows the frequency of extramyocardial coronary artery thrombosis in 446 cases in which CHD was found at death and could be classified as to whether it was the principal cause of death or an incidental finding and whether it consisted of coronary occlusion without myocardial infarction or scar, fresh infarction (with or without myocardial scar), or myocardial scar without fresh infarction. The frequency of thrombosis was several times higher when CHD was the principal cause than when it was an incidental finding, and that was irrespective of the kind of CHD.

One explanation might be that the way a person dies from CHD is different from the way a person dies from another disease with incidental CHD, and that the difference is related to thrombus formation or disappearance. One aspect of this—sudden versus nonsudden death—can be examined in the WHO data.

Table 11 shows the prevalence of thrombosis in sudden and nonsudden deaths due to CHD and deaths in which CHD was incidental. Again,

TABLE 10 Prevalence of Thrombosis in Extramyocardial Coronary Arteries in Subjects Who Died of Coronary Heart Disease and Subjects in Whom Coronary Heart Disease Was an Incidental Finding at Death (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

Type of CHD	Number of Cases [a]	Number with Thrombosis	% with Thrombosis
All deaths in which CHD was Principal (P) cause	297	143	48
All deaths in which CHD was Incidental (I)	149	16	11
(a) Coronary occlusion alone:			
P	26	17	65
I	17	3	18
(b) Fresh infarct all:			
P	176	87	49
I	28	6	21
(c) Old scar alone:			
P	95	39	41
I	104	7	7

[a] An additional 27 cases could not be categorized in this way.

thrombosis was more common in the deaths due to CHD, whether sudden or not, than in the deaths in which CHD was incidental.

The conclusion is that the association between thrombosis and death from CHD is strong. However, causation is not proved; it is clear that, in half the cases, CHD death occurs in the absence of thrombosis of the type that has been assessed.

Another way of looking at this question is to consider the cases in which coronary thrombosis is found in the absence of myocardial infarction, and vice versa.

TABLE 11 Prevalence of Thrombosis in Extramyocardial Coronary Arteries in Deaths Due to CHD and Deaths in Which CHD Was Incidental, According to Suddenness of Death (Men and Women Aged 50–59 Years; Malmö, Prague II, Yalta)

Type of Death	Number of Deaths	Number with Thrombosis	% with Thrombosis
Sudden, CHD principal	178	83	47
Sudden, CHD incidental	35	4	11
Not sudden, CHD principal	119	60	50
Not sudden, CHD incidental	114	12	11

CORONARY THROMBOSIS AND MYOCARDIAL INFARCTION

It is difficult to obtain information on this question in the population-related autopsy study, because myocardial infarction that occurred within 12 hr of death might have been missed, not yet being macroscopically apparent. In this study, histologic examination was not always made when infarction was not seen, and enzyme tests and nitrotoluidine blue tests were not made. The presence of fresh infarction cannot be excluded in the 14 sudden deaths in which no infarction was found but in which coronary thrombosis was present. Nor can it be excluded in the 34 sudden deaths in which myocardial scar, but no fresh infarction, was found and in which coronary thrombosis was present. These deaths could have been due to thrombosis without fresh infarction, but all that can be said is that infarction was not seen. In Table 12, these 48 cases are placed in column 1, because the presence of infarction is in doubt.

There were 18 nonsudden deaths in which thrombosis was found but no fresh infarction. In these cases, the records have been examined to see whether the onset of acute symptoms occurred sufficiently long before death to give the myocardial infarction time to make itself macroscopically apparent at autopsy.

In four cases, symptoms began 22 hr, 24 hr, 32 hr, and 3 days before death. Two of these were CHD deaths, one was due to cancer of the breast with complications, one was due to postoperative shock. These four are in column 3 of Table 12, because myocardial infarction was absent. In six cases, the interval between onset of symptoms and death was unknown (column 2), and in the other eight cases, symptoms began less than 12 hr before death (column 1).

In the 176 CHD deaths with fresh infarction, thrombosis was not found in 89, of which 47 were sudden.

Table 12 shows that, in the 446 deaths in which CHD was found, thrombosis without fresh infarction was definitely present in four (column 3) —less than 1%—and might have been present in 62 others (columns 1 and 2)—14% of all CHD cases. Fresh infarction without thrombosis in the main extramyocardial coronary arteries occurred in 111 deaths (column 4)—25%.

COMPARISON OF CHD DEATHS IN WHICH FRESH INFARCTION WAS PRESENT, WHEN CORONARY THROMBOSIS AND OCCLUSION WERE PRESENT OR ABSENT

Of the 176 CHD deaths with fresh infarction, coronary thrombosis was found in 87; of the remaining 89, nonthrombotic coronary occlusion was

TABLE 12 Fresh Infarction and Extramyocardial Coronary Artery Thrombosis in CHD (Men and Women Aged 40–59 Years; Malmö, Prage II, Yalta)

Type of Death	Thrombosis but No Fresh Infarction Found			Fresh Infarction but No Thrombosis Found (4)	Fresh Infarction and Thrombosis Found (5)	No Fresh Infarction or Thrombosis Found (6)	Total (7)
	Time from Symptoms to Death <12 hr, ? No Fresh Infarction (1)	Time from Symptoms to Death Unknown, ? No Fresh Infarction (2)	Time from Symptoms to Death >22 hr (3)				
CHD principal	46[a] + 4[b]	4	2	89	87	65	297
CHD incidental	2[b] + 4[b]	2	2	22	6	111	149
TOTAL	56	6	4	111	93	176	446

[a] Symptoms ≤6 hr before death.
[b] Symptoms >6 <12 hr before death.

found in 43, and neither occlusion nor thrombosis was found in 46. Table 13 shows that these three groups were similar in age, sex, weight, height, thickness of subcutaneous fat, and heart weight. The group with neither thrombosis nor occlusion consisted of subjects who died suddenly more often (63%) than those with thrombosis or occlusion (42%) and had medical care before death less often (41% compared with about 75%). These differences are statistically significant ($p<0.01$).

ATHEROSCLEROSIS AND MYOCARDIAL INFARCTION ACCORDING TO PRESENCE OR ABSENCE OF CORONARY THROMBOSIS

In Table 14, atherosclerosis of the left anterior descending coronary artery is compared for type, extent, and stenosis in CHD deaths with fresh infarction and coronary thrombosis, fresh infarction with nonthrombotic coronary occlusion, and fresh infarction with neither coronary thrombosis nor occlusion. A similar analysis is made for CHD deaths with either fresh or old myocardial infarction.

Atherosclerosis is assessed as generalized fibrous plaque, complicated, and calcified and in terms of the percentage of the surface of the vessel involved. "Stenosis" means one or more areas of narrowing of the lumen by 50% or more.

Generally speaking, the subjects with thrombosis and nonthrombotic occlusion were similar and had, on average, more "total amount," more fibrous plaque, more complicated lesion, and more often stenosis than subjects with neither thrombosis nor occlusion. The differences are significant in the recent infarction group for total amount and complicated lesions, and in the recent or old infarction group for total amount, complicated lesions, and stenosis ($p<0.05$).

CORONARY THROMBOSIS AND EXTENT OF ATHEROSCLEROSIS

Extent of atherosclerosis in the aorta and coronary arteries is influenced considerably by age, sex, and cause of death and to a lesser degree (in this material) by source. Some of these factors may also influence the frequency of observation of coronary thrombosis. To assess the association between extent of atherosclerosis and coronary thrombosis, it is necessary to make some sort of allowance for these factors. The procedure can be illustrated by reference to Table 15, which is concerned with the total amount of atherosclerosis in the left anterior descending coronary artery.

All cases in which CHD was found at autopsy are divided and ranked by deciles of extent of atherosclerosis (column 1). The number of cases

TABLE 13 Comparison of Factors in Subjects with Fresh Infarction with and without Thrombosis and Nonthrombotic Occlusion [a] (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

				No Thrombosis					
	Thrombosis			Nonthrombotic Occlusion			No Occlusion		
Factor	No. Cases	Mean	S.E.	No. Cases	Mean	S.E.	No. Cases	Mean	S.E.
Age, years	87	54.1	0.5	43	53.8	0.7	46	53.5	0.7
Males, %	87	73.6	4.7	43	86.1	5.3	46	76.1	6.3
Medical care before death, %	87	73.6	4.7	43	76.7	6.4	46	41.3 [c]	7.3
Death sudden, %	87	42.5	5.3	43	41.9	7.5	46	63.0 [c]	7.1
Weight, kg	85	73.6	1.4	42	70.7	2.0	45	74.4	2.1
Height, cm	85	168.1	1.0	42	169.8	1.3	45	167.2	1.3
Subcutaneous fat, mm	76	25.5	1.4	35	21.5 [b]	1.8	22	22.9	2.1
Heart weight, g	87	446.2	13.1	43	458.6	16.7	45	447.4	17.5

[a] Derived from Kagan *et al.*[8]

[b] Significantly different from thrombosis group ($p < 0.01$).

[c] Significantly different from thrombosis and occlusion group ($p < 0.01$).

TABLE 14 Comparison of Coronary Atherosclerosis (Left Anterior Descending Coronary Artery) and Stenosis in Subjects with Infarction with and without Thrombosis and Nonthrombotic Occlusion [a] (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

				No Thrombosis					
	Thrombosis			Nonthrombotic Occlusion			No Occlusion		
Atherosclerosis Factor	No. Cases	Mean	S.E.	No. Cases	Mean	S.E.	No. Cases	Mean	S.E.
RECENT INFARCTION									
Total amount	84	62.6	2.4	42	62.3	3.6	45	53.4[d]	3.7
Fibrous plaque	84	50.2	2.5	42	51.3	3.3	45	45.5	3.8
Complicated	84	3.9	0.7	42	2.4	0.8	45	1.5[b]	0.9
Calcified	84	6.9	1.2	42	6.7	1.8	45	4.5	1.5
Stenosis	87	67.8	5.0	43	67.4	7.2	46	60.9	7.2
RECENT OR OLD INFARCTION									
Total amount	123	62.4	2.0	60	63.6	2.9	81	56.7[c]	2.9
Fibrous plaque	123	50.9	2.0	60	49.6	2.8	81	47.7	2.8
Complicated	123	3.4	0.5	60	3.1	1.1	81	1.3[b]	0.5
Calcified	123	6.4	0.9	60	9.0	2.0	81	6.2	1.4
Stenosis	126	69.1	4.1	61	67.2	6.0	84	56.0[b]	5.4

[a] Derived from Kagan *et al.*[5]

[b] Different from thrombosis group ($p < 0.05$).

[c] Different from nonthrombotic occlusion group ($p < 0.05$).

[d] Different from both other groups ($p < 0.05$).

TABLE 15 Extent of Atherosclerosis (Left Anterior Descending Coronary Artery) and Frequency of Thrombosis of Extramyocardial Coronary Arteries (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

Decile of Atherosclerosis (1)	No. of Cases with CHD (2)	No. of Cases of Coronary Thrombosis Observed (O) (3)	No. of Cases of Coronary Thrombosis Expected (E) [a] (4)	O/E (5)
⩾1%, <10.8%	24	2	8	0.24 [b]
⩾10.8%, <20.6%	16	1	5	0.19 [b]
⩾20.6%, <30.4%	37	13	12	1.11
⩾30.4%, <40.2%	48	16	16	0.98
⩾40.2%, <50.0%	36	14	12	1.2
⩾50.0%, <59.8%	64	23	23	0.99
⩾59.8%, <69.6%	49	21	16	1.32
⩾69.6%, <79.4%	63	25	23	1.1
⩾79.4%, <89.2%	51	21	20	1.1
⩾89.2%, <100%	65	26	24	1.1

[a] Based on all cases of CHD of same age, sex, source, and cause of death as in column 2, but irrespective of extent of atherosclerosis; rounded to nearest whole number.

[b] $0.01 < p < 0.05$.

in each decile is shown in column 2. The number of these cases in which coronary thrombus was observed is shown in column 3. A calculation is now made of how many cases of thrombus would be expected in the same number of subjects of the same age, sex, source, and cause of death as those in column 2, but based on all the subjects with CHD at autopsy, irrespective of extent of atherosclerosis; this is the expected number of thrombosis cases, recorded in column 4 as the nearest whole number. Column 5 records the actual (unrounded) observed:expected ratio. Only for the two lowest deciles is the ratio significantly different from 1.

We would expect that, if atherosclerosis had no effect on thrombosis, the O:E ratio figure would not differ from 1. It is clear from Table 15 that thrombosis occurs much less frequently than expected when the total amount of atherosclerosis of the left anterior descending coronary artery is less than 20%, but otherwise there is no substantial difference.

A similar analysis of fibrous plaque in the left anterior descending coronary artery showed a less-than-expected frequency of thrombosis at levels below 10%, but no difference from the expected with greater degrees of fibrous plaque. Table 16 shows a similar analysis for total amount of atherosclerosis of the abdominal aorta. The ratio of observed

TABLE 16 Extent of Atherosclerosis (Abdominal Aorta) and Frequency of Thrombosis of Extramyocardial Coronary Arteries (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

Decile of Atherosclerosis (1)	No. of Cases with CHD (2)	No. of Cases of Coronary Thrombosis Observed (O) (3)	No. of Cases of Coronary Thrombosis Expected (E) [a] (4)	O/E (5)
⩾4%, <13.5%	14	4	5	0.79
⩾13.5%, <23.0%	19	9	6	1.4
⩾23.0%, <32.5%	27	8	9	0.87
⩾32.5%, <42.0%	31	8	10	0.8
⩾42.0%, <51.5%	30	12	9	1.3
⩾51.5%, <61.0%	57	15	21	0.73
⩾61.0%, <70.5%	50	24	18	1.30
⩾70.5%, <80.0%	45	19	17	1.1
⩾80.0%, <89.5%	75	34	27	1.24
⩾89.5%, <100%	118	33	41	0.81

[a] Based on all cases of CHD of same age, sex, source, and cause of death as in column 2, but irrespective of extent of atherosclerosis; rounded to nearest whole number.

to expected does not differ significantly from 1 at any level of atherosclerosis. Similar figures are obtained (not shown) for fibrous plaque. This type of analysis cannot be used for calcified and complicated lesions because there are too few cases in the higher deciles.

MYOCARDIAL INFARCTION AND CORONARY THROMBOSIS AT LOWEST LEVELS OF CORONARY ATHEROSCLEROSIS

The material has been examined to find the lowest levels of atherosclerosis in the three main coronary arteries (taken together) at which myocardial infarction and extramyocardial coronary thrombosis occur (Table 17).

Of the cases in which the total amount of atherosclerosis in the three main coronary arteries averaged 2–3% of the surface, there were 10 cases of myocardial infarction, including one with thrombosis. There were no cases of thrombosis without infarction.

Of the cases in which the proportion of fibrous plaque averaged 2–3% of the surface, there were 13 cases of myocardial infarction, including one with thrombosis. Again, there were no cases of thrombosis without infarction.

TABLE 17 Minimal Coronary Artery Atherosclerosis and Myocardial Infarction and Thrombosis [a] (Men and Women Aged 40–59 Years; Malmö, Prague II, Yalta)

	Total Atherosclerosis 2–3% [a]	Fibrous Plaque 2–3% [b]	No Calcification or Complication [b]	All CHD Cases
No. cases with myocardial infarction	10	13	70	403
No. cases with thrombosis	1	1	13	159
Ratio of infarction cases to thrombosis cases	10	13	5	2.5

[a] Derived from Kagan *et al.*[8]

[b] Average percentage of surface area in three main coronary arteries.

In the absence of complicated or calcified lesion in all three main coronary arteries, myocardial infarction was found in 70 cases. In 12 of these cases, there was thrombosis, and in another case thrombosis was found without infarction.

This assessment does not take account of atherosclerosis in the branches of the three main coronary arteries, nor was any assessment made of the coronary wall at the site of the thrombus. Nevertheless, the data demonstrate that myocardial infarction may occur with very little coronary atherosclerosis, usually without thrombosis of extramyocardial vessels. When thrombosis is seen, it is almost always associated with infarction.

MYOCARDIAL NECROSIS IN THE ABSENCE OF CORONARY THROMBOSIS

Carlos Marigo of São Paulo (personal communication, 1967) has examined hearts, from hospital deaths, at autopsy. Using multiple serial sections and histologic techniques, he has found many cases of myocardial disease without evidence of coronary thrombosis, in subjects of non-European origin and subjects in whom there is little coronary atherosclerosis. The myocardial lesions were macroscopically and histologically indistinguishable from large myocardial infarcts.

PLATELET ADHESIVENESS AND FIBRINOLYSIS

Comparison of platelet adhesiveness and fibrinolysis in CHD-prone and non-CHD-prone subjects may throw some light on the role of coronary thrombosis in CHD.

Fodor *et al.*[6] examined a sample of rural male and female Jamaicans

aged 35–64 and showed that approximately 14% had evidence of cardiac pain on effort, electrocardiographic signs thought to be indicative of ischemic heart disease, or both. There was good reason to believe that these symptoms and signs were indicative of a different kind of disease when found in Jamaicans than when found in Western Europeans and North Americans. Thus, in autopsy studies of hospital deaths, myocardial infarction is rarely found in Jamaicans,[13] whereas cardiomyopathy is seen often.[16]

D. E. Christian of the Department of Medicine, University of the West Indies, writes (personal communication):

> In view of these observations, it was decided to try and assess the thrombogenic tendency by estimating platelet stickiness in a randomly selected sample of the same population.
>
> A questionnaire with specific questions about ischaemia was filled out for each subject. Their height and weight were recorded and an examination of the CVS, including fundoscopic examination, carried out. A standard 6-foot PA chest film was taken for heart size and configuration, and a 12 lead ECG recorded. The haemoglobin, ESR, PCV, VDRL, RPCFT, Serum Cholesterol and fibrinogen levels in addition to platelet stickiness were estimated and the urine examined for protein and reducing substances. Altogether, 165 persons were examined of whom 143 had blood tests suitable for analysis.
>
> There were 48 normal males and 39 with evidence of ischaemia either on questioning or on ECG. All were in the 55–64 age group as it was felt that this was the age at which the highest incidence of ischaemia was to be expected. There were 37 normal females and 19 with ischaemia. All were in the 60–64 age group. This was to ensure that all would be postmenopausal as platelet stickiness has been shown to vary with the phases of the menstrual cycle.
>
> Because of the small numbers in some of the age groups, in the analysis which follows, the series is divided into normals and abnormals—the abnormal group being those with evidence of ischaemia.
>
> There was no correlation between cholesterol levels, serological tests for syphilis, haemoglobin levels and platelet stickiness.

Table 18 shows mean values and ranges of platelet adhesiveness found in Christian's series and compares them with those found by McDonald and Edgill [10] in patients who had recovered from CHD and in controls in the United Kingdom. The methods used for assessing platelet stickiness were the same in Jamaica and the United Kingdom, and Dr. Christian had studied the technique with Dr. McDonald.

The most interesting finding is that the average platelet adhesiveness of rural male and female Jamaicans is much the same as that of men in the United Kingdom who have recovered from overt CHD; and the range is as wide as that found in patients and controls in the United Kingdom.

A. G. Shaper—WHO Research Professor at the WHO Research and Training Centre for Cardiovascular Diseases, Kampala, Uganda—has

TABLE 18 Platelet Adhesiveness in Rural Jamaicans with and without Symptoms or Signs of Myocardial Ischemia, Compared with Postmyocardial Infarction Cases and Controls in the United Kingdom

	Rural Jamaica [a]				United Kingdom [b]	
	Male		Female			
	"Ischemic"	"Normal"	"Ischemic"	"Normal"	Patients	Controls
Age group, years	55–64		60–64		42–71	41–61
No. of cases	39	48	19	37	47	22
Platelet adhesiveness, mean, % of initial count remaining at 20 min	61	64	62	63	59	80
Platelet adhesiveness, range, % of initial count remaining at 20 min	31–77	36–90	30–88	41–78	36–76	57–88

[a] Data from D. E. Christian (personal communication).
[b] Derived from McDonald and Edgill.[10]

carried out studies of platelet adhesiveness and fibrinolysis in Africans, Asians, and Europeans. He writes (personal communication):

> In the indigenous African community, coronary artery disease (myocardial ischaemia, coronary thrombosis, myocardial infarction) is an extreme rarity while in the Asian community (mainly originating from the Bombay area of India) coronary heart disease with myocardial infarction is apparently as common as in any advanced Western country. The European community (mainly American and British) tend to behave as they would in their areas of origin, but are a highly selected group in that frank hypertensives, diabetics or those with cardiovascular disease are excluded from service in this country.

Some of the findings from the Centre are as follows:

Platelet adhesiveness, platelet aggregation, and factors influencing fibrinolytic activity have been studied in African, Asian, and European men aged 40–60 years. The most striking finding was the complete lack of difference in platelet adhesiveness between the three ethnic groups, despite their marked difference in tendency to develop atherosclerosis and coronary heart disease. This challenges the suggestion that platelet adhesiveness plays a significant role in the predisposition to atherosclerosis and its complications.

Fibrinolysis has been studied in relation to body fatness, serum lipids, and CHD in African and Asian men in Uganda.[14] The Asiatic men were divided into "coronary" and "noncoronary" groups; the African men were all "noncoronary." No difference in fibrinolytic activity was found between the "coronary" and "noncoronary" Asiatics, but fibrinolytic activity was greater in the Africans than in the Asiatics. Fibrinolytic activity decreased with decreased glucose tolerance and increased age, skinfold thickness, or serum cholesterol in the Asiatics, but not in the Africans. There was no association of fibrinolytic activity with serum triglycerides in the Asians or Africans.

Fibrinolytic activity in pregnancy, during parturition, and in the puerperium has been studied.[15] The precise time at which the markedly delayed fibrinolysis seen in pregnancy returns to normal has been determined. Normal fibrinolytic activity returns abruptly as the child is delivered and before the cord is clamped, and it has been suggested that delayed lysis time in pregnancy is associated with the placental secretions of steroid hormones. This finding is clearly of significance in the whole study of thrombosis in relation to the use of female steroid hormones in contraceptive tablets, and possibly of wider significance in the thrombosis problem.

Dr. Shaper and his colleagues would be the first to point out that results cannot be extrapolated beyond the material of their study. However, their findings, with data from other sources, indicate a swing of interest

from platelet adhesiveness to fibrinolysis in the study of the role of thrombosis in proneness to CHD.

SUMMARY OF DATA FROM STUDIES PROMOTED BY WORLD HEALTH ORGANIZATION ON CORONARY THROMBOSIS AND CORONARY HEART DISEASE

In summary, the points that emerge from the WHO-promoted studies on thrombus in the main extramyocardial coronary artery and CHD are:

1. Thrombus is more common in CHD deaths than in deaths in which CHD is an incidental finding, irrespective of the kind of CHD (Table 10) or the suddenness of death (Table 11).
2. Proved cases of thrombosis without fresh infarction are rare, but fresh infarction without thrombosis is common (Table 12).
3. Cases of CHD death with infarction and thrombosis or occlusion are similar to cases of CHD death with infarction without thrombosis or occlusion, with respect to age, sex, weight, height, and heart weight. Persons who die of CHD with thrombosis or occlusion are more likely to have medical care before death and less likely to die suddenly than persons who die of CHD without thrombosis or occlusion (Table 13). The thrombosis or occlusion cases also have, on average, more extensive atherosclerosis of the coronary arteries and coronary stenosis more frequently than those of infarction without thrombosis or occlusion (Table 14).
4. At low levels of coronary atherosclerosis, thrombosis occurs less frequently than would be expected if extent of atherosclerosis had no effect (Table 15), and, although infarction not uncommonly occurs at low levels of atherosclerosis in the coronary circulation taken as a whole, thrombosis rarely does (Table 17).
5. Myocardial lesions, histologically indistinguishable from large myocardial infarctions, have been found in Brazilians of non-European origin in cases in which extensive serial sections show no coronary thrombosis or atherosclerosis.
6. Platelet adhesiveness is much the same in rural Jamaicans and Africans, who are not prone to CHD, as in Asians and Europeans, who are.
7. Fibrinolysis time is shorter in middle-aged Africans than in Asians. It does not increase with age in Africans, but does in Asians, and in the Asians it also increases with factors that are thought to be related to CHD, such as rise in serum cholesterol, decrease in glucose tolerance, and increase in obesity.

DISCUSSION

Point 1 in the summary indicates a close association between thrombosis and CHD death. But this association does not necessarily imply causation. Point 2 indicates that death without thrombosis but with fresh infarction is common, and that suggests that thrombosis may not be necessary for a myocardial lesion and resulting death. This finding has several possible explanations. The first is that thrombosis was present in branches. But points 3 and 4 of the summary suggest (but do not prove) that in some cases thrombosis occurs after the myocardial lesion, or not at all, and that it is more likely to occur when there is substantial coronary atherosclerosis. Point 5 indicates that a myocardial lesion that is indistinguishable from infarction can occur without coronary thrombosis or atherosclerosis. That raises the question of whether thrombosis can be the *result* of a myocardial lesion in the presence of coronary atherosclerosis, rather than its *cause*. The data could be explained in this way just as well as the reverse.

That thrombosis often follows infarction, rather than precedes it, is supported by observations on age of infarct and thrombus, such as those of Branwood and Montgomery [2] and Baroldi.[1] In their judgment, thrombus when found was often older than the infarct. Also, Vihert, Boguslavski, and Gudeem (personal communication, 1962), studying 2000 forensic deaths in which time of onset and nature of symptoms were clear, found that the frequency with which thrombosis was found in CHD deaths increased as the interval between onset of symptoms and death increased from 2 hr up to 3 days.

The strongest evidence against thrombosis as the cause of death is the failure of anticoagulant treatment (heparin, coumarin, phenindione), directed against the fibrinogen-to-fibrin type of thrombus, to prevent more than a small proportion of CHD deaths (around 5%) or onset or recurrence of myocardial infarction. Much of the small benefit is due to the prevention of embolism. This evidence would apply only to platelet fibrin thrombi, the type we have assessed in the autopsy study. It is conceivable that platelet aggregates forming at the site of defects in the coronary artery intima, as a result of the exposure to subintimal collagen or to adenosine diphosphate, could form in spite of anticoagulant therapy, obstruct a coronary vessel, and trigger an acute CHD attack and death. Platelet aggregation induced by adenosine diphosphate has been shown to induce myocardial ischemia in swine.[11] Such thrombi can resolve quickly and might disappear before death.

Another way in which myocardial lesion and CHD death might be triggered is suggested by data from coronary-care units. There is strong

evidence that, shortly after an acute attack, arrhythmias precede death, and that, if the arrhythmia is prevented, death is prevented. It is conceivable that an arrhythmia would trigger the initial attack. This may lead to or arise from a local myocardial metabolic disturbance or local ischemia. These three factors might indeed form a vicious circle begun by any one or a combination of them. The effect might spread and, in the presence of a sufficiently impaired coronary circulation, give rise to the type of platelet fibrin thrombus so often seen.

This is, of course, speculation. The possibility that thrombus gives rise to infarction, as is classically taught, is not excluded by our data; but it does seem likely that, if this is so, we are treating the wrong phase of thrombus formation with our anticoagulants or using the wrong test of anticoagulant activity. The data would fit equally the possibility that thrombus is secondary to myocardial lesion. Even so, thrombosis might contribute to death.

The WHO-related studies contribute little to the answer to the question of whether thrombosis plays a part in the pathogenesis of atherosclerosis. Points 6 and 7 in the summary indicate that it would be wise to study fibrinolytic activity in relation to the question.

LIMITATIONS OF PRESENT STUDIES AND FUTURE APPROACH

The data from WHO studies have been examined particularly from the point of view of the role of thrombosis as a precipitating cause of CHD death. A similar role in death from "cerebral infarction" has been only mentioned. Another, perhaps even more important aspect is the role of thrombosis in causing atherosclerosis. Both these aspects need clarification. Studies are hampered by:

1. Difficulty in accurately assessing early thrombosis of different kinds, early myocardial infarction (or necrosis or metabolic myocardial change), the "hypercoagulable" state, factors contributing to and associated with thrombosis and fibrinolysis, CHD and its various manifestations, "cerebral infarction," and atherosclerosis;
2. The frequency with which subjects die from CHD before medical observation is available;
3. Lack of suitable substances to depress or stimulate different stages in the thrombocoagulation process (e.g., known drugs that will depress the formation of platelet aggregation are too toxic for use [3,4]); and
4. Difficulties in understanding the way in which multiple factors interact to result in thrombotic or myocardial death.

The data discussed indicate that, at autopsy but not yet during life, some of the factors mentioned in (1) can be accurately determined. Today, most persons who die in an acute attack of CHD do so before the doctor sees them. The growing use of coronary-care units and the consequent interest in getting the "coronary case" under skilled supervision at the earliest possible moment will make it possible to study more of the acute attacks, and under conditions that will permit good diagnosis and continued assessment of physiologic and pathologic change. This, with nontoxic means of intervening, should permit a better understanding of the thrombogenic process and its role in causing acute coronary attacks and death in CHD.

More precise methods of assessing anatomic, physiologic, and pathologic factors and endpoints will make possible a fresh approach in the analysis of data that will show the role of factors, singly or in combination, in the development of CHD.

The ability to identify the coronary-prone person is improving. So is the ability to monitor and analyze data from such subjects continuously. It is conceivable that, with further advances, it will someday be practical to do this. If so, it will provide a useful means of identifying the factors that precipitate CHD attacks.

In the study of the role of thrombosis in the development of atherosclerosis, the evidence points to the need to assess fibrinolytic activity, as well as tendency to thrombosis or "hypercoagulable" state.[10,14,15] Even more emphasis must be placed on precise assessment of factors and endpoints than in the study of the acute attack, because, unless that is done, studies will be prolonged and will require large numbers of cases, data will be crude, and results will be indefinite. As for studies of the acute attack, analysis that will permit an understanding of the interplay of factors in producing a pathologic result is needed, and it should not depend on preconceived ideas of normal distribution or linear relationships; it should show up threshold, catalytic, and sequential effects.

With better ways of assessing thrombofibrinolytic factors and with more suitable means of intervention, studies in which population groups are assessed during life, re-examined when they become ill, and examined according to a detailed protocol at autopsy when they die are likely to be productive. Such studies are difficult to organize, but one that has been going on for several years is that of Katsuki *et al.*[9] on cerebrovascular disease. Others are now being developed in several areas by WHO for the study of atherosclerosis, CHD, hypertension, cerebrovascular disease, and chronic respiratory disease.

The fundamental aspects of the thrombofibrinolytic process have been mentioned. Thromboembolic phenomena are not all of the same type

and origin, and the evidence is that, even if cerebral infarct, CHD, and atherosclerosis are due to TEP, the three do not have precisely the same pathogenesis and they are not due to a general derangement of the thrombofibrinolytic mechanism. The latter is, in all probability, an important fundamental mechanism that has served biologic organisms well for millions of years. Granting this, and I think it is necessary to define the circumstances under which it is useful, it is unlikely that an optimal remedy for modern disease will involve affecting this fundamental process. The aim should be to find out which conditions need to be altered, so that the fundamental mechanism will not be impaired and will not be caused to misfunction. Such conditions are likely to be outside the fundamental mechanism.

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Appraisal of Epidemiologic Studies

ABRAHAM M. LILIENFELD *

The position of "appraiser" of the epidemiologic studies of the broad spectrum of cardiovascular diseases is a difficult one. Several of the presentations have sifted and evaluated the epidemiologic data on specific diseases or groups of diseases. We realize that our epidemiologic knowledge of several forms of vascular disease is conspicuous by its absence, and that, too, has been clearly stated. Consequently, all that I can do is make some personal observations on a few points and attempt to indicate some future directions for epidemiologic research on this important group of problems.

First, when one views epidemiologic activity in this field, one is struck by the overwhelming concentration on coronary heart disease. This has been confirmed by a recent review of the extramural epidemiologic research projects supported by the National Heart Institute. It is, to some extent, not unexpected, in view of the public-health importance of this disease entity. Nonetheless, none of the contributors to this volume would question the urgent need for epidemiologic investigation of the other forms of vascular disease, particularly those in which thrombosis may play an important role. Some means must be found for stimulating and broadening epidemiologic research interest in these areas.

When one focuses on the epidemiologic studies of coronary heart disease, a picture of sameness emerges. One immediately notices the continued interest in possible etiologic factors, such as lipids, cigarette smoking, blood pressure, and obesity. Although it has been repeatedly pointed out that additional factors—genetic factors, psychosocial factors, physical activity, etc.—may very well play an etiologic role, well-designed and adequately controlled studies concerned with these factors are virtually absent.

* Recipient of Public Health Service research career award K6 GM 13901 from the National Institute of General Medical Sciences.

It seems quite clear from the discussion of coronary disease that risk factors—elevated serum cholesterol, cigarette smoking, etc.—carry with them high probabilities for the later development of coronary disease. In addition, epidemiologic data are not clear on the etiologic role of thrombosis; the possible role of thrombosis has not been adequately studied. Epidemiologists who have attempted to evaluate the role of thrombosis in coronary disease have been continually frustrated in their efforts by the unavailability of a valid and precise measurement of thrombotic phenomena. Epidemiologic investigations would be immensely accelerated if useful methods of measurement were available.

In considering the potential role of thrombosis in the etiology of coronary heart disease, two possibilities can be hypothesized. First, the acknowledged risk factors, such as elevated serum cholesterol and cigarette smoking, may affect the risk of developing coronary disease by producing changes in the coagulation mechanism or platelet activity; i.e., the thrombotic effect may be intermediate or it may represent one of the pathogenetic mechanisms. As has been mentioned, there is some evidence that this may be the case with respect to some of the risk factors, but more systematic observations are necessary. It would be extremely helpful to obtain some quantitative estimate of the relative importance of such a mechanism, thereby providing another potential avenue for preventive intervention. In general, one can safely say that epidemiologic studies have not provided the best means of elucidating pathogenetic mechanisms of a disease. However, it would be useful to speculate on the possible contribution that the present on-going prospective studies of population groups might make to our knowledge on this particular problem. In man, the principal modes of studying the role of thrombosis are the postmortem examination and the evaluation of blood coagulation, including the study of platelet activity. What can be done in the prospective-study populations is to select population segments with the highest risks of developing coronary heart disease, i.e., those wtih high serum cholesterol levels, heavy cigarette smoking, and high blood pressures, and, as a control group, those at the other end of the disease probability spectrum, i.e., with the lowest probabilities in terms of these risk factors. One can then follow these groups more intensively and include the necessary laboratory determinations of the different components of blood coagulation. In working with these selected groups of subjects one can also develop some procedures for obtaining immediate reports of death and autopsy. The number of deaths, as well as the number of cases of coronary heart disease, would probably be quite small in any one community; it would therefore be necessary to establish a collaborative arrangement among several such investigative groups. I do not know

whether this type of study is feasible in this country; perhaps it would be necessary to conduct it on an international basis.

Second, thrombosis, or the thrombotic tendency, may serve as an independent risk factor with respect to coronary heart disease. (The two possible roles of thrombosis are not mutually exclusive.) Several years ago, Morris suggested that there is no one-to-one correspondence between coronary heart disease and coronary atherosclerosis. Elaborating on this concept, Acheson suggested that coronary heart disease in middle-aged men differs from that in older men. Furthermore, in middle-aged men, thrombosis serves as the pathogenetic mechanism for such risk factors as cholesterol, cigarette smoking, body build, and physical inactivity, whereas, in older men, the major pathogenetic mechanism is the development of mural atheroma, which is influenced by hypertension. Of course, this distinction may become somewhat blurred, depending on one's view of the thrombus–atherosclerotic plaque relationship—and, needless to say, the pathologic evidence is rather confusing.

In considering the possible role of the thrombotic tendency as an independent risk factor, it would be desirable to estimate how much of coronary heart disease, as determined in the prospective epidemiologic studies, can be explained in terms of definitely established risk factors. The published data from these studies are reported in a way that permits one to make only crude estimates limited to three risk factors: serum cholesterol, cigarette smoking, and blood pressure. The data suggest that about 30–40% of coronary heart disease can be explained by these three factors. It should be noted that the very nature of the data on which this estimate is based suggests that it is a minimal estimate for at least two reasons. (1) In the prospective studies of the relationship of these risk factors to the development of coronary heart disease, the data thus far reported are limited to information obtained on the first examination of the study group, even though the population was examined at regular intervals. The integration of the periodically obtained information into the analysis poses several statistical problems that have not yet been resolved; those problems are now under active investigation. (2) These studies have also shown that there is a greater degree of relationship between these risk factors and coronary disease in the younger groups than in the older groups. Therefore, the relative contribution of these factors should be estimated for the different age groups. Unfortunately, the published reports do not present the data in a form that allows one to make such estimates by age. However, it is quite possible that, in the younger groups, these risk factors may well account for 75–80% of coronary disease, in contrast with possibly 10–20% in the older groups. Thus, if one speculates on the role of thrombosis as an indepen-

dent risk factor, it is more likely to be so in the older groups. It would seem, therefore, that any investigator interested in pursuing this problem will find the study of those in the older groups more profitable.

Admittedly, all this is speculative; but this type of reasoning is now necessary to plan more adequately future programs that will attempt to explore new hypotheses. It underscores the need for presenting the findings of these prospective studies in terms that would be meaningful for the development of new hypotheses.

It should be pointed out that the study of the low-risk group segment of the investigation suggested earlier could be designed to test hypotheses other than that of thrombosis. In fact, it might be generally useful to evaluate the cases of coronary heart disease that occur in the very-low-risk group from that viewpoint.

In the case of the cerebrovascular diseases, epidemiologic studies were virtually absent 3–4 years ago; much of what we know has been gained through recent studies and from several that are still in progress. Much of the work presently being done includes the examination of risk factors that have been found to be related to coronary disease and also examines the relationship of transient ischemic attacks to stroke. My comments on the possible role of thrombosis with respect to coronary heart disease apply equally well to cerebrovascular disease.

The situation with respect to other manifestations of thromboembolic disease is most depressing: except for mortality statistics (and rather crude ones at that), our knowledge of these disorders is virtually nonexistent. Epidemiologic studies of such diseases as thrombophlebitis, pulmonary embolism, and various forms of peripheral vascular disease, particularly with respect to possible associations with various factors that may be of etiologic significance, are practically absent. The conduct of epidemiologic studies of this group of disorders presents several problems. There are difficulties in establishing definitive diagnoses of several of the individual disease conditions in this group. It is essential for clinicians dealing with these disease conditions to develop diagnostic criteria for epidemiologic use. Another problem results from the low frequency of several of these conditions in the population, making it difficult to obtain a sufficient number of cases in a single medical center or even in an entire community for study. This necessitates the development of collaborative studies in several communities.

A more difficult problem stems from the fact that the hospitalized cases of several such conditions represent the top of the iceberg, so to speak, and any adequate epidemiologic investigation would have to include samples of the general population, in turn requiring methods of case-finding that can be applied on a mass basis. It seems clear that informa-

tion is needed on the distribution of several of these disease entities by population characteristics, such as age, sex, race, socioeconomic status, and occupation. Studies on the familial aggregation of the specific vascular disease entities, as well as their totality, may prove interesting; these can be readily conducted. Useful data will no doubt be forthcoming from current studies that are attempting to assess the role of the contraceptive pill in the etiology of thromboembolic disorders.

The over-all strategy adhered to in epidemiologic studies of these vascular disorders has been a concentration on possible etiologic factors with respect to specific clinical entities. However, epidemiologic data available on thrombosis itself and on the thrombotic tendency would also be extremely valuable. For example, do we have any information on the distribution in the population of such phenomena as platelet aggregation? Do we know the factors, even such basic ones as age, sex, and race, that influence the frequency of abnormalities of platelet aggregation? There is also a need for detailed autopsy studies of thrombosis in various vessels by specific population characteristics.

The preceding presentations have been oriented mainly toward individual diseases. However, we recognize that there are interrelationships between these clinical entities. Thus far, our knowledge of the degree of relationships between them is fragmentary, and the underlying reasons for any relationship are based on such general concepts as the "atherosclerotic process." Epidemiologic data may well be able to contribute useful information on these relationships in two ways. First, one can compare the distributions of the various types of vascular diseases according to such population characteristics as age, sex, and race; differences in distribution may lead to the development of hypotheses that would help to explain the differences themselves, both qualitatively and quantitatively. Second, one can study the relationships by detailed studies of the natural history of these diseases. By a follow-up of cases with a given disease, as unselected as possible, one can determine the probability, over time, of developing other types of vascular disease. Although there are published reports of such studies, they usually involve highly selected patient groups from single medical centers with varying degrees of follow-up. If they were conducted on a concurrent basis, one could obtain information with regard to the possible role of thrombosis. Such studies are not easy to initiate or conduct; much careful planning is necessary.

It is quite clear that much remains to be done. In a vast majority of the disease entities related to thrombotic phenomena, there has been only an initial effort at an epidemiologic approach. One might well ask: "What is the best organizational means to encourage epidemiologic re-

search in this area?" Probably the best approach would be to establish one or two epidemiologic research centers in this country, which could provide a focal point for studies of these disorders. An excellent start would include rigorously designed and controlled case-control studies, conducted collaboratively in several communities, of specific disease entities in which epidemiologic work has not been done. These centers should include experienced clinicians in these specific areas, as well as laboratory workers concentrating on the development of tests for measuring thrombotic disorders for use in large-scale epidemiologic research programs. In addition, more detailed analyses of the available national mortality data could be carried out; they may very well provide some leads for more detailed investigation. A focused and intensive research effort cannot help but bring us closer to a solution of these disease problems.

III
THE NATURE OF A THROMBUS

The Anatomy of a Thrombus

A. B. CHANDLER

The concept of thrombosis was developed largely through anatomic observations. For centuries, the development of the concept was hindered by the lack of general recognition that blood in the heart and blood vessels may solidify after death as well as during life.[42,60] The confusion that existed until well into the nineteenth century is best illustrated by the well-known history of the problem of heart polyps. The problem concerned the distinction between polyps that were true thrombi and possessed "vitality" [11] and polyps that were simply postmortem coagula. The pathologic nature of a thrombus was determined mainly by these anatomic observations: evidence of degeneration during life, lamination, and adherence to the vessel wall.[6,11,19] The recognition by Virchow [65] and Kirkes [39] of the effects of ischemia caused by thromboembolization made it quite clear that thrombi have vitality.

Experimental studies established that thrombi of a distinctive structure form in flowing blood from constituents of blood (Figure 1).[9,22,70] Natural thrombi were found to have the same basic structure and constituents, indicating a similar mode of formation.[22,26,70] Variations in structure, although observed and beautifully illustrated as early as 1805 by Jones [38] (Figure 2), were not fully understood until it was shown how disturbances of blood flow influence the structure and development of a thrombus.[2,59,66,69]

Welch, in his noted review of 1899,[69] summarized the experimental and morphologic studies that led to the concept of thrombosis as we know it today. His definition of a thrombus, "a solid mass or plug formed in the living heart or vessels from constituents of blood," is widely used.[56] Although the main aspects of the nature of a thrombus were worked out by the end of the nineteenth century, there have been contributions since then, especially by the application of recently developed techniques—

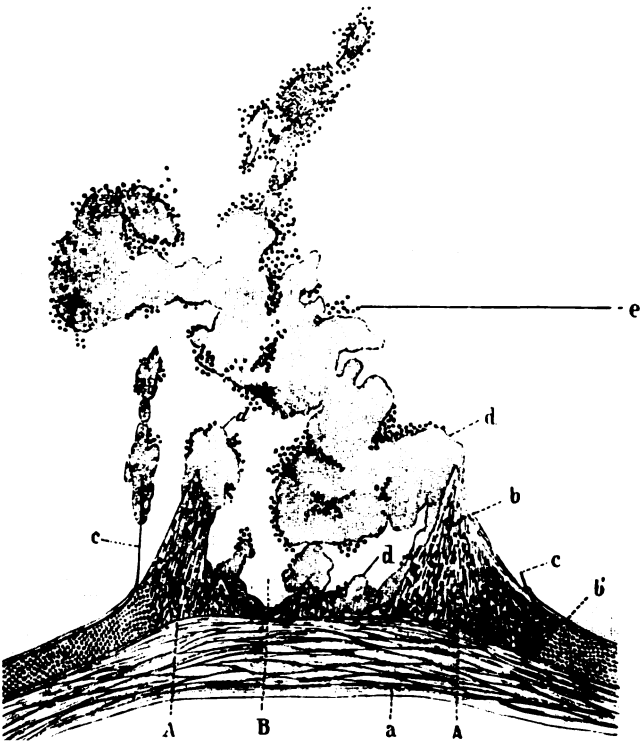

FIGURE 1 Experimental thrombus of a femoral artery of a dog at site of injury produced by a ligature, which was immediately removed. The thrombus was examined 45 min after the injury. Masses of aggregated platelets (d) are stuck to the edges of the arterial wound and project into the lumen. Numerous leukocytes (e) adhere to the platelet columns. Fibrin is not evident in this fixed but unstained preparation. (Reprinted from Eberth and Schimmelbusch.[22])

electron microscopy, enzyme histochemistry, and immunofluorescent microscopy.

Anatomic studies are not an end in themselves but give insight into the pathogenesis of a lesion and its pathologic consequences. The purpose of this review is to bring together old and recent observations with these two points in mind and to assess still unanswered questions that may be clarified by anatomic studies.

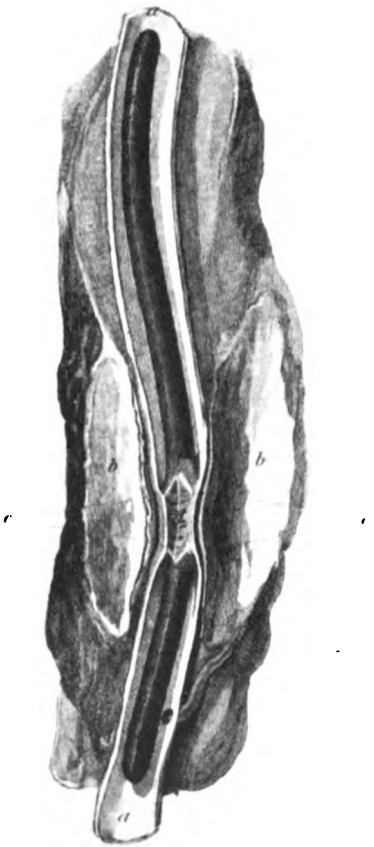

FIGURE 2 Experimental thrombus of a carotid artery of a horse at the site of an injury produced 3 days previously by ligatures, which were immediately removed. The white mass at (c) was interpreted as coagulated lymph, which had effused from the injured arterial wall, rather than as a thrombus. Extending from the white mass in both directions along the vessel is probably a propagated clot. (Reprinted from Jones.[38])

GENERAL CHARACTERISTICS

All cellular elements of the blood may be found in thrombi.[69] However, there is variation in their normal concentration, because their accumulation is in part selective.[22,70] Selective accumulation of platelets and leukocytes, the major cellular elements that form a thrombus, can occur as long as flowing blood brings them to the site of formation. The cellular elements are held together principally by fibrin. Even though there is little fluid in a thrombus, derivatives of plasma other than fibrin can be identified. For example, plasma lipoproteins in close association with fibrin have been detected by immunofluorescent techniques.[71]

The thrombic mass is further characterized by the specific organized

arrangement of its constituents.[22,70] In contrast, a clot that forms in stagnant blood contains the elements of blood in their normal concentration and lacks an organized structure.[18,43]

Some part of the surface of a thrombus usually is attached to the vessel wall over a variable area.[18,64] Subsequent growth of the thrombus may lead to a greater area of attachment.[18] There may or may not be an underlying causative lesion of the vessel wall.[2,52,53,69] However, the nature of the base of attachment seems not to influence the structure and development of a thrombus as much as the conditions of blood flow that exist at the site of formation.[2]

DETAILS OF STRUCTURE

One can distinguish between *simple* thrombi, composed predominantly of one element, such as platelets, and *complex* thrombi, composed of several elements, often with only a few predominating.

SIMPLE THROMBI

These may consist of either platelets or fibrin, although they rarely occur in pure form.[2,45,69] A small amount of fibrin is often present in platelet thrombi,[5,32] and a few platelets in fibrin thrombi.[45,69] Some leukocytes may be found in either type. Pure leukocyte thrombi have been described, but they are rare and relatively unimportant.[24,69] When it is not possible to identify the predominant element, the descriptive term "hyaline" usually is used.[24,73]

In platelet thrombi, fibrin is deposited on the surface of platelet masses in the same relationship as in larger complex thrombi, suggesting that both are basically the same type of lesion.[32] Occlusive platelet thrombi in small vessels have no opportunity for growth or development into larger and more complex lesions, as do microthrombi, their counterparts, on the walls of large vessels.

Fibrin thrombi are distinctly different from other thrombi in several respects. In addition to being composed mostly of fibrin, they are always small and occur almost exclusively in small vessels, especially in capillaries.[44,45] The few platelets in these thrombi are unaggregated and appear to be trapped and attached to fibrin strands.[45] Occlusion and distention of the vessels by closely packed strands of fibrin help to differentiate these thrombi from the loosely arranged fibrin strands of postmortem coagula.[45]

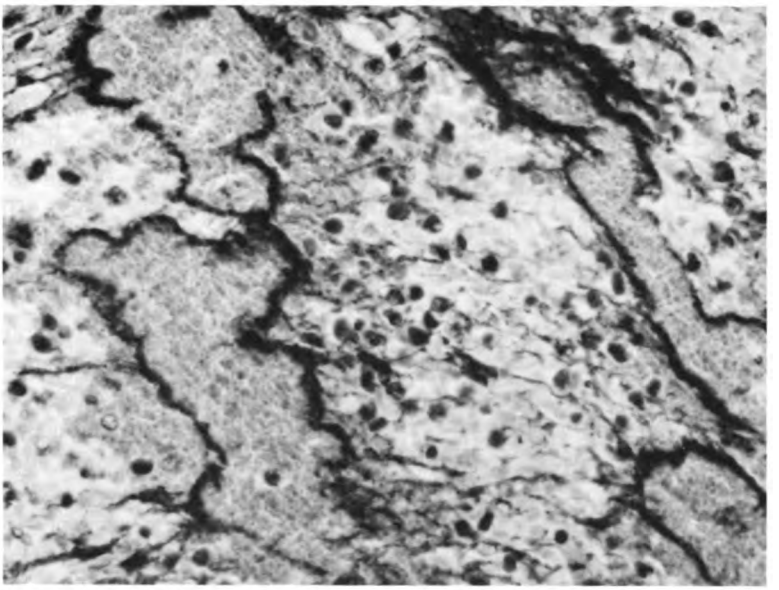

FIGURE 3 Structural unit of a complex thrombus. Aggregated platelets are enclosed by a rim of fibrin (black). The platelets within the aggregates are distinct; a few have spilled out along the surface. Outside the fibrin rim are numerous leukocytes, mostly granulocytes and monocytes. Anastomosing fibrin strands connect adjacent units. Phosphotungstic acid–hematoxylin. (× 100)

COMPLEX THROMBI

Although the proportion of the constituents of complex thrombi varies greatly, the basic structure can always be recognized and is similar at any site of formation. Complex thrombi form in arteries, veins, and the heart. Platelets, leukocytes, and fibrin are the basic elements of these thrombi.[70] Aggregates of closely packed platelets are surrounded by fibrin and leukocytes in a distinctive arrangement [69] that forms the structural unit or "building block" (Figure 3). The platelets within the aggregates are usually well delineated, and their membranes appear intact.[40] The platelet aggregates are covered in part or completely by a layer of dense fibrin (the hyaline rim of Hanau *).[33,67,69] Strands and filaments

* Hanau [33] thought that the hyaline rim was composed of fibrin and altered platelets. However, the rim probably represents only fibrin, inasmuch as it is absent from thrombi from cases of congenital fibrinopenia [35] and from artificial *in vitro* thrombi from cases of congenital afibrinogenemia.[17]

of fibrin radiate from the surface of the aggregates and incorporate leukocytes along the fringe.[70]

Small aggregates connected by fibrin in a linear chain form columns or beams. Other columns are composed of large aggregates of uninterrupted masses of platelets. Studies of *in vitro* thrombi suggest that this type of column can form by coalescence of smaller adjacent aggregates.[17] Occasional broad-beamed columns are composed of aggregates arranged side by side and in a linear chain. Leukocytes may be present on the border of these thick beams but not between the individual platelet aggregates.

Platelet columns may be separated by no more than a few strands of fibrin, or they may be more widely separated by variable numbers of red blood cells and leukocytes. The cells are contained in the spaces between the columns by a fibrin mesh aligned in parallel strands connecting the columns (Figure 4).[43,73] The columns or beams often branch and anastomose with one another to form a structure likened by Aschoff [3] to branching coral or a sponge. The free surface of mural thrombi is often marked by ripples caused by the formation of ridges at the tips of platelet columns (the lines of Zahn).[3,72]

The close approximation of platelets to each other in a thrombus makes this a curious lesion—no morphologic basis to account for platelet cohesion has been found. It has been suggested that the bond between apposing platelets is electrostatic and is related to changes in the electrokinetic charge on the surface of the platelet.[61] Most morphologic studies of this problem have been done on recently formed experimental thrombi.[29,34] The platelets are not fused. They are not stuck together by fibrin, nor has any other extracellular substance been identified between them. Indeed, electron microscopic studies of these experimental thrombi have shown that the platelets may be as close together as epithelial cells. Recently Rambourg and Leblond [58] found carbohydrate-rich material between epithelial cells by means of a PAS–methenamine stain, but observations on thrombi using this stain have not been reported. In histologic sections of natural thrombi, platelets in aggregates usually are separated from each other and well delineated (Figure 3). They may even be "spilled out" along the margins of the aggregates. These arrangements of platelets may be in part artifactual and in part degenerative. Nevertheless, they suggest that the bond between platelets is not necessarily firm or permanent and point out the importance of fibrin to the integrity of a thrombus.

Fibrin coagulation has been considered a secondary reaction that occurs within a thrombus largely after it has formed.[2,3,10] This restricted view of the role of fibrin in thrombosis is misleading. It is well known that

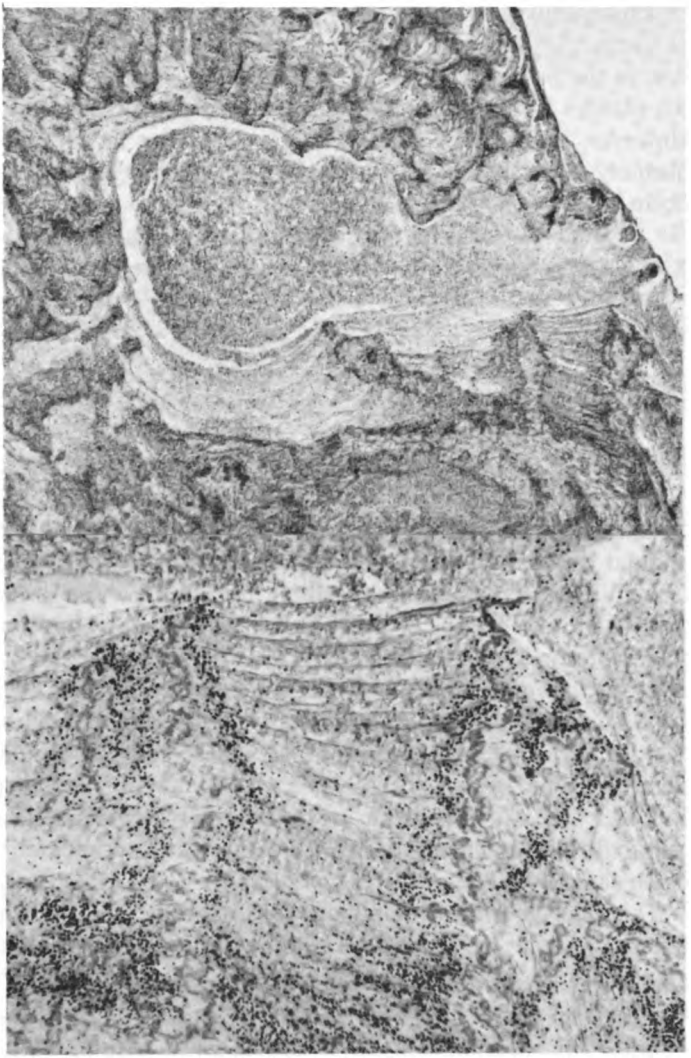

FIGURE 4 Top: Surface of thrombus at the site of a gap. Platelet columns of varying dimensions project along the surface. The central part of the gap is filled with a retracted clot. Bottom: High-power view of the two platelet columns in right lower corner of picture above. The branching columns are outlined by a covering of numerous leukocytes. Parallel strands of fibrin connect the platelet columns out to their tips and entrap circulating blood cells. Periodic acid–Schiff hematoxylin. (Top, × 5; bottom, × 20)

experimentally induced thrombi are transient and unstable in the absence of fibrin.[4,27,50] Fibrin formation is intimately related to platelet aggregation in the building of a complex thrombus. Fibrin surrounds and links the platelet aggregates to stabilize the thrombus as it forms (Figure 4). However, its presence does render a thrombus susceptible to lysis by fibrinolysins. MacCallum [43] clearly pointed out the important role of fibrin in the formation of a thrombus:

> It must not be supposed that they [the platelet lamellae] rise up alone and unsupported in the current. Instead of that, they quickly catch the passing leucocytes . . . ; and at the same time they seem to liberate thromboplastic substance, so that filaments of fibrin spread out from them on all sides, and, meeting with filaments from the next lamella, hang in festoons between them. In this way the branching and anastomosing lamellae are guyed and braced together by fibrin . . . .

By quickly following platelet aggregation, the formation of fibrin around the aggregates defines and limits further growth of the individual structural units that make up a complex thrombus. Finally, it should be pointed out that complex thrombi do not form without fibrin. In two recently reported cases of congenital fibrinopenia,[35] large thrombi in leg veins were found to be composed of masses of aggregated platelets with some entrapped circulating cells. These thrombi were not stable, in that extensive pulmonary thromboembolism occurred in both cases.

Although it is generally accepted that red blood cells are passively trapped in a thrombus during its formation and contribute only to its bulk,[3,69] some studies indicate that they may be active participants. Johnson [37] has presented electron microscopic evidence that red blood cells participate actively in the formation of experimental thrombi and hemostatic plugs by undergoing lytic changes.

The incorporation of numerous leukocytes in freshly formed thrombi was first noted by Virchow,[64] who described both multinucleate and uninucleate forms. Leukocytes not only are trapped in thrombi with red blood cells, they are also selectively incorporated. Granulocytes and monocytes,[56] but not lymphocytes, usually border the platelet aggregates outside the fibrin layer. Granulocytes predominate.[70] Although leukocytes collect along the platelet aggregates before or at the same time that fibrin forms in experimentally induced thrombi,[56] they are usually outside the fibrin layer in completed thrombi. Our studies of *in vitro* thrombi indicate that fibrin formation along the edge of aggregates takes place between the platelets and leukocytes. Neutrophilic granulocytes are rich in proteolytic enzymes [54] and contribute to the puriform liquefaction of thrombi.[24,64] Eosinophilic granulocytes, which are reported to contain plasminogen,[7] may also contribute to the lysis of thrombi. Mono-

cytes participate in resolution and organization.[16,24,36] An explanation of the paucity of lymphocytes might provide a clue to the stickiness of other cellular elements.

POSTMORTEM CHANGES

In studies of human disease, one must be aware of the postmortem and postsurgical changes that can alter the structure of a lesion. Empty spaces between platelets in histologic sections are common and probably artifactual. Degenerative postmortem cellular changes are at times difficult to distinguish from antemortem changes. However, in many thrombi, degenerative changes can be correlated with zones of differing age [69] to indicate their antemortem nature. Activation of the fibrinolytic system occurs in cases of sudden death [46] and may cause postmortem fibrinolysis within a thrombus.[28] Although it is not always possible to differentiate postmortem from antemortem lysis, fibrinolysis before death is often associated with liquefaction and necrosis of leukocytes.

Postmortem coagula that form casts in stagnant blood of the heart and vessels contain the cellular elements of the blood in their normal concentration and fibrin needles dispersed at random.[18,43] Occasional small, dense aggregates of platelets surrounded by thick radiating strands of fibrin, but not leukocytes, serve as centers of coagulation.[21] Settling of cells before coagulation produces a layered clot.[43,73] Postmortem coagula often form on the surfaces of thrombi and are easily stripped away.[43] Lytic holes or gaps in thrombi may be filled with liquid or clotted blood.

STRUCTURAL FEATURES IN RELATION TO SITE AND MODE OF ORIGIN

In general, the site of thrombus formation seems to influence structure only because of the properties of flow at that site.[2] Both rate of flow [66] and irregularities of flow [59] at a particular site may influence the composition and structure of a thrombus. Thrombi that form in fast-moving pulsatile blood of arteries contain fewer entrapped red blood cells and are more compact than thrombi that form in slower currents of veins.[28,57] Turbulence and eddy formation are important, not only in initiating thrombus formation [59] but in imparting directional and whirling patterns to its structure. In small vessels, such as venules, simple thrombi composed predominantly of one element may contain few red blood cells, even though flow is low. Thus, with few exceptions, the factors of flow and location are difficult to separate.

Although most thrombi probably form on a vessel wall,[18,64] some form in the bloodstream [33,63,73] and may become attached to the wall after development has begun. Therefore, it is important to consider the structural features of a thrombus in relation to its mode of origin.

FORMATION ON THE VESSEL WALL

Complex thrombi that form on a vessel wall tend to develop columns of platelets oriented at right angles to the wall or bent in the direction of flow.[3] Although there may be much variation in structure, this over-all pattern generally can be discerned (Figure 5). The structural features of the thrombus at the site of attachment to the vessel wall, however, are not clearly worked out.

From the earliest experimental studies, it has been shown repeatedly that the initial thrombus on an injured vessel wall is composed of platelets, which are soon followed by the accumulation of leukocytes and the formation of fibrin.[9,22,29,70] Although completely formed natural and ex-

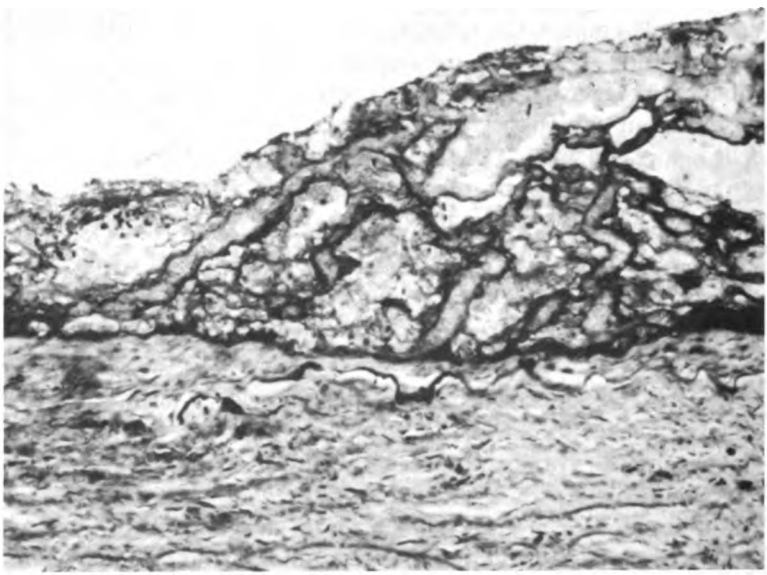

FIGURE 5 Microthrombus on the wall of a carotid artery near the site of a needle puncture wound made 22 hr before death. Thin platelet columns project into the lumen and are surrounded and connected by fibrin (black). The base of attachment at this stage of development appears to be largely fibrin. Endothelium was not identified at this site. Tannic acid–phosphomolybdic acid–buffalo black, modified. (× 50)

perimental thrombi are structurally similar,[26,70] the relation of platelets and fibrin to the vessel wall in human lesions is not so clearly established. One reason is that it is almost impossible to obtain recent thrombi that are accurately dated and unaltered by postmortem changes.[26] Furthermore, thrombi may form in the blood and then become attached by either fibrin or platelets.

The acceptance, without adequate proof, of the idea that platelets always form the initial seat of attachment in natural thrombosis has perhaps retarded our understanding of this disease.[2,57] Only recently was the first detailed report made of the site of attachment of a human thrombus by electron microscopy. Angrist and his associates [1] studied thrombi on heart valves and found that platelets adhered to collagen in areas of denuded endothelium. Immunofluorescent techniques for the identification of platelets,[12] as well as fibrin,[13] are now available and may help to clarify this subject further.

FORMATION IN THE BLOODSTREAM

Complex thrombi that form entirely in the bloodstream are composed of a central platelet-fibrin core covered by circumferential layers of fibrin. In the core, small platelet aggregates surrounded by fibrin and leukocytes are closely packed; few red blood cells are entrapped. Such thrombi can be produced in the experimental animal at sites of disturbed blood flow when the blood is made hypercoagulable.[49] They bear a striking resemblance to artificial *in vitro* thrombi that form entirely in the blood.[14,17]

Söderström [63] has called attention to unattached "recess" thrombi in the trabecular meshwork and appendages of the atria but does not describe their structure in detail. Thrombi whose structure is consistent with formation in the blood are occasionally observed in veins and pulmonary arteries (Figure 6). Thrombi may also form in the blood around fragments of bone marrow in cases of bone marrow embolism.[68] Because a thrombus that forms in the blood may become attached to the vessel, recognition of this mode of formation may be obscured unless the structure of the thrombus is evaluated.

INFLAMMATION AND INFECTION

Thrombi that form in association with angiitis basically are no different in structure from other thrombi. When a thrombus is infected, necrosis and lysis are prominent features that probably account for frequent embolism.[69] Valvular endocardial lesions deserve special comment. Bland vegetations on heart valves are thrombi composed almost entirely of

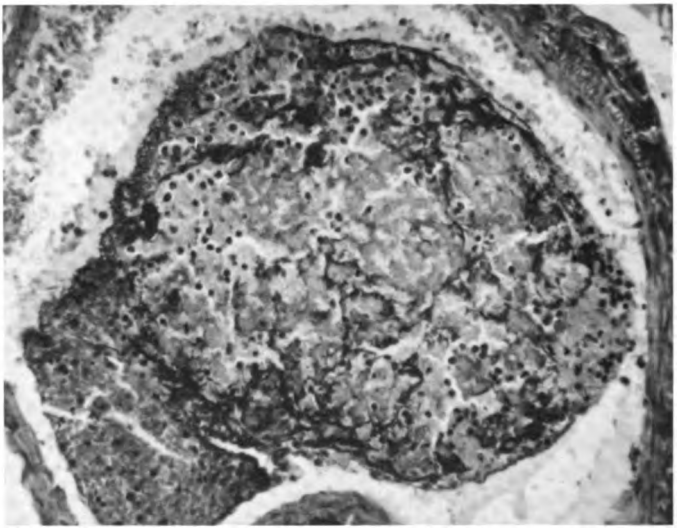

FIGURE 6 Thrombus in a pulmonary artery. This thrombus is structurally consistent with its formation entirely in the bloodstream. It is composed of a core of closely arranged platelet aggregates covered and connected by fibrin, and a few leukocytes. Its perimeter is enclosed in a membrane of fibrin. This thrombus is structurally identical with an artificial *in vitro* thrombus. Phosphotungstic acid–hematoxylin. (× 50)

platelets massed into closely packed clumps (Figure 7).[41,51] Other cellular elements are sparse, and there is usually little fibrin. Infection by microorganisms on the surface of these thrombi is thought to be one of the most important mechanisms for the production of bacterial endocarditis.[1] It is of interest to note that mural thrombi of the atria and aorta, although common, rarely become infected. Perhaps the distinctive structure and location of valvular thrombi are related to their susceptibility to infection.

RELATION TO ANTICOAGULANT THERAPY

Experimental studies, both *in vivo* [4,8] and *in vitro*,[30,55] have shown that thrombi may form in the presence of anticoagulated blood. These thrombi contain little fibrin. In this regard, it is probably significant that *in vitro* much less thrombin is required for aggregation of platelets than for fibrin formation.[74] The structure of thrombi in anticoagulated patients is difficult to interpret, because the level of anticoagulation when

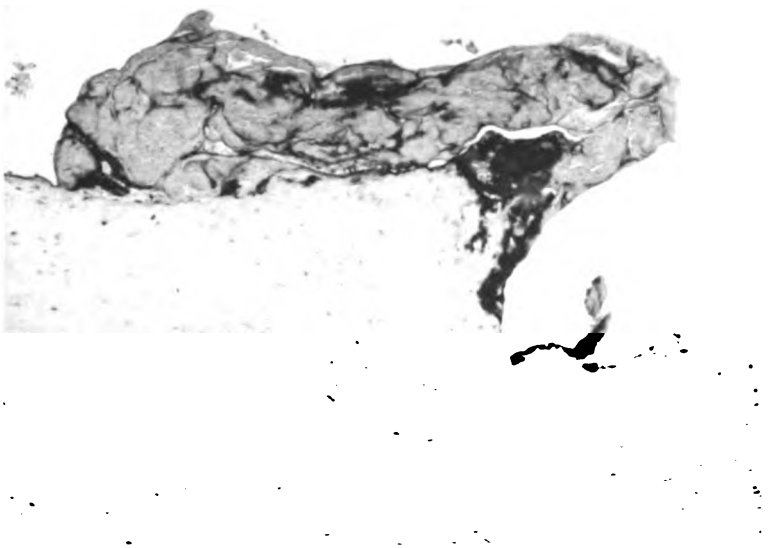

FIGURE 7 Vegetation of an aortic valve. The vegetation is a thrombus composed of compact masses of aggregated platelets and little fibrin (black). A fibrin membrane partially covers the surface. Endothelium was not identified at the seat of attachment, which appears to be made up of both platelets and fibrin. Tannic acid–phosphomolybdic acid–buffalo black, modified. (× 100)

thrombosis occurs is rarely known. It is known, however, that anticoagulation is effective in reducing pulmonary embolism [62]; most pulmonary emboli are propagated thrombi or propagated clots that contain much fibrin.

STRUCTURAL FEATURES IN RELATION TO GROWTH

Thrombi grow in a variety of ways: by progressive uninterrupted accretion, in episodes, by prolongation or propagation along the vessel, and by increase in any one constituent.

The rate of growth of a thrombus is difficult to measure by morphologic studies alone. In some thrombi, all the cellular elements appear to be the same age and the over-all structure is fairly uniform, indicating that formation can take place over a short period. The occurrence of suddenly developing thrombocytopenia in cases of massive venous thrombosis suggests that thrombosis can be quite rapid at times.[47] In other

thrombi, a layered appearance reflects episodic growth (Figures 8 and 9).[18,64,69] The layers often have different structural patterns.[69] Layers of differing age indicate that growth can occur in episodes at variable intervals.[69] The classic example is the layered thrombus of an aortic aneurysm. Oka and Angrist [51] demonstrated by enzyme histochemistry that growth of platelet thrombi on heart valves often occurs in episodes. The diphosphopyridine nucleotide diaphorase reaction is intense in the platelets of fresh deposits on the surface of the thrombi and becomes progressively diminished in the older, deeper layers.

Growth of thrombi in episodes is an old observation,[6] yet perhaps the least questioned. The observation underscores two points: a thrombus can stop forming before it occludes the lumen, and a new thrombus can form on it. Crawford [20] emphasized this aspect of thrombosis. He pointed out that an occlusive thrombus in a coronary artery often forms on an older thrombus. What marks the surface of a thrombus to indicate that

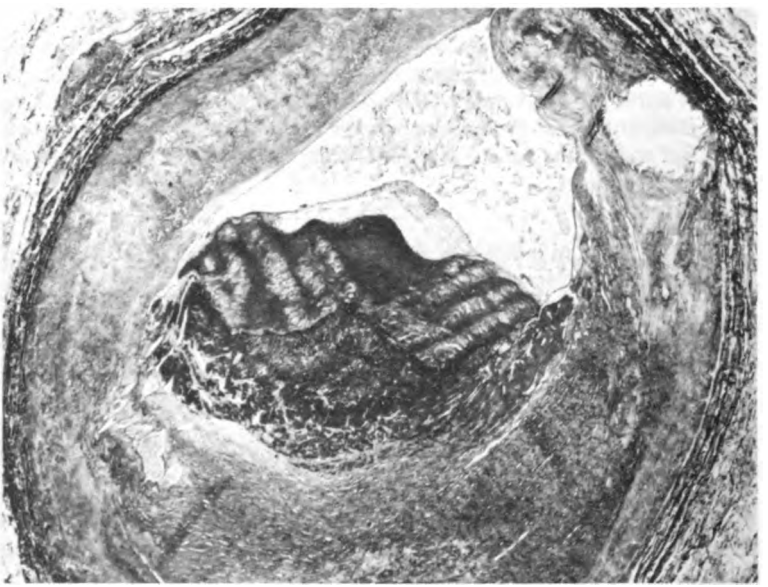

FIGURE 8 Layered thrombus of a coronary artery. Three distinct layers of differing age can be seen. In the most recent top layer, there is much fibrin between the radiating platelet columns. The surface is well demarcated by a layer of fibrin. Capillaries (on the left) are growing into the organizing base of the deepest older layer of the thrombus. Phosphotungstic acid–hematoxylin. (× 5)

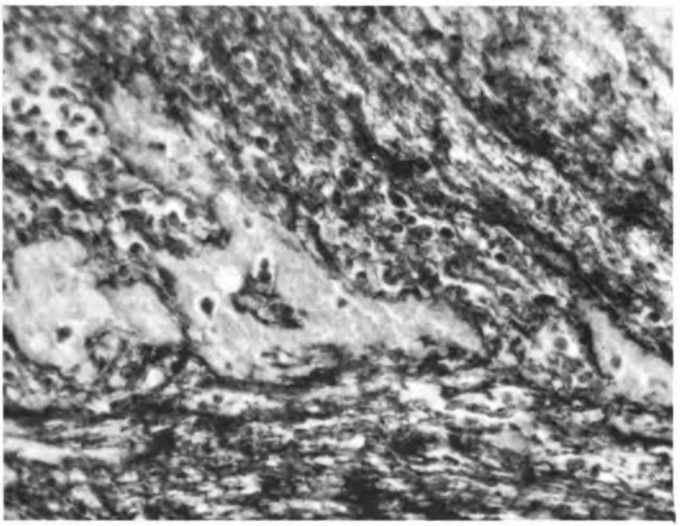

FIGURE 9 Junctional zone between recent and older layers of a thrombus of a pulmonary artery. The two layers are clearly demarcated by the plane of direction of the fibrin strands. Platelet aggregates of the more recent thrombus on top are attached along part of the surface of the older thrombus, which is composed of compressed layers of fibrin at the junction. Phosphotungstic acid–hematoxylin. (× 100)

growth, at least temporarily, has stopped? What changes, if any, take place on the surface to induce the formation of a new thrombus?

The free surface of a thrombus is usually covered by a layer or membrane of fibrin [51,63] that may be oriented in a different plane from the fibrin strands within the thrombus. It has been suggested that traces of residual thrombin on the surface may induce the formation of a new thrombus,[48] but that could not explain the presence of layers of widely differing ages. Detailed studies of the structure and histochemical properties of the surface of thrombi that bear on this problem have not been reported. Perhaps changes of an episodic nature in the blood itself, such as transient hypercoagulability or increased platelet stickiness, are responsible. The relation of platelets and fibrin to the seat of attachment of a new thrombus to an older one has not been worked out.

Growth of thrombi by propagation along the vessel occurs in both veins and arteries but is usually more extensive in the slower currents of veins.[57,64] The rate of formation of thrombin relative to flow is probably an important factor in determining the extent of growth.[28,48] During

gradual occlusion, slowing of flow before complete arrest allows the primary thrombus to propagate from its base of attachment along the vessel in both directions.[2] Usually, many red blood cells are entrapped. With cessation of flow by occlusion of the lumen, the building of a primary and propagated thrombus is completed, because platelets and leukocytes can no longer be brought to the thrombus by flowing blood.[2] However, growth can continue by the formation of a propagated clot.[2,43,69] (The red thrombus described in the older literature was probably what is now considered a propagated clot that forms in stagnant blood.[24]) Thromboplastic substances can diffuse from the thrombus into the contiguous stagnant blood on each side of the thrombus and cause it to coagulate. Because propagated clots and postmortem clots are identical in structure, it may be difficult to establish the antemortem nature of a recent propagated clot unless its connection to the causative thrombus is disclosed.[2,69]

The growth of a thrombus may result from an increase in any of its constituents. The content of fibrin in thrombi may increase, owing to infiltration of plasma.[70] However, collapse and retraction of fibrin strands in a resolving thrombus may give a false impression of increased fibrin formation. It is also reported that leukocytes increase in numbers by invasion from the blood,[70] but again there are other factors to consider. Lysis of granulocytes in the deeper, older layers of a thrombus and their preservation in the superficial, newer layers could cause an apparent increase in leukocytes near the surface. Monocytes persist and increase,[24,36] but whether they infiltrate from the blood or increase by multiplication is not known.

Resolution and organization may be taking place in one part of a thrombus, while growth is continuing in another part. The structure and fate of thrombi are especially important to understand in relation to therapy [23,25,81] and the pathogenesis of atherosclerosis.[15] We have barely explored the changes that take place during resolution and organization by the application of newer histochemical and immunofluorescent techniques and electron microscopy.

SUMMARY

Thrombi of a distinctive structure are built from constituents of blood in a way that, during life, could occur only in flowing blood. Selective accumulation of platelets and leukocytes, the major cellular elements that form a thrombus, can occur as long as flowing blood brings them to a site appropriate for thrombus formation. When flow is stopped by thrombotic occlusion of a vessel, thromboplastic substances can diffuse from

the thrombus into a contiguous column of stagnant blood and cause it to coagulate. This coagulum, like a postmortem clot, contains the cellular elements of blood in their normal concentration and lacks the specific organized structure of a thrombus. Thus, the structure of thrombi (and of clots) is related to the mechanism of their formation.

All cellular elements of the blood may be found in thrombi. They are held together principally by fibrin. Although the proportions of the constituents vary, the basic structure of a thrombus can always be recognized, and it is similar at any site of formation. The basic structural unit, or building block, is composed of aggregated platelets surrounded by fibrin and leukocytes in a characteristic arrangement. The platelets in aggregates are not fused. In histologic sections, the loose arrangement of platelets suggests that the bond between them is not necessarily firm or permanent and points to the importance of fibrin in maintaining the integrity of a thrombus. Fibrin surrounds and links aggregates to stabilize the thrombus, yet renders it susceptible to lysis by fibrinolysins. Granulocytes and monocytes, but not lymphocytes, usually border the platelet aggregates outside the fibrin rim. Granulocytes contribute to the puriform liquefaction of thrombi, and monocytes participate in resolution and organization. An explanation of the paucity of lymphocytes in thrombi might provide a clue to the stickiness of other cellular elements.

The site of origin influences the structure and development of a thrombus, largely by way of the conditions of flow that obtain at a particular site. Structural variations related to the mode of origin, whether on the vessel wall or in the bloodstream, also occur.

Thrombi grow in a variety of ways: by progressive uninterrupted accretion, in episodes, by prolongation or propagation along the vessel, and by increase in any constituent. An important yet poorly understood mechanism of growth is the episodic formation of new layers of thrombus on older layers. Little is known about the structural and histochemical properties of the surface of thrombi in relation to this mechanism.

Studies of the composition and structure of thrombi recently have been expanded by the use of several techniques. The identification of plasma lipoproteins in thrombi by immunofluorescence, the detection of enzymatic reactions in platelet thrombi by enzyme histochemistry, and electron microscopic observations of structural alterations of platelets, including their apposition to the vessel wall and to each other, are some of the recent advances. Correlation of these new observations of the structure of thrombi with the many recent advances concerning the biochemical mechanisms should provide added insight into the pathogenesis of thrombosis.

Studies of *in vitro* thrombi reported here were supported in part by Public Health Service research grant HE 03973 from the National Heart Institute. I am indebted to Drs. G. I. C. Ingram, D. J. McBrien, and J. Spencer for kindly allowing me to review histologic sections of material from their cases of congenital fibrinopenia.

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The Fine Structure of Experimental Thrombi

J. E. FRENCH

The general structure of a thrombus and the distribution of its components can be studied best at the level of the light microscope. The use of relatively large histologic sections of fixed material allows the configuration of the mass within a vessel to be defined and to be oriented with respect to blood flow. In addition, direct microscopic observations can be made on living preparations to provide information about dynamic aspects of thrombus formation that otherwise could at best only be deduced from the appearance of fixed specimens.

The use of the electron microscope is supplementary to these other procedures; what can be said about the fine structure has to be based on the large body of information already obtained by other means. However, with the greater resolving power of the electron microscope, it is possible to describe points of detail that are beyond the range of the light microscope and to illustrate with clarity some aspects of thrombus structure that were previously uncertain.

EXPERIMENTAL MODELS USED IN ELECTRON MICROSCOPY

In studies of fine structure, it is usually preferable to use experimental thrombi, or other models of comparable structure, rather than the natural thrombi found in man, because the age and site of origin of the thrombus can be controlled and tissues can be selected in such a way that they allow optimal conditions for sampling and fixation.

EXPERIMENTAL THROMBOSIS *in Vivo*

Direct Injury The classical studies by light microscopy on thrombus structure were made on small vessels that had been injured by chemical

or physical means.[54] This type of procedure has now been followed in several studies by electron microscopy, including injury by irradiation of small vessels in the pituitary [9] and lung [32]; injury by crushing of small vessels in the mesentery and cerebral cortex of the rabbit [22] and in the tongue of the frog [15]; injury to the marginal ear vein with a nylon sound in the rabbit [51]; mildly traumatized vessels in the carotid body of the cat [49]; and injury to small arteries and veins in the cheek pouch of the hamster by an electrical stimulus.[18] Occlusive thrombi in veins have been induced by injecting a sclerosing agent into a temporarily ligated segment of the femoral vein in rats.[56] Electron micrographs of small thrombi have also been published as incidental observations in other studies on injured vessels.[43] Recent examples include the platelet aggregates that form in small vessels in healing muscle wounds [46] and in skin injured by heat.[8]

Introduction of Foreign Surfaces A thrombus can be induced *in vivo* by deliberately placing a foreign surface in contact with the bloodstream. A convenient method is to pass a suture of silk, catgut, or collagen through the lumen of a vessel, and this method has been used in electron microscopic studies in veins in the rat,[3] in the aorta of the rabbit,[11] and in the carotid artery of the pig.[27] The deposit that forms on the inner surface of a fabric graft inserted into the arterial circulation is essentially a mural thrombus [13] and has been used as a model for studying some of the stages of thrombus organization by electron microscopy.[40,41]

Infusion of Platelet-Aggregating Agents Some agents that induce the aggregation of platelets *in vitro* will also induce aggregation when infused into the circulation of living animals. Because the aggregates are trapped, at least temporarily, in small vessels in the lung or kidney, they provide a model for the study of some aspects of intravascular thrombosis. The fine structure of aggregates of this type has been described following infusion of thrombin,[47,53] adenosine diphosphate,[37] and various types of small particles, including an artificial lipid emulsion.[17] The extensive thrombosis that can be induced by injecting small amounts of some unesterified fatty acids has also been investigated by electron microscopy.[21]

The Hemostatic Plug The hemostatic plug closely resembles an experimental thrombus in structure and provides a satisfactory model, in that it can easily be located and is relatively immovable once formed. Accounts have been given of the fine structure of the plug that forms at the cut ends of small arteries and veins in the mesentery in rabbits, rats,

guinea pigs,[25,28] and dogs [34] and at the cut ends of small arteries in the hamster cheek-pouch.[16,18]

EXPERIMENTAL THROMBI *in Vitro*

Attempts have been made to bridge the gap between the clotting of blood in a test tube and the formation of a thrombus in living vessels by constructing model systems in which the effects of blood flow on platelet behavior and blood coagulation can be observed. These so-called artificial thrombi can be obtained from extracorporeal shunts, with the animal itself acting as the pump,[35] or in tubes of glass or plastic through which the blood is circulated by an artificial mechanism. The method devised by Chandler,[7] in which fresh whole blood or recalcified citrated plasma is made to flow through a circular loop of plastic tubing until a solid object forms at the leading edge, provides a convenient and easily produced model that is strikingly similar in its structure to other forms of experimental thrombi.[39] Artificial thrombi produced in this way have been examined directly by electron microscopy [19] or have been introduced into the circulation as emboli, which permits their organization to be observed after their impaction in the pulmonary vessels.[6,50] Contributions to the study of thrombus structure have also been made on the basis of changes observed in platelets during the course of blood coagulation in more static systems,[45] but these will not be considered in detail here.

STAGES OF THROMBUS DEVELOPMENT IN RELATION TO STRUCTURE

Microscopic observations on experimental thrombi have established that the gross structure of a fully developed thrombus is the end result of an ordered sequence of events.[36,42] These events can be considered as distinct stages in thrombus growth, although the stages often overlap and the rate at which they occur varies widely in different experimental situations. The first stage is the accumulation of platelets at the site; platelets then attach to the wall, become highly packed, and form a mass consisting predominantly of platelets (Figure 1), which may rise to multiple small emboli or continue to build up until it impedes blood flow. When the latter occurs, platelets at the periphery of the mass undergo morphologic changes, leukocytes adhere to the platelets, and the presence of fibrin becomes obvious. Coagulation may now extend into the stagnating column of blood; the formation of the fresh thrombus is complete. With the pas-

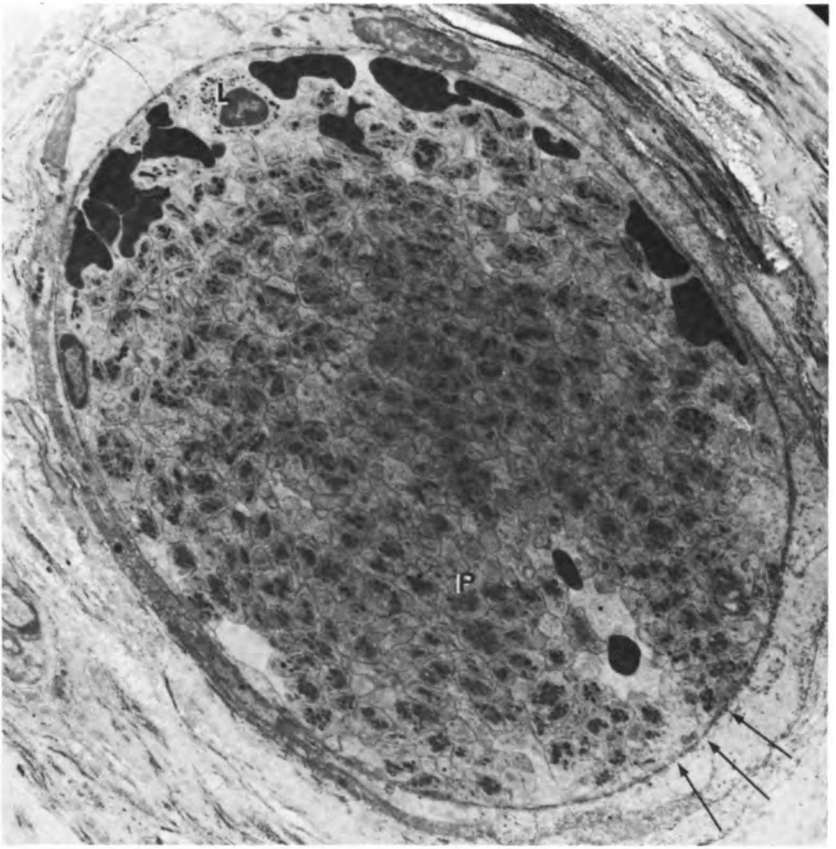

FIGURE 1 A thrombus at the site of an electrical injury (arrows) in a small artery in a hamster cheek pouch consists almost entirely of closely packed platelets. A leukocyte (L) and a few erythrocytes are seen at the edges of the platelet mass. P, platelets. (× 2825) (Reprinted with permission from French *et al.*[18])

sage of time, further changes occur: there is an increase in the amount of fibrin and in the number of leukocytes, to be followed by autolytic and degenerative changes and eventual organization. The contributions of electron microscopy to an understanding of thrombus structure will be considered in relation to these various stages.

THE INITIAL STAGES

Platelet Accumulation A local accumulation of loosely arranged platelets is the first detectable event in thrombus formation in injured vessels.[43]

The individual platelets are well preserved at this stage; they have retained their normal shape and appear to touch one another only at a few points.[32] Some change in the morphology of the granules in the loosely aggregated platelets has been described, and there is a suggestion that there may be an increase in the concentration of some amorphous material outside the plasma membranes at points of platelet–platelet contact.[49]

Adhesion of Platelets to the Wall Platelets do not normally adhere to the endothelial lining of the blood vessels. In most of the techniques used to induce experimental thrombosis, there is an actual destruction of endothelium or a deliberate placement of a foreign surface in contact with the blood. In the former case, the site of attachment of the thrombus is seen to be in the region where endothelium is lost and platelets have made contact with exposed subendothelial tissue [18,51]; in the latter, platelets adhere to the foreign surface.[35]

In the injured vessels, the exposed tissue consists of elastin, collagen, and intimal ground substance, so it is not possible to say precisely to which element the platelets are adhering (Figure 2). However, in some of the other models, it is more clear that collagen fibers are primarily involved. Apparent adhesion of platelets to *extravascular* collagen fibers has been observed in the hemostatic plug [18,28] and in arterial thrombi induced by a collagen suture.[27] From other studies on the properties of platelets *in vitro,* it also seems likely that collagen, rather than elastic tissue, is responsible for the effect.[48] A special property of the collagen surface in relation to adhesion is further suggested by the finding that in *in vitro* systems the approximation of the platelet plasma membrane to the collagen fiber can be extremely close (100–200 Å), which may indicate some specific interaction of the two surfaces.[12] The adherent platelets, either at the base of the thrombus [51] or in contact with collagen fibers in other situations,[18,27,28] have undergone structural changes that indicate swelling and loss of granules. Some of the platelets in contact with collagen fibers may show breaks in the continuity of their surface membranes.[27]

In some situations, the adhesion of platelets to subendothelial tissue can be achieved as a consequence of minute defects in the endothelial lining. In mildly injured vessels, a gap may be present between adjacent endothelial cells that allows only a single platelet to become interposed (Figure 3). Several electron micrographs illustrating this phenomenon have been published [18,31,33,38]; it may have some relevance to the attachment of thrombi to the wall in veins, in circumstances in which it is not possible to demonstrate endothelial defects by light microscopy.

There is no clear evidence that platelets can adhere tenaciously to a

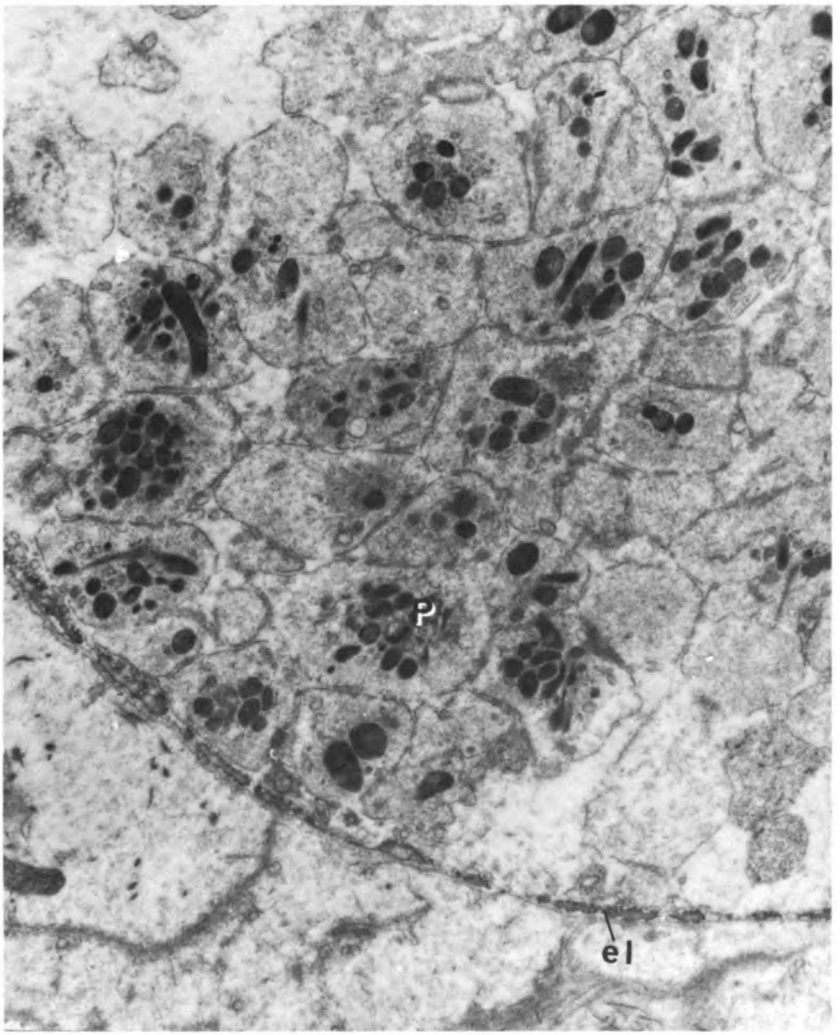

FIGURE 2 Attached edge of the thrombus shown in Figure 1. The endothelium is missing and platelets (P) are in contact with the exposed subendothelial tissue, which includes elastic fibers (el). (× 11,600) (Reprinted with permission from French *et al.*[18])

morphologically intact endothelial surface. Degenerate or vacuolated endothelial cells have been observed in injured vessels, but adherence of platelets apparently does not occur until the injured cells have desquamated.[32,52] A layer of electron-dense material, between endothelial cells

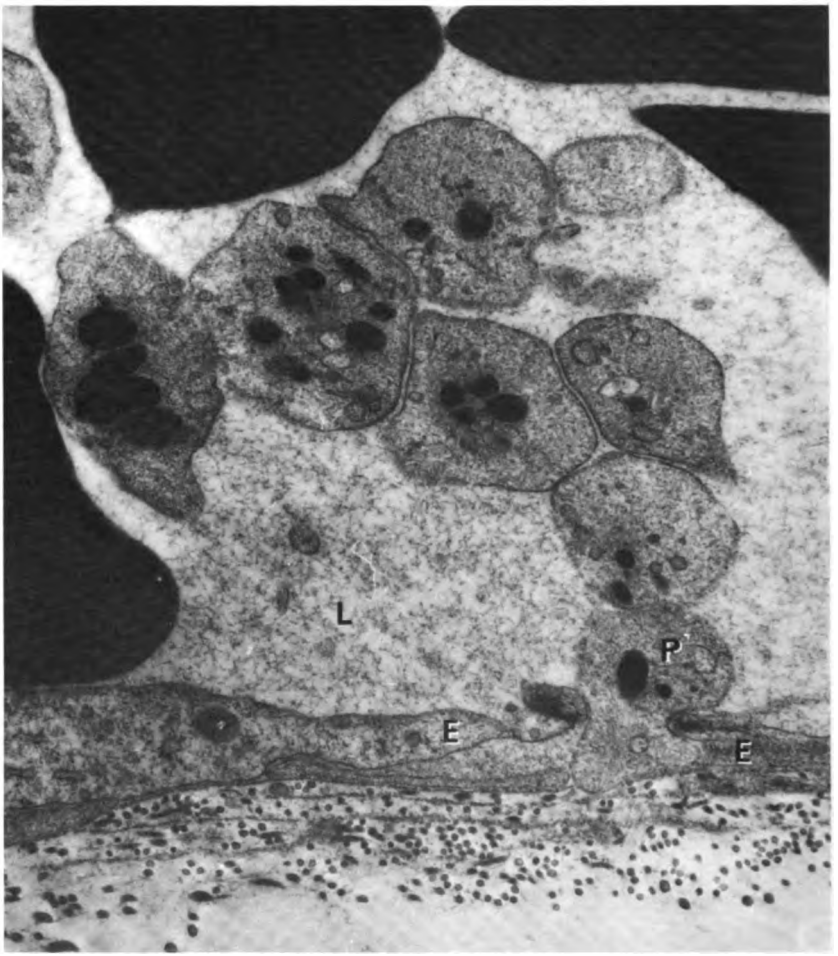

FIGURE 3 Part of the wall of a small vein in a hamster cheek pouch shows a group of platelets (P) in the lumen (L). One of the platelets occupies an intercellular gap in the endothelium (E). (× 22,900) (Reprinted with permission from French *et al.*[18])

and apparently adherent platelets, has been described in small vessels in rat skin after mild injury by heat,[8] but the significance of that finding is not yet clear. Sometimes when a group of platelets appear, in a particular section, to be adhering to normal endothelium, sections at different levels reveal a nearby endothelial defect, around which the platelets are grouped.[1,14]

Platelet Aggregates A mass of aggregated platelets forms the central core of the developing thrombus. On the basis of light microscopy, it was thought that platelets had fused together at this stage, but electron microscopy has shown that the appearance is due to a very tight packing of the platelets. In thin sections, a close and mainly regular approximation of adjacent plasma membranes gives a characteristic mosaic pattern (Figure 4), which is mentioned in most accounts of the fine structure of experimental thrombi of the various types. It has been illustrated, for example, in hemostatic plugs,[28] in artificial thrombi produced by Chandler's method,[19] and in thrombi within injured vessels.[18,32] It has also been described in sections of the platelet aggregates induced by adenosine diphosphate (ADP), both *in vitro*[24] and *in vivo*.[37] In the center of the mass, the individual platelets have in general retained their internal structure, but there are some irregularities in surface contours and some platelet profiles that appear less dense than others. This can be taken as evidence that, within the limitations of such a compact structure, the platelets are swollen and have formed outward projections of their hyaloplasm.[11,34,49] These changes are, however, much less pronounced than the platelet changes at the periphery of the aggregates.

The apparent gap between the apposed plasma membranes is some 200 Å, widening somewhat in the angles formed where three platelets come together. It is generally agreed that there is no material identifiable as polymerized fibrin within this narrow space between the platelet surfaces in the center of the mass, but the question of what does occupy this space has still to be settled. Regularly arranged threads or filaments have been described as bridging the gap in platelet aggregates induced by addition of ADP to blood *in vitro,* and it has been suggested that these filaments represent extracellular material, possibly fibrinogen. A further possibility is that the gap is more apparent than real, and that a much closer approximation is achieved by a component of the platelet membranes that is not shown by the standard electron microscopic techniques.

It is now recognized that there is an outer coating, probably rich in carbohydrate, on the outer surface of the plasma membrane of many different types of cell; this has been termed the "glycocalyx" or "cell coat." The coat is not readily seen by standard methods, but can be shown up by adaptation of histochemical techniques to electron microscopy[44]; its density is increased by addition of lanthanum salts to the fixative[29] or by use of methods that are thought to stain acid mucopolysaccharides—namely, addition of ruthenium red dye to the fixative[30] and the colloidal iron procedure.[20] Evidence is accumulating that platelets have a cell coat of this type. Several reports on the fine structure of individual platelets have mentioned the presence of poorly defined amor-

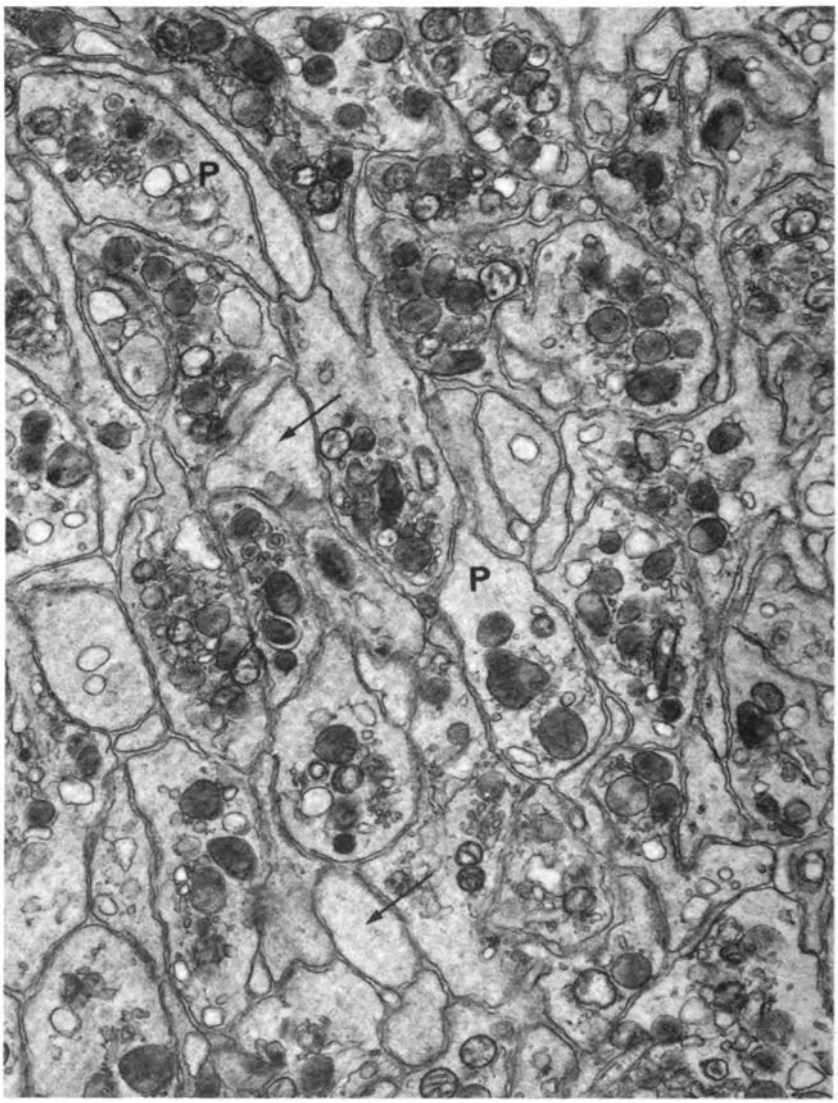

FIGURE 4 Mosaic arrangement of tightly packed platelets (P) at the center of a platelet aggregate in an artificial thrombus. Platelets have retained their organelles and show intact boundary membranes, but a few less dense profiles (arrows) indicate the formation of pseudopodia. (× 14,200)

phous material at the surface of the plasma membranes, and it appears on the basis of negatively stained preparations that this amorphous material corresponds to a cell coat with an adsorbed cloud of plasma protein.[5] The amorphous material at the platelet surface has the ability to bind colloidal iron,[2] and its density is increased by ruthenium red (Figure 5), which suggests that it, too, has a polysaccharide component. Further support comes from the finding that polysaccharide material is released into the medium when platelet suspensions are incubated in the presence of some carbohydrases (e.g., neuraminidase and α-amylase) or proteases that disrupt carbohydrate–protein complexes (e.g., pronase and trypsin).[23]

If those findings can be substantiated, then it seems probable that at least part of the apparent gap between the platelets in the aggregates is occupied by the outer coating or glycocalyx of the adjacent plasma membranes. We have therefore attempted to stain, with ruthenium red and with lanthanum nitrate, the interspace in the platelet aggregates in artificial thrombi prepared from native rat blood. These stains were added in the fixative and apparently penetrated the platelet mass with difficulty, but a positive result was obtained at the edges of the block. The increased density of the interspace that we observed is illustrated in Figures 6 and 7. However, until more is known about the histochemical specificity of these methods, it is unwise to conclude too much about the nature of the material displayed.

Stabilization of Platelet Thrombi The morphologic changes in platelets that apparently increase the stability of an early thrombus occur principally at the periphery of the platelet mass. These changes occur at variable intervals in intravascular platelet aggregates, presumably depending on the effectiveness of the platelet mass in reducing blood flow,[11,18,34] but similar changes occur rapidly at the edges of the hemostatic plug,[18,28] at the edges of platelet aggregates in artificial thrombi,[19] and in the platelet aggregates that may form during the coagulation of blood.[45] They consist of platelet swelling and loss of granules; the platelet aggregates become surrounded by a fringe of saccular bodies that have a lower density than deeper platelets in the mass and contain no organelles other than vesicles of irregular size.

Some of these nongranular bodies may represent platelets from which the organelles have been lost, but, when the plane of section is favorable in the artificial thrombi, some can be recognized as outward projections, or pseudopodia, of hyaloplasm from deeper platelets (Figure 8). The mechanism by which such pseudopodia are formed is not clear, but it has been shown in glutaraldehyde-fixed material from venous thrombi

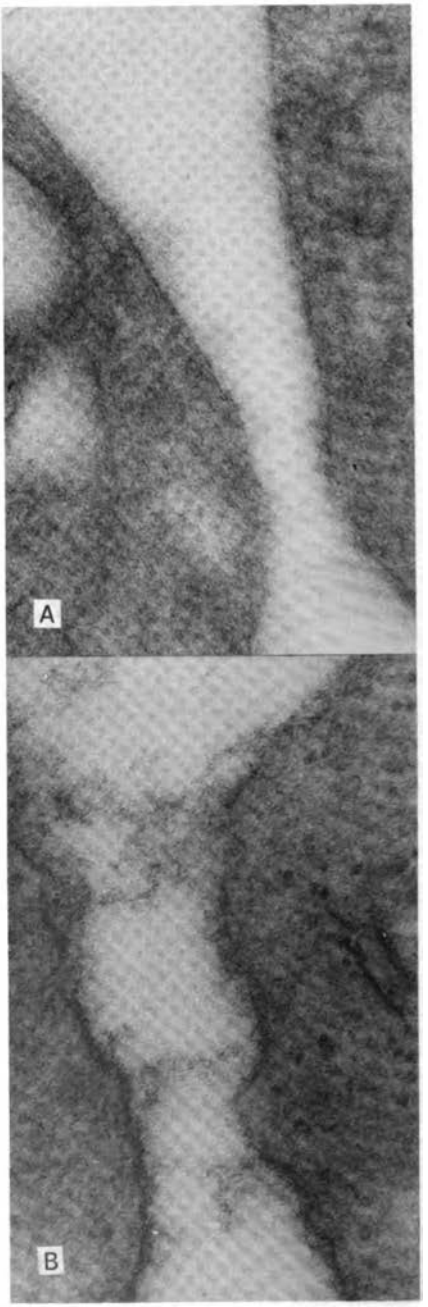

FIGURE 5 The surface of platelets in platelet-rich plasma from a rat, as seen after fixation in glutaraldehyde followed by osmium tetroxide. (A) Poorly defined amorphous material at the surface in an unstained preparation. (B) Increased density of this material can be seen when ruthenium red is added to the fixatives. (× 94,000)

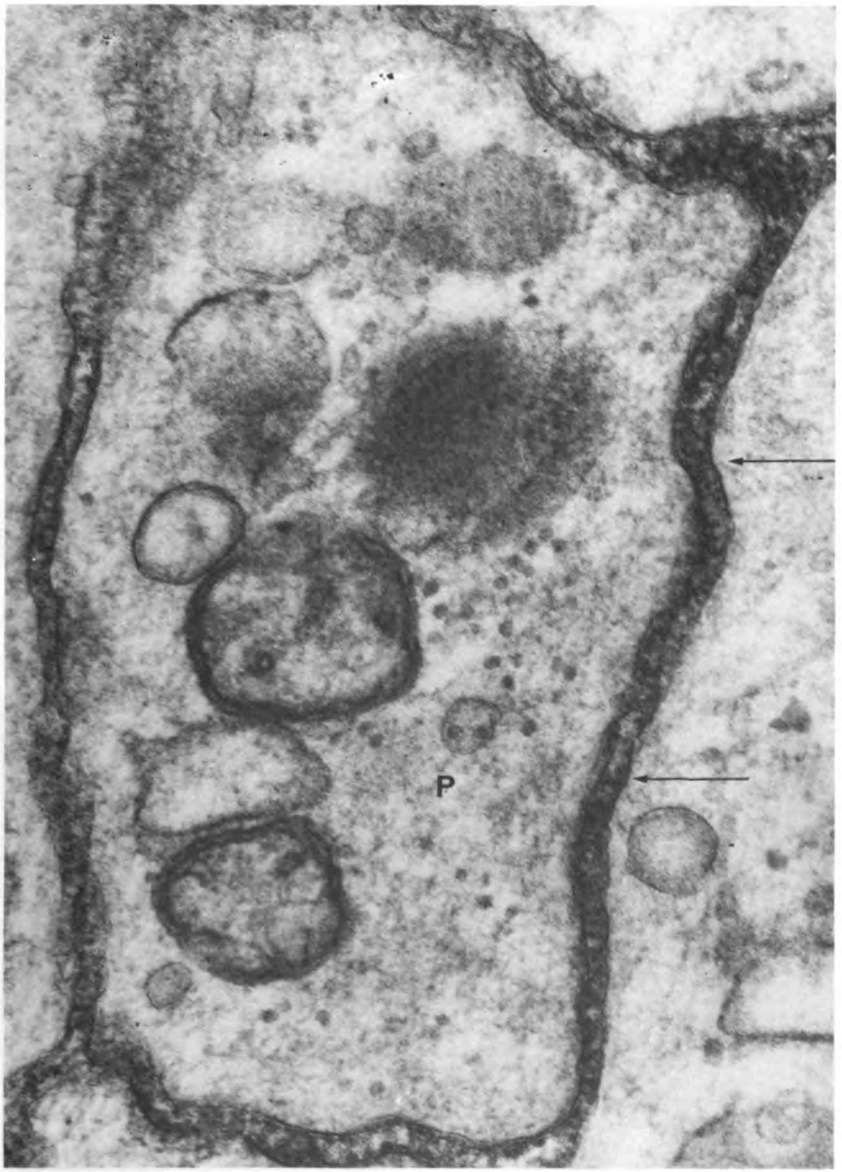

FIGURE 6 A platelet (P) in a compact aggregate in an artificial thrombus that was fixed in osmium tetroxide containing ruthenium red. The apparent space separating the plasma membrane from adjacent platelets (arrows) is occupied by densely stained material. (× 99,000)

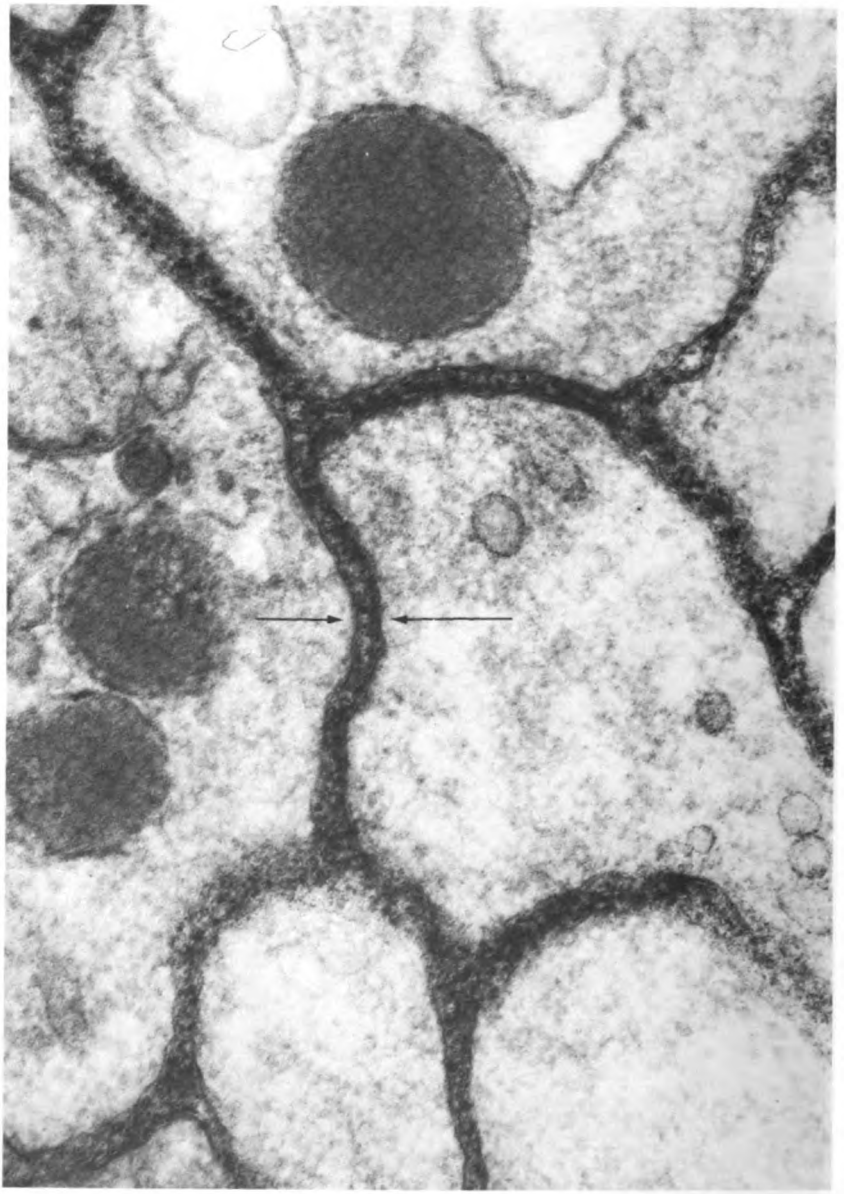

FIGURE 7 A group of tightly packed platelets in an artificial thrombus that was fixed in osmium tetroxide buffered with *s*-collidine and containing 0.75% lanthanum nitrate. The apparent space between the apposed plasma membranes (arrows) is irregularly stained. (× 107,600)

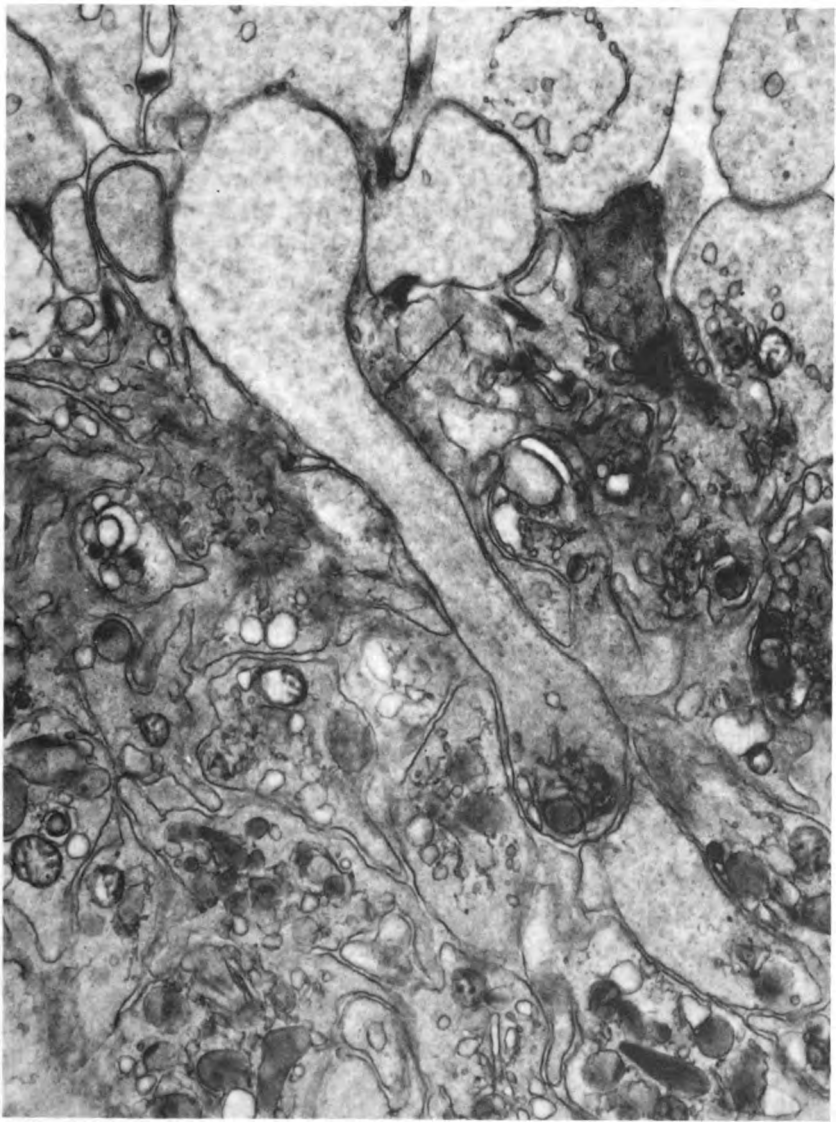

FIGURE 8 The edge of a platelet aggregate in an artificial thrombus shows an outward projection of hyaloplasm (arrow) from a platelet situated deeply in the mass. (× 22,700)

that the bundle of microtubules, which is curved around the perimeter in fresh platelets, becomes reoriented linearly in the pseudopodia.[3]

There is also uncertainty about the way in which the granules are lost from the platelets. In clotting blood, some granules appear to disintegrate within the hyaloplasm by loss of their limiting membrane and coalescence of their contents into a loose osmiophilic mass.[55] Others may be discharged at the surface, although there is little evidence that complete granules are ever observed outside the platelet membrane. In the artificial thrombi, we have seen examples of an apparent fusion of the limiting membrane of a granule with the platelet plasma membrane, so that a flask-shaped structure is formed, whose contents have access to the exterior.[19]

Concurrently with the morphologic changes in the peripheral rim of platelets, fibrin makes its first appearance in the developing thrombus as a network of fine strands around the edges of the nongranular bodies. These strands are most prominent at the extreme edges, where the spaces between nongranular bodies appear to be wider, but may extend inward for a short distance among the more tightly packed part of the fringe. There is, however, no extension of visible fibrin into the central parts of the main platelet mass at this stage.[11,19,34] The formation of fibrin may have a specific spatial relationship to the altered platelets and to the disintegration of their granules, but investigators of this problem have so far been concerned more with blood coagulation *in vitro* than with experimental thrombi.[55] It is usually considered that thrombin is activated at this stage of thrombus formation, and this belief is supported by the finding that a platelet–fibrin aggregate with similar structural features is formed *in vitro* when thrombin is added to agitated platelet-rich plasma [24] or *in vivo* when thrombin is infused intravascularly.[53]

Leukocytes are not distributed at random in the thrombus but are concentrated at the edges of the aggregates, where they appear to adhere to nongranular bodies rather than to intact platelets. This relationship is seen clearly in the artificial thrombi, in which granulocytes, monocytes, and (more rarely) lymphocytes are seen adhering to the nongranular fringe. They are separated from the nongranular bodies by narrow gaps about the size of those between adjacent platelets.[3,19]

THE LATER STAGES

Further alterations in the platelets and in the distribution of fibrin and leukocytes occur when thrombi persist *in situ*. Within a few hours, the platelet aggregates appear less densely packed, and the fibrin at the edges of the aggregates forms coarser strands.[27] By 24 hr, platelets

throughout the mass show a varying degree of degeneration of internal structure but can still be recognized by the persistence of their boundary membranes and, to a lesser extent, of their organelles. The platelets or platelet remnants are more widely spaced at this stage and, in contrast with fresh thrombi, fibrin is readily seen in the spaces between them.[11,16,27,56] This gradual extension of fibrin into the platelet mass as it disintegrates accounts for the apparent fibrinous transformation of hemostatic plugs and thrombi, which has been observed by light microscopy.[26] The additional fibrin is probably derived from plasma that infiltrates the platelet mass as it shrinks or breaks up; but, inasmuch as the platelets themselves are known to contain fibrinogen,[4] platelet fibrinogen is a possible alternative source.

It is not known how long recognizable platelets can persist in the thrombus. Apparently intact platelets have been demonstrated in mural thrombi in aortic grafts that had been inserted 1 week or more previously.[10,13] However, in some experimental thrombi, an accumulation of intact platelets has been observed outside the zone of platelet degranulation and dense fibrin formation in an older deposit.[11,27] Thus, it appears that the growth of a thrombus can be episodic, and that the age of the platelet deposits observed by sampling does not necessarily correspond to the age of the thrombus as a whole.

The leukocytes that are incorporated into the thrombus as it forms or invade it later probably play an important part by the release of autolytic enzymes and the phagocytosis of debris in the later stages. The number of granulocytes increases during the first few hours, but these cells soon show degenerative changes and few can be recognized after 1 week.[6,27] Macrophages, derived from blood monocytes, appear early in thrombi and emboli. These cells are active in the removal of cell debris, and, from about the third day on, they are seen to contain granular material and breakdown products of ingested red cells and hemoglobin.[27,50,56] The ability of macrophages to ingest whole platelets in resolving thrombi, which was recognized by light microscopy, has been confirmed by electron microscopy in the mural thrombi lining aortic grafts,[40] in mural arterial thrombi induced by a suture,[27] and in platelet-rich thromboemboli.[50] In the emboli, the macrophages are gradually transformed into highly vacuolated foam cells whose lipid is presumably derived from the ingested platelets,[6,50] but this change is less striking in the mural thrombi. The reason for the difference is not clear; it may be related to the more extensive extracellular disintegration of platelets that is observed in mural thrombi.[27]

There is a progressive reduction in the amount of fibrin during the first week [56] and the fibrin that remains becomes less fibrillary and more

amorphous.[6] The loss of fibrin, presumably as a result of fibrinolysis, is most marked where the thrombus is in contact with circulating blood,[27] but, inasmuch as clear zones are seen around leukocytes, particularly macrophages, these cells probably play a role in the fibrinoyltic process.[6,27,50] Phagocytosis of fibrin by macrophages has been described [6,50] but does not appear to be on a scale that could account for a major part of the removal of fibrin.[27,56]

The final stage in the natural history of a thrombus is organization, with penetration of its substance by mesenchymal cells and growth of endothelium over its free surfaces. Cells with the morphologic features of fibroblasts have been described in the first few days; but, by 1 week, cells that contain bundles of peripherally arranged myofibrils are seen, and thereafter most of the cells organizing the thrombus have the features of vascular smooth muscle.[6,34,41] Because these cells apparently have an ability to synthesize collagen and elastin,[14] the thrombus may gradually be transformed into a tissue that is rich in collagen and elastic fibers, in which smooth muscle is the predominant cell type. The development of new endothelium appears to be rapid; in small experimental thrombi or emboli, the surface is covered by the end of the first week by cells that seem to be actively growing and to have some phagocytic properties [27,50]; but there is some doubt about the true nature of these cells. In the aortic grafts, growth of endothelium has been shown to occur from pre-existing endothelium at the edges and from the mouths of small vascular channels that open into the lumen of the graft.[41] In other accounts of the fine structure of organizing thrombi, it has been said that the appearance of the new endothelium and of fibroblasts near the surface is compatible with an origin in bloodborne cells.[27,50] It seems likely, however, that a definitive answer to this difficult question will not be provided by electron microscopy but will require further investigation by other techniques, including cell-labeling.

SUMMARY

The use of electron microscopy in the investigation of thrombus structure supplements observations with the light microscope. It has enabled some points that were already known to be illustrated with greater clarity, and it has added new points of detail.

In the initial stages of thrombus formation, adhesion of platelets to the vessel wall is associated with exposure of subendothelial tissue, either by complete loss of endothelium or by microscopic defects that interrupt endothelial continuity. Platelets in the center of the thrombus

are not fused, as had been thought, but very tightly packed. An apparent space of approximately 200 Å between adjacent platelets is probably occupied by a coating on the surfaces of the apposed membranes, analogous to the cell coat or glycocalyx in other types of cell.

At a later stage, changes in the platelet mass apparently increase its stability. Platelets at the edges become swollen and form outward projections of their cytoplasm; some lose their granules. Fibrin appears first at the edges of the platelet aggregates and eventually forms a network in the spaces between them. There is no obvious fibrin in the center of the aggregates at this stage.

Still later, when light microscopy shows a wider distribution of fibrin in the thrombus, platelets or platelet remnants can still be identified by electron microscopy; but they are now more widely spaced and strands of fibrin can be seen in the spaces between them. Finally, when the thrombus undergoes resolution or organization, electron microscopy has been used to confirm the phagocytosis of platelets and other debris by macrophages, the development of lipid-filled foam cells, and the gradual replacement of the mass by fibroelastic tissue in which the predominant cell type has the morphologic features of vascular smooth muscle.

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The Pathology of Venous Thrombi

J. C. PATERSON

Venous thrombi are ubiquitous: they can be demonstrated microscopically in areas of tissue injury from practically any cause, usually as microthrombi in capillaries or in small venules.[4] They may also be found in larger veins not necessarily in relation to obvious injury, e.g., in the prostatic plexus of veins in elderly men, in hemorrhoids, in the portal vein system in some cirrhotics,[6] and in the right atrial appendage in patients with congestive heart failure. Exceeding all those in frequency and in embolic potential are the thrombi that occur in the deep veins of the pelvis and legs, and most of the comments that follow will pertain to such thrombi.

The incidence of venous thrombi of the legs varies considerably in different hospital populations; and there is reason to believe that it varies with age, with sex, and perhaps even with race and diet. However, the greatest variable seems to be the way in which venous thrombi are looked for in the course of routine postmortem examination. It is generally admitted that complete dissection of the veins of the legs at autopsy gives the only accurate picture of the incidence of the disease. Unfortunately, few complete dissections are performed, because of the restrictions that are placed on postmortem venous dissection of the legs by most hospitals.[2] Using careful dissection, however, McLachlin and I [9] reported a 34% incidence in unselected autopsies on male patients over the age of 40, whereas Sevitt and Gallagher [18] found a much higher incidence (86%) in elderly persons who had recently suffered fractures of the head of the femur.

Venous thrombi of the legs are of two general types, inflammatory and bland. The distinction is usually made on the basis of microscopic study: when thrombi are associated with an obvious injury in the underlying vein wall, such as an inflammatory reaction or a laceration, the condi-

tion is classified as inflammatory and called "thrombophlebitis," and when they are found in normal-appearing veins, the condition is classified as bland and called "phlebothrombosis." The distinction is thought by some to be important, in that, it is claimed, the inflammatory variety rarely results in embolism, presumably because the attachment of the thrombus to the vein wall is enhanced by the inflammatory process. However, there is some evidence that patients with thrombophlebitis are by no means immune to the catastrophe of pulmonary embolism [2,9]; either of the two varieties of thrombus may have a floating tail that is susceptible to fracture and embolism.

Over the last century opinions as to the exact site of initiation of venous thrombi of the legs have gone full-circle. The main point at issue is whether the thrombi arise proximal or distal to the point at which the deep and superficial femoral veins join to form the common femoral vein. Virchow [19] originally advanced the idea that pulmonary emboli arise from thrombi that are laid down on the central sides of valves of the femoral and iliac veins. Approximately 100 years later, McLachlin and I [9] voiced a similar opinion after complete dissection of the veins of the legs in a large series of deaths of men over the age of 40; we found that the point of origin of thrombi was proximal to the origin of the common femoral vein (37 instances) as often as distal to it (39 instances). These findings have been confirmed in a general way by Sevitt and Gallagher.[18] However, between 1846 and 1951, most investigators stressed the importance of the small veins of the calf muscles as sites of primary thrombosis —notably, Rössle,[16] Neumann,[10] and Hunter and co-workers.[7] Bauer,[1] on the basis of phlebographic studies, concluded that, in 98% of cases of deep venous thrombosis, the process begins in calf veins. The only dissenting voice during that period was that of Frykholm,[5] who, although admitting the importance of the primary site in the calf veins, found an equal number of thrombi that began above the point of origin of the common femoral vein.

MACROSCOPIC CHARACTERISTICS

When suspected thrombi are found in veins during the course of an autopsy, the pathologist can often tell them from postmortem clots by their macroscopic appearance. True thrombi are dry and granular, friable, variegated, corrugated on some part of their surface (lines of Zahn), and adherent to the vein wall at their point of initiation (Figure 1). Postmortem clots, on the contrary, are moist and shiny, elastic, smooth, reddish-black, and readily detached from the vein wall. These macro-

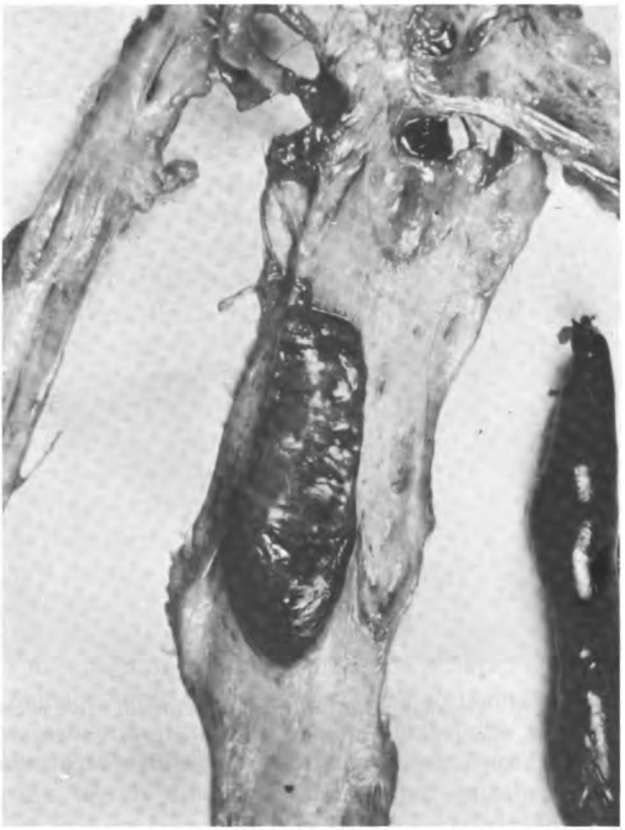

FIGURE 1 Photograph of a thrombus that arose in a valve pocket at the upper end of the superficial femoral vein. A postmortem clot is shown at the right for comparison. The thrombus had fractured, embolized to the lung, and caused sudden death. (Reprinted with permission from McLachlin and Paterson.[9])

scopic distinctions apply particularly to sizable thrombi. In early incipient lesions, particularly those in capillaries, venules, and the valve pockets of veins, only the microscopic criteria can be used.

A second major macroscopic feature of bland venous thrombi is that they are laid down almost invariably within valve pockets of the veins of the leg, thigh, and pelvis [12,19] (Figure 2). However, not all incipient lesions remain confined to the valve pockets; as they grow by accretion, they blossom out from the pockets until they fill, or almost fill, the lumen of the vein. When this occurs, propagation probably is in both directions;

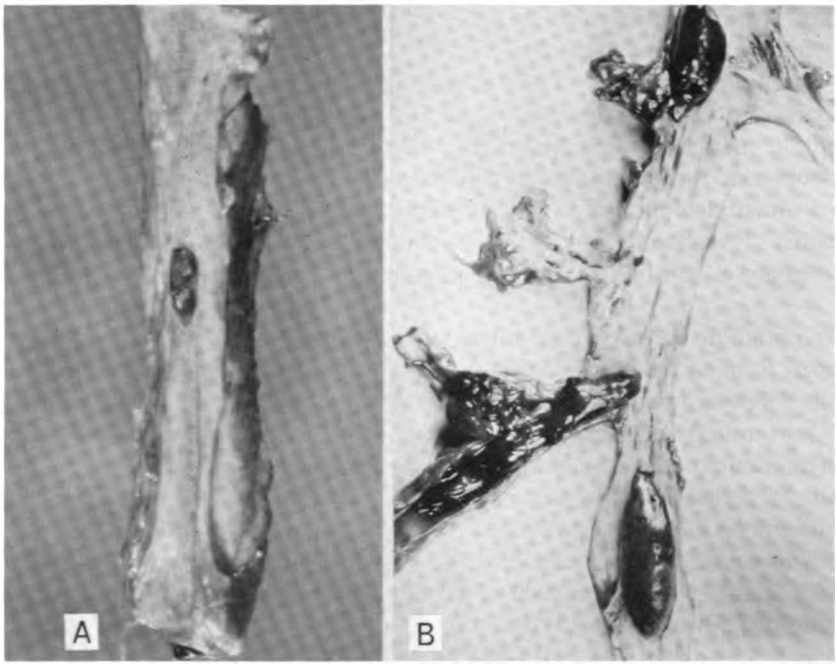

FIGURE 2 Photographs of thrombi that arose in valve pockets in the deep veins of the legs. Blood flow is upward. The lesion in A was the only one found in the patient. (Figure 2B reprinted with permission from McLachlin and Paterson.[9])

as a result, the primary site of deposition is obscured. Macroscopically, most parts of these propagated masses are often more like postmortem clots than like true thrombi, suggesting that the role of hypercoagulability has in large part superseded that of platelet conglutination. Stasis of blood flow seems to have considerable importance in the formation of early lesions within valve pockets. Usually, the valve pocket is the part of the vein in which stasis is most severe. McLachlin and co-workers [8] injected radiopaque materials into the ankle veins of normal subjects, and showed: (1) that, although the leg veins empty fairly quickly when the subject is horizontal and supine, the valve pockets do not; (2) that the valve pockets empty quickly when the legs are 15 deg above the horizontal; and (3) that the valve pockets empty more quickly in young than in elderly patients. Along the same lines, Wright and Osborn [20] had previously shown by tracer techniques that posture plays an important part in the speed of venous return from the legs: the flow rate is doubled when the legs are as little as 10 deg above the horizontal.

MICROSCOPIC CHARACTERISTICS

The microscopic appearance of venous thrombi at their points of origin is best studied in the small lesions that are seen on occasion in valve pockets of the deep veins. The words "on occasion" are used advisedly: G. L. Medina and I have identified such small thrombi only five times during microscopic examinations of 146 valve pockets of the superficial femoral veins in 26 unselected autopsies on male patients over the age of 40. Four additional suspected lesions that distended valve pockets were interpreted microscopically as postmortem clots; and there were also three examples in the series of full-blown propagated thrombi, either recent or organized. The impression from this small series, that not all incipient thrombi progress to the propagated casualty-producing stage, may well have practical implications.

The distinction between a thrombus and a postmortem clot within the valve pocket of a vein can be made only by microscopic study; the macroscopic criteria described above cannot be applied, because the masses are so small as to be hidden from view. In general, the diagnosis of "thrombus" is justified (1) if definite clumps of platelets are seen, whether they are or are not encircled by condensed skeins of fibrin (Figure 3), or (2) if signs of organization can be made out (Figure 4). Occasionally, the platelet–fibrin components may be so compressed or so old that platelets cannot be identified; but thrombi of this type will usually show invasion by fibroblasts, capillaries, or both. Loose red-cell and fibrin masses that merely distend a valve pocket, so-called stasis thrombi, Figure 5, should not be regarded as thrombi, nor should loose fibrin collections ("chicken-fat" clots, Figure 6), homogeneous plasma precipitates, or aggregates of leukocytes or of red cells (sludged blood). In fact, the criteria for the diagnosis of small venous thrombi are like those listed recently by Eeles and Sevitt [4] for the identification of microthrombi in capillaries or small arteries.

A thrombus that has propagated to the orifice of a valve pocket presents less difficulty in diagnosis; the advancing floating tail will show the classical platelet–fibrin architecture (Figure 7), although in some areas a pure fibrin mesh with trapped red cells and leukocytes may be the only distinctive feature. These propagating lesions will show, almost invariably, evidence of organization at their points of attachment, indicating that venous thrombi, like thrombi in arteries, are usually built up slowly.[13]

It should be mentioned that venous thrombi do not originate invariably at the apexes of valve pockets: initiation may be anywhere on the lateral surface of the vein wall within the pocket but never on the inner surface of the valve curtain (Figures 3 and 4). Furthermore, our studies involv-

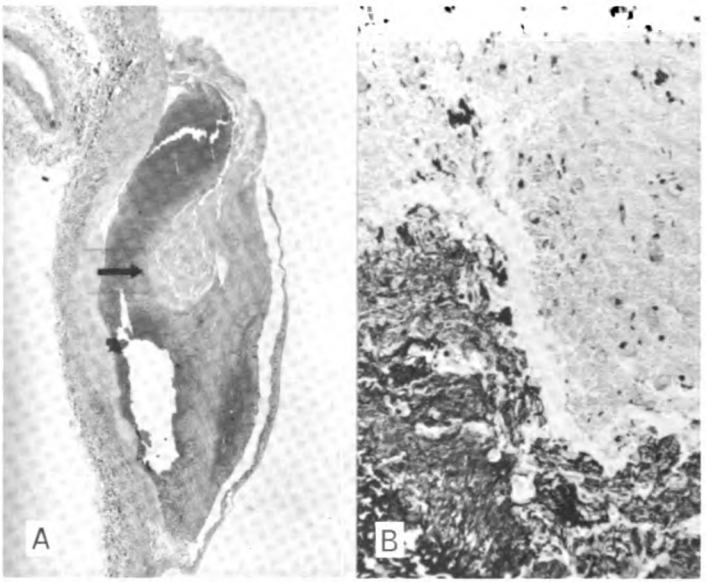

FIGURE 3 Photomicrographs of a fairly early thrombus that originated at the apex of a valve pocket in the superficial femoral vein. A, The entire lesion. The darker areas consist of fibrin coagulum with enmeshed red cells, whereas the paler (central) portions consist of platelet clumps and fibrin. Masson's trichrome. (× 7) B, A higher magnification of the area in the vicinity of the arrow in A. The lower portion consists mostly of fibrin, and that at the top of platelets and occasional phagocytic cells. Phosphotungstic acid–hematoxylin. (× 400)

ing complete serial sections through early venous thrombi have failed to yield convincing evidence of injury to the vein wall next to the area of thrombotic attachment.[14] These studies, however, were done using conventional light microscopy, and it is conceivable that surface endothelium impregnations, as used by O'Neill,[11] Samuels and Webster,[17] and Robertson and co-workers,[15] or even more sophisticated techniques, will reveal a lesion in the vein wall that will explain why platelets clump at one point in the valve pocket and not at another.

End-stage propagated venous thrombi, the kind that have such disastrous effects when they fracture and embolize, present a microscopic picture of blood coagulation in most parts and of platelet conglutination here and there (Figure 7). That is to say, there are many microscopic fields in which a fibrin mesh with entrapped red cells and leukocytes is

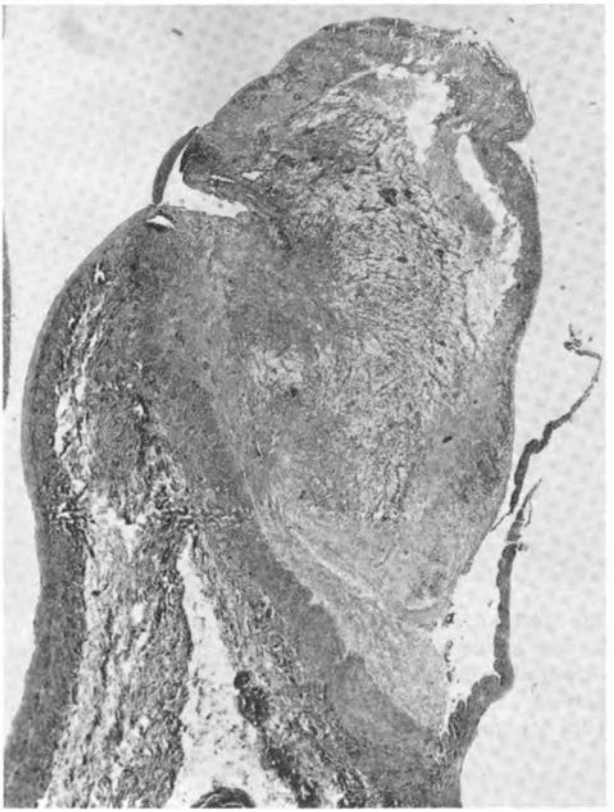

FIGURE 4 Photomicrograph of an organizing thrombus that is bulging slightly from a valve pocket of the superficial femoral vein. The entrance of a branch vein is shown on the left. Only the tail of the thrombus is recent, the remainder being organized and vascularized. The oldest point of organization is at the left, close to the orifice of the pocket. Masson's trichrome. (× 18)

all that is seen, and others in which the presence of granular platelet masses, rimmed by skeins of fibrin, establishes the antemortem nature of the lesion.

The fate of venous thrombi depends on the stage to which they have progressed. Those which remain confined to valve pockets will organize and eventually result in valvular incompetence. Propagated thrombi will either embolize to the lungs or organize, with recanalization; if they organize, there will be some obstruction to venous return, pending the opening of collateral channels.

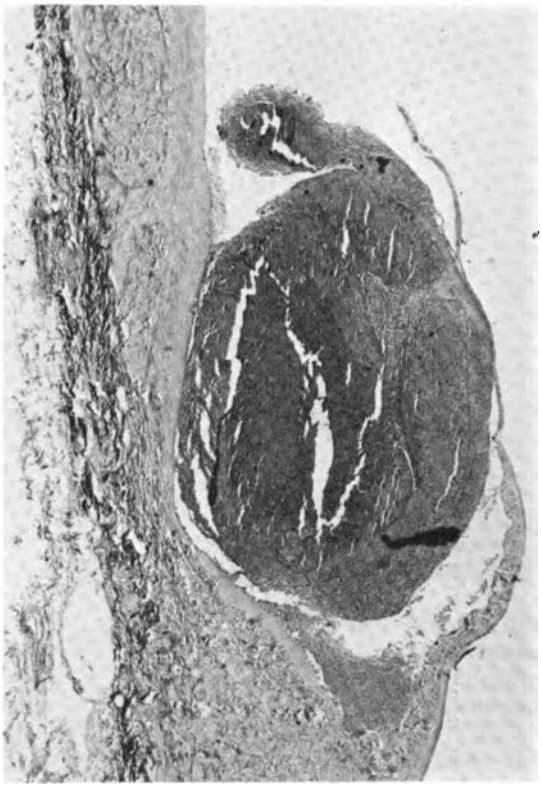

FIGURE 5 Photomicrograph of a fibrin clot with many enmeshed red cells, simulating a thrombus in a valve pocket. Microscopically, no platelet clumps were seen; the microscopic picture resembled that in Figure 6B, with the addition of many enmeshed red cells. Masson's trichrome. (× 23)

MORPHOLOGIC DIFFERENCES BETWEEN ARTERIAL AND VENOUS THROMBI

There seems to be a common impression that the pathogenesis of arterial and venous thrombi is fundamentally different. Deykin,[3] for example, stated that red thrombi occur almost exclusively in the venous circulation, that they are composed primarily of red cells and fibrin, and that stasis of blood flow is necessary for their formation. This description, acceptable in part, must be corrected in two respects: (1) it applies to the propagated parts of venous thrombi, not to their points of initiation; and

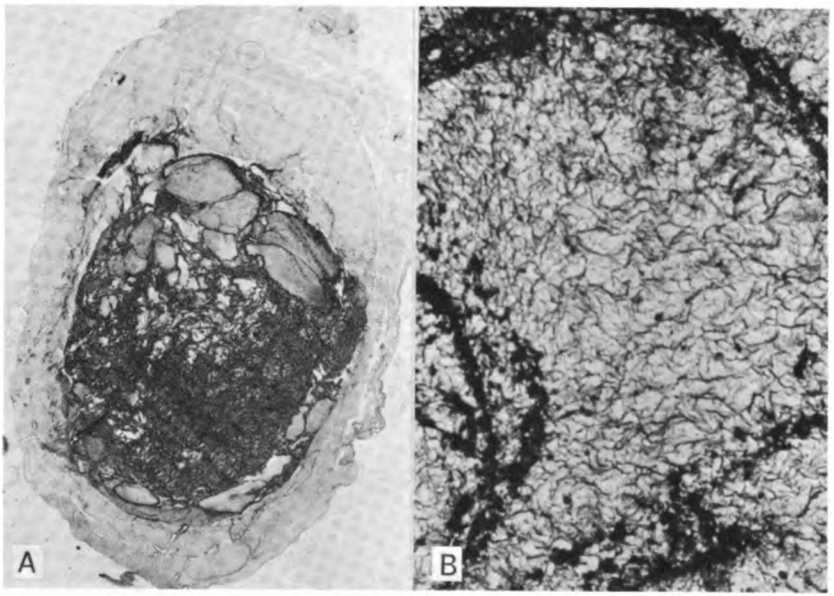

FIGURE 6 Photomicrographs of a postmortem ("chicken-fat") clot from the left ventricle of a patient without known cardiac disease. A, A low-power view of part of the clot whose pseudocoralline appearance is reminiscent of a thrombus. Phosphotungstic acid–hematoxylin. (× 8) B, A high-power magnification, showing that the clot consists only of fibrin. Phosphotungstic acid–hematoxylin. (× 350)

(2) it ignores the essential feature of platelet conglutination, which is always present in some part of a venous thrombus—indeed, in my opinion, the diagnosis of an early thrombus, red or white, cannot be made without it.

Thus, arterial and venous thrombi are not fundamentally different in their morphology, although they do show architectural variations that sometimes are striking. For example, condensation of the platelet–fibrin masses is not nearly so impressive in veins as in arteries, owing presumably to the lower pressure of blood in the venous circulation. As a result, lamination of the thrombus is not so evident, and the looser fibrin mesh allows more red cells and leukocytes to be caught in its interstices. But the platelet component is always present to some degree; it is seen particularly at the thrombus head, but portions are also scattered here and there throughout the propagated tail, where they impart a pale color and a friable texture to the mass. Indeed, it is suspected that it is at these points of friability that fractures of the thrombus occur, with resulting embolism. Nevertheless, most of the tail of a propagated venous

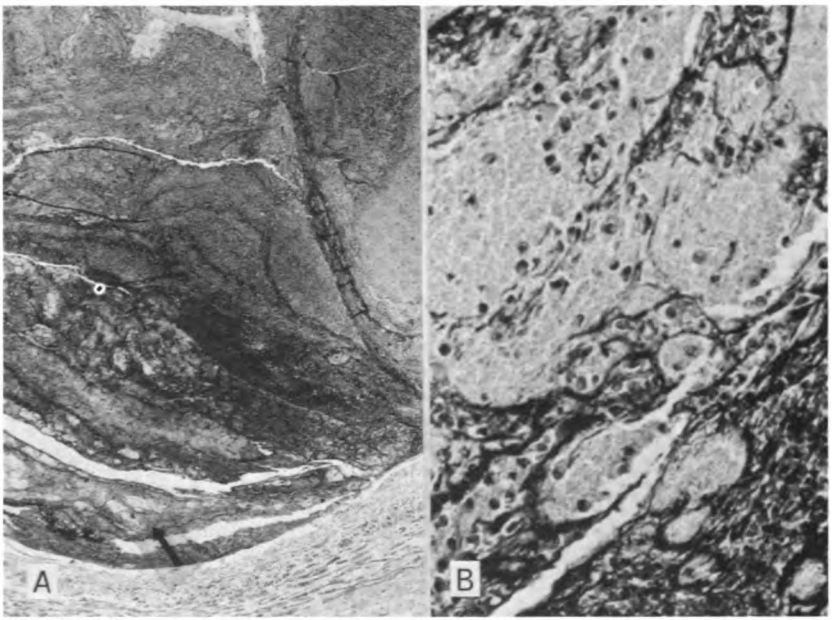

FIGURE 7 Photomicrographs of the propagated portion of a long venous thrombus. A, A low-power view showing the typical coralline appearance of the lesion. Phosphotungstic acid–hematoxylin. (× 6) B, A higher magnification of the area to which the arrow points in A, showing clumps of unstained platelets rimmed by fibrin, and, in the lower part, a fibrin mesh that makes up the great mass of the propagated tail. Phosphotungstic acid–hematoxylin. (× 360)

thrombus is composed of fibrin and enmeshed red cells, and this portion, theoretically, should be preventable by anticoagulant therapy. This inference is supported by the findings of Sevitt and Gallagher,[18] who assessed the effect of anticoagulants on patients with recent fractures of the hip; although propagation of venous thrombi with embolism was prevented, small thrombi, presumably of platelet origin, remained in valve pockets.

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IV
THE PATHOGENESIS OF THROMBOSIS

The Problem in Perspective

KENNETH M. BRINKHOUS

It was nearly a century and a quarter ago that a 23-year-old German pathologist, Rudolf Virchow, gave what was considered to be a revolutionary lecture on the pathogenesis of thrombosis and the mechanisms of embolism.[15] A photostatic copy of the first page of the original manuscript is shown in Figure 1. This manuscript was reported to have been lost,[1] until our discovery of it in 1966, now reported for the first time. Virchow's ideas have been the subject of both praise and denigration from the time of that lecture to the present day. The lecture itself was delivered in a low voice, so that many in the audience could not hear it completely.[5] And a geheimrat considered the lecture presumptuous and on leaving the hall was overheard to remark to this effect: "Did you hear it? We don't know anything any longer." [15] Virchow laid down in this speech and in his writings of the next decade [17] a crude conceptual framework of the pathogenesis of thrombosis that has come to be known as Virchow's triad. Although it was never presented succinctly by Virchow, thrombosis was held to be the result of (1) changes in the vessel wall or surface, (2) changes in the blood and its coagulability, and (3) changes in blood flow or stasis. It is interesting that these same three factors are considered in some detail in the presentations that follow.

The next major step forward in the study of thrombosis followed the recognition of the blood platelet about a century ago [14] and the realization that the white thrombus is made up of agglutinated platelets. The names of Eberth and Schimmelbusch,[6] Zahn, Bizzozero, Hayem, and William Henry Welch still stand out for their contributions to this period.

The modern era of understanding of fibrin clotting is often considered to have had its beginnings in the quantitative studies on prothrombin in the middle 1930's.[8,13,18] A remarkable spurt in basic new knowledge appears to have reached its zenith in the 1950's, when many new clotting

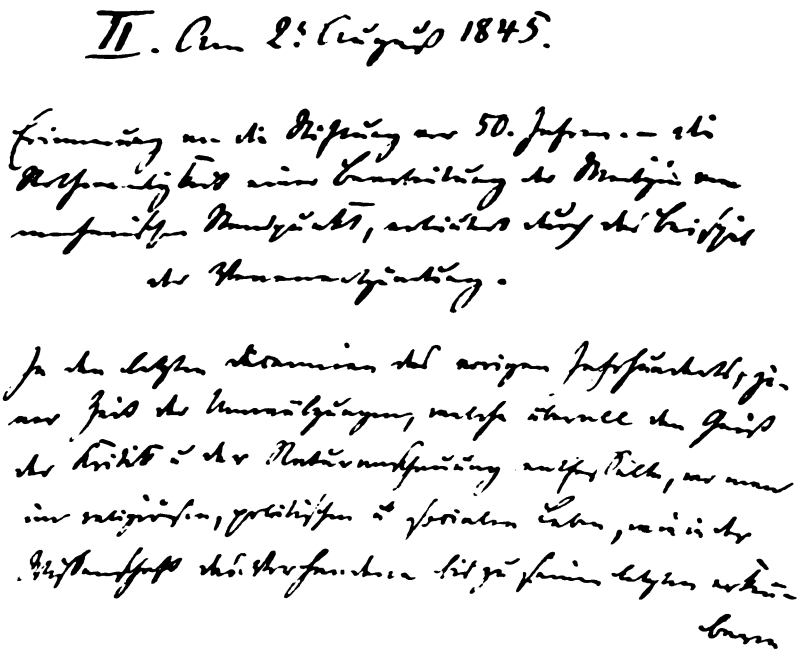

FIGURE 1 Holograph of first page of manuscript of Virchow's lecture on thrombosis (the second lecture of his career), delivered on August 2, 1845. Discovered in 1966 with the help of Mrs. Elisabeth Sommer, University of North Carolina. The assistance of the Literatur-Archiv des Instituts für deutsche Sprache und Literatur, Nachlass Rudolf Virchow, Deutsche Akademie der Wissenschaften zu Berlin, is acknowledged.

factors were recognized. This new information has been applied effectively to the field of hemorrhagic diseases,[3,4] but only indirectly and relatively ineffectually to the field of thrombosis, except for the studies with heparin [11] and dicumarol.[2,9,12]

Another major step forward was taken in the present decade and dealt with the basic mechanisms of platelet function.[7,10] Although these newer data on platelets seem to bear directly on the pathogenesis of thrombosis, it appears that not all the pieces needed to solve the puzzle of the sequence of events in the initiation of a thrombus have been identified.

Although blood coagulation and platelets have been and are being studied intensively, less effort has been given over the years to the interrelated problems of the pathology of the vessel wall and disturbances in

blood flow, in relation to the development of thrombi. It is encouraging that increasing attention is now being directed to all these factors, as reflected in the papers that follow. All the papers that make up this section have been written by persons who have made basic contributions to the understanding of the biologic and pathophysiologic processes that determine whether thrombosis will develop.

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The Role of the Blood-Clotting Mechanism in the Pathogenesis of Thrombosis

OSCAR D. RATNOFF *

Any inquiry into the nature of thrombosis is, in its essence, a search for the devices that transform fluid blood into a gel. This presentation will focus on the nature of the gelation process itself, that is, on the nature of the clotting mechanism; and, in a sense, this entire publication is devoted to the same problem.

The burden of this essay will be to ask a question that is crucial to our future understanding of the thrombotic state: Does a thrombus form because some localized change in the blood-vessel wall initiates the clotting process? Alternatively, is the clotting mechanism disturbed at a distance from the site of thrombosis so that the blood is abnormally likely to clot; that is, does thrombosis result from a hypercoagulable state of the circulating blood? This second question implies that a thrombus forms because local conditions, such as stasis, foster the clotting of hypercoagulable blood. These two possibilities, that thrombosis is a local process and that it is the local result of a generalized change in the blood, are, of course, not mutually exclusive. An examination of what is known about the mechanisms that bring about blood clotting may help us to elucidate the thrombotic state.

Nearly all we know about the coagulation of fluid blood has been gained, not in the living animal, but under artificial conditions, in the test tube. Such studies have led to the view that fibrin, the fundamental element of clotted blood, is derived enzymatically from a soluble precursor, fibrinogen. Two enzymes, evolved in plasma during coagulation, bring about this transformation. The first, thrombin, a derivative of prothrombin, breaks four arginyl–glycine bonds in each fibrinogen molecule, splitting off four small fragments, the fibrinopeptides.[2] The re-

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maining monomeric units of fibrin polymerize to form a macroscopic, insoluble network of fibrin; this process is accelerated by calcium ions. Normally, the monomers are bonded to each other by a second enzyme, fibrin-stabilizing factor (factor XIII). Fibrin-stabilizing factor is a transamidase that is probably inert in circulating blood. In the presence of calcium ions, it can be changed by thrombin to a form in which it creates firm chemical bonds between adjacent fibrin molecules.

The mechanisms by which thrombin is elaborated during blood clotting remain the central enigma, despite an extraordinary volume of research. Several recent reviews epitomize different hypotheses proposed to explain the available information.[1,5,15,16] Two principal physiologic pathways for the formation of thrombin have been described. When blood comes into contact with injured tissue, one or more entities released by the tissues seem capable of initiating the changes in plasma that lead to the evolution of thrombin. These entities, known generically as thromboplastin, interact with factor VII and calcium ions to form a clot-promoting agent. This agent then "activates" Stuart factor (factor X); that is, it changes Stuart factor into a form that can participate in the formation of thrombin. Activated Stuart factor reacts with proaccelerin (factor V), phospholipid, and calcium ions to form a prothrombin converting principle. The phospholipid for this reaction appears to be derived at least in part from the complex of substances described as thromboplastin.

The nature of the prothrombin converting principle is disputed. At one time, my colleagues and I thought that this substance was derived from proaccelerin. More recently, evidence has accumulated that both activated Stuart factor and proaccelerin may be needed to convert prothrombin to thrombin, and that they function only when adsorbed to phospholipid.[9] In one intriguing experiment, Prentice and I [14] were able to show that the prothrombin converting principle, after it had been elaborated, could be inhibited by an antibody directed against activated Stuart factor. Similar experiments have been performed by Denson.[7] Denson also showed that an antiserum directed against proaccelerin did not inhibit the prothrombin converting principle.[7] These various observations suggest that our earlier view was incorrect, and that the active agent in the prothrombin converting principle is derived from activated Stuart factor. But the data to support this view, however appealing, are still incomplete. In any event, in the presence of phospholipids, the prothrombin converting principle releases thrombin from prothrombin, through an enzymatic process.[13] The sequence of events through which tissue thromboplastin acts has been called the extrinsic pathway for the formation of thrombin, because thromboplastin is not part of the circulating blood.

Plasma, separated from blood cells, clots when placed in glass tubes. The route through which thrombin forms in cell-poor plasma has been designated as the intrinsic pathway, because all the elements needed are present in the plasma itself. One view of the steps involved in the intrinsic pathway has been called the "waterfall" [6] or "cascade" [10] hypothesis. Clotting is initiated by exposure of plasma to glass or some other foreign surface. Possibly, glass induces a molecular rearrangement in a specific component of plasma, Hageman factor (factor XII). This rearrangement makes Hageman factor behave in isolated systems as if it were a giant macromolecule.[8] The altered Hageman factor next changes another plasma component, plasma thromboplastin antecedent (PTA, factor XI) to a clot-promoting agent, activated PTA, which has many of the characteristics of a hydrolytic enzyme. Activated PTA, in turn, in some way alters still another factor, Christmas factor (factor IX), so that this, too, behaves as if it were in an activated form. The transformation of Christmas factor to its activated state takes place only if calcium ions are present. In the next step, activated Christmas factor alters antihemophilic factor (factor VIII) in such a way that it can convert Stuart factor to its activated form; calcium ions and phospholipid are needed for this reaction to proceed. Thereafter, the steps in clotting appear to be the same in the intrinsic and extrinsic pathways. The phospholipid needed at the several steps in the intrinsic pathway is probably furnished both by plasma itself and by the platelets. Thrombin, the product of the intrinsic pathway, so alters both antihemophilic factor and proaccelerin that their participation in clotting is enhanced.

Thus far, I have described two pathways that have received much attention in recent years. Perhaps other routes to the formation of fibrin exist, at least under pathologic conditions. A number of substances, foreign to the blood, can bring about its coagulation—for example, trypsin and Russell's viper venom, which can initiate the formation of fibrin by activating Stuart factor; tiger snake venom, which, in the presence of proaccelerin, can convert prothrombin to thrombin; staphylocoagulase, which can change prothrombin to thrombin in the absence of proaccelerin; and the venoms of the Malayan pit viper or the Brazilian jararaca, which clot fibrinogen directly. The importance of many of these agents is that they may be introduced into the circulation. But beyond that lies the possibility that similar agents, not yet discovered, may arise in our bodies and bring about thrombosis.

To return to the question posed at the start: Does thrombosis ordinarily arise because of changes in the endothelial surface at the point at which the thrombus attaches or because the circulating blood has become hypercoagulable? In the laboratory, endothelial damage is often followed by local thrombosis, but how this comes about is not at all

clear. One possibility is that the injured endothelial surface acquires glass-like properties, and can then activate Hageman factor. Marin *et al.*[11] reported that they were able to demonstrate such a change in isolated venous segments. Conceivably, injury to endothelium exposes the circulating blood to collagen, a substance that Niewiarowski *et al.*[12] found to be an activator of Hageman factor. This appealing view is not yet substantiated. Preparations of collagen available to us have been contaminated with tissue thromboplastin, so we have been unable to tell whether this protein acts on Hageman factor. Botti and I [4] were unable to demonstrate that injured vascular endothelium activated Hageman factor, again because the damaged vessels released tissue thromboplastin. Our experiments suggested that thrombosis after local injury arises via the extrinsic pathway of coagulation. The importance of the extrinsic pathway is emphasized by the clinical observation that thrombi may form in the blood vessels of patients with hereditary defects of the intrinsic pathway of blood clotting, that is, deficiencies of Hageman factor, PTA, Christmas factor, or antihemophilic factor.

In any event, clot-promoting substances, accumulating in the vicinity of vascular injury, may be important in the evolution of a thrombus. Note the emphasis on the *accumulation* of clot-promoting substances. It is well known that vascular stasis increases the likelihood of thrombosis after experimental vascular injury, probably because stasis prevents the dispersion of activated factors by the flowing blood. Yet another local factor important in thrombosis is the accumulation of platelets at the site of vascular injury. Perhaps platelets are not sufficient in themselves to initiate thrombosis, but these cells may release clot-promoting agents, such as phospholipids, which accelerate coagulation started by some other means.

Evidence exists, then, that thrombosis may be initiated by local vascular injury. Surprisingly, however, such local changes have been hard to find in many clinical situations. We must therefore turn to the second possibility. Can thrombosis come about because the circulating blood has become hypercoagulable? It has been known for many years that the intravenous injection of tissue thromboplastin or thrombin brings about intravascular clotting, massive or minute, depending on the dose administered. But in such experiments, the vascular tree is merely a living test tube, filled with blood. Much more is to be learned from the experiments first performed by Feissly and then elaborated by Wessler.[17] In essence, these investigators found, as had their predecessors, that blood would clot only very slowly or not at all in an isolated segment of vein. They injected serum intravenously into experimental animals; again, no thrombi appeared. But if serum was injected, and a segment of vein was

immediately isolated between ligatures, a clot promptly formed in the static blood. Presumably, the injection of serum brought about a change in the circulating blood that allowed it to clot once its flow was arrested. Wessler has demonstrated that the responsible agents in the injected serum are activated forms of the factors that participate early in the intrinsic pathway of coagulation. Botti and I [13] extended their observations. In the test tube, solutions of a compound called ellagic acid can activate Hageman factor. The intravenous injection of solutions of ellagic acid so altered the circulating blood that, when it was removed from the body, it clotted rapidly. Indeed, after the injection of ellagic acid, the animal's blood clotted as rapidly in silicone-coated tubes as in glass tubes. Remarkably, intravascular clotting did not ensue. Clotting occurred only when we tied off a segment of vein after the ellagic acid had been injected.

These experiments epitomize some of the problems yet to be solved to achieve an understanding of the thrombotic state. The intravenous injection of ellagic acid makes the blood hypercoagulable. Why, then, will coagulation occur only in static blood? The glib answers are inadequate. Blood contains anticoagulant substances that might be thought to protect against thrombosis. But the hypercoagulable state induced by ellagic acid may be maintained for many hours, so circulating inhibitors do not seem to explain away the failure to induce thrombosis. Similarly, circulating procoagulant substances may be removed or inactivated by passage through the liver or by the reticuloendothelial system. Again, the fact that the hypercoagulable state may be sustained for many hours seems to minimize the role of these clearance mechanisms. Nor is there evidence that the absence of thrombosis during the experimental hypercoagulable state is in any way related to the action of such plasma proteolytic enyzmes as plasmin. In short, we must seek some new explanation for the failure of the hypercoagulable state to induce thrombosis in the absence of stasis. Whatever this explanation turns out to be, it must be consistent with the fact that the injection of such agents as thromboplastin and thrombin will bring about intravascular coagulation, whereas the injection of ellagic acid or serum will not.

In this short review, I have tried to emphasize how little we understand about the relationship between the clotting process and thrombosis, and to demonstrate the inconsistencies in what we think we know. Ironing out these inconsistencies may well be a major step in gaining an appreciation of the pathogenesis of this important process.

Heretofore-unpublished studies were supported by Public Health Service grant HE 1661 from the National Heart Institute and by a grant from the American Heart Association.

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The Tissue Factor System and Its Possible Role in Thrombosis

WILLIAM J. WILLIAMS

Aqueous extracts of tissues have long been known to accelerate the coagulation of blood, and a number of studies have demonstrated that such extracts may fulfill two functions in clotting. The first is due to phospholipids in the extracts. The phospholipids may be free or bound to protein and function in coagulation in the same manner as platelets; the material responsible for this effect is referred to as "lipid activator." The second is due to "tissue factor." This material is a phospholipoprotein and requires both protein and lipid moieties for its activity. It is commonly called "tissue thromboplastin," but, because of the vagueness of "thromboplastin," the term "tissue factor" will be used here.

It is the purpose of this presentation to review some of the newer work on tissue factor and its mode of action and to relate this to the problem of thrombosis.

CHEMISTRY AND DISTRIBUTION OF TISSUE FACTOR

PURIFICATION OF TISSUE FACTOR

The early work of Howell [14] suggested that a complex of lipid and protein was necessary for maximal coagulant activity of tissue extracts. More recently, Chargaff and his associates [6] purified the coagulant activity of bovine lung extracts and found that it was associated with subcellular particles composed of protein, phospholipid, and cholesterol. I carried out similar studies [35,36] on aqueous extracts of fresh bovine lung, fresh human placenta, and acetone-dried human brain. The coagulant activity of fresh bovine lung was found in subcellular particles behaving on centrifugation as would be expected for microsomes. The final puri-

fied product was composed of 48% protein, 38% phospholipid, and 10% cholesterol. The phospholipid was a mixture, including phosphoinositide, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and sphingomyelin. The coagulant activity of the lung preparations was purified about 500-fold by the procedures used. The final product sedimented on isopyknotic density-gradient centrifugation on a single band with a density of 1.09–1.14 g/cc and was therefore suitable for use in experiments designed to detect binding of protein to the tissue factor.

The coagulant activity of fresh human placenta was purified by a procedure essentially the same as that used for the lung. The final product was composed of 49% protein, 42% phospholipid, and 7% cholesterol. Again the phospholipid was a mixture similar to that found in lung. This material was purified only 38-fold.

The brain particles were prepared from acetone-dried tissue. The final product contained 44% protein and 56% phospholipid, and was purified 10- to 43-fold.

These three preparations were capable of inducing rapid clotting of recalcified plasma, and of activating factor X in protein fractions containing factors VII and X from human serum. All three preparations were extremely active. For example, reaction mixtures containing as little as 1 μg of lung tissue factor per milliliter developed coagulant activity (activated factor X) rapidly.

LIPID AND PROTEIN COMPONENTS

It is well established that the coagulant activity of tissue extracts is largely destroyed by heating, although the residual phospholipids are still capable of functioning as platelets (lipid activator) in coagulation.[14,16,30] These results indicate a heat-labile component of tissue factor, which might be a protein, possibly an enzyme.

A number of studies have been done on the dissociation of the lipid and protein moieties of the tissue factor. Kuhn and Klesse [22] demonstrated that the lipid and protein components could be separated by extraction of lung tissue factor with organic solvents, with loss of coagulant activity. By recombination of these components the coagulant activity was restored.

More recently, Deutsch *et al.*[8] have studied dissociation and recombination of the lipid and protein moieties of tissue factor. They demonstrated that the extracted protein had no coagulant activity and that the phospholipid could substitute for platelets in coagulation systems. On recombination of the phospholipid and protein components, full coagu-

lant activity was recovered. Similar results have been reported by Nemerson.[25]

The results of Deutsch *et al.*[8] have been extended to a study of the species specificity of tissue factor.[15] It has been recognized for some time that tissue factors are species-specific in that tissue extracts activate homologous plasma faster than heterologous plasmas. Mann and Hurn [23] reported that the species specificity was related to a plasma protein, and Hecht [12] found no species specificity for the lipid activators from various sources. Irsigler *et al.*[15] compared human, bovine, and chicken brain tissue factors and found that each activated the homologous plasma best. These workers dissociated the tissue factors from the three species and confirmed that the extracted lipids were not species-specific, whereas the recombined tissue factors were. When protein from one species was combined with lipid from another, the specificity was found to reside in the protein component.

Nemerson and Spaet [27] studied the activity of tissue factor of rabbit brain treated with butanol to remove the phospholipid. They obtained a water-soluble product that was capable of activating factor X. It was concluded that this material may represent a protein moiety of brain tissue factor with enzymatic activity. In all probability, the factor X used in these experiments was contaminated with factor VII, so that possibly the soluble protein did not activate factor X directly but reacted with factor VII before the factor X was activated.

It must be noted that Hecht *et al.*[13] have reported the preparation of brain tissue factor that was biuret-negative and presumably contained lipids, and Hecht [11] has recently presented further evidence of this type of "thrombo-plastin." The relationship of this material to those described above is unknown.

DISTRIBUTION OF TISSUE FACTOR

Astrup [1] has reported on the distribution of tissue factor in various organs—brain, lung, kidney, heart, liver, and muscle—of a number of species. Considerable difference in thromboplastic activity was found among the various organs, with lung and brain having the greatest activity. Astrup *et al.*[2] also examined the thromboplastic activity of human aorta and found considerable activity in the intima and media. Later this study was extended to other species.[3] Blood-vessel intima was found generally to have thromboplastic activity, although the concentration varied considerably between species. The thromboplastic activity of the intima of human aortas ranged from $\frac{1}{3}$ to $\frac{1}{2}$ that of brain to twice that of brain.

Kirk [21] has also studied the thromboplastic activity of human vascular tissues. He found that the intima from both arteries and veins contained thromboplastic activity. The values for arteriosclerotic vessels were somewhat lower than for normal vessels. Kirk [21] found the activity of vascular intima to be much lower relative to brain than did Astrup *et al.*[2] but explained this on the basis of a difference between the brain preparations used in the two laboratories. In absolute terms the thromboplastic activities found in the two studies were apparently quite similar.

"THROMBOPLASTIC" ACTIVITY OF BLOOD CELLS

Thromboplastic materials released from the formed elements of blood could play a role in thrombosis and must be considered. The thromboplastic activity of platelets and red cells has been studied by several workers.[24,29,31] These cells contain phospholipoprotein that functions as a lipid activator rather than as tissue factor.

THE MECHANISM OF ACTION OF TISSUE FACTOR

It has already been noted that incubation of tissue factor with serum preparations containing factors VII and X leads to activation of factor X. We attempted to separate factors VII and X and determine how they interact with lung tissue factor.[37] Bovine plasma was used as starting material. Factors VII and X could be readily separated by treatment with barium sulfate followed by column chromatography on DEAE–cellulose. Factor VII so prepared was free of factor X but was contaminated with prothrombin, which could be removed by rechromatography on DEAE–cellulose. Factor X was free of prothrombin but contained factor VII, which could also be removed by rechromatography.

We studied the reaction of lung tissue factor with purified preparations of factor VII.[37] Calcium was found to be an essential cofactor. Marked coagulant activity developed in reaction mixtures containing these three components, but attempts to study the kinetics of the reaction were unsuccessful when we used the approach that had been used to study the activation of factor X by Russell's viper venom.[10] In the experiments on factor VII, incubation of the reaction mixtures for periods ranging from 30 sec to 5 min did not result in any increase in activity, which suggested that the reaction occurred extremely rapidly. By using a simplified assay system, we shortened the incubation period to as little as 5 sec and could then demonstrate that the coagulant activity developed very rapidly. Full activity appeared in 10–20 sec in nearly all experi-

ments. The rate of development and the yield of coagulant activity were proportional to the concentration of either tissue factor or factor VII, depending on which was present in limiting concentration.

These results are inconsistent with a classical enzyme–substrate reaction and suggest that the coagulant activity was due to formation of a complex of tissue factor, factor VII, and calcium. There is evidence to support this concept. The activity was sedimentable, suggesting that it was bound to the tissue factor particles. If the tissue factor concentration in the reaction mixtures was increased, the activity of the sediment increased and residual factor VII in the supernatant fluid decreased. If factor VII were bound to the particles, one would expect an increase in the nitrogen content of the tissue factor sediment, and that was observed experimentally. Finally, binding of protein to the tissue factor would be expected to increase the density of the particles, and that could be demonstrated by isopyknotic density-gradient centrifugation.[37] The coagulant activity could not be eluted from the sediment by a variety of methods commonly used to dissociate protein–phospholipid complexes.

Nemerson [26] has similarly studied the reaction between brain tissue factor and factors VII and X. He was also able to show that the reaction between tissue factor and factor VII occurred extremely rapidly, and that the product of the reaction was tightly bound to the tissue factor particles.

The factor VII used in the experiments described above [37] was prepared from plasma and was purified from 100- to 500-fold. Nemerson gave no purification data in his report.[26] Others have purified factor VII from serum, and have reported purification of 2300-fold [28] and from 400- to 1300-fold [9] from this source. The relationship between factor VII from serum and that from plasma is unclear, but it is known that serum factor VII has greater activity than that from plasma.[19]

THE ACTIVATION OF FACTOR X IN THE TISSUE FACTOR SYSTEM

In reaction mixtures containing tissue factor, factors VII and X, and calcium, the product is activated factor X.[35,36] Tissue factor does not activate factor X in the absence of factor VII.[37] Because the initial reaction in this system is between factor VII and tissue factor, it seemed that the product of this initial reaction must activate factor X. In order to test this concept, tissue factor–factor VII complex was prepared and reacted with purified factor X and calcium. The product of this reaction was activated factor X, and the kinetics of the reaction indicated that the tissue factor–factor VII complex was functioning as enzyme and factor X

as substrate.[26,37] The tissue factor–factor VII complex is inhibited by diisopropylfluorophosphate [26,34] and by soybean trypsin inhibitor.[26]

The physical state of the factor X activated by tissue factor–factor VII complex depended on the source of the tissue factor used.[35,36] Thus, with bovine lung tissue factor, about 70% of the activated factor X was soluble, and the remainder was bound to the sediment but could be recovered by treatment of the sediment with EDTA. With brain particles, almost no activity was present in the supernatant fraction, but full activity could be recovered from the particles by washing with EDTA. With human placenta particles, the coagulant activity appeared to be firmly bound to the tissue factor, although the activity was unstable, and experiments similar to those with lung and brain could not be performed. Nemerson and Spaet [27] similarly demonstrated binding of activated factor X to brain particles and its elution by washing with EDTA. The bond between tissue factor and factor X is clearly different from that between tissue factor and factor VII.

THE NATURE OF THE TISSUE FACTOR–FACTOR VII COMPLEX

Although the tissue factor–factor VII complex could be shown to act as enzyme in blood coagulation, the question of whether the enzymatic activity is due to tissue factor or to factor VII is unanswered. As has already been stated, attempts to dissociate the complex were unsuccessful.[26,37] Indirect evidence indicating that the activity may be due to activated factor VII was obtained from experiments with a preparation of factor VII that underwent "spontaneous" activation on storage and was then able to activate factor X without prior incubation with tissue factor.[37] (Spontaneous activation on storage has been reported for factor X, as well as for factor VII.[10]) The spontaneously activated factor VII functioned as enzyme and factor X was substrate in these reactions, which suggested that the activity of the tissue factor–factor VII may be due to activated factor VII.

If activated factor VII is indeed responsible for the enzymatic activity of the complex, the question of how it is activated arises. It is possible that the tissue factor has an enzymatic activity that converts factor VII to an active form, which is then bound tightly to the phospholipid moiety. The experiments of Nemerson and Spaet [27] suggest an enzymatic activity for tissue factor. Alternatively, the factor VII may be activated by binding to the surface of the tissue factor without chemical change. The activity could then be due to some alteration in the molecule consequent on its adsorption to the surface of the particle. Further studies will be necessary to decide between these alternatives.

REACTIONS AFTER ACTIVATION OF FACTOR X

Once factor X is activated, it reacts with calcium, phospholipid, and factor V to form a product that can catalyze the rapid conversion of prothrombin to thrombin.[4,17] Thrombin then catalyzes the formation of fibrin from fibrinogen, and coagulation ensues. In addition, thrombin alters factors V and VIII to make them more susceptible to reaction with their activators or cofactors [5,33] and thus can accelerate coagulation by this feedback mechanism. Thrombin also has a marked effect on platelets, including aggregation and consolidation of the platelet plug.[20,32] The reactions between activation of factor X and the formation of thrombin occur very rapidly, and thrombin also reacts rapidly with fibrinogen and with platelets.

THE POSSIBLE ROLE OF TISSUE FACTOR IN THROMBOSIS

The widespread distribution of tissue factor activity, including significant concentrations in the intima of blood vessels, suggests that it could play a role in thrombosis. Furthermore, the functional properties of the tissue factor system are consistent with this concept in that it leads to the rapid formation of thrombin, and only small concentrations of tissue factor are needed for this to occur.[7,26,35,37] Thus, in experimental systems, coagulant activity developed in reaction mixtures containing less than 1 μg of tissue factor per milliliter, and full activity could be developed in periods as short as 10 sec.[37] This concentration of tissue factor could reasonably be expected to exist in the immediate vicinity of a damaged intima, and the few seconds required for the development of marked coagulant activity would probably be available, especially in regions of stasis of blood flow.

Further properties of the tissue factor system that may bear on thrombosis are the formation of a complex with factor VII and the binding of activated factor X. Thus, if tissue factor were merely exposed in a vessel wall, rather than being released into the circulation, coagulant activity could develop at the site of damage to the vessel wall and induce clotting in this local area, perhaps even without stasis.

After the tissue factor–factor VII reaction has occurred, the reactions leading to thrombin may be complete within a few seconds, and the thrombin so generated is then able to convert fibrinogen to fibrin and to aggregate platelets. These last reactions occur promptly in normal hemostasis.[18] The same reactions could occur equally rapidly as a result of tissue factor activity in thrombosis.

Thrombin also reacts with factors V and VIII to increase their reac-

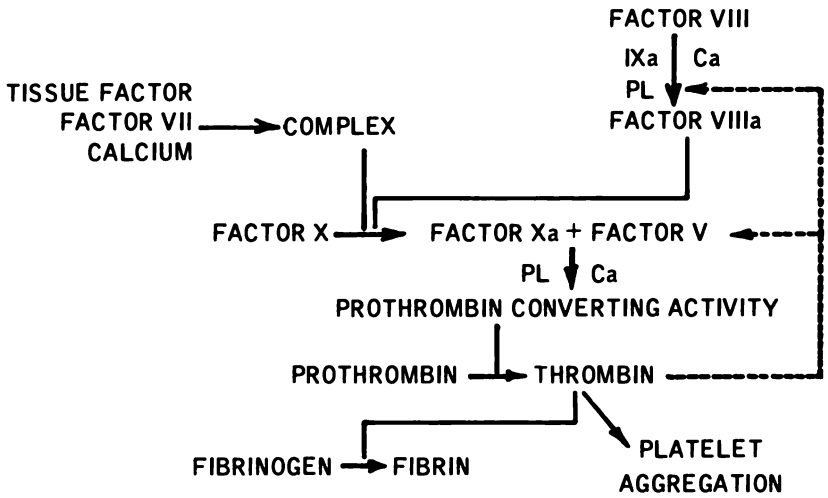

FIGURE 1 Schematic representation of the possible participation of the tissue factor system in thrombosis. Thrombin is formed via the reactions beginning with that of tissue factor with factor VII and calcium (upper left). The product of the reaction involving factor Xa, factor V, phospholipid, and calcium is prothrombin converting activity. Thrombin can then convert fibrinogen to fibrin and cause platelet aggregation. Thrombin also "activates" factors V and VIII (broken lines) and thereby further accelerates some of the reactions, leading to more thrombin formation. In the figure, "a" following a roman numeral indicates an activated coagulation factor. PL, phospholipid.

tivity and therefore has an accelerating effect on other reactions in coagulation that could aggravate a tendency to thrombosis. Another possible means of accelerating the reactions leading to thrombin may be related to the observation that serum has two to three times as much factor VII activity as does plasma. Possibly in some *in vivo* situations, sufficient "plasma factor VII" could be converted to "serum factor VII" to cause a significant increase in the rate of reaction of tissue factor with factor VII and thereby accelerate the production of thrombin.

The reactions that may result from the activity of the tissue factor system are illustrated in Figure 1. Although all this discussion is of necessity highly speculative, the properties of the tissue factor system are such that it can initiate the rapid production of thrombin *in vivo* and therefore can reasonably be suspected of playing a significant role in thrombosis.

The work reported here was supported by Public Health Service research grant HE 06757 from the National Heart Institute, training grant T1 AM-05228 from

the National Institute of Arthritis and Metabolic Diseases, and research career program award HE K3-2629 from the National Heart Institute.

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Plasma Free Fatty Acids, Hypercoagulability, and Thrombosis

WILLIAM E. CONNOR, JOHN C. HOAK, AND EMORY D. WARNER

The hypothesis that the blood may become hypercoagulable and thrombogenic as a result of endocrine and metabolic imbalance dates back at least to the experiments of Cannon and Gray,[3] who produced shortening in the whole-blood clotting time of cats by epinephrine injections. Our view has been that a plasma lipid fraction might well be one factor involved in the pathogenesis of endocrine-metabolic hypercoagulability. The lipid fraction of plasma that changes most dramatically in response to a variety of stimuli is that consisting of free fatty acids (FFA).[10] Normally, plasma FFA circulate in the range of 300–600 μEq/liter. Under conditions of stress, starvation, diabetic acidosis, and pregnancy, and after injections of ACTH, growth hormone, glucagon, and catecholamines, plasma FFA may rise threefold to sixfold as the result of endogenous mobilization from adipose tissue. At times, these changes in plasma FFA may occur in a matter of minutes.

Lipids have long been known to exert important effects on blood coagulation.[29] Howell, in 1912, demonstrated that crude cephalin accelerated clotting.[23] When lipids were extracted from plasma by Macfarlane *et al.*, its subsequent coagulation was retarded.[25] Phosphatides, released from platelets or supplied artificially, are essential for thromboplastic activity. Potential thrombogenic effects may arise from lipids of at least three sources: the lipids of the blood cells (platelets, erythrocytes, and leukocytes), lipids circulating as plasma lipoproteins, and the lipids of the vessel wall (especially those of arterial atheroma). Although chylomicrons and phospholipids may enhance *in vitro* coagulation,[29] *in vivo* thrombogenic effects from these lipids have not been conclusively demonstrated. Such lipids as triglyceride and cholesterol have had little or no effect on blood coagulation. Triglyceride emulsions, when given intravenously to patients for nutritional purposes, have not usually caused hypercoagulability of the blood.[12]

The concept that the plasma FFA constitute a unique circulating moiety that might cause thrombosis is based on evidence from a variety of *in vitro* and *in vivo* experiments. The observations of Stuber and Heim in 1916,[31] of Poole in 1955,[28] and later of Poole and Connor [8] called attention to the clot-promoting effects of fatty acid salts when added to citrated plasma or when tested in a system that produced clots with the morphologic features of thrombi. Some fatty acids especially accelerate the coagulation of blood that has been carefully collected to avoid "contact" activation.[4] For example, as shown in Table 1, a long-chain saturated fatty acid, such as stearic acid, shortened the clotting time of whole blood in silicone-coated tubes from 31 to 8 min. The concentration of fatty acid used was within the range of the FFA circulating in the blood. Native plasma spun to contain different numbers of platelets clotted rapidly after stearic acid but poorly with saline controls.[5] In the rotating tube of a modified Chandler apparatus, the "thrombus"-formation time was similarly accelerated by fatty acids, from 8.3 to 2.4 min.[4] The advantage of the Chandler apparatus is that artificial thrombi are formed that closely resemble those occurring in thromboembolic disease. These thrombi have a white platelet head and a red fibrin tail. As will be emphasized later, fatty acids affect both platelet aggregation and fibrin formation. This system permits the study of both the cellular and the fibrin components of the thrombi.

It is noteworthy that all long-chain, saturated fatty acids produced a significant shortening of the thrombus-formation time, i.e., stearic and palmitic acids (Figure 1). The short-chain fatty acids, caproic and lauric, had minimal action, and all unsaturated fatty acids—oleic, linoleic, lino-

TABLE 1 Clotting Time and Thrombus-Formation Time of Human Blood Collected in Silicone-Coated Glass [a]

	Time, min	
	With 0.9% NaCl	With long-chain saturated fatty acids
Clotting of whole blood [b]	31	8
Clotting of native plasma [c]		
4,000 rpm	36	10
15,000 rpm	No clot after 24 hr	13
Thrombus formation in rotating tube (Chandler apparatus) [a]	8.3	2.4

[a] Derived from Connor *et al.*[7]

[b] Test system: 1 ml blood and 0.1 ml of 0.1% fatty acid salt (C22:0) or saline.

[c] Test system: 0.2 ml of native plasma and 0.05 ml of 0.1% sodium stearate or saline.

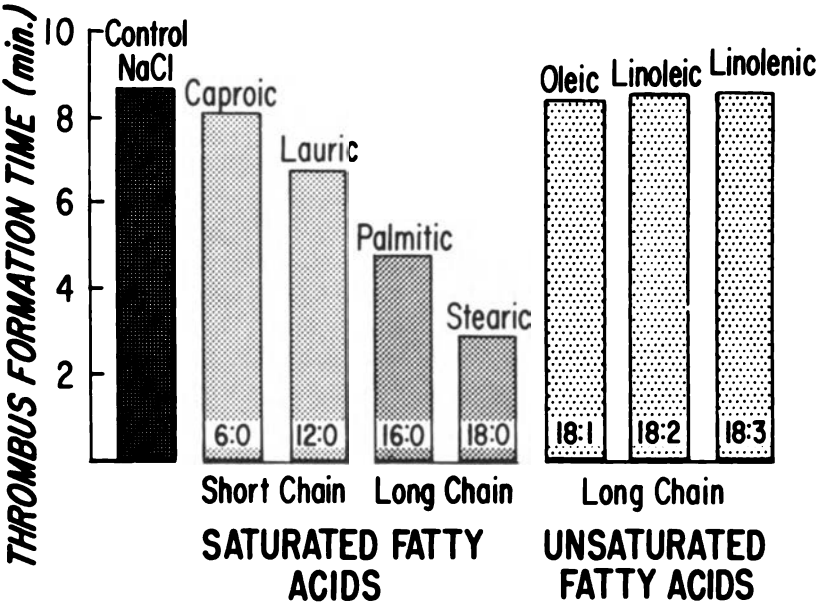

FIGURE 1 The effects of various fatty acids on thrombus-formation time. Other long-chain, saturated fatty acids (arachidic, C20, and behenic, C22) gave test results like those of stearic acid, whereas the long-chain, unsaturated arachidonic acid (C20:4) behaved as linoleic acid in this test system.

lenic, and arachidonic—were without effect in this system.[4,8] As will be indicated later, this differential effect of various fatty acids probably resulted from different rates of binding to the plasma albumin.

All long-chain fatty acids aggregated platelets, both as washed platelets and when present in platelet-rich plasma, as indicated in Table 2.[22] Note that stearic acid invariably produced gross clumping. Unsaturated fatty acids (oleic, linoleic, and linolenic acids) had lesser but still demonstrable effects, especially when the platelet aggregation was checked by phase-contrast microscopy (Figure 2). The aggregation of platelets by fatty acids required the presence of calcium ions. The aggregation was irreversible. The addition of albumin diminished the aggregating effects of fatty acids, but microscopic aggregates still formed in most instances.

INTRAVENOUS INFUSION OF FATTY ACIDS

An important question about the thrombogenic potential of a lipid is the result of injecting it into experimental animals. In our studies, massive

TABLE 2 Effect of Fatty Acids on Washed Platelets and Platelet-Rich Plasma [a]

Fatty acids	Number of observations	Aggregates		
		Gross	Microscopic Only	None
WASHED PLATELETS				
Stearic (C18:0)	24	24	0	0
Oleic (C18:1)	6	4	1	1
Linoleic (C18:2)	4	0	4	0
Linolenic (C18:3)	8	1	7	0
PLATELET-RICH PLASMA				
Stearic	34	27	5	2
Oleic	8	3	3	2
Linoleic	6	1	4	1
Linolenic	12	2	4	6

[a] Derived from Hoak *et al.*[22]

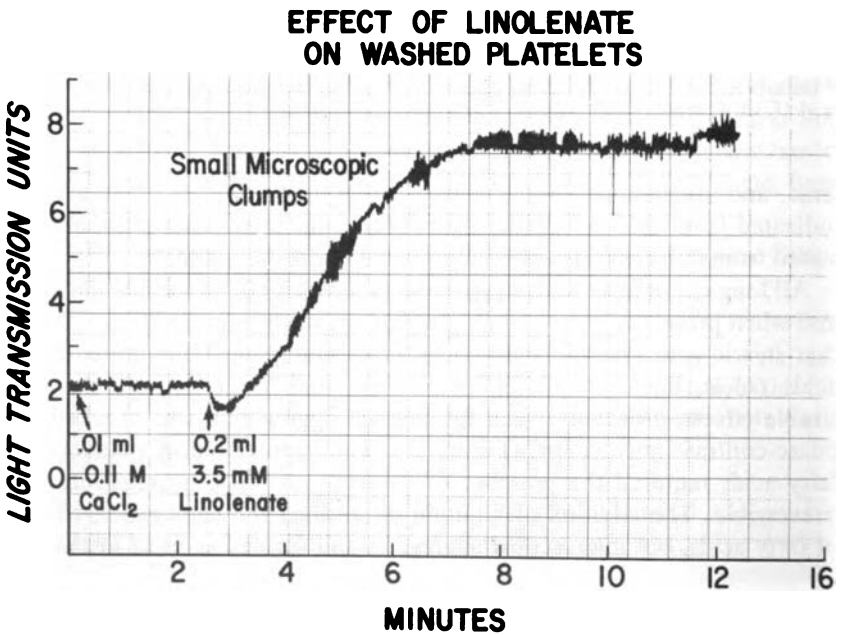

FIGURE 2 The platelet-aggregating effects of linolenic acid (sodium linolenate) on washed platelets. (Reprinted with permission from Hoak *et al.*[22])

thrombosis and death usually resulted when long-chain, saturated fatty acids were given intravenously to dogs, rabbits, mice, and rats. The results in dogs are summarized in Table 3.[6] Ten of 11 dogs given long-chain, saturated fatty acids had massive thrombosis. Before thrombosis occurred, the circulating blood became hypercoagulable, as evidenced by the production of stasis thrombi in isolated jugular vein segments in nine of 11 animals and by shortening of the whole-blood silicone clotting time from 32 to 17 min. Short-chain fatty acids and long-chain, unsaturated fatty acids did not cause death or massive thrombosis. That they have some hypercoagulable effect, however, should not be ignored. After intravenous injection of unsaturated fatty acids, thrombi developed in the jugular veins in 14 of 16 dogs, and the whole-blood clotting time in silicone tubes was shortened from 34 to 25 min.

Undoubtedly, stasis was an important contributing factor in the massive thrombosis found in the dogs given long-chain, saturated fatty acids intravenously. It has been shown that these fatty acids cause a profound reduction of cardiac output in the intact animal,[19] and that they depress the function of the perfused isolated rabbit heart (Severeid, L., unpublished observations).

Like Zbinden,[32] we observed thrombocytopenia in animals given long-chain fatty acids, either saturated or unsaturated (Table 4).[7] The platelet counts decreased to 71,000 and 55,000/mm^3 immediately after the completion of the infusion. At autopsy, platelet–fibrin thrombi were widespread.[18] The platelets in the thrombi found at autopsy and the thrombocytopenia correlated well with the platelet aggregation occurring after the addition of long-chain fatty acids *in vitro*. An electron micrograph of a thrombus that formed after the injection of linoleic acid is shown in Figure 3. A clump of platelets surrounded by red cells and not adherent to the vessel wall can be seen illustrating that hypercoagulability of the blood in this instance was associated with platelet aggregation.

ENDOGENOUS MOBILIZATION OF FATTY ACIDS

In the previous experiments, we had infused fatty acids in the unbound form. Because fatty acids circulating in the body are usually bound to the plasma albumin, experiments were performed to produce a high level of the plasma FFA fraction by endogenous mobilization. We produced high plasma FFA concentrations by administering either adrenocorticotropin (ACTH) or anterior pituitary hormone. The hormones were given subcutaneously to rabbits, and a saline injection was given to a control group of rabbits. Two hours after hormonal or saline injection, a jugular seg-

TABLE 3 Results of Intravenous Injection of Fatty Acids into Dogs (3.5 mM Fatty Acid, 10 ml/kg of Weight)[a]

	Dogs	Massive Thrombosis	Jugular Vein Thrombi	Whole-Blood Clotting Time (Silicone-Coated Tubes), min	
				Before	After
Saturated fatty acids					
Short-chain (C6–C12)	13	0	5	28	24
Long-chain (C14–C22)	11	10	9	32	17[b]
Unsaturated fatty acids (C18–C22)	16	0	14	34	25
Control NaCl	10	0	0	36	37
Soybean phosphatide	2	0	0	—	—

[a] Derived from Connor *et al.*[7]
[b] $p < 0.001$.

TABLE 4 Long-Chain Fatty Acid Infusions and Thrombocytopenia [a]

	Average Platelet Count/mm³	
	Before	After
Saturated fatty acids (7 dogs) (stearic acid)	302,000	71,000
Unsaturated fatty acids (8 dogs) (oleic, arachidonic, and erucic acids)	264,000	55,000

[a] Derived from Connor *et al.*[7]

ment was isolated and clamped for 15 min and then examined for the presence of a thrombus. Then the rabbits were heparinized, killed 5 min later with Nembutal, and autopsied.

In the initial experiments, Hoak and Robinson [21] used 50 units of ACTH per kilogram of weight, and plasma FFA concentrations increased from 552 to 2411 μEq/liter (Table 5). The clotting time in silicone-coated tubes declined from 41 to 22 min, a significant decrease. Injections of anterior pituitary extract had similar effects. Because the dose of ACTH was large, smaller doses were given to ascertain whether the same rise in FFA and hypercoagulability of the blood could be attained.[7] The effects of injections of 2 and 10 units/kg on clotting time and FFA concentration were similar to those of 50-unit/kg injections. Even with a dose as low as 0.5 unit/kg, a rise in FFA resulted 1 or 2 hr later. It seemed, however, that the peak effect on the blood was attained after this smallest dose at 1 hr, inasmuch as the silicone clotting time shortened at 1 hr, but did not change at 2 hr, when the FFA concentration was lower.

All doses of ACTH produced comparable amounts of thrombosis. In fact, with the 0.5-unit/kg dose, five of 10 animals had jugular thrombi and three of the 10 had lung thrombi (Figure 4). Even with this small dose of ACTH, a definite thrombogenic effect was demonstrated. There were no such effects after saline injection.

Our hypothesis is that the thrombogenic effect of ACTH is mediated through a sudden rise in the FFA fraction of the blood. In all probability, the FFA fraction of the blood must increase beyond some point before thrombogenic effects occur. In this connection, the work of Goodman and associates,[13–15] dealing with the binding of fatty acids to albumin, lipoproteins, and blood cells is of importance. Goodman has shown that albumin has two tight binding sites for fatty acids, such that it effectively competes with other plasma proteins and takes up available fatty acids until these two binding sites are completely filled. For the usual plasma albumin concentration, this would mean that the two "first-class" sites

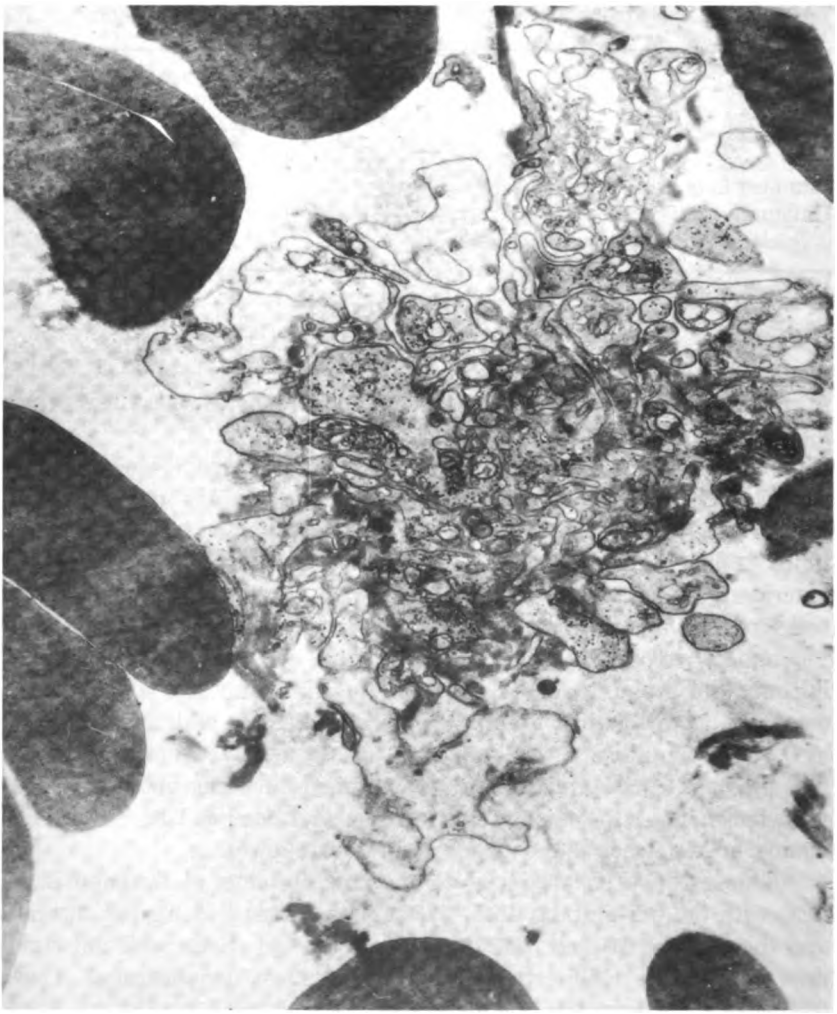

FIGURE 3 An electron micrograph of a thrombus that formed after the injection of linoleic acid into a mouse. (× 10,400)

would bind fatty acids up to a level of 1160 μM/liter or a molar ratio of fatty acid to albumin of 2. If the FFA concentration increased further, perhaps to 1740 μM/liter or a ratio of 3, the next five binding sites of albumin for fatty acid might not completely take up the fatty acid. Then the binding sites of other plasma proteins and red blood cells might compete effectively with plasma albumin. Theoretically, then, when the molar

TABLE 5 ACTH-Induced Plasma Free Fatty Acid Increase, Hypercoagulability, and Thrombosis [a]

ACTH Injected Subcutaneously, units/kg of Weight	No. of Rabbits	Plasma FFA Concentration, μEq/liter		Silicone Clotting Time, min			Number with Jugular Thrombi	Number with Lung Thrombi
		Before	After	Before	After	Change		
0 (saline)	5	472	483	31	36	+5	0	0
0.5 (1 hr)	5	315	1277	42	28	−14	3	1
0.5 (2 hr)	5	448	970	34	33	−1	2	2
2 (2 hr)	5	431	1917	39	25	−14	2	2
10 (2 hr)	5	360	1985	45	18	−27	3	3
50 (2 hr)	10	552	2411	41	22	−19	6	7

[a] Derived from Connor *et al.*[7]

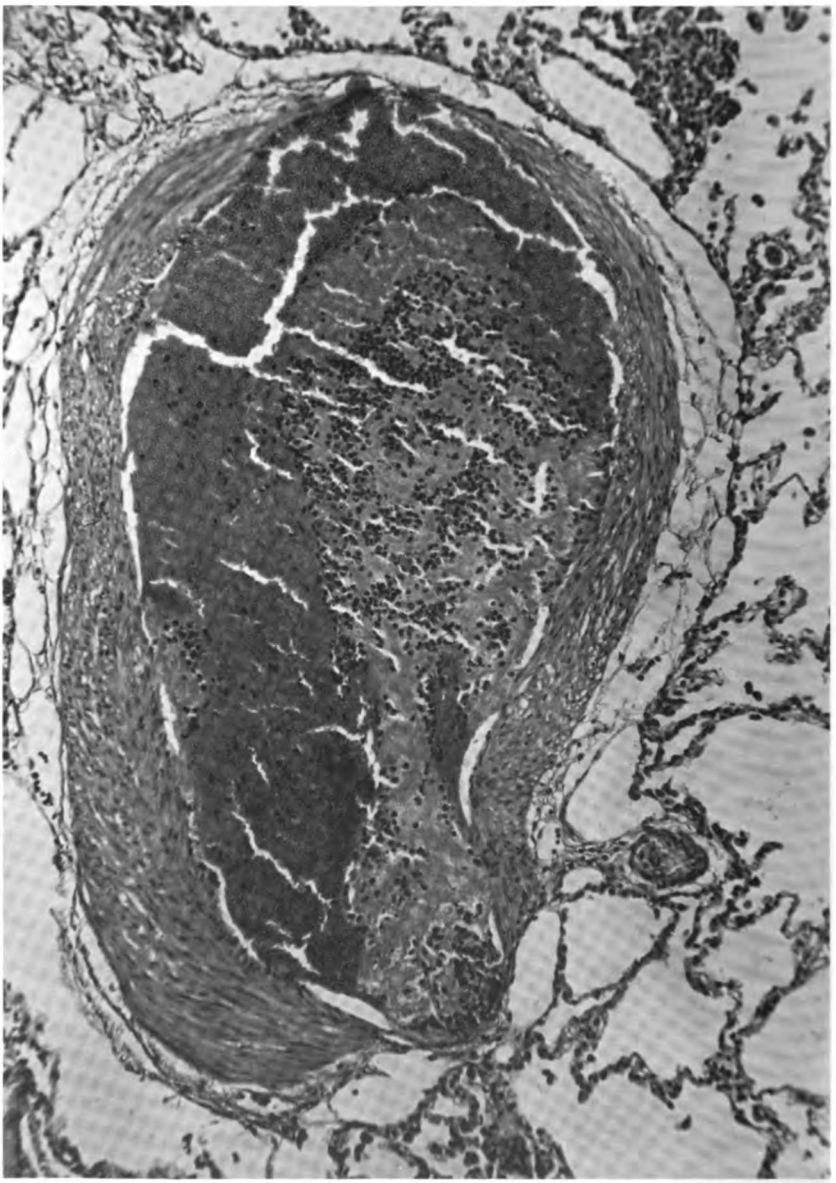

FIGURE 4 Thrombus in a branch of a pulmonary artery from a rabbit that received an injection of ACTH. (× 255)

ratio of fatty acids to albumin exceeds 2, the fatty acid might be bound to other plasma proteins and blood cells. Our speculation is that, when this happens, fatty acid may affect the coagulation mechanism through the activation of some clotting proteins and through platelet aggregation.

STUDIES OF BINDING OF FATTY ACIDS TO ALBUMIN

As has already been mentioned, the plasma FFA are transported in the blood bound chiefly to the plasma albumin. Would fatty acids bound to albumin before intravenous infusion still have thrombogenic effects? The infusion of albumin alone without added fatty acid but containing the 0.3–0.6 molar ratio of intrinsic fatty acid had some activity in the production of jugular segment thrombi (Table 6).[20] Infusions of fatty acid previously mixed and presumably bound to albumin caused lung and jugular segment thrombi. The infusion of albumin plus stearic acid had a fatty acid:albumin molar ratio of 1.9 to 2.9 and the infusion of albumin plus oleic acid had a fatty acid:albumin molar ratio of 2.7. Both infusions exceeded for the most part the two tight binding sites of the albumin molecule. Eight of the 15 rabbits given stearic acid had lung thrombi; four of the 10 rabbits given oleic acid had lung thrombi. The blood became hypercoagulable after these fatty acid–albumin infusions, as evidenced by the shortening of the whole-blood clotting time (Table 7). In these studies, also, unsaturated fatty acids were less potent than saturated fatty acids in their thrombogenic effects.

Spector and John have shown recently that a greater concentration of plasma FFA exists in an unbound form than was thought previously.[30] For example, 8.5 μM/liter of stearic acid was unbound in a solution having a stearic acid:albumin molar ratio of 3 (Spector, A. A., personal communication). When fatty acids were added to platelet-rich plasma in concentrations similar to those found to be unbound, the whole-blood clotting time was little affected, but microscopic platelet aggregation occurred for all three fatty acids tested, including both saturated fatty acids and the unsaturated oleic acid, but not for buffered saline (Table 8). A typical pattern of aggregation after the addition of oleic acid is shown in Figure 5. Platelets were not aggregated by 0.9% NaCl (Figure 6). The thrombus-formation time has previously been shown to be shortened by fatty acids in a concentration as low as 3.5 μM/liter,[4,8] compared with the 8.5 μM/liter found by Spector to exist in the unbound form in blood. These results suggest that fatty acids, in concentrations within the physiologic range, might well induce hypercoagulability of the blood.

We next conducted experiments to indicate why saturated fatty acids

TABLE 6 Incidence of Thrombosis after Infusions of Albumin and Fatty Acids [a]

	FFA Concentration of Infusate μEq/liter	Molar Ratio of Fatty Acid to Albumin	No. Rabbits in Group	No. Rabbits with Lung Thrombi	No. Rabbits with Jugular Segment Thrombi			
					Grade [b]			
					0	1	2	3
5% albumin (control)	200–400	0.3–0.6	10	0	5	4	0	1
5% albumin + stearic acid	1400–2100	1.9–2.9	15	8 [c]	5	2	4	4
5% albumin + oleic acid	1960–2060	2.7	10	4 [d]	6	3	0	1

[a] Derived from Hoak *et al.*[30]

[b] Grading of jugular thrombi: 0, none; 1, <5 mm; 2, 5 mm; and 3, larger than 5 mm.

[c] $p < 0.02$.

[d] $p < 0.05$.

TABLE 7 Effect of Infusions of Albumin and Fatty Acids[a] on Whole-Blood Silicone Clotting Time

Infusion	Clotting Time (Mean Values), min: Before Infusion	After Infusion	Change	p
5% albumin (control)	35.5 ± 2.5[b]	30.8 ± 3.7	— 4.7	>0.10
5% albumin + stearic acid	37.8 ± 3	23.2 ± 3	—14.6	<0.01
5% albumin + oleic acid	38.0 ± 2.6	23.9 ± 2	—14.1	<0.01

[a] Derived from Hoak *et al.*[20]
[b] Standard error.

had more pronounced activity than unsaturated fatty acids in accelerating blood coagulation, causing platelet aggregation, and inducing thrombosis. We suspected that the rate and amount of fatty acid binding to proteins, and especially to albumin, might be the critical factor. Goodman had previously studied protein binding but had used a 48-hr incubation system.[14] We designed a dynamic system in which the rate of fatty acid binding to bovine albumin in different molar ratios could be studied from minute to minute.[2] Figure 7 indicates the rate of binding of different fatty acids to the serum albumin at a fatty acid:albumin molar ratio of 4. Note the rapid binding, within 10 min, of the short-chain fatty acids, palmitic (C16:0) and oleic (C18:1). Stearic (C18:0), arachidic (C20:0), and behenic (C22:0) acids were poorly bound. In man, of these very-long-chain saturated fatty acids, only stearic acid is quantitatively important.

The polyunsaturated fatty acids, linoleic and linolenic, were bound extremely rapidly. The binding curve of linoleic acid at a molar ratio of 8 is

TABLE 8 Effect of Fatty Acids on Platelet Aggregation in Platelet-Rich Plasma

Test Solution	Whole-Blood Clotting Time, min	Platelet Aggregation
Buffered saline	22	No
Palmitic acid (0.6×10^{-3} μM)	26	Yes
Stearic acid (8.5×10^{-3} μM)	26	Yes
Oleic acid (1.6×10^{-3} μM)	23	Yes

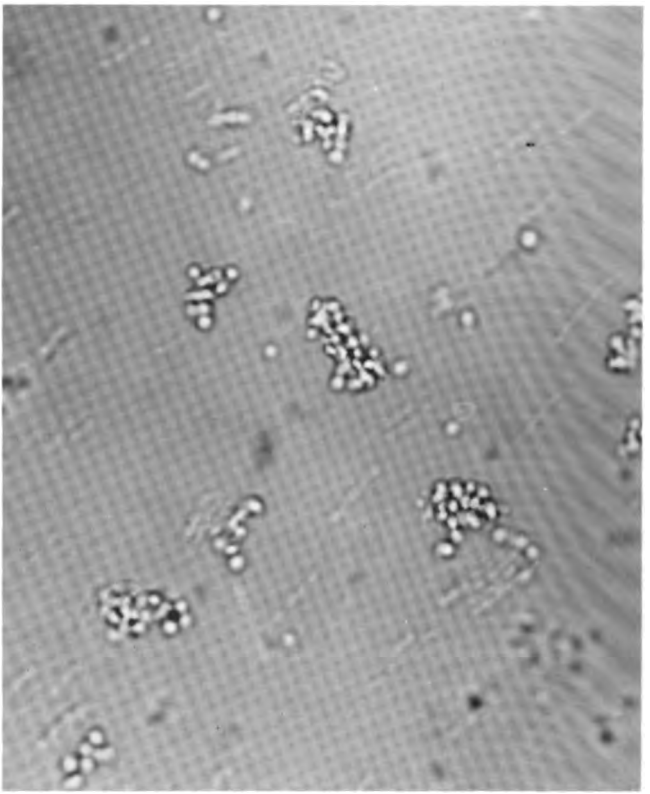

FIGURE 5 Microscopic aggregation of platelets in platelet-rich plasma after the addition of oleic acid in a concentration of 1.6×10^{-3} μM/liter. Palmitic and stearic acids produced similar results. ($\times$ 300)

complete at 4 min (Figure 8). In only 5 sec, remarkable changes are manifest (the steep portion of the curve). Short-chain fatty acids (C12 and below) are bound immediately and up to a fatty acid:albumin molar ratio of 12; unsaturated, long-chain fatty acids (i.e., oleic) are somewhat less rapidly bound and up to a molar ratio of 8; and saturated long-chain fatty acids (i.e., stearic) are bound slowly and incompletely and in molar ratios from 2 to 8, depending on chain length.

THE MECHANISMS OF FATTY ACID EFFECTS ON BLOOD COAGULATION AND CELLS

The salts of fatty acids probably initiate blood coagulation in the early phases by the activation of the factor XII–XI system.[1,4,9,26,27] It has been

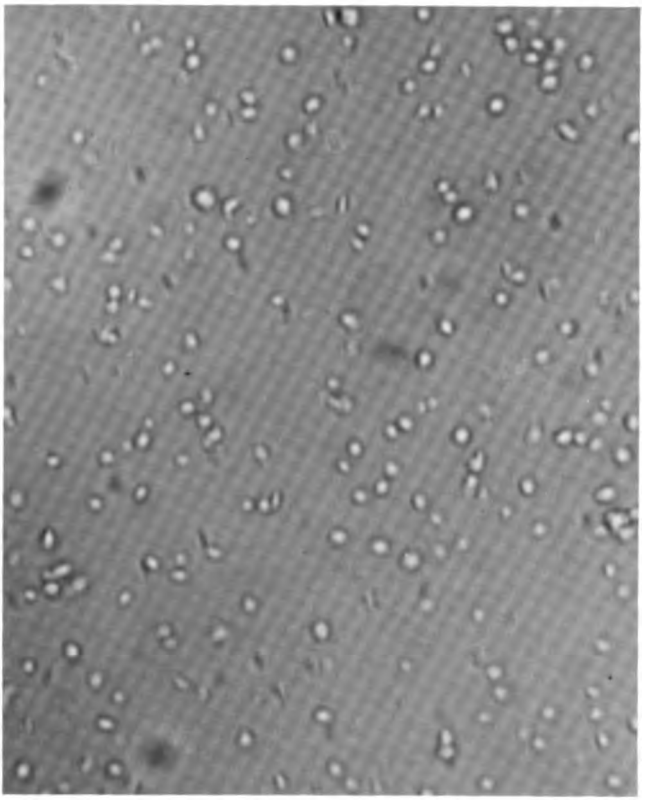

FIGURE 6 The failure of platelets to aggregate when 0.9% NaCl was added to platelet-rich plasma. (× 600)

suggested that fatty acids act analogously to other "contact" or surface factors, of which glass is a most conspicuous example. Like glass, bentonite, and kaolin, fatty acid salts are negatively charged and may exert their effects by virtue of this negative charge.[24]

In addition, the effects of fatty acids on cells may operate through metabolic pathways. Haslam has evidence that fatty acids aggregate platelets through the release of adenosine diphosphate.[16] Furthermore, fatty acids promote hemolysis [11] and block metabolic activity in liver mitochondria by uncoupling oxidative phosphorylation.[17]

These diverse actions of fatty acids, as well as their major importance in supplying substrate for the energy metabolism of the body, point to the possibility that fatty acids might at times be pathogenic, especially thrombogenic. Fatty acids are a unique group of substances circulating in the blood and changing rapidly in response to metabolic needs and

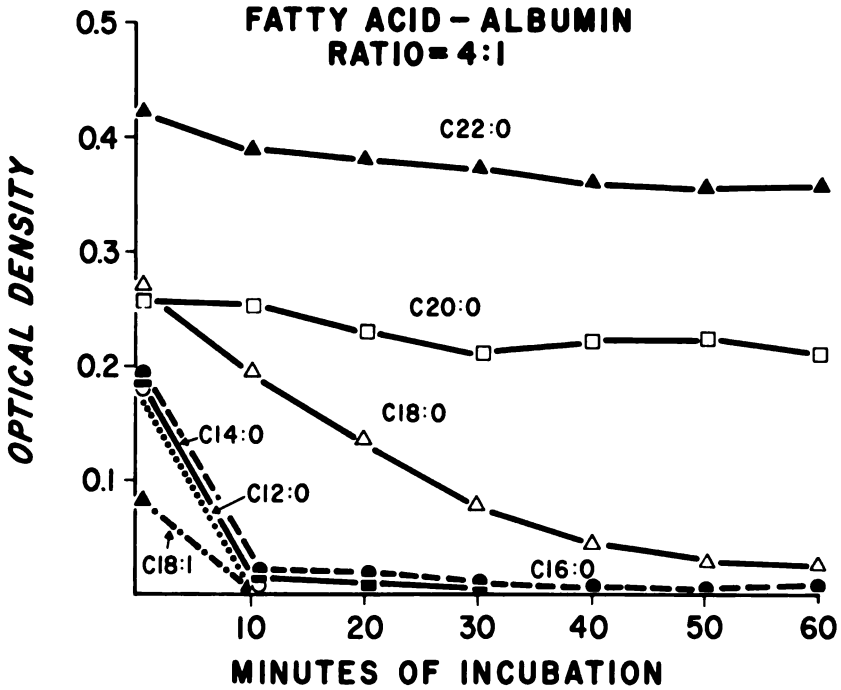

FIGURE 7 The rate of fatty acid binding to bovine albumin at a fatty acid : albumin ratio of 4. Various fatty acids were tested. The sodium salts of the fatty acids and bovine albumin were incubated at 37 C in an aqueous system of Krebs-Henseleit buffer. At zero time the mixtures were turbid and had a high optical density. Binding was indicated by a decrease in optical density.

humoral stimulation; they also have the capacity to activate the blood-clotting proteins, aggregate platelets, and injure cells. Probably, these pathogenic actions of fatty acids are not normally operative. It is hypothesized, however, on the basis of the experiments described in this presentation, that metabolic and humoral factors may produce transient hypercoagulability of the blood and subsequent thrombosis, and that these effects are likely mediated through high plasma levels of FFA. The hypercoagulability may be not only transient but localized to the blood perfusing a single vascular bed.

The work reported here was supported by Public Health Service research grants HE 11485, HE 7239, and HE 9862 from the National Heart Institute and grants from the American Heart Association and the Iowa Heart Association; research career award HE K3-18406 from the National Heart Institute (Dr. Connor); and research career award HE K3-19370 from the National Heart Institute (Dr. Hoak).

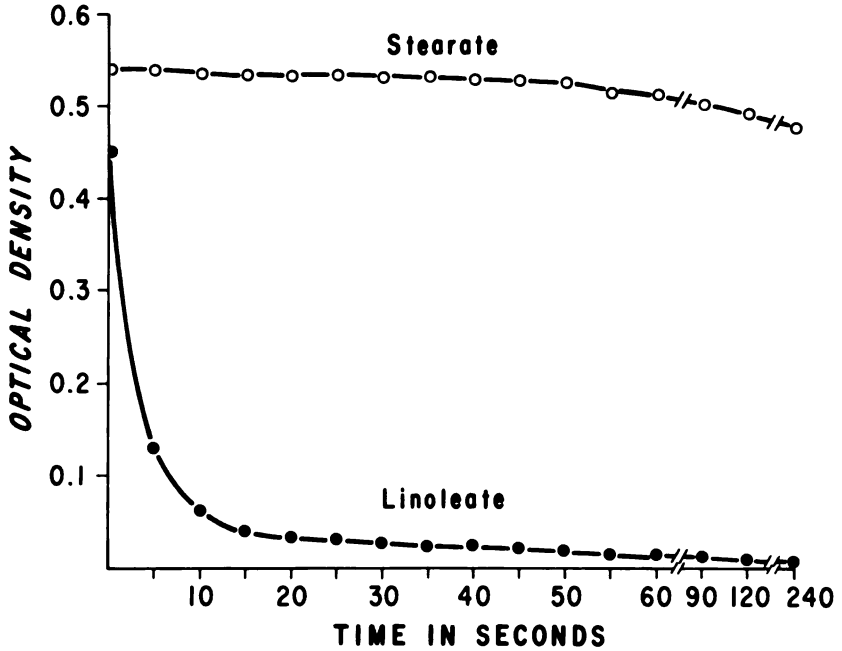

FIGURE 8 The rate of binding of linoleic acid and stearic acid to albumin. The fatty acid : albumin molar ratios were 8. Note the almost complete lack of binding of the saturated stearic acid, compared with the polyunsaturated linoleic acid.

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The Role of Formed Elements in Thrombosis

TORSTEIN HOVIG

Transient thromboemboli and freshly formed mural thrombi are composed of aggregated blood platelets, with a few red and white cells interspersed, but usually with no evidence of polymerized fibrin. Because the platelets are the key elements in the formation of the thrombus, their role will be discussed first.

PLATELETS

NORMAL PLATELETS

When blood is drawn directly into a fixative, such as glutaraldehyde, without the addition of an anticoagulant ("native blood"), the platelets have a disk or rod shape and few or no pseudopods (Figures 1 and 2). This is in sharp contrast with the shape of the platelets in native blood that has been shaken for 30 sec in a siliconized tube before fixation is initiated. In the latter case, most of the platelets become spheric and have several pseudopods of various shapes, some of which may appear to be swollen (Figures 3 and 4).

The surface membrane is surrounded by a fluffy coat that probably contains mucopolysaccharides [5] and proteins, e.g., fibrinogen (Figure 5). Whether this coat corresponds to the *atmosphère plasmatique plaquettaire,* suggested by Roskam,[30] is not known. Electron microscopic histochemical studies have failed to demonstrate specific adenosine triphosphatase (ATPase) activity at the platelet surface.[6,38] If these observations are valid, they will raise several questions concerning processes assumed to take place on the platelet surface.

The electron-dense granules of the platelets from normal persons are of various sizes, shapes, and electron densities (Figures 5 and 6). Their

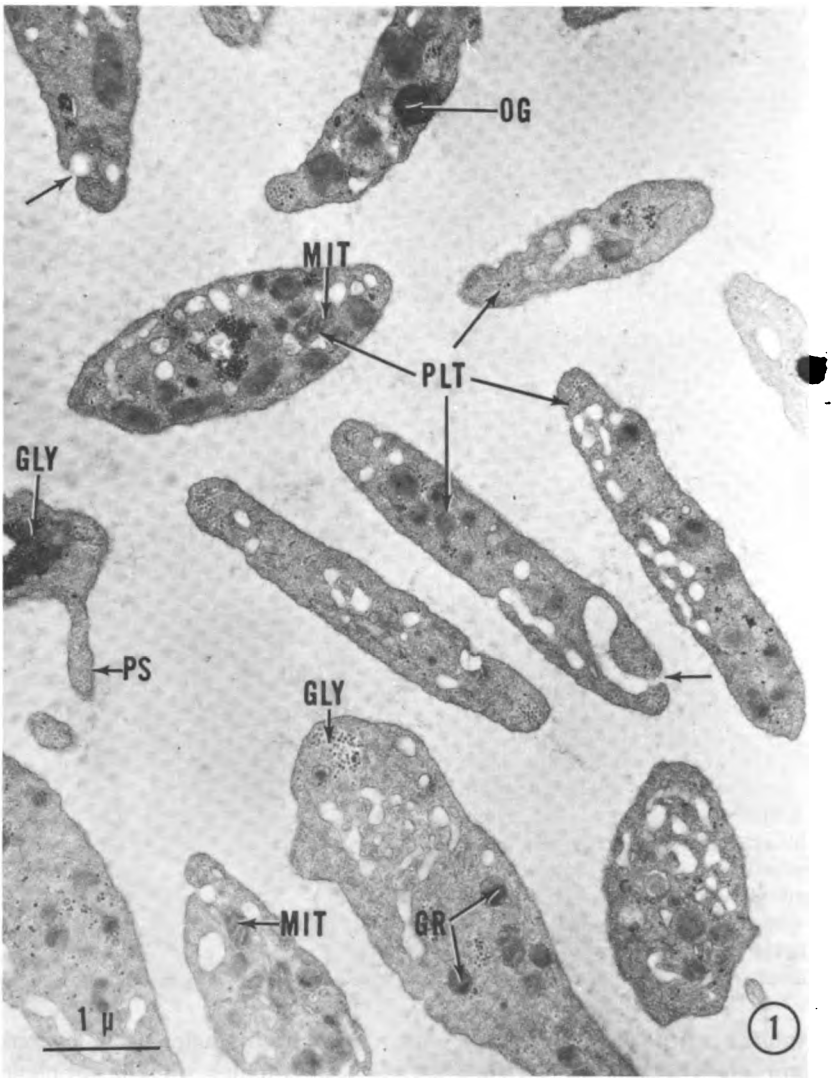

FIGURE 1 Normal human blood platelets (PLT) rapidly fixed by drawing blood directly into the fixative, 2.5% glutaraldehyde in phosphate buffer. Note the regular elliptic or rodlike shape of the platelets. A single pseudopod (PS) can be seen. GLY, glycogen; GR, electron-dense granules; OG, osmiophilic granule; MIT, mitochondria. The arrows indicate apparent communication between vacuoles and the surrounding medium.

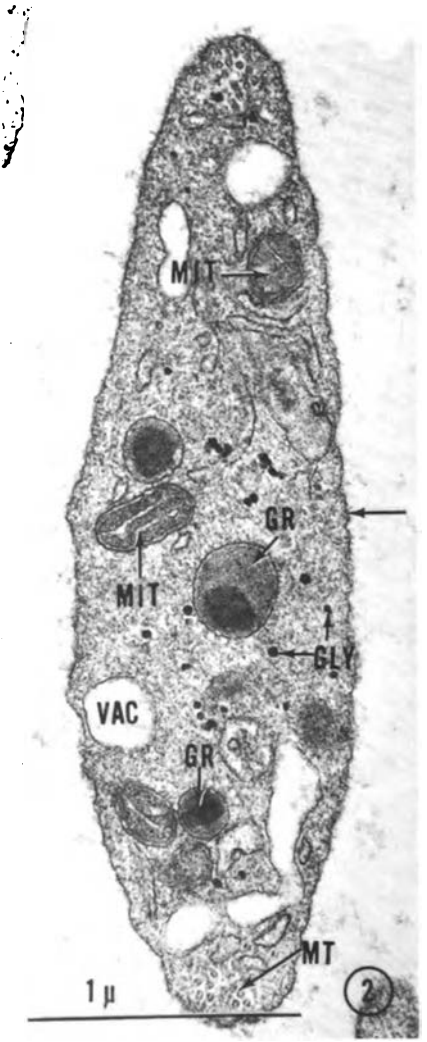

FIGURE 2 Normal human platelet rapidly fixed in glutaraldehyde. GR, electron-dense granules, with bull's-eye; MIT, mitochondria; MT, microtubules; VAC, vacuoles. Scattered glycogen granules (GLY) can be observed. The arrow indicates the irregular fluffy coat at the platelet surface.

common feature is that they are surrounded by a single, triple-layered membrane. The nature and function of these granules are still not clear and, to judge by the literature, they may be different structures. Thus, granules have been reported to be lysosomes,[25] containing the "hidden" form of platelet phospholipids active in coagulation (platelet factor 3).[39] They have also been found to contain fibrinogen.[33]

Some osmiophilic and very electron-dense granules (Figure 1) have also been observed, and these have been found to contain 5-hydroxytryptamine (serotonin).[1,38,40]

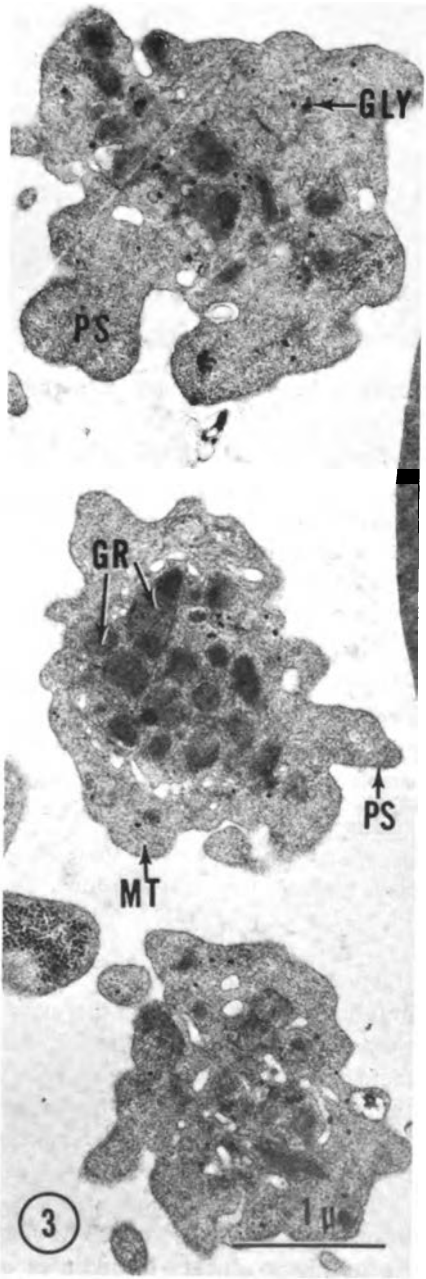

FIGURE 3 Blood platelets from "native blood" fixed after having been shaken in a siliconized tube for 30 sec. There is no aggregation of the platelets, but alteration of their shape to more nearly spheric. Pseudopods (PS) are formed. Similar alterations occur in citrated blood.

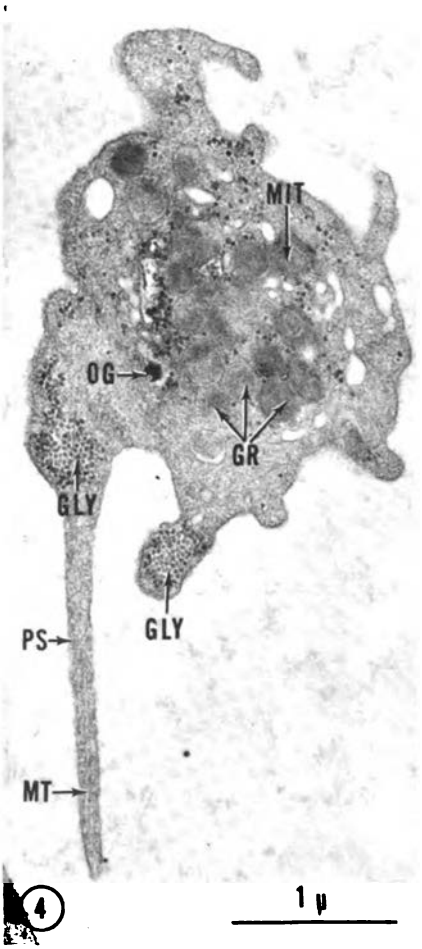

FIGURE 4 A platelet from a blood sample prepared as described in Figure 3. Note the pseudopod (PS) containing microtubules (MT). GR, electron-dense granules; OG, osmiophilic granule; MIT, mitochondrion.

The mitochondria of the platelets probably do not differ fundamentally in function from those in other cells. By electron microscopic histochemistry, they have been shown to contain ATPase activity. It is striking that the platelets contain relatively few mitochondria, although the ATP content of the platelets is very high. This might indicate that the ATP in the platelets may be present in other structures in addition to the mitochondria.

It has been suggested that the contractile protein of the platelets, thrombosthenin, plays a role in clot retraction, as well as in thrombosis. According to Zucker-Franklin *et al.*,[42] this protein appears ultrastructurally as filaments. Similar filaments are seldom found in unaltered

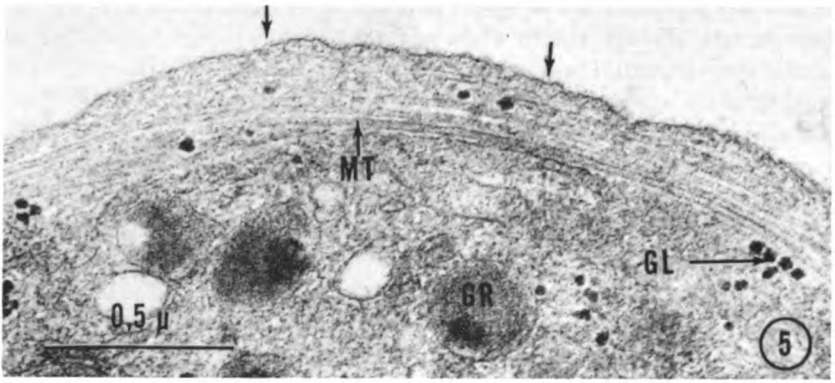

FIGURE 5 Platelet surface showing a fluffy coat. Submarginal band of microtubules (MT). GR, electron-dense granules.

platelets but are frequently seen in altered platelets, especially in pseudopods.[19,23] Whether a relationship exists between the filaments and the microtubules that can be seen in normal glutaraldehyde-fixed platelets is not clear, although such a relationship is indicated by ultrastructural studies. Microtubules in the platelets are found especially at the poles of sectioned platelets (Figure 6), but they may also be seen in pseudopods (Figure 4). Synthesis of thrombosthenin has been found to occur in the

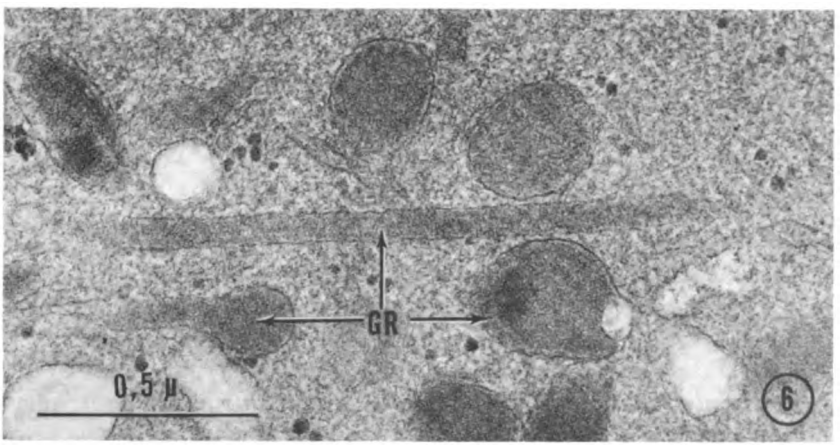

FIGURE 6 Part of a normal human platelet. The shape of the electron-dense granules (GR) may show great variation.

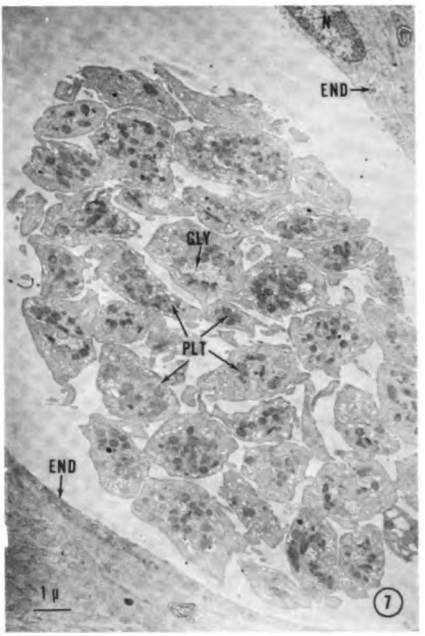

FIGURE 7 Cerebral vessel from cat containing loosely packed platelets (PLT) more spheric than normally seen and with pseudopod formation. There is no clear evidence of degranulation of the platelets. END, vessel endothelium, with a nucleus (N); GLY, glycogen.

platelets,[7] and they should be expected to contain ribosomes as other protein synthesizing cells. However, ribosomes are rarely found in normal circulating platelets.

The vacuoles that can be observed within platelets sometimes appear to be in communication with their surroundings (Figure 1). Particles of various size may be taken up by this system, as seen in phagocytosis, but it is also possible that intracellular components may escape from the platelets through the same system. These vacuoles or channels are coated on the inner surface with a fluffy coat similar to that on the platelet surface.

TRANSIENT THROMBOEMBOLI

The first main step in the thrombotic process is the aggregation of platelets at the site of vessel injury. Such aggregation probably may occur in flowing blood. Figure 7 shows a platelet aggregate in a small cerebral vessel of a cat. Fixation was carried out by formaldehyde perfusion.* The stimulus for aggregation is not known, but it may be related to the operative procedure that was carried out. The early stage of a thrombus seen in Figure 7 is composed of loosely packed platelets that have been

* In an experiment carried out at The Anatomical Institute, University of Oslo, Norway.

shown by serial section to be floating freely in the lumen of the vessel. The platelets in this small thrombus were mostly spheric and showed some pseudopod formation, but otherwise no clear ultrastructural alterations were found. As seen in Figure 8, the platelets were covered by a fluffy coat, and threads of electron-dense material appeared to bridge the gap between adjacent platelets where they were lying close to each other (at a distance of about 300–600 Å).

Platelet aggregation, both *in vivo* and *in vitro,* may be induced by numerous physiologic stimuli. As far as is known today, platelet aggregation induced by any of the different stimuli is mediated via ADP. Several theories have been put forward concerning the mechanism whereby ADP acts, but none has so far been proved to be valid. The effect of ADP on platelet ultrastructure has been described and ADP has been found to cause only minor ultrastructural alterations of the platelets in citrated or native plasma *in vitro* [17,29] or no such alterations at all.

When studying the effect of ADP on the ultrastructure of platelets in plasma, the blood has to be centrifuged before the addition of ADP and the fixative. In order to avoid this possible source of damage to the platelets, we have studied the effect of ADP on platelets in native blood. Normal blood was drawn directly into a siliconized glass tube containing ADP (final concentration, 0.8 μg/ml) and continuously stirred for 30 sec before the fixative was added. The specimens were fixed in 2.5% glutaral-

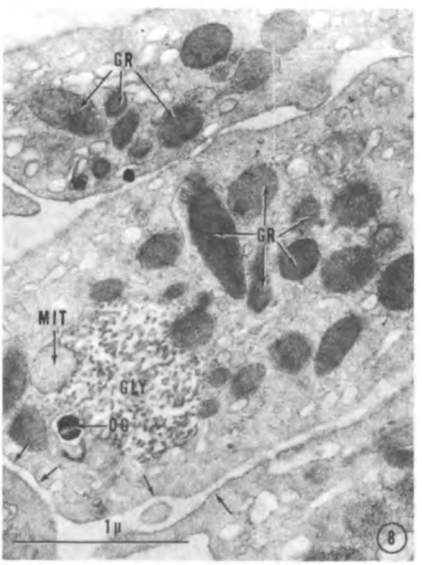

FIGURE 8 Platelets within the same aggregate as in Figure 7, at higher magnification. There appears to be some connecting material between adjacent platelets (arrows). GR, electron-dense granules; OG, osmiophilic granule; GLY, glycogen; MIT, mitochondrion.

dehyde in phosphate buffer for 1 hr (0–2 C) and then postfixed in buffered 1% osmic acid for 1 hr (0–2 C).

Platelet aggregates could be observed by light microscopy on the 0.5-μ-thick sections stained with toluidine blue. No red or white blood cells were adhering to these aggregates. Platelet pseudopod formation could be seen. In ultrathin sections, the platelets were found to be aggregated with a distance between adjacent platelets of about 200–600 Å. In agreement with earlier observations, pseudopod formation was the only structural alteration of the platelets that could be clearly demonstrated (Figure 9). However, this was also the case without ADP in the sample. There was no evidence of degranulation of the platelets; this situation is similar to that observed in the *in vivo* aggregate (Figure 7).

At higher magnification, threadlike material appeared to bridge the gap between adjacent platelets. These threads, which have a diameter of about 60–100 Å, appeared to radiate from the platelet surface almost perpendicularly, and they could be clearly followed from one surface membrane to the other (Figure 10). At times, two threads from different platelets appeared to be overlapping. They were not evenly distributed around the platelet surface. In zones where the distance between

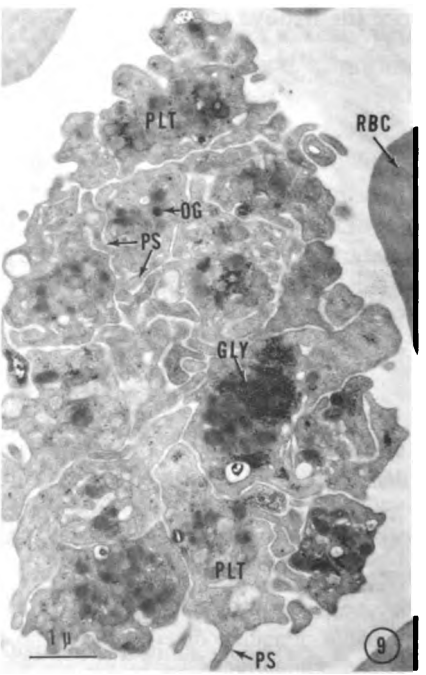

FIGURE 9 ADP-induced platelet aggregate in native blood *in vitro*. Human blood was drawn directly into a siliconized glass tube containing ADP (final concentration, 0.8 μg/ml) and shaken for 30 sec before fixation was started. The appearance of the platelets is similar to that in Figure 3. No adherence of red blood cells (RBC) or white blood cells. GLY, glycogen; OG, osmiophilic granule; PS, pseudopod.

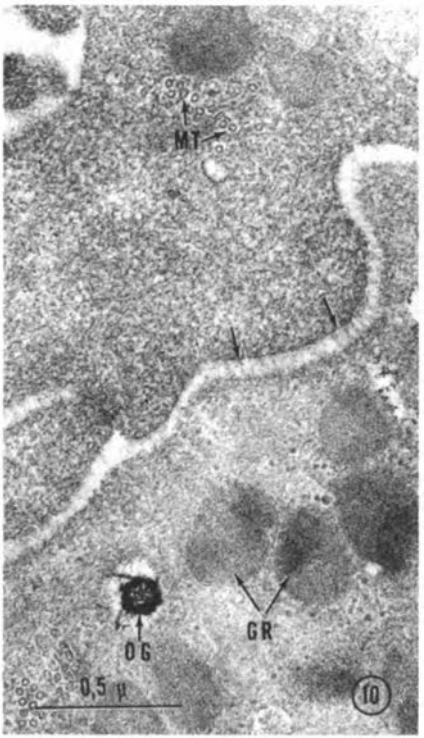

FIGURE 10 The contact zone between adjacent platelets in an aggregate induced by ADP in native blood. Note the connecting material appearing to bridge the gap between platelets (indicated by arrows). GR, electron-dense granules; OG, osmiophilic granule; MT, microtubules.

neighboring platelets was greater than usual, no connecting material was found. Similar material was also found in ADP-induced aggregates in citrated blood prepared in the same manner. Inasmuch as this connecting material was almost perpendicular to the platelet surface and ran in various directions, depending on the direction of the platelet surface, it did not represent cutting artifacts due to the knife.

There seemed to be direct continuity between adjacent platelets by the connecting material, and it may represent the binding forces between aggregated platelets. If so, these are not permanent bindings; it is well known that ADP-induced platelet aggregates tend to disintegrate.

These observations of induced *in vitro* aggregates are similar to those reported by Stehbens and Biscoe,[35] who found bridging material between adjacent platelets in spontaneously occurring aggregates in the carotid body of cats. In these studies fixation was obtained by perfusion with glutaraldehyde. The authors stress the possibility that this material is concerned with the initial adhesion between platelets and between platelets and leukocytes.

This bridging material originates from the fluffy coat at the surface of

unaggregated platelets. Its nature is not as yet clear. It is known that fibrinogen is important for ADP-induced aggregation of washed platelets. [8,26,34] It is therefore possible that it is fibrinogen or partially polymerized fibrin. It is interesting to note that, when polystyrene latex particles are suspended in a solution of fibrinogen (300 mg/100 ml) and stirred, the latex particles aggregate, and structures similar to the bridges can be observed radiating from the latex particles (Figure 11). If the observed phenomenon in the platelet aggregates is analogous to that in aggregated latex particles, the former may also be interpreted as a surface phenomenon independent of intracellular reactions.

Calcium is known to be of fundamental importance in ADP-induced platelet aggregation, as it is in cell adhesion in general. It may be assumed that the calcium ions are important for the arrangement of the fluffy coat

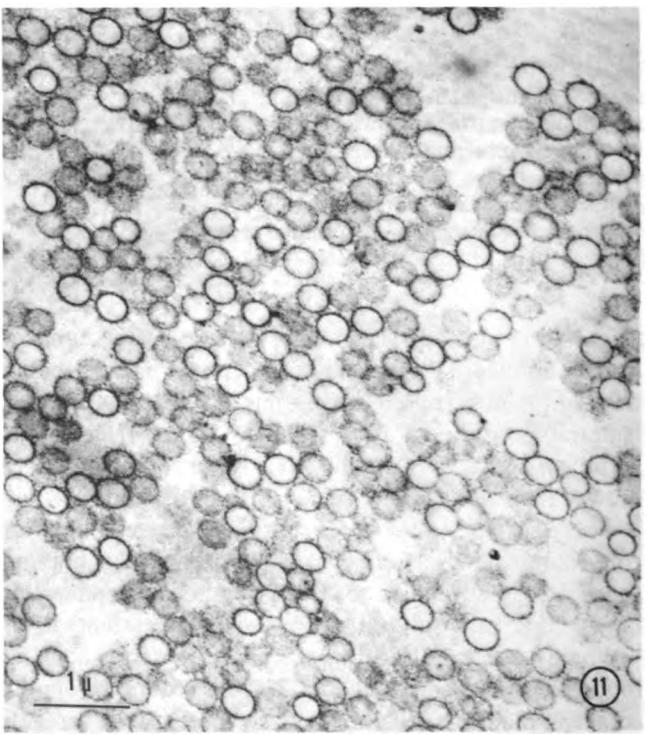

FIGURE 11 Polystyrene latex particles suspended in a fibrinogen solution (300 mg/100 ml) and stirred for 3 min. Aggregation of the particles occurred. Note the material coating the particles and appearing to bridge the gap between aggregated particles.

on the platelet surface. Whether calcium ions play a role as a link in this bridging material cannot be ascertained from the present studies.

Platelet thrombi in flowing blood may break up and the platelets may enter into the circulation, but the aggregates may also become attached to sites of damaged or denuded vessel endothelium. By release of various substances—such as lysosomal enzymes, serotonin, and histamine—they may also cause damage to the vessel wall and more generalized tissue damage. Mustard's group found that production of platelet aggregates in the myocardial circulation by infusion of ADP into the coronary circulation caused myocardial infarction.[22]

EXPERIMENTAL MURAL THROMBI

Even when a thrombus is produced by trauma to the vessel wall, fragments of the thrombus are broken off and can be observed to pass away with the bloodstream. However, new platelets adhere to those already attached to the site of the damage, and finally a stable mass, occluding or not occluding the vessel lumen, is built up. Within 1 hr after the initiation of the trauma, marked structural alterations of the platelets in the thrombus can be found.[23] The platelets are closely packed, the distance between neighboring platelets being around 200–300 Å. The platelets usually show marked pseudopod formation, but in other regions they may appear swollen and devoid of organelles and bear little evidence of pseudopod formation. The surface membrane of the platelets usually appears to be ultrastructurally intact. The most striking alterations are often found in areas of close contact between the platelet mass and the vessel wall and at the surface of the thrombus facing the vessel lumen. At sites of contact between platelets and the collagen of the vessel wall, breaks in the platelet surface membrane may be found. All these platelet alterations strongly suggest that the stimulus influencing them must be more potent than that due simply to ADP.

One component of the vessel wall has been shown to have a pronounced effect on the platelets—collagen. Platelets first readily adhere to collagen fibrils, whereby morphologic alterations of the platelets are induced and intraplatelet substances released; later, platelet aggregation occurs.[15,16,18,41] There may be other components of the vessel wall that under some conditions have a similar effect, but so far that has not been convincingly demonstrated.

Likewise, thrombin has a profound effect on blood platelets. Thrombin may be produced in plasma via both the extrinsic (tissue thromboplastin) and the intrinsic pathways of coagulation. Aggregated platelets themselves may serve as a focus of initiation and activation of the coagulation

mechanisms.[28] Thus, thrombin may be produced at the site of a thrombus formation.

The presence of polymerized fibrin at the periphery of a thrombus clearly indicates thrombin activity. Because small amounts of thrombin induce platelet aggregation in plasma *in vitro* before fibrin occurs, it may be suggested that traces of thrombin may have exerted an effect on platelets *in vivo* before polymerized fibrin can be found. Thus, the ultrastructural alterations of the platelets at the periphery of a thrombus with degranulation and pseudopod swelling most likely are due to the presence of thrombin. The increasing amounts of fibrin between the platelets during the 24 hr after the formation of the thrombus show that, in time, thrombin comes to act not only at the surface but also within the thrombus.

It is widely accepted that the stabilization of the platelet mass in a thrombus is mediated via thrombin, with the fibrinogen at the platelet surface as the substrate. The fibrin cap usually found at the periphery of a thrombus may help to keep the platelet mass together. But thrombi, as well as hemostatic platelet plugs, appear to be fairly stable when such a cap is lacking, and even when the coagulation is greatly impaired. The view that fibrinogen is the substrate for thrombin, thereby providing the "glue" holding the platelets together, is supported by the findings of increased bleeding times in some subjects with severe congenital deficiency in fibrinogen.[11] Other observations, however, contradict that view. Davey and Lüscher [9] found that a snake venom clotted fibrinogen without having any effect on the platelets. They also found that platelets that had been incubated with chymotrypsin, rendering dissolved fibrinogen incoagulable within a short time, still reacted normally with thrombin. It must therefore be concluded that an essential step in thrombosis—the mechanisms whereby the platelets are kept together—is still poorly understood.

Many of the platelets appear empty and swollen soon after the initiation of a mural thrombus, so intracellular substances must have been released. Electron-dense granules have been reported to be extruded from the platelets,[22] but they are very rarely found outside the platelets, especially if the great number of these elements found within intact platelets is considered. The organelles are therefore most probably dissolved within the platelets, whereby they pass out through the membrane. Altered platelets themselves may thus be the source of substances—lysosomal enzymes, serotonin, histamine, adenine nucleotides, platelet factor 3, etc. —that can influence processes taking place in the thrombus, in adjacent tissue, and in the blood passing by the site of thrombosis.

Twenty-four hours after the formation of a mural thrombus, large

amounts of polymerized fibrin can be found between the platelets, which now show marked structural changes.[23] They usually appear shrunken, and at this stage many of them appear to be separated once more. The bindings between the platelets in a thrombus are therefore not necessarily permanent; they keep the platelets together until the thrombus becomes organized.

Clinical and experimental evidence has accumulated that thrombosis, as well as hemostasis, may occur even when blood coagulation is greatly impaired. This would indicate that, if the stimulus of the vessel wall or of flowing blood is sufficient, platelet thrombi may be formed independently of blood coagulation. Anticoagulant treatment can therefore not be expected to represent a final solution of the problem of thrombosis. Another way to attack this problem would be to suppress platelet–surface and platelet–platelet interaction, inasmuch as these reactions are involved in the initial steps of the process. The search for substances that influence the platelet surface and its coating should therefore be intensified.

THE RED CELLS

Red blood cells contain adenine nucleotides, and ADP can be released from red cells by various types of damage in a concentration sufficient to induce platelet aggregation.[10,13] By quantitative estimation of the nucleotides in the blood from wounds it has been found that the amount of nucleotides in plasma exceeded that found in the total number of platelets, indicating a possible release from red cells.[20] If these findings are valid, it may be suggested that the contact between the damaged vessel wall or the thrombus and the flowing blood may lead to release of ADP from the red cells, which would cause growth of the platelet thrombus. It is also possible that damage to the red cells in flowing blood may lead to the formation of transient platelet thromboemboli.

The red tail of a thrombus may be produced by trapping of red cells in a fibrin meshwork. Red cells are known to contain phospholipids,[2,31] which can promote coagulation. Whether such phospholipids as can be obtained by red-cell lysis are available in the red tail of the thrombus is not known. Some lysis of the red cells may occur and, thus, further fibrin may be formed.

When collagen fragments are inserted into an extracorporeal shunt and exposed to blood flow, both platelets and red cells appear to be associated with the collagen.[18] The red cells may be trapped between collagen fibrils. When polystyrene latex fibers forming a similar meshwork are inserted, instead of collagen, only few red cells are found. It is therefore

possible that the red cells have a particular affinity for collagen and interact with components of a damaged vessel wall. This problem needs clarification.

An important effect of red cells in thrombosis may be their influence on the rheologic properties of the blood. As pointed out by Merrill *et al.*,[27] the fibrinogen and red cells in whole blood cause human blood to act as a non-Newtonian fluid. The viscosity of non-Newtonian fluids may increase markedly under conditions of low flow, and the slowing effect of a platelet thrombus on blood flow is itself enhanced by the apparent increase in viscosity.

THE WHITE CELLS

When platelet aggregation is induced by ADP in native blood *in vitro*, white blood cells are usually not associated with the aggregates. White cells may be closely related to *in vivo* ADP-induced platelet aggregates, but this finding is not particularly common and the numbers of white cells are low.[21] It may therefore be concluded that white blood cells are not essential in the formation of transient thromboemboli.

White blood cells contain ADP-degrading enzymes, and it has been found *in vitro* that the presence of white cells produces disaggregation of ADP-induced platelet aggregates.[12] Whether a similar effect is significant *in vivo* is not known.

Although the number of leukocytes is low in an early mural thrombus, it will increase in time, and by 24–48 hr a marked invasion of leukocytes will have taken place.[14,23] Some of the neutrophilic granulocytes may have vacuoles containing platelets or platelet debris. Fibrin can also occasionally be found within the granulocytes.

The granulocytes possess fibrinolytic activity, probably due to their content of hydrolytic enzymes, such as cathepsins. Cathepsin concentrates rich in cathepsin B have been found to activate profibrinolysin to fibrinolysin [24] and may contribute to fibrin dissolution and thrombus instability. It has been suggested that the activity of neutrophils may lead to disruption of a thrombus, which will then be fragmented by the pressure of blood flow. Thus, it seems that, although platelets and red cells contribute to the buildup of a thrombus, the white cells mainly have the opposite effect.

SUMMARY AND CONCLUSIONS

Platelets and red and white cells all participate in the thrombotic process, but the platelets appear to be the key elements. The coat on the platelet

surface is important; it may be essential in the initial phase of thrombus formation—platelet adhesion and aggregation. As demonstrated by electron microscopy, there appears to be connecting material radiating from the platelet surface and bridging the gaps between adjacent platelets in aggregates induced by ADP in native blood *in vitro* and at the early stage of thrombosis *in vivo*. The nature of this connecting material is not known. If the adhesive forces are associated with this material, it cannot represent a stable binding, inasmuch as ADP-induced aggregates are known to break up rapidly. The mechanism of the more permanent binding, probably induced by thrombin, is poorly understood.

The only component in the vessel wall known to interact readily with the platelets is collagen. When collagen is exposed to blood flow, platelet deposition takes place (in spite of an impaired coagulation mechanism), leading to structural changes of the platelets. However, thrombin may be formed locally in the thrombus by various initiating stimuli when coagulation is normal, leading to growth and stabilization of the thrombus.

Red blood cells may participate in thrombus formation, especially the "red tail," by providing phospholipids, which promote coagulation. Furthermore, red cells may be important in thrombus formation because they contain adenine nucleotides, which can be released under some circumstances.

The role of white blood cells during the early stages of thrombosis is probably of little importance, but these cells may be essential later, in the lysis of a thrombus.

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Formed Elements and Thrombus Stability

NATHANIEL F. RODMAN

Agglutination or aggregation of blood platelets is important in the formation of a white thrombus. Stability of platelet agglutinates and, therefore, stability of the thrombus depend on several factors related to agglutination. These factors include the extent to which morphologic changes progress, the formation of a fibrillar support for the platelet agglutinates during rhexis and lysis, and the permanency of platelet–platelet adherence within the agglutinates. Permanency or transiency of platelet–platelet adherence and the extent to which morphologic changes progress are determined by the agglutinating agent to which the responding platelets are exposed.[11] Development of the fibrillar support requires fibrinogen and fibrin polymerization.[14]

We have developed a model for the study of the formation and evolution of white thrombi.[13,15] Citrated platelet-rich plasma (PRP) is prepared from human blood. To the PRP may be added calcium chloride to initiate clotting or agglutinating agents to induce platelet agglutination directly. Aliquots of the reaction mixture are then fixed at various intervals and prepared for study with the electron microscope.

By these time-lapse techniques, platelet responses to agglutinating agents have been investigated in detail. Early and late responses to such agglutinating agents as thrombin, adenosine diphosphate (ADP), and collagen and the effects of these agents on the ultrastructure of normal and afibrinogenemic platelets have been observed.[11,13–15] This presentation describes responses to these agglutinants by platelets from normal and afibrinogenemic subjects and relates them to the stability of the white thrombus.

EXPERIMENTAL SUBJECTS

NORMAL SUBJECTS

Several subjects were found to be within normal limits, on the basis of the absence of a history of abnormal bleeding and several hemostatic

and clotting tests. These subjects had normal prothrombin time,[9] partial thromboplastin time,[5] whole-blood clotting time,[6] and thromboplastin generation[1] tests. Their platelet counts, bleeding times,[3] and clot retraction[16] were within normal limits, and tourniquet tests[16] were negative.

AFIBRINOGENEMIC SUBJECT

The congenitally afibrinogenemic subject is a Caucasian male, who was 13–16 years old during the period of study. His disorder has been the subject of previous reports.[8,11,14] He has had many hemorrhagic episodes since birth, beginning with protracted bleeding from the umbilical cord. Finger and venipuncture sites have been known to ooze for days, with a bleeding time of longer than 20 min. The subject's blood and plasma failed to clot in whole-blood clotting time, prothrombin time, and partial thromboplastin time tests. The partial thromboplastin time test for inhibitors[10] was negative. Results of the thromboplastin generation test, using afibrinogenemic plasma, "serum," and platelets, were within normal limits. Immunodiffusion determinations 11 weeks after fibrinogen transfusion revealed very small amounts of fibrinogen in the subject's plasma and platelets: less than 2 mg of fibrinogen per 100 ml of plasma and 3.5–7 μg of fibrinogen per 10^9 platelets.[4] The fibrinogen level of normal platelets has been reported to be 150–180 μg per 10^9 platelets.[7]

PLATELET RESPONSES

RESPONSE TO THROMBIN

In each experiment, citrated PRP was prepared from normal or afibrinogenemic blood and then recalcified. Following recalcification, the platelets of each subject progressed through an orderly sequence of events, which led ultimately to destruction of the platelet as an intact cell. This orderly sequence of morphologic events has been divided into four stages.[12] Stage 1 consists of organelle reorientation and pseudopod formation. These are preagglutination changes that occur in individual platelets shortly before the onset of agglutination, which is stage 2. Stage 3, thrombocytorhexis, is a continuum of changes, which is easier to discuss as though it consists of two parts: part a, organelle disintegration, sometimes seen even in unagglutinated platelets with centrally apposed organelles; and part b, plasma-membrane disruption, which occurs in normal platelets at about the time a solid clot is formed. In afibrinogenemic platelets, plasma-membrane disruption is markedly

delayed, compared with normal platelets. Stage 4, thrombocytolysis, or generalized cytoplasmic disintegration, follows plasma-membrane disruption.

The pattern of morphologic change in normal and afibrinogenemic platelets is similar during stage 1 and the early part of stage 2. The later stages, including the timing of rhexis and the onset and effects of lysis, are different. Agglutinate stability is likewise different.

Stages 1 and 2 In each PRP there is an initial lag phase that follows recalcification and lasts for several minutes. During this time, the platelets are morphologically indistinguishable from those recovered from citrated nonrecalcified plasma (Figure 1). The platelets are oval or round, and organelles are randomly distributed in the cytoplasm. Several minutes after recalcification, the end of the lag phase is manifested by the sudden appearance of preagglutination (stage 1) changes in individual platelets (Figure 2). Pseudopods are formed, and organelles become reoriented from random distribution to close apposition in the center of the platelet. A short time later, the altered platelets adhere to one another, and agglutination (stage 2) occurs (Figure 3). Organelles are gathered within the centers of platelet bodies in the agglutinate, and pseudopods are oriented toward the periphery. Organelles are sharply defined, except that, in Figure 3 (arrow), rhexis (stage 3a) has begun in one platelet, as evidenced by the granular area of osmiophilia.

Up to this time, the early phase of agglutination, morphologic changes within the platelets, and interaction between platelets are identical in normal and afibrinogenemic plasmas.

Stages 3 and 4 During the later stages there is a marked difference in the ultrastructural pattern of normal and afibrinogenemic PRP. Once disintegration of centrally apposed organelles has begun, the pattern differs in the two types of thrombi, both as to timing and as to ultimate effects of the agglutinates on stability.

As normal platelets agglutinate to form small and then larger agglutinates, fibrin fibrils slowly form at the periphery of the agglutinate and in the ambient fluid medium. During this time, gradual disintegration of centrally apposed organelles (stage 3a) begins. Suddenly, the rate of fibrin fibril formation increases sharply, and a solid clot forms. Simultaneously disintegration of centrally apposed organelles rapidly goes to completion, and plasma membranes in the centers of platelet agglutinates become disrupted (stage 3b). Plasma-membrane disruption signals the completion of thrombocytorhexis, and individual platelets

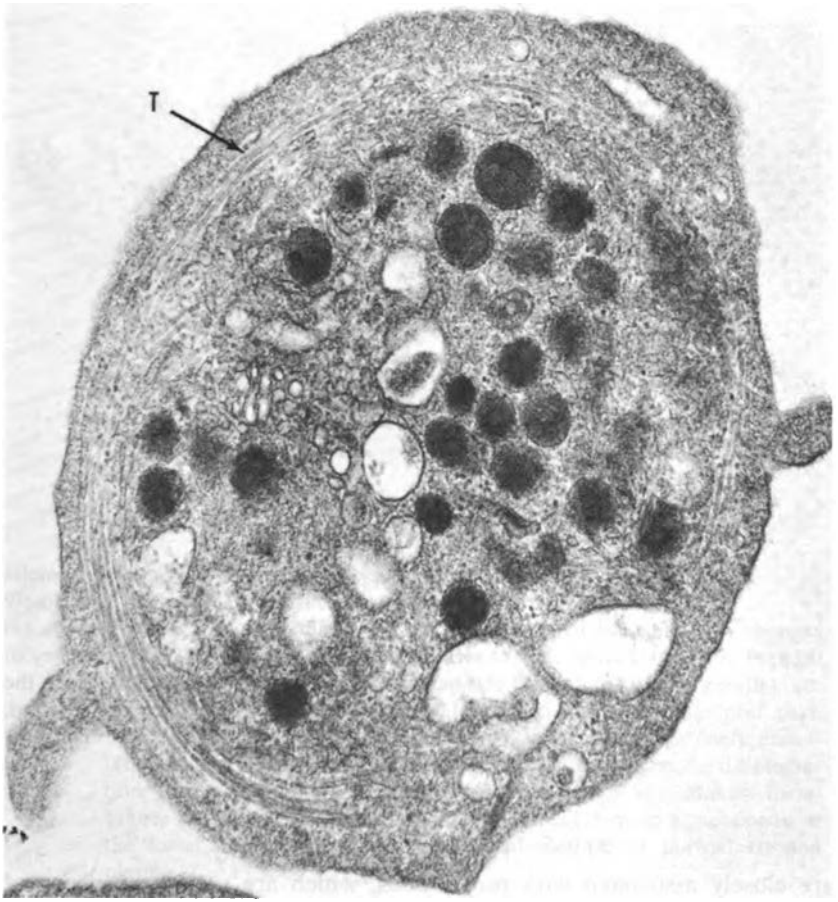

FIGURE 1 A platelet recovered from normal citrated platelet-rich plasma (PRP) to which one-fifth volume saline was added. Platelets from this sample are thought to be similar to the resting cells in the circulating blood. The platelet is round, with short, stubby projections. Microtubules (T) are at the periphery and extend about 315 deg around the circumference, indicating that the plane of section is in the circular plane of the disk-shaped cell. Cytoplasmic organelles are randomly distributed. Most organelles are within the area enclosed by the microtubule bundle, although several vacuoles are outside this enclosure, as in the upper and lower right. (× 35,500)

are no longer recognizable in the platelet mass (Figure 4). Thrombocytolysis (stage 4) proceeds gradually during the period of clot retraction. During this period, fibrin fibrils closely surround individual masses of agglutinated, disintegrating platelets (Figures 4 and 5). Fibrin fibrils

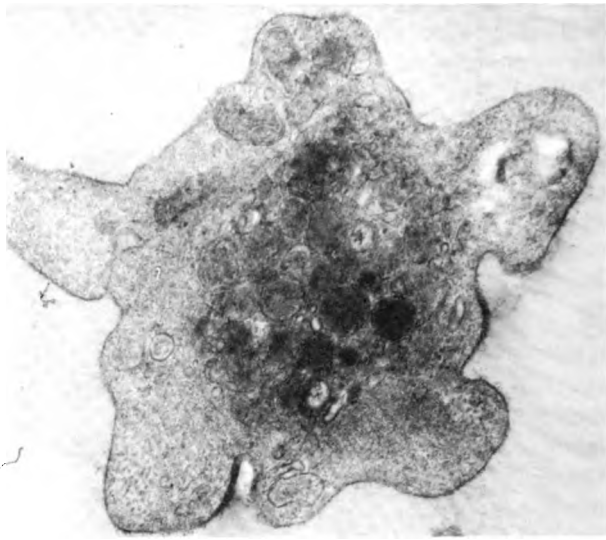

FIGURE 2 A single, unagglutinated afibrinogenemic platelet with preagglutination, or stage 1, changes. Organelles are closely apposed in the center of the platelet, and several pseudopods extend from the platelet body. For the most part, pseudopod cytoplasm is free of organelles, except for several mitochondria in the upper part of the platelet. Organelles are still sharply defined, indicating that rhexis has not yet begun. Fixed 10 min after recalcification of afibrinogenemic plasma. (× 30,200)

are closely associated with pseudopods, which are at the periphery of the mass of disintegrating platelets (Figure 4), and retain this orientation to the platelet mass at least until disintegration is far advanced in a late, well-retracted clot (Figure 5).

The failure of "clotting" afibrinogenemic PRP to form fibrin fibrils has far-reaching effects on the stability of the platelet agglutinates. Extremely large, composite platelet agglutinates form,[14] but, in the absence of fibrin polymerization, plasma-membrane disruption (stage 3b) and lysis (stage 4) are markedly delayed. Figure 6 illustrates definite organelle disintegration (stage 3a) in platelet bodies in an agglutinate in which plasma and other membranes are intact throughout. This agglutinate was fixed at a time when lysis would be moderately advanced in the partially retracted clot of a normal subject (see Figure 4).

Definite lysis is seen at the end of 1 hr [14] and is widespread at the end of 2 hr (Figure 7). The occurrence of lysis in the absence of fibrin fibrils about platelet masses results in agglutinate dissolution. In this

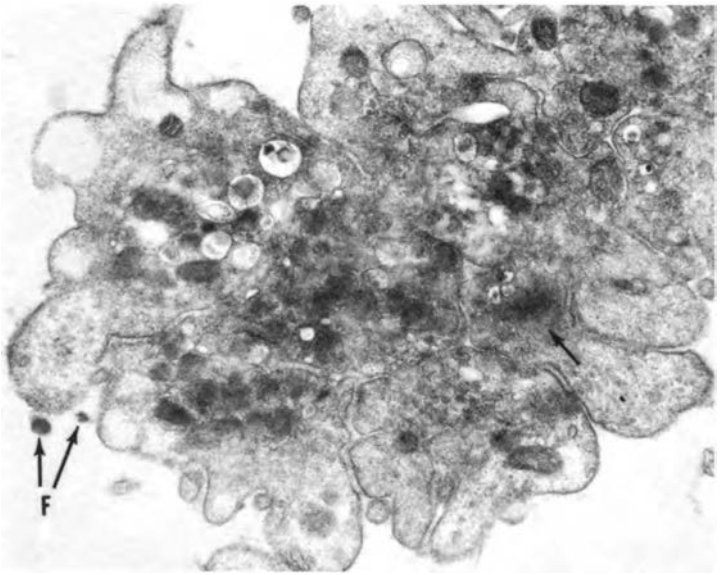

FIGURE 3 Part of an agglutinate of normal platelets with changes of stages 1 and 2 and, in one platelet, those of early stage 3. Pseudopods extend toward the periphery of the agglutinate. Organelles are closely apposed in the centers of platelet bodies. For the most part, organelles remain well defined, although in one platelet a finely granular, densely osmiophilic area (*arrow*) indicates organelle disintegration, or the first part of thrombocytorhexis (stage 3a). Plasma membranes are intact. Two fibrin fibrils (F) are adjacent to a pseudopod in the lower left. Fixed 8 min after recalcification of normal citrated plasma. (× 19,300)

process of dissolution, the agglutinates break apart into many small, unagglutinated vesicles. Figure 7 illustrates one of the many very small agglutinates seen in the presence of advanced lysis, with cytoplasmic debris (D) in the central portion and pseudopods at the periphery. Several unagglutinated vesicles are present nearby and separate from the small agglutinate. These have presumably separated from agglutinates that are disintegrating as a result of lysis. Such vesicles constitute most of this specimen.

RESPONSE TO COLLAGEN

The response to collagen was tested by adding finely divided collagen [2] to citrated PRP without added calcium. The response by afibrinogenemic platelets was identical with that of normal platelets.

Morphologic changes are observed within 1 min after addition of

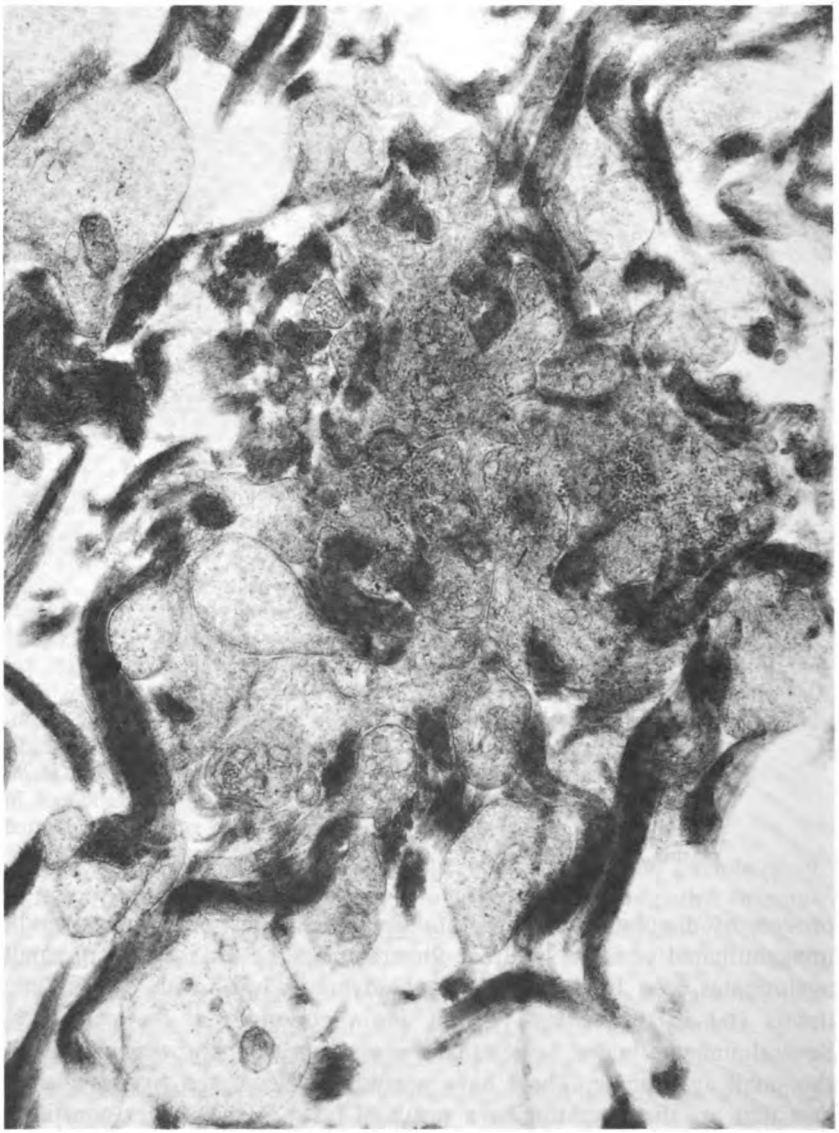

FIGURE 4 A mass of disintegrating platelets in a partially retracted clot of a normal subject. In the central portion of the mass, rhexis is complete and lysis is moderately advanced. Only a few membranes and organelles are identifiable. Pseudopods are at the periphery of the mass. Many fibrin fibrils are among the peripheral pseudopods. They are also abundant in the ambient fluid at a distance from the platelet mass. Fixed 20 min after recalcification, or 7 min after formation of a solid clot. (× 18,600)

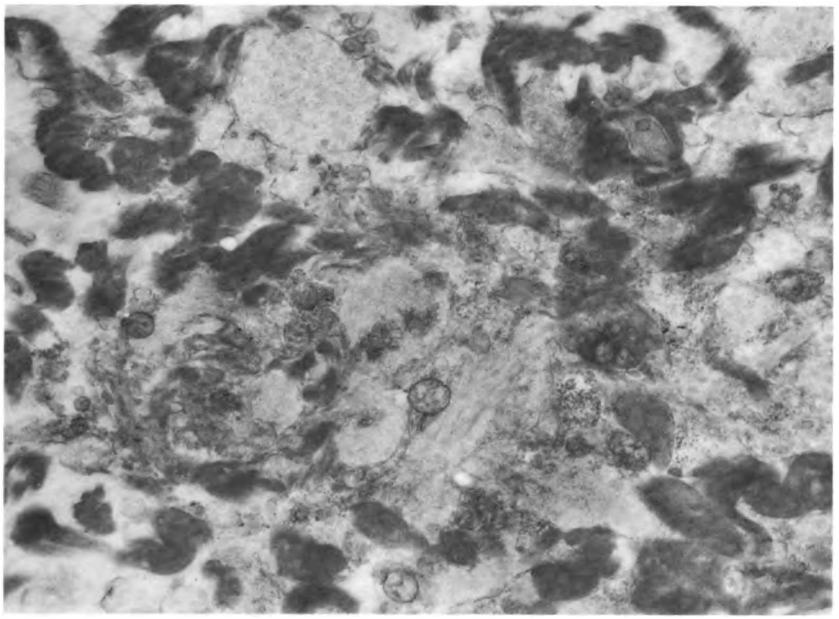

FIGURE 5 A mass of platelet debris with advanced lysis (stage 4) from a well-retracted clot. The mass of debris contains structures resembling mitochondria in the center, and fibrin fibrils surround the platelet mass at the periphery. Among the fibrin fibrils are several membrane-bound vesicles, as in the upper left. These vesicles are remnants of peripheral pseudopods such as were seen in earlier samples (see Figures 2–4). Fixed 140 min after recalcification. (× 12,160)

collagen to PRP. Preagglutination changes are observed, as well as many small agglutinates. Shortly thereafter, evidence of thrombocytorhexis is noted. At the end of 1 hr, extensive thrombocytolysis is observed in the central portions of the agglutinates (Figure 8). Only a few organelles—such as vesicles and mitochondria—and occasional segments of membrane, presumably plasma membrane, are recognizable centrally. Numerous pseudopods are present peripherally. The cytoplasm of some pseudopods is seen to be continuous with the area of lysis in the center. Fibrin was not observed in this or any other sample of collagen-induced agglutination.

RESPONSE TO ADP

Platelet response was observed after adding ADP without additional cation to citrated PRP. The final concentration of ADP was 0.002 M.

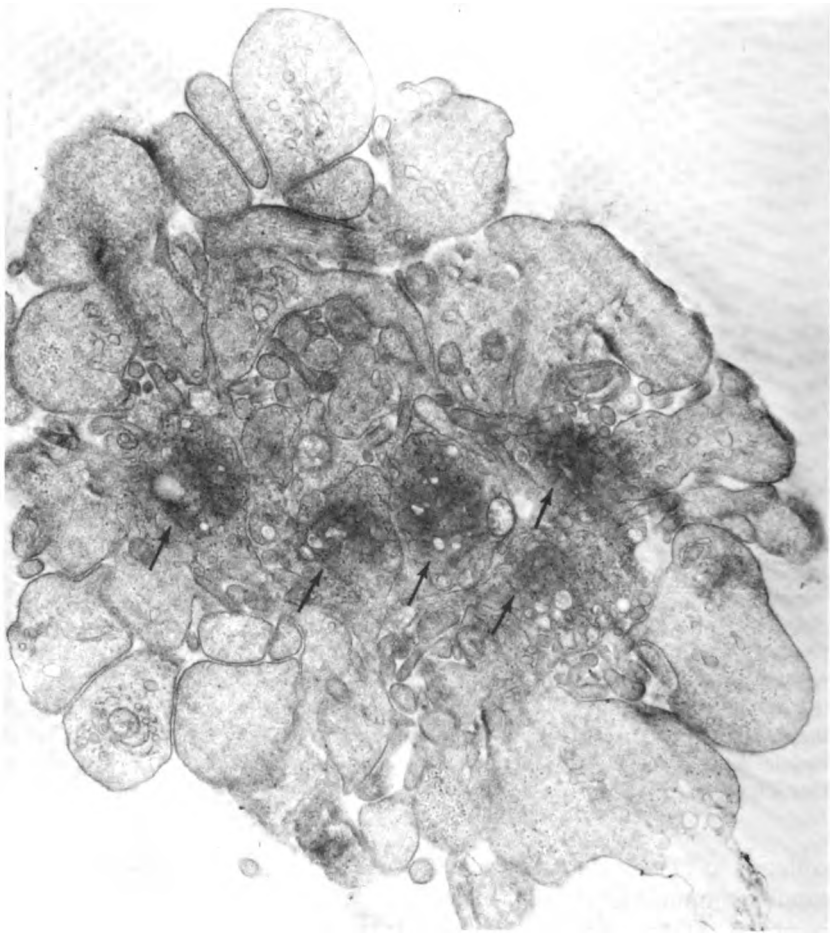

FIGURE 6 A small agglutinate of afibrinogenemic platelets with stage 2 and early stage 3 changes. Organelles in the centers of platelet bodies have disintegrated, as evidenced by the five granular, densely osmiophilic areas (*arrows*) in platelet bodies in the central part of the agglutinate. Plasma membranes are still intact throughout. Pseudopods are oriented toward the periphery of the agglutinate. Fixed 20 min after recalcification. (× 15,165)

The response to ADP by normal and afibrinogenemic platelets is similar, in that a rapid response occurred and was followed in both instances by significant loss of the ADP effect.

More than half the platelets exhibit the changes of stages 1 and 2 at

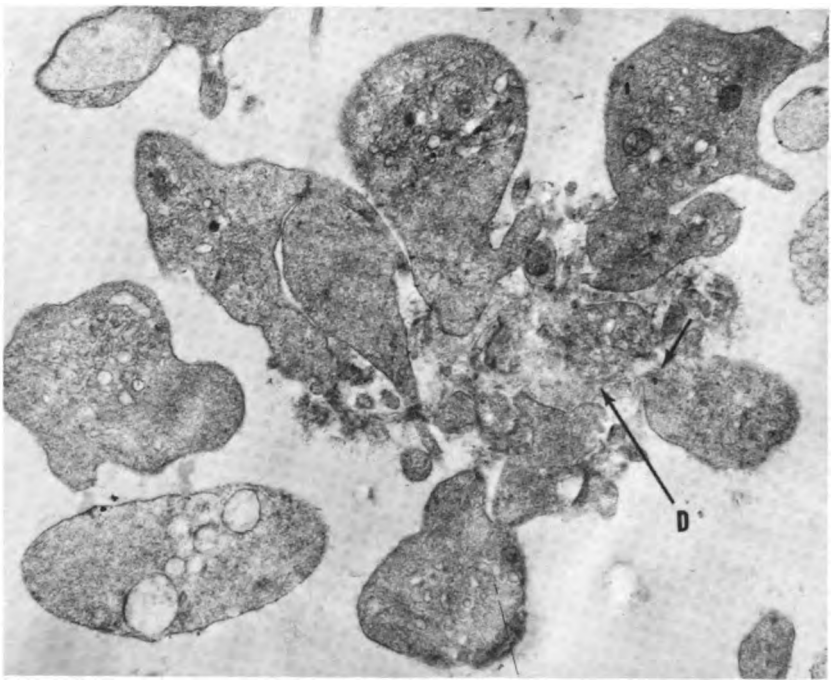

FIGURE 7 A small agglutinate of afibrinogenemic platelets, with two disconnected vesicular remnants of platelet pseudopods in the lower left. In the center of the agglutinate there is advanced lysis, as evidenced by the debris (D). Cytoplasm of some of the vesicular pseudopods about the periphery is seen to be continuous with the central area of lysis (*arrow*). Others presumably are continuous in an adjacent plane or have already separated from the area of lysis as part of agglutinate dissolution. Fixed 120 min after recalcification. (× 16,140)

the end of 1 min. Many large agglutinates are observed in a sample of normal platelets fixed 4 min after addition of ADP to the plasma (Figure 9). The platelets are closely apposed within the agglutinate, presenting a mosaic pattern. Organelles within the platelets are sharply defined. The response is limited, in that the sequence of morphologic events does not go beyond stage 2. Neither organelle disintegration nor plasma-membrane disruption occurs. The response to ADP is transitory: at the end of 1 hr, more than half the platelets are deagglutinated. The deagglutinated platelets are round or oval, with random organelle distribution (Figure 10), and thus morphologically resemble the resting platelet.

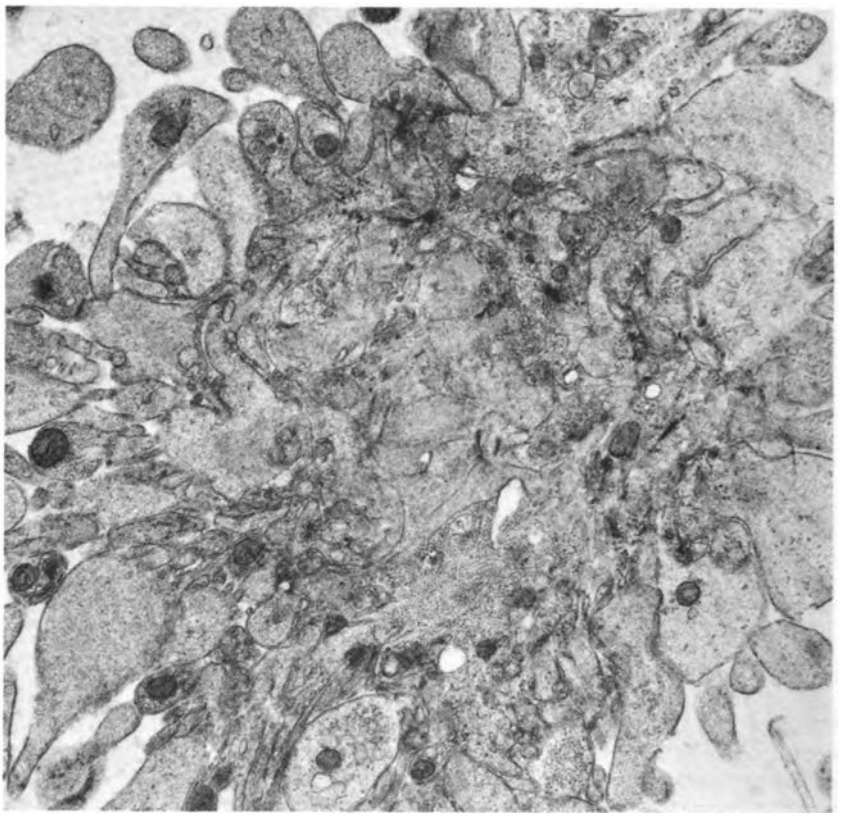

FIGURE 8 An agglutinate of normal platelets, illustrating late effects of collagen on platelets in citrated PRP. There is advanced lysis in the central portion of the agglutinate, with pseudopods extending toward the periphery from this large area of lysis. Although none appears in this plane of section, fragments of collagen are occasionally seen within such agglutinates. Fibrin is not observed. Fixed 60 min after addition of collagen to normal citrated PRP. (× 14,540)

DISCUSSION

Two kinds of variation in platelet responses to agglutinating agents have been described. One illustrates the specificity of platelet response to the agent. The other illustrates the effect of an atypical platelet response, compared with the response of normal platelets. Each kind of variation affects thrombus stability as mediated by formed elements.

The specificity of platelet response relative to the agglutinating agent used markedly affects thrombus stability. Platelet agglutination occurs

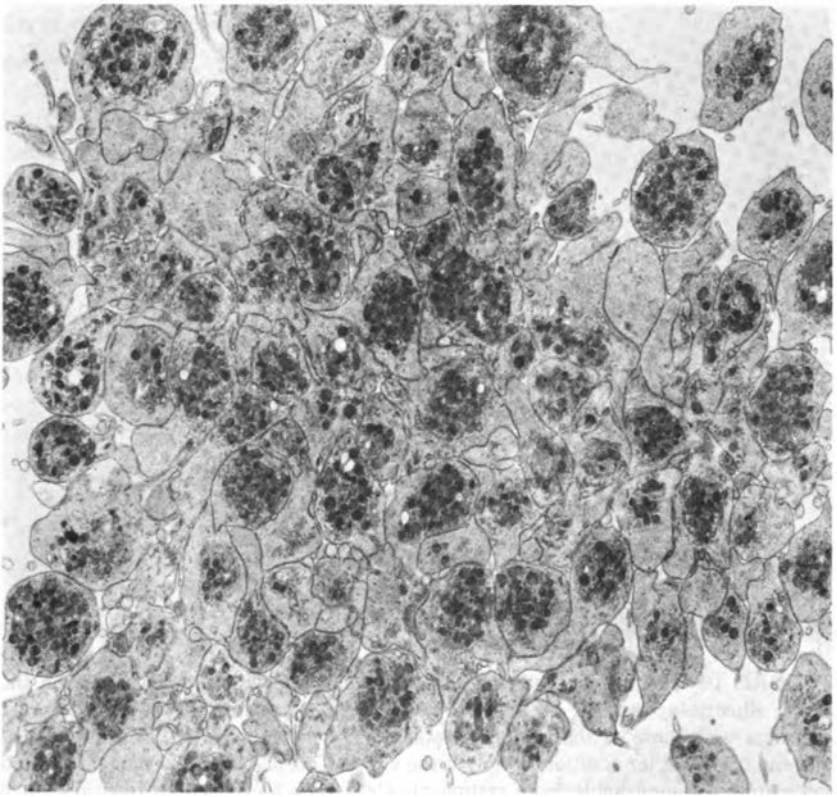

FIGURE 9 An agglutinate of platelets induced by addition of ADP to citrated normal PRP. Final ADP concentration in the plasma was 0.002 M. Fixed 4 min after addition of ADP to the plasma. The platelets form a mosaic pattern in the agglutinate. Organelles, gathered in the centers of the platelets, are intact, as are plasma membranes. (× 5,700)

in response to thrombin, to collagen, and to ADP. Once agglutination has occurred in response to thrombin or to collagen, it is permanent, in the sense that deagglutination does not occur. However, the response to ADP fails to go beyond simple agglutination (stage 2). Progression through rhexis to lysis does not occur, and the response to ADP is transitory. Partial deagglutination occurs, and many single, unagglutinated platelets are observed at the end of 1 hr. This partial deagglutination, with reduction in the number of platelets in an agglutinate (compare Figures 9 and 10), would result in an unstable thrombus. Thus, a thrombus that formed under the influence of ADP only would

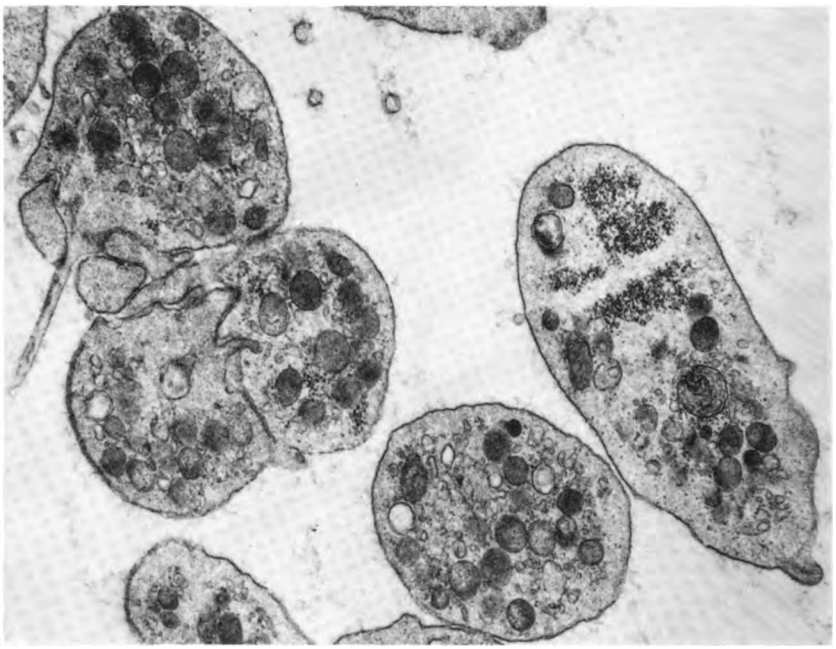

FIGURE 10 A small agglutinate on the left and unagglutinated platelets on the right, illustrating partial loss of the ADP effect. Experimental conditions were identical with those of the sample shown in Figure 9, except that fixation was at the end of 1 hr after addition of ADP to the citrated normal PRP. The unagglutinated cells are indistinguishable from resting platelets; they are round or oval and have random distribution of cytoplasmic organelles. The small agglutinate of three platelets is typical of those seen late in the reaction, and in marked contrast with the larger ones seen earlier (see Figure 9). Note that, even in these still-agglutinated platelets, organelles are distributed at random in the cytoplasm. (× 15,600)

form an ineffective hemostatic plug. Loss of the ADP effect, with resulting deagglutination, would allow such a thrombus to be swept away in a stream of flowing blood.

In the morphologic sequence observed in clotting in normal PRP, stages 1 and 2 are accompanied by gradual formation of fibrin fibrils among and adjacent to pseudopods at the peripheries of agglutinates and also in the ambient fluid medium (Figure 3). During stages 3 and 4, fibrin fibrils remain at the periphery of platelet agglutinates (Figures 4 and 5). In this position, they support and contain the disintegrating platelet masses during lysis, which progresses as the clot retracts.

The importance of peripheral fibrin fibrils is emphasized by the

effects of their absence around agglutinates of afibrinogenemic platelets (Figures 6 and 7). Plasma-membrane disruption (stage 3b) and the onset of lysis (stage 4) are delayed [14] (Figure 6), although once lysis occurs, it results in an unstable agglutinate or thrombus (Figure 7). As generalized cytoplasmic disintegration, or lysis, becomes increasingly extensive, agglutinates undergo dissolution.[14] Aggregates break up into many small, unagglutinated vesicular remnants, which originated as peripheral pseudopods. Such a series of events in a mass of agglutinated platelets forming a thrombus in a transected blood vessel would lead to an ineffective hemostatic plug. It seems logical to conclude that a stable thrombus requires a fibrin meshwork to support and contain the masses of disintegrating platelets during lysis. Because fibrin fibrils are not observed in the platelet response to collagen, it seems logical to conclude, furthermore, that at least small amounts of thrombin must be present in order to generate the fibrin fibril support.

The work reported here was supported in part by Public Health Service grants HE 01648 and HE 06350 from the National Heart Institute, GM 92 from the National Institute of General Medical Sciences, and FR 46 from the General Clinical Research Centers Branch of the Division of Research Facilities and Resources, and in part by funds from The Kurt Orban Foundation.

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Platelet Aggregation and Release Reactions Induced by Adenosine Diphosphate and Other Physiologic Substances

MARJORIE B. ZUCKER

The accumulation of blood platelets at the site of vascular injury was observed *in vivo* during the late nineteenth century. Its causes have been significantly, although incompletely, elucidated mainly in the last two decades—particularly through *in vitro* study of platelet aggregation in platelet-rich plasma.

EFFECTS OF THROMBIN

Thrombin evolution is a striking reaction to blood shedding, so it was natural to speculate that thrombin could cause platelet aggregation. Lack of aggregation in early studies involving the addition of thrombin to washed platelets can be ascribed to use of too low a pH,[36] and it is now agreed that thrombin causes aggregation of washed platelets suspended in saline buffered to a pH near 7.4.

Aggregation induced by thrombin is accompanied by other effects, e.g., tight packing of the platelets, which gives the clumps a homogeneous glassy appearance under the phase microscope (probably due to contraction); release of serotonin [34] and adenosine diphosphate (ADP)[13] from the platelets; and shape change and degranulation, reviewed by Rodman in the preceding paper. These changes have been referred to collectively as "viscous metamorphosis."

Although aggregation induced by thrombin does not require exogenous fibrinogen, the role of the fibrinogen within or firmly bound to platelets is not clear. According to the Baltimore group,[21] platelet fibrinogen is the substrate for thrombin's action on platelets, but some other enzymes that cause fibrinogen to clot have less effect on platelets,[3,20] and the finding that platelets of patients with congenital afibrinogenemia

and virtually no platelet fibrinogen react normally with thrombin in most respects [11] also suggests that platelet fibrinogen is not the substrate for thrombin.

Heparinized platelet-rich plasma is useful in studying the effect on platelet aggregation of agents other than thrombin, because heparin blocks the evolution and action of this enzyme. Incidentally, platelet aggregation induced by thrombin or a number of other aggregating agents occurs optimally at a physiologic concentration of ionized calcium but can also take place when the concentration of calcium ions is around 0.05 mM, as in ordinary citrated plasma with a citrate concentration of about 23 mM.[17] In contrast, the calcium-dependent steps in blood coagulation need a concentration of calcium ions approximating that in native-blood plasma (1.2 mM) and therefore cannot take place in citrated plasma. This difference permits the use of citrated plasma in studies of platelet aggregation *in vitro*. In plasma with the usual anticoagulant concentration of ethylene diamine tetraacetic acid (EDTA) (4.5 mM), the concentration of calcium ions is too low to permit platelet aggregation.

EFFECTS OF SUBSTANCES OTHER THAN THROMBIN

In citrated or heparinized platelet-rich plasma, platelet aggregation has been induced by a number of substances other than thrombin.[16,22,33] These substances can be divided into several groups: (1) some proteolytic enzymes, such as trypsin; (2) particulate material, such as connective-tissue (CT) particles, collagen fibers, latex particles, kaolin, and antigen–antibody precipitates; (3) long-chain saturated fatty acids; (4) some amines, namely, epinephrine, norepinephrine, and serotonin (5-hydroxytryptamine); and (5) ADP.

Like thrombin, at least some of the above agents have effects on platelets other than aggregation. For example, antigen–antibody precipitate and aggregated immunoglobulin G cause platelet contraction and the release phenomenon.[1,22] CT particles cause degranulation,[9] as well as release of ADP [10,29] and serotonin.[29,*] Some authors therefore

* Our recent studies have been carried out with platelets in human citrated or heparinized platelet-rich plasma incubated for 15 min with ^{14}C-labeled serotonin in a concentration of about 1 μM.[32] Although over 80% of this serotonin is taken up by the platelets, it may have a different distribution from serotonin normally found within platelets. The latter may be largely bound to granules,[30] whereas Rosenthal *et al.*[27] found that tritiated serotonin, incubated with platelets in a concentration of about 1 μM, is taken up by the platelets but remains largely outside of platelet granules.

state that these aggregating agents cause viscous metamorphosis. Although it is convenient to have a term to indicate that these substances do more than cause aggregation, it may obscure differences between the actions of different agents. Thus, EDTA prevents release of ADP and serotonin by thrombin and kaolin, but not by CT [28,33]; and anti-inflammatory agents prevent release by particulate material, including collagen, but not by thrombin.[26,33,38] I consider it preferable to supplant the term "viscous metamorphosis" with a simple statement of what has been determined about a substance, e.g., that it induces aggregation, contraction, release of ADP and serotonin, or degranulation.

Another characteristic sometimes altered during aggregation is the availability of platelet factor 3 (PF-3) activity. This term describes the platelets' ability to behave as phospholipid in some clotting tests. Thus, the platelets in platelet-rich plasma do not accelerate coagulation induced by Russell's viper venom (Stypven) unless they have been exposed to such substances as kaolin.[28] Development of PF-3 activity is difficult to study in platelet-rich plasma, because some of the substances under investigation affect coagulation in ways other than through platelets. It is also difficult to study PF-3 with isolated platelets, however, because it is activated to some extent simply by centrifugation and resuspension of the platelets.

ADP-MEDIATED PLATELET AGGREGATION

The discovery that platelet aggregation could be induced by ADP concentrations below 1 μM, and the knowledge that ADP is present within platelets, raised the possibility that release of endogenous ADP actually causes the aggregation due to the first four types of substances listed above. Demonstration of the release of ADP in sufficient concentration to cause aggregation after the addition of thrombin [13,29] and CT [10,29] lent strong support to the theory; it was also supported by the finding that platelets of patients with congenital thrombasthenia failed to aggregate, not only with ADP but also with substances in the other categories.[6,37] Furthermore, adenosine, a competitive inhibitor of ADP-induced clumping,[32] inhibited aggregation caused by many of these agents.[8,23,33] The most convincing evidence of ADP's role as the final common causative agent in aggregation is Haslam's demonstration [8] that thrombin, collagen, fatty acids, serotonin, and epinephrine cause much-decreased platelet aggregation if appropriate enzymes are added to metabolize ADP as soon as it is released.

The mechanism of platelet aggregation by ADP is not known. Hovig

has summarized the rather scanty knowledge (pp. 381–385). The impression that ADP acts only at the platelet surface gained support by the finding that it failed to cause degranulation.[9] It is now clear, however, that ADP affects platelets in two ways besides inducing aggregation: it causes platelet swelling and shape change, and, at least at times, it causes release of an aggregating agent (presumably ADP) and serotonin and development of PF-3 activity.

ADP-INDUCED SWELLING

The platelets in carefully collected, heparinized or citrated platelet-rich plasma kept at 37 C are disk-shaped, as in the circulation.[35] When 1-μM ADP is added, they become irregular in shape, although essentially spheric, within 15 sec.[15,39] Because of the change from disk shape to a spiny sphere, the "swirl" seen when fresh, warm platelet-rich plasma is shaken disappears when ADP is added,[10] as does the oscillation in some types of recordings of optical density.[24] The shape change is inhibited by adenosine, but occurs normally in the presence of calcium antagonists and with thrombasthenic platelets.[33,37,39] It also occurs under other circumstances, such as chilling and collection in EDTA.[35] It is accompanied by swelling of about 25%.[2]

ADP-INDUCED RELEASE

Macmillan [14] observed that aggregation often occurs in two phases after the addition of ADP to platelet-rich plasma kept at 37 C, and that the second phase of aggregation depends on the platelets' release of an aggregating agent, presumably ADP. Earlier evidence of a parallel release of ADP and serotonin induced by either thrombin or CT [29] led to our discovery that, when ADP caused secondary aggregation, the platelets also released serotonin. In addition, the platelets developed PF-3 activity.[38] Secondary aggregation is seen only after a critical concentration of ADP is added to platelet-rich plasma at 37 C.[14,38] Too low concentrations cause only reversible aggregation; concentrations above the critical level cause marked and virtually irreversible aggregation in which primary and secondary aggregation are fused. Below the critical concentration of ADP, there is no serotonin release and little PF-3 activation; at the critical and higher concentrations, serotonin is released and PF-3 activity develops.[38]

These release reactions to ADP occur only under particular conditions,

not yet fully elucidated or always predictable. We have never observed secondary aggregation when ADP has been added to platelet-rich plasma from ordinary ACD blood tested at room temperature.[32] Macmillan [14] noted secondary aggregation in about 75% of the normal subjects he tested, using platelet-rich plasma from citrated blood at 37 C. We noted ADP-induced secondary aggregation, serotonin release, and development of PF-3 activity under the same conditions in eight of 10 normal subjects.[38] When the two nonreacting subjects were retested, these reactions to ADP were subnormal. Another subject exhibited excellent ADP-induced serotonin release on five occasions and none at all on another occasion. The type of tube influenced the results. Serotonin release was usually investigated in glass tubes, using platelet-rich plasma that had not previously been in contact with glass. Release also occurred in several types of plastic tubes, but not in thick-walled tubes made of polypropylene. There was no correlation between the effects of tube composition on serotonin release and on acceleration of blood clotting. The role of glass contact is reminiscent of the change in electrophoretic mobility noted by Hampton and Mitchell [5] when platelet-rich plasma was placed in glass containers. The cause and meaning of both observations are obscure.

DISCUSSION

O'Brien observed that aggregation induced by epinephrine can occur in a single phase, with the platelets remaining as disks,[24] or in two phases.[23] The second phase resembles the second phase of aggregation produced by ADP, in that it is accompanied by shape change, is irreversible, and is attributed to released ADP.[14] Epinephrine does not induce secondary aggregation in all blood samples from all persons [25]; the reacting samples also show secondary aggregation with ADP.[14]

Mills and Roberts [18] observed that release of an aggregating agent by ADP, epinephrine, or collagen (but not thrombin) is readily inhibited by several drugs, including chlorpromazine and desmethylimipramine. We found that ingestion of 1.3 g of acetylsalicylic acid, in a divided dose 1 and 2 hr before the blood sample was drawn, inhibited secondary aggregation, serotonin release, and development of PF-3 activity induced by ADP.[38] This and other anti-inflammatory agents also affect platelet response to other aggregating agents. For example, aggregation induced by collagen is inhibited by adding phenylbutazone or sulfinpyrazone *in vitro*.[22,26] Acetylsalicylic acid given orally prevents release induced by CT [4,31] and by kaolin.[31] However, aggregation and serotonin release induced by thrombin are unaffected by acetylsalicylic acid or other

anti-inflammatory agents.[26,33,38] Investigators in other laboratories [4,26,31] stated that these drugs do not impair ADP-induced aggregation; they observed no secondary aggregation induced by ADP, finding only its primary aggregating activity, which is *not* affected by anti-inflammatory drugs.

Table 1 shows the effect of a number of compounds and conditions on ADP-induced shape change, aggregation, and release reactions of platelets. When aggregation is prevented by EDTA or by diguanidinodiphenylsulfone,[32] serotonin release is also prevented. When aggregation is only partially inhibited by these substances or by adenosine, serotonin release is usually only partially impaired. These data suggest that the platelets must be in actual contact to promote release of serotonin. Two additional observations substantiate this theory. Platelets of a patient with congenital thrombasthenia, which fail to aggregate with ADP, did not release serotonin with the addition of ADP (unpublished observation), although release occurred with CT or thrombin.[37] Even with ADP concentrations as high as 100 μM, serotonin is not released unless the platelet-rich plasma is shaken so that aggregation takes place. These results agree with the conclusions of Hardisty and Hutton,[7] that PF-3 activity is induced by ADP only when aggregation occurs, and contrast with observations on the ADP-induced shape change that is noted in unshaken samples and in platelets of thrombasthenic patients.

The release of serotonin and an aggregating agent, and the PF-3 activation induced by ADP, has important implications for the formation of a hemostatic platelet plug or a thrombus. Platelets initially adhering to CT or basement membrane [33] release ADP, which causes other

TABLE 1 The Effect of Various Compounds and Conditions on the Three Types of Platelet Response to Adenosine Diphosphate

	Effect on ADP-Induced Response		
Compound or Condition	Shape Change	Aggregation at Room Temperature	Serotonin-^{14}C Release
Adenosine	Inhib.	Inhib.	Inhib.
EDTA	Normal	Inhib.	Inhib.
Diguanidinodiphenyl-sulfone	Normal	Inhib.	Inhib.
Thrombasthenia	Normal	Inhib.	Inhib. (one expt.)
Unshaken	Normal	Inhib.	Inhib.
Acetylsalicylic acid	Normal	Normal	Inhib.

platelets to adhere to the original layer. As suggested by Mills and Roberts,[18] the process would then stop if ADP did not cause platelets in the second layer to release their aggregating agent(s), resulting in the collection of more platelets. Furthermore, development of PF-3 activity on the platelets presumably enhances the local evolution of thrombin by the intrinsic pathway, helping to consolidate the platelet mass. Failure to induce secondary aggregation, serotonin release, and PF-3 activation with ADP in all samples of platelet-rich plasma from normal subjects (and imperfect understanding of the causes of this failure and the conditions required for positive results) makes it difficult to assess the physiologic role of these ADP-induced effects. As detailed elsewhere,[38] the observation that ingestion of acetylsalicylic acid prolongs the bleeding time in normal persons, and markedly impairs hemostasis in persons or animals that are taking oral anticoagulants or that have hemorrhagic disorders, suggests that ADP-induced secondary aggregation and release are important. They do not prove the point, however, because the inhibitory effects of this drug on CT-induced aggregation and release [4] may be responsible for its effect on hemostasis. Nevertheless, the findings do suggest that it would be worthwhile to investigate the effect of such drugs as acetylsalicylic acid on the incidence and progress of thrombosis.

The work reported here was supported in part by Public Health Service grant 05003-08 from the National Heart Institute.

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Vascular Endothelium and Thrombogenesis

THEODORE H. SPAET AND CHUNG-HSIN TS'AO

In a previous publication, evidence concerning the various theories of thrombogenesis was reviewed, and it was concluded that the primary factor leading to pathologic thrombotic deposit is a vascular lesion.[4,8] This point of view is by no means novel; various investigators have espoused it for over a century.[5] However, in recent years, emphasis has shifted so that changes in blood composition receive an ever-increasing degree of attention. Variations on the theme of the "hypercoagulable state" have been proposed by numerous authors to account for thrombogenesis. As understanding of hemostasis has grown, more elaborate accounts of "hypercoagulability" have appeared, and such concepts as increased plasma clotting factors, platelet "stickiness," and a multitude of altered plasma clotting states have been emphasized.

Our view on the primacy of the vascular lesion in thrombogenesis is based on several considerations, which may be briefly summarized as follows:

1. In experimental animals and in man, deliberate production of intimal injury alone is an effective and reliable method for producing thrombotic deposits. Although the magnitude and duration of the thrombi so produced are variable, some thrombotic material will deposit on adequately damaged vessels of all sizes and in both the arterial and venous systems.

2. Experimental measures designed to change the state of the blood have been uniformly unsuccessful in producing thrombi. Marked local or systemic acceleration of blood coagulation fails to elicit the formation of a structured thrombus and has no effect even on already established microthrombi produced by vascular injury. Similarly negative results are obtained when platelet reactivity is augmented by the local

intravascular or systemic administration of adenosine diphosphate (ADP), although high local concentrations of the compound may produce temporary enlargement of a traumatic microthrombus.

3. When measures are taken to overwhelm the ability of the organism to maintain blood fluidity, generalized clotting may occur. When this condition is produced by such agents as thrombin, tissue extracts, or introduction of massive amounts of particulate matter, the resulting lesion is not thrombotic, in the usual sense of the term. Instead, widespread embolization occurs. Masses of blood clot do not form on a free wall of a major vessel. Rather, these clots are trapped in the first vessel which is too small to allow passage. This is embolization, not thrombus formation.

4. The structure formed in the presence of blood hypercoagulability combined with complete stasis is a whole blood clot that forms a cast of the vessel in which it resides. Although this type of structure may form under some clinical conditions, it differs from the complex and well-described lesion that is generally recognized as a thrombus.

5. The view that fibrin may deposit progressively as a result of imbalance between clotting and fibrinolysis has not been supported by a body of recent data. Two lines of evidence appear to minimize this possibility: with increasing application of electron microscopy to the study of vascular morphology, the lack of fibrin deposits on the normal endothelium is becoming more certain; and the concept that blood coagulation is a continuous process has not been confirmed.

Our recent studies have therefore proceeded according to the hypothesis that thrombosis of the usual clinical variety is a lesion of vascular origin, in which endothelial cells are sloughed from the vessel wall. The consequence of this denudation is the accumulation of hemostatic material at the affected site, with platelet adhesion onto collagen as the initial thrombotic event. We hold that the subsequent growth and consolidation of the thrombus is a process analogous to normal hemostasis, but that it is inappropriately applied.

Considerable attention has been given in other publications to the reactions involving blood components in the hemostatic process, and this subject will not be discussed here. It is the purpose of this presentation to consider the vascular reactions that may lead to endothelial-cell desquamation. The subject to be covered will be mainly that relating to larger vessels, because they are generally the sites of clinically significant thrombi. The discussion is intended as a companion piece to our earlier paper.[48]

ANATOMIC CONSIDERATIONS

With the introduction of electron microscopy into the study of blood vessels, many of the older concepts based on light microscopy have had to be revised or discarded. The status of the field before the advent of ultrastructural studies is extensively covered in the monograph of Altschul,[1] and an account of small-vessel endothelium is elaborately presented in the admirable review of Majno.[27] The following remarks are intended to establish a basis for the picture of vascular injury to follow.

Figure 1 shows electron micrographs of normal rabbit aorta and inferior vena cava, which correspond with descriptions given by earlier authors.[6,8,14–17,20,25] Several features deserve special emphasis. The cell appears to be actively engaged in transport, as evidenced by numerous pinocytotic vesicles, both at the luminal surface and at the site of basilar attachment. The importance of this transport activity to the remainder of the vessel wall is uncertain, although it is generally assumed that passage of nutrients through the endothelial cell provides at least some of the medial nourishment. This is difficult to reconcile with the isolation of the intima from deeper structures by the extremely wide internal elastic lamina, particularly notable in the aorta. Nevertheless, Sawyer and his associates [44,45] reported sodium and chloride ion flux from the intimal to the adventitial surface of the aorta, although it was from adventitia to intima in the inferior vena cava. Interpretation of these data is difficult, because they are based on the use of isolated vessel strips *in vitro*. Endothelial cells of larger vessels are similar in most respects to those of their smaller relatives, and one of these similarities is their ability to ingest colloidal material, such as thorotrast [8] and ferritin.[15] Such material will not pass into deeper structures unless there is concomitant vascular injury, a phenomenon in keeping with similar findings in smaller vessels.[11,28]

The metabolic and synthetic activity of endothelial cells appears to be modest, compared with those in such active organs as the liver and pancreas. The Golgi apparatus is not particularly prominent, mitochondria are relatively sparse, and there is only a moderate endowment of rough endoplasmic reticulum and ribosomes.[14] Morphologic evidence that synthetic structures are present in endothelial cells is in keeping with the observations of other authors that these cells do indeed manufacture compounds. Histochemical observations suggest that endothelial cells synthesize chondroitin sulfate,[32] a view supported by the finding that the cells can incorporate ^{35}S.[12,26] It also appears that at least some endothelial cells can manufacture basement membrane and may be responsible for the observed incorporation of tritiated proline into this structure.[27,50]

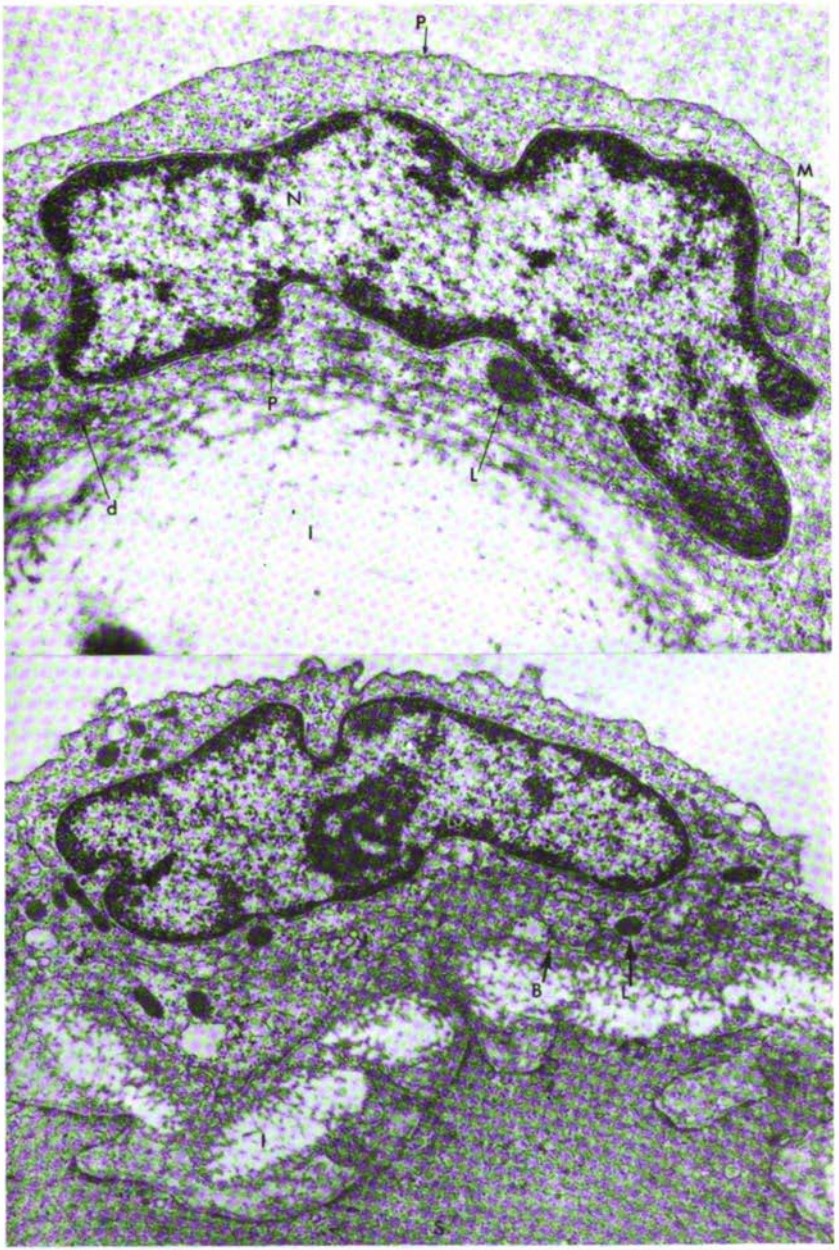

FIGURE 1 Normal rabbit endothelial cells. *Top*, From aorta. d, half-desmosome; I, internal elastic membrane; L, lysosome-like granule; M, mitochondrion; N, nucleus; p, pinocytotic vesicle. (× 25,500) *Bottom*, From inferior vena cava. B, basement membrane. (× 14,600)

Another structure of interest in the endothelial cells is the body described as "lysosome-like" by Rhodin.[41] Acid phosphatase-positive granules have long been recognized by light microscopists in vascular endothelium, but, surprisingly, it has been difficult to establish such structures by electron microscopic histochemical methods. The presence of lysosomes in these cells has been denied,[22] and Marchesi was able to demonstrate acid phosphatase-positive granules only in injured cells.[29] By using short fixation and long substrate incubation times, we have been able to demonstrate the probable presence of acid phosphatase in the lysosome-like granules in all types of vascular cells, including those of the endothelium, as shown in Figure 2. These structures do not appear to be abundant, compared with hepatic cells and leukocytes.

Endothelial cells contain features generally accepted as being related to their mutual attachment. These include paired desmosomes and terminal bars.[33,53] In addition, there appear to be specialized structures that fasten the cells to the basement membrane. There is perhaps loose attachment throughout the adjacent surfaces by strands of fibrillar ma-

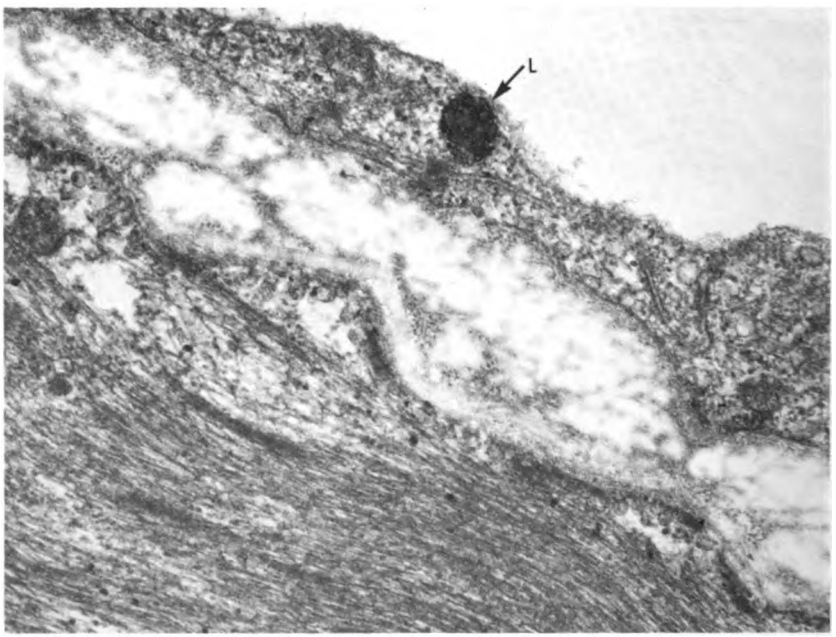

FIGURE 2 Acid phosphatase-positive lysosome-like granule (L) in rabbit inferior vena cava. (× 48,150)

terial of unknown composition, and desmosome-like densities, seen at intervals, appear to provide a more tenacious bond. Because the basement membrane does not have an opposing twin, these structures (shown in Figure 1) have been termed "half-desmosomes" by Stehbens.[53]

Underlying the endothelial cells is the basement membrane, seen as a narrow, electron-dense line of varying thickness and uncertain structural features. Whether the basement membrane is discontinuous in larger vessels, as suggested by French,[17] or has a continuity difficult to demonstrate remains to be determined. Also a subject for further investigation is the material of which the basement membrane is composed. Inasmuch as the basement membrane of glomerular capillaries contains proline,[50] it is possible that the structure is collagenous or contains allied compounds. However, ultrastructural demonstration of typical collagen striation has not been forthcoming. The basement membrane is probably in a state of dynamic equilibrium, and its presence may depend on activity of viable endothelial cells; if these cells are injured or desquamative, the basement membrane may soon disappear in that area.[1]

Between the endothelial cells and the internal elastic membrane is another type of cell, commonly called the "pericyte," and more recently labeled the "myointimal cell." As illustrated in Figure 3, these cells morphologically appear to be transitional forms between endothelial cells and medial smooth-muscle cells. Indeed, recent data suggest that smooth-muscle cells migrate through the internal elastic membrane to become transformed into myointimal cells,[7,58] and that myointimal cells in turn may complete the migration and transformation to end as endothelial cells.[58] The myointimal cells contain myofilaments, and they may be related to the contractile elements of smaller-vessel pericytes or Rouget cells.[27] In normal rabbits, myointimal cells are seen more frequently in the aorta than in the inferior vena cava, but they appear in both vessels following trauma.

Underlying the intima, with its endothelial and myointimal cells, is the internal elastic membrane. Although this structure is grossly continuous, it appears to have numerous potential and actual fenestrations through which even cells can penetrate with relative ease. The internal elastic membrane is not very electron dense, owing to its abundant elastin; but it is also endowed with electron-dense components, particularly at the inner and outer margins. These probably are largely collagen fibrils.

Beneath the internal elastic membrane is the media, which is composed of smooth-muscle cells and connective tissue. Although the pri-

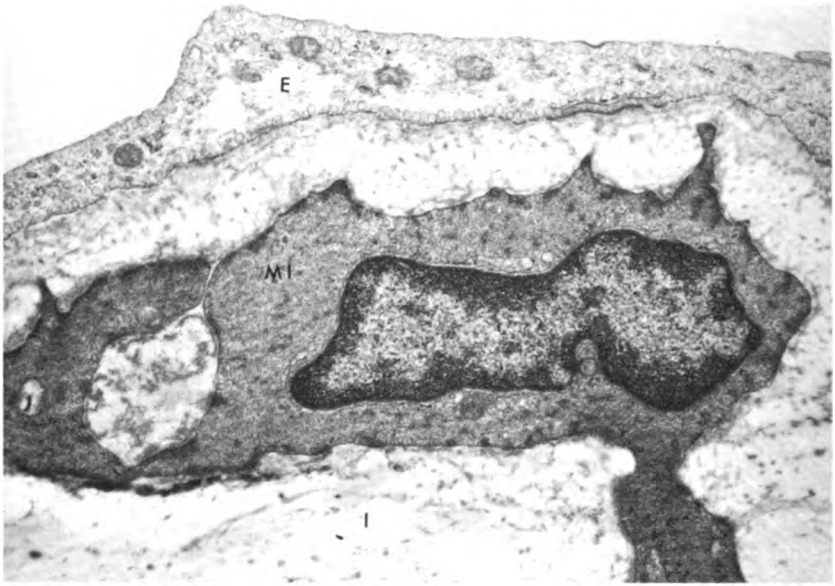

FIGURE 3 Myointimal cell (MI) in rabbit aorta. E, endothelial cell; I, internal elastic membrane. (× 19,260)

mary subject of this presentation is the intima, a few remarks concerning medial nutrition may be relevant. It is generally stated that the walls of large blood vessels are supplied by vasa vasorum. Capillaries are thought to penetrate to the inner third of the media, and the remainder is considered to be nourished by blood in the vascular lumen. The preparations leading to these conclusions have been specimens that have received injections of agents that can be visualized when cleared or demonstrated by x-ray.[9,19,59] However, even these "classical" techniques have given conflicting results and have suggested less vascularity of the media, especially in veins, than was formerly suspected.[59] Electron microscopy appears to be revealing a different picture; it has been repeatedly noted that capillaries in the media are conspicuously absent in a variety of species studied.[8,14] One exception is in a report by Tedder and Shorey,[55] who observed capillaries penetrating from the lumen through the intima. Our own material from rabbit aortas and inferior vena cavas similarly appears to be devoid of blood supply, although adventitial capillaries are abundant. Similar findings appear

to prevail in the limited human material that we have studied. It is therefore possible and likely that much of the tissue of major blood vessels is far removed from convenient blood supply, despite its content of smooth-muscle cells, which give morphologic evidence of being metabolically active.

It has long been an open question whether the vascular system is a stable tissue in which cell turnover and mitotic activity are seen only in response to injury as a repair reaction, or whether replacement of effete vessel cells is normal.[1] Earlier studies based on identification of mitotic figures by light microscopy were conflicting and indeterminate. The first use of tritiated thymidine as a more sensitive index of mitotic activity was reported by Spraragen and associates, working with the rabbit aorta.[51] These investigators used pulse labeling and failed to find mitotic activity in normal vessels. We used similar techniques but prolonged the infusion of tritiated thymidine.[49] In adult rabbits, labeled cells were found in vessels of all sizes and in all layers of the vessel wall. However, cells in mitosis were extremely sparse. In the aorta, less than 0.1% of intimal cells and less than 0.01% of medial cells were labeled. It appears that slow replacement of vessel cells does indeed occur; but even this must be only a tentative conclusion, because the laboratory rabbit continues to gain weight even after complete maturity has been attained. One is therefore moved to wonder with Altschul [1] at the durability of vascular cells, particularly those of the endothelium, which are subjected to so much stress and strain in their exposure to circulating blood.

DEVELOPMENT OF EARLY VASCULAR LESIONS

Until recently, only relatively late and advanced damage to blood vessels could be identified. In endothelium without demonstrable abnormalities by standard histologic techniques, electron microscopy may reveal profound changes. This is perhaps best illustrated by the necessity for extremely rapid fixation in electron microscopy, whereas by standard methods even postmortem material may look normal. The use of *en face* preparations has provided an advance, with demonstration of earlier endothelial damage; but even the lesions seen by this method represent marked progression, compared with those visible by ultramicroscopy. It therefore appears that establishment of the morphologic basis of the onset of thrombosis must emerge from electron microscopic studies. This field is in its early infancy, and only a few studies have been performed. These have been with experimental animals and with highly

contrived situations; nevertheless, some valuable information on the reaction of the blood vessel to injury has been obtained.

Three groups of investigators have inflicted mechanical trauma on major vessels of small animals. Ashford and Freiman used pinching of rat femoral veins,[2] whereas Tedder and Shorey[54,56] and we[46,57] partially constricted aortas and inferior vena cavas of rabbits. Ultramicroscopic analysis of the material obtained by each group has revealed similar but supplementary findings, although the changes reported by Tedder and Shorey occurred considerably more slowly than the ones we saw.

In our experiments, the early manifestations of endothelial-cell injury were not uniform from cell to cell; some changes were seen in some cells but not in others, even though the damage appeared to be at least as great in the latter. Nevertheless, a progression of changes associated with damage was generally evident. Staging of injury progression was obtained by varying the duration of ligation and procuring specimens at different distances from the site of application.

One of the first manifestations of injury often encountered is edema, an observation made by Shimamoto[46] and illustrated in Figure 4. The degree of separation in some areas between endothelial cells and the underlying connective tissue should be noted. Concurrently, there is disappearance of cell organelles and swelling of the endoplasmic reticulum, as seen in Figure 5. These changes are reminiscent of platelet degranulation, and it might therefore be suspected that there is an accompanying discharge of cellular contents into surrounding areas. Also at this stage there is the appearance of amorphous and electron-dense material in the subendothelial space. The nature of this material is unknown, but it is often associated with obscuring of the basement membrane.

As damage proceeds, there is additional evidence of autolysis. Large areas of cytolysis are shown in Figures 6 and 7, and the extracellular electron-dense material appears to be subjected to a similar lytic process. When the inferior vena cava reaches this stage of damage, neutrophils are seen penetrating the wall (Figure 8), and these cells often enter the deeper layers. This reaction is considerably less prominent in the aorta. Nuclear damage is seen as condensation of the chromatin and loss of its typical structural pattern, so that an amorphous density results.

At some stage in endothelial-cell damage, attachment of the cell to underlying structures is no longer possible, and desquamation follows. Platelets appear not to react with damaged cells but only with the underlying connective tissue.[2,48,57] Johnson and associates[24] have recently reported observations suggesting the alternative possibility, that cellular debris remains on the wall and is the site of platelet accumulation; but

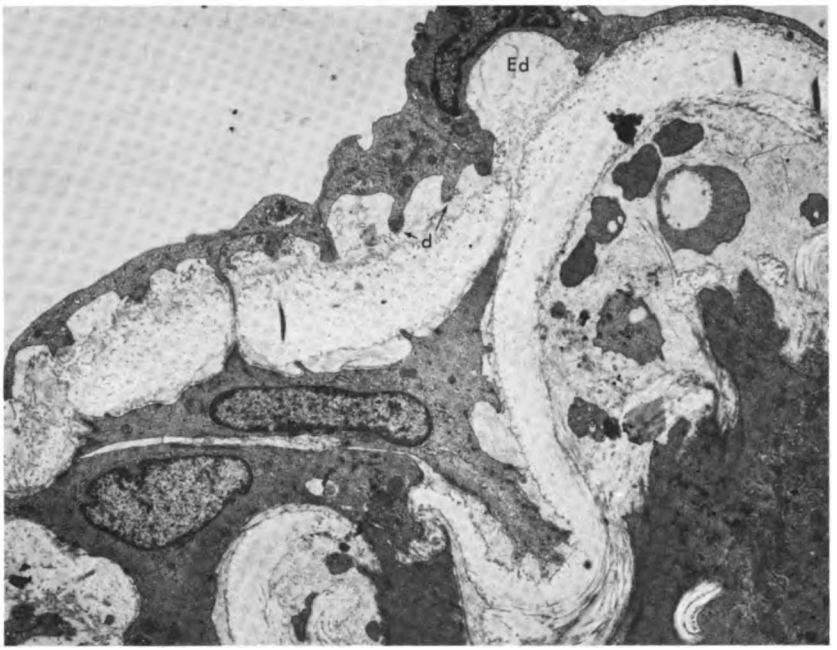

FIGURE 4 Rabbit aorta subjected to brief partial ligation, with fixation a few minutes after injury. Area about 2 mm proximal to ligature site. Note partial separation of endothelial cell from underlying structures. d, half-desmosome; Ed, edema. (× 6840)

most investigators probably are in agreement with the concepts expressed here. We have never observed platelets adhering to endothelial cells, no matter how severely damaged the cells were. Once desquamation has occurred, the accumulation of platelets at the denuded site begins promptly, and minute microthrombi are demonstrable, as seen in Figure 9. Desquamation is evidently preliminary to thrombus formation, so the mechanism of this reaction is a matter of considerable interest. What must occur is a weakening or lysis of the material that binds the cells to the subendothelial structures. Two phases of desquamation may exist. First, there may be loss of the looser strands of material between the cell and the basement membrane, leaving the cell attached at the sites of denser bonds or half-desmosomes. Cells so attached have been reported by Fulton and Berman [18] and by Stehbens [53]; the phenomenon is illustrated in Figure 4. For final desquamation to occur, the firmer attachments must also be destroyed. Although the nature of the material providing cellular attachment is not known, it is possible that

it is liable to lysis following exposure to endothelial-cell contents. The endothelial-cell degranulation that accompanies injury might be responsible for discharge of lysosomal enzymes into the subendothelial space that are active against binding structures, basement membrane, or both. Collagenase has been demonstrated in hepatic lysosomes [64]; endothelial-cell lysosomes may be a similar source of such enzymes. It is possible that the leukocytic infiltration accompanying injury also contributes to desquamation, inasmuch as it has been shown that neutrophils contain cathepsin-like enzymes capable of lysing basement membrane.[10] Rapid desquamation of endothelial cells in response to injury

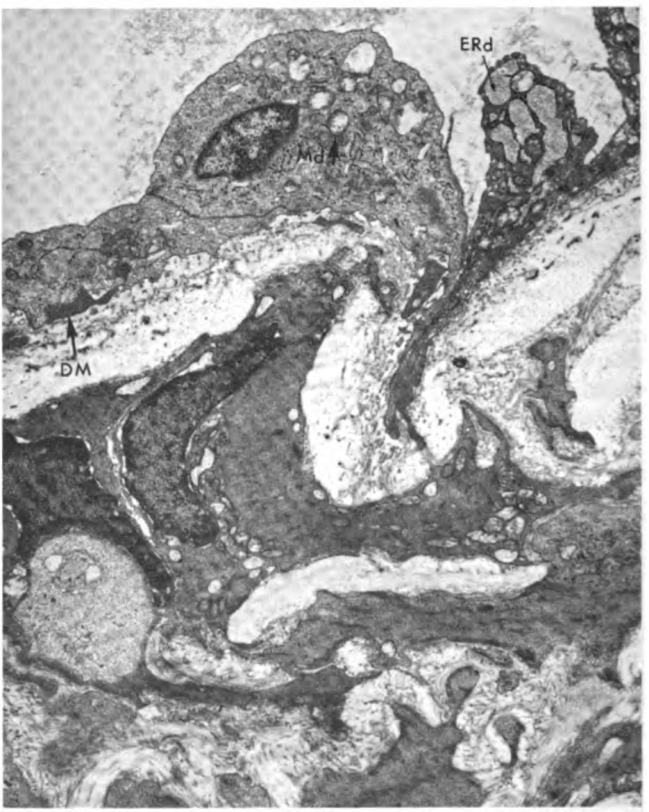

FIGURE 5 Early degenerative changes in rabbit aortic endothelial cells. Partial ligation for 1 min, and fixation a few minutes after injury. Area about 2 mm distal to ligature site. DM, dense material; ERd, dilated endoplasmic reticulum; Md, degenerated mitochondrion. (× 8800)

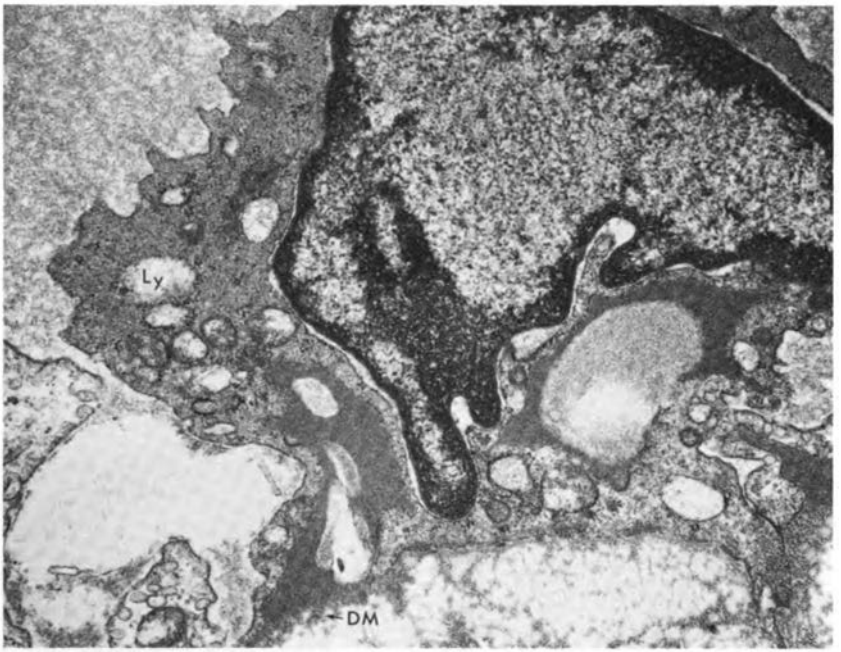

FIGURE 6 Progress of endothelial-cell lesion in rabbit inferior vena cava. Partial ligation for 1 hr, area about 2 mm distal to ligature. DM, dense material; Ly, lytic area. (× 19,600)

may be important in normal hemostasis. If the hemostatic plug is to form with greatest efficiency, exposure of subendothelial collagen must be maximal to present an adequate base on which platelets can accumulate. Intervention of cellular debris might hinder the process.

In the experimental conditions described above, mechanical trauma produces a series of progressive endothelial-cell changes leading to ultimate cell destruction and desquamation. Few if any of these lesions are evident by light microscopy. Thrombosis evidently follows the presentation of exposed collagen to circulating blood elements, particularly platelets.

A reasonable picture thus emerges, but several important questions remain to be answered. Not the least of these concerns the comparability of this acute and relatively artificial lesion with spontaneous disease in man. Clarification will follow only when suitable studies are performed on vessels from patients liable to thrombotic disease. Whether the sequence of events following mechanical trauma is universal or highly

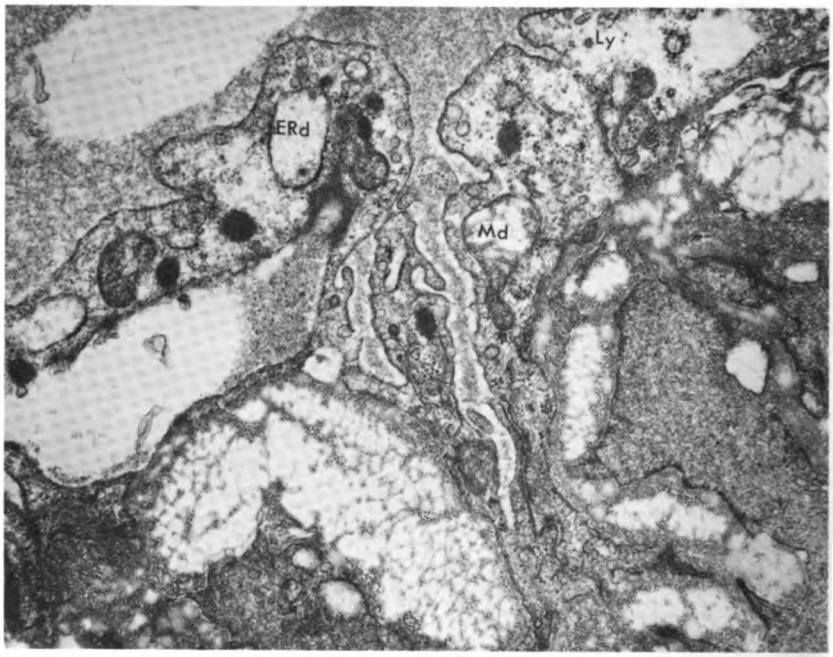

FIGURE 7 Advanced cytolysis in endothelial cells of rabbit inferior vena cava. Partial ligation for 1 hr, area about 4 mm distal to ligature. ERd, dilated endoplasmic reticulum; Ly, lytic area; Md, degenerated mitochondrion. (× 21,000)

specialized is of prime importance. Growth of the thrombus to the point of clinical significance and possible sources of vascular damage are two additional problems of interest, and these may be discussed with the aid of a few clues from available experimental data.

THROMBUS GROWTH

Vascular injury with endothelial-cell desquamation does not universally guarantee the development of an occlusive thrombus. Blood vessels can tolerate a considerable degree of chemical or mechanical trauma without forming thrombi, provided they are of sufficient diameter or the flow characteristics are adequate to dislodge the forming hemostatic mass. Poole and associates [40] emphasized this concept by demonstrating that even severe injury from an intraluminal brass rod failed to elicit significant thrombus formation in the rabbit aorta. Data of this type have

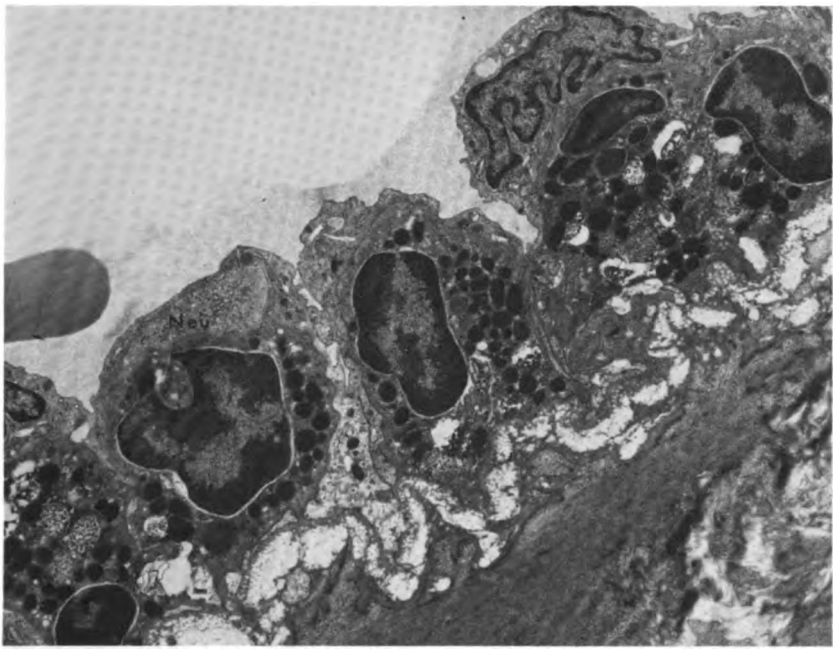

FIGURE 8 Leukocytic infiltration of partially ligated rabbit inferior vena cava. Two hours of tie application, area about 10 mm distal to ligature site. Neu, neutrophil. (× 7300)

been reported by many authors, and they appear to raise a valid question. However, they are subject to some difficulty in interpretation, in that the completeness of endothelial-cell desquamation has never been fully assessed. It is not impossible that only patches of collagen are denuded, and that large intact areas persist even when trauma is assiduously applied.

Nevertheless, the hemostatic elements are capable of a remarkable degree of thrombus formation and can occlude vessels of considerable diameter. This is illustrated by early studies on extracorporeal shunts made with artificial materials,[47] and by the more recent difficulties encountered by surgeons in keeping vascular prostheses patent when synthetic materials or even grafts are inserted. The extent of vascular injury is certainly an important determinant of thrombus growth, and studies that have achieved a degree of quantitation indicate that the greater lesion gives a larger thrombus.[3]

Stasis has long been considered a factor favoring thrombus growth, although a degree of flow preservation is necessary for thrombus forma-

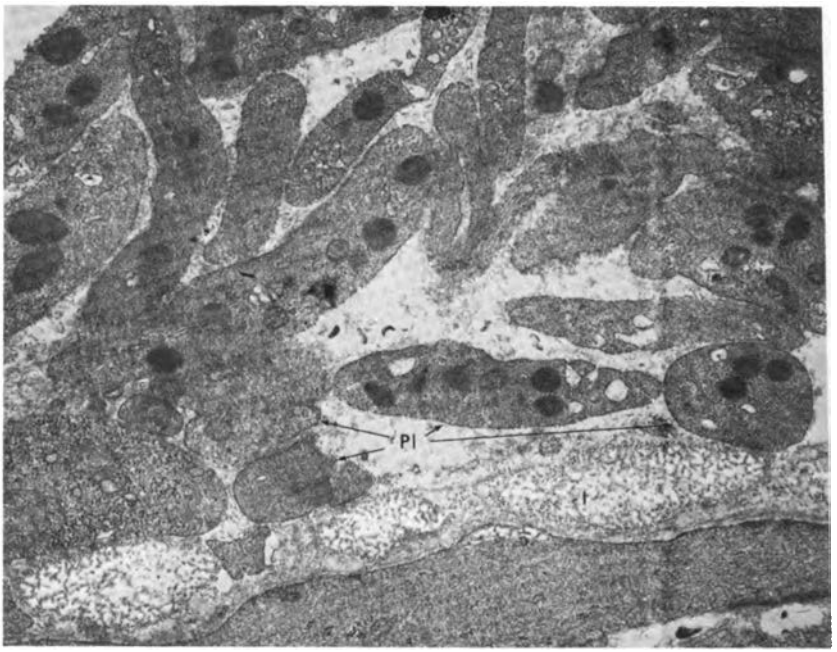

FIGURE 9 Microthrombus at injury site in 17-hr partially ligated rabbit inferior vena cava. I, internal elastic membrane; Pl, platelet. (× 14,600)

tion. Welch noted in 1899 [61] that, in the presence of complete stasis, blood remained fluid for long periods, but that thrombi formed readily when sluggish flow was combined with vascular injury. The cast of the blood vessel that forms in the presence of complete stasis and hypercoagulability [62] is a whole-blood clot and does not resemble the usual pathologic thrombus.[30] Recent authors[30,39,63] also have emphasized the importance of stasis in thrombogenesis, but there is little quantitative work to define more exactly the degree and types of flow alteration that may have the greatest effect on thrombus growth. It is readily observable that a growing microthrombus formed by mechanical injury in an otherwise normal blood vessel shows such a small degree of platelet embolization that complete vascular occlusion may never occur,[23] and it is possible to produce extensive chemical injury to a vein and obtain an occlusive thrombus without further intervention.[36] Between these extremes is a vast expanse of ignorance. Although a thrombus will probably continue to grow as long as flowing forces are insufficient to disrupt it, analyses of factors that influence these forces has not been systemati-

cally undertaken. A start in this direction has been made by Rodbard and Johnson,[42] who used agar-coated plastic tubes to ascertain the effect of turbulence on deposits in a fluid system. Acetic acid vapor was forced through the tubes, and blackening of the agar indicated deposition of the chemical. The data indicated that turbulence was a major factor in determining deposition, an observation in conformity with one's intuitive feeling that a similar relationship prevails in thrombosis. Simple stasis appears to be insufficient to account for the varied preconditions that favor thrombus growth.

CONDITIONS PRODUCING VASCULAR INJURY

The vascular endothelium is extremely sensitive to trauma, and minor irritation or manipulation may produce grossly visible damage [52]; but how this sensitivity is specifically related to thrombogenesis in the common clinical thrombotic states is largely unknown. In some situations it is easy to picture how extensive vascular lesions lead to endothelial desquamation and the consequent buildup of thrombotic material. The thrombophlebitis accompanying local inflammation, various vasculitides, and the lesions underlying major atherosclerotic plaques or aneurysms are familiar examples of dramatic vascular pathology. It is less obvious how endothelial damage might develop in the thrombotic diatheses that accompany some malignancies, postoperative thrombosis, infections, and other apparently unrelated conditions. How varied these conditions may be is emphasized by the extensive monograph on the subject by McKay.[31]

Many different noxious stimuli probably can lead to the final common pathway of endothelial damage. A specific example is that accompanying infection. The multiple small-vessel thrombi often seen in sepsis and even less severe bacterial disease have been related to the generalized Shwartzman reaction, and it is widely held that these lesions are the result of embolization following diffuse intravascular clotting. An alternative possibility is suggested by the experiments of Rubenstein and associates,[43] who studied the distribution of endotoxin injected into dogs, by means of fluorescent antibody. It was shown that endotoxin became widely attached to vessels in various organs, and that it was often transported by leukocytes. Although vascular pathology was not specifically studied, it is evident that a combination of endotoxin and leukocyte lysosomal enzymes is potentially highly vasculotoxic.

At present, the status of other circulating vasculotoxic agents is speculative. Breakdown products of leukocytes [10] and platelets,[35] and

presumably other tissues, may prove to be important. Tumor metabolites or autoimmune products are other possible candidates. In the presence of such circulating agents, the endothelial cell would be particularly vulnerable. This vulnerability would arise both from the marked sensitivity to injury noted above and from the location of these cells, which places them first in line of exposure to any bloodborne material.

An additional factor that might be important in the pathogenesis of vascular injury is the state of nutrition of the vascular wall. As has already been noted, it appears that the endothelial cell derives its blood supply from the lumen, and that the adventitia is the only component that is endowed with a microcirculation. The presence of any thrombotic deposits overlying endothelial cells might produce further compromise of blood supply. Endothelial damage could thus follow circulatory alterations within the lumen, or medial damage following reduction of its own precarious blood supply. That the intima is sensitive to lesions in deeper portions of the vessel is suggested by the endothelial damage that results from outer-wall trauma.[52]

Whatever the etiologic factors may be, there is evidence that diseases associated with a thrombotic diathesis have accompanying endothelial pathology. Two groups of investigators have demonstrated the presence of endothelial cells in the circulating blood, which are presumably the result of desquamation.[4,21] Even basement-membrane and small-vessel fragments were identified.[21] Malignancies, infections, and allergic disorders were some of the more common underlying disorders in the patients presenting these findings. A second line of evidence in the same direction is provided by data on gastric vessels obtained at surgery and studied by the *en face* method, which reveals endothelial detail. Ohta and his colleagues [37] showed that veins and arteries from patients with gastric malignancies had platelet microthrombi far in excess of those from patients with benign peptic ulcers. The lesions resembled those produced by O'Neill [38] and others in experimental animals subjected to vascular trauma.

CONCLUSIONS

Therapeutic efforts in the management of thrombosis have taken two major directions. The older and better known has been the development of drugs designed to prevent the formation of fibrin or to cause its removal. Success with these agents has been greatest in the case of venous disease; that is to be expected, inasmuch as fibrin formation is favored in the slowly flowing venous system, where it is associated with considerable amounts of clotted whole blood. In arteries, thrombi are com-

posed of larger masses of white and mixed structure, suggesting that platelet aggregation is of greater importance. It would be anticipated that such structures would be less vulnerable to anticoagulant or fibrinolytic attack; and clinical observation indicates that anticoagulant or fibrinolytic management of arterial disease is considerably less satisfactory.

A more recent approach, still in its infancy, shows considerable promise. It is designed to inhibit platelet reactivity at its various stages and thereby prevent the development of the earliest thrombotic mass. Compounds are being sought that prevent the aggregating effect of adenosine diphosphate, and already some that evidently interfere with the platelet collagen reaction have been studied. Two drugs that demonstrate the latter effect are aspirin [13,60,65] and butazolidine.[34]

It seems reasonable to assume that, as the pathogenesis of vascular damage is elucidated, therapeutic concepts based on specific components of the reactions will emerge. For example, if the enzyme activity responsible for endothelial-cell desquamation is identified, it may be possible to achieve inhibition of this enzyme and thus delay desquamation until repair is established. Of course, with present knowledge, future therapeutic directions are unpredictable. Ideally, prevention of the vascular lesion by elimination of the underlying factors is the ultimate answer. But any approach based on such considerations must follow concentration on vascular studies, a hitherto neglected area of investigation.

The work reported here was supported by Public Health Service grant HE 05415-07 from the National Heart Institute.

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Ultrastructural Studies of Endothelial Injury in the Microcirculation, with Special Reference to Thrombosis

RAMZI S. COTRAN

The role of vascular injury in thrombosis—recognized since Wharton Jones and Virchow—has been emphasized by a number of recent electron microscopic studies. In this publication the problem is reviewed with regard to thrombosis in larger vessels, and the importance of endothelial-cell injury in the initiation of the thrombotic process has been substantiated.

In the last few years, much has been learned about the submicroscopic appearance of endothelial cells injured by a variety of chemical, physical, bacterial, and immunologic stimuli. Most of the studies were done on the endothelium of the microcirculation—venules, capillaries, and small arterioles—but did not deal specifically with the relationship of the endothelial changes to thrombosis. They have, however, provided rather precise information on the events involved in increased vascular permeability, cellular exudation, endothelial phagocytosis, and regeneration; and they have contributed as much to the understanding of the phenomena of inflammation as can be expected from an essentially morphologic technique. The literature on normal and pathologic endothelium was reviewed in detail by Majno in 1965 [35] and more recently by French [19] and myself.[14] In this presentation, I will deal with only the most recent developments and emphasize points that may be relevant to the problem of thrombosis.

ULTRASTRUCTURAL BASIS OF INCREASED VASCULAR PERMEABILITY

In 1961, shortly after the fine structure of normal endothelium had been described in some detail,[3,18,47] Majno and Palade reported that in-

creased vascular permeability induced locally by histamine and serotonin is due to the formation of discrete gaps in the normally continuous endothelial lining of the vessel.[38] Such gaps occur principally in venules (rather than capillaries) and are apparently formed by a separation in the junctions between endothelial cells.[39,*] Electron-dense tracer particles, recognizable circulating macromolecules (such as chylomicrons), erythrocytes, and platelets are often found in these gaps and presumably exit into the vessel wall through them.† These elements then encounter the basement membrane, which forms a continuous external layer around the endothelial cells and pericytes. The basement membrane allows the passage of most molecules 100 Å or less in diameter (most plasma proteins) but retains tracer molecules of the size of carbon (300 Å) and, at least temporarily, erythrocytes and platelets. Fibrinogen also seems to pass across the basement membrane, inasmuch as the interstitium contains recognizable fibrin strands; at times, however, polymerization of fibrinogen does appear to occur within the vascular wall, internal to the basement membrane.[13,38] Factors responsible for the differential deposition of fibrin inside and outside the vessel wall have received little attention and deserve further study.

Endothelial gaps at or near intercellular junctions have been found to be the morphologic basis of increased vascular permeability in virtually all the models of acute inflammation studied: thermal,[10,13,16,43] chemical,[21,27,43] bacterial,[27] toxic,[12] immunogenic,[8,44,56,58,60] and traumatic.[40] In most of these models, there is evidence that the phenomenon is also chemically mediated, but not by histamine or serotonin.[59] However, gaps may also occur as a result of direct injury to the endothelium.

POSSIBLE MECHANISMS FOR FORMING ENDOTHELIAL GAPS

It is clear that the mechanism of formation of endothelial gaps is of utmost importance. In the case of histamine-induced endothelial gaps, several mechanisms have been proposed: a change in the nature of the

* Until recently, intercellular junctions in normal endothelium were considered to be closed and thus to form a barrier to the transendothelial passage of solutes.[85] However, recent evidence,[80] based on the use of horseradish peroxidase as a tracer, showed that the junctions are normally permeable to peroxidase (molecular weight, 40,000) and presumably to smaller molecules but not to tracer molecules of the size of ferritin (100 Å) or carbon (300 Å).[19,85]

† Leukocytes also cross the endothelium intercellularly,[41] but the phenomena of increased vascular permeability and leukocytic exudation are separate and probably have different mechanisms.[18,26,26]

intercellular material,[6] a direct action on the membranes at the intercellular junction,[38] rupture of endothelium due to increased hydrostatic pressure,[53] and endothelial contraction.[42] The most exciting development in this field has been the recent demonstration by Majno and Leventhal [37] that the gaps are caused at least in part by contraction of endothelial cells. These authors found by electron microscopy that nuclei of endothelial cells adjacent to gaps exhibited numerous infoldings and pinches and looked like nuclei seen in contracted smooth muscle. Rigid controls showed that this phenomenon was present very infrequently or not at all in normal endothelium.

The ability of endothelial cells (and pericytes) to contract was suggested by many earlier investigators, but their evidence was challenged and the problem remained controversial.[5,35] However, the ultrastructure of endothelial cells is compatible with the notion of endothelial contraction. Even the earliest electron microscopic study of endothelium [47] showed the presence of cytoplasmic filaments scattered throughout the cytoplasm. Subsequently, many authors reported filaments 60–100 Å in diameter, in a variety of endothelia from different species [1,4,5,18,22,23,32,46,50–52,57,63] and regarded such filaments as "contractile," "supportive," "tonic," or "elastic." Ancla and de Brux [1] reported a "spotty" endothelial fibril in the arterioles of human endometrium, and more recently Röhlich and Oláh [52] described cross-striated fibrils in the endothelium of rat myometrial arterioles. On occasion, we have found various striated structures in normal and pathologic endothelium.[12,14] Although the presence of filaments is not proof of contractility, recent reports,[36] using fluorescent-antibody techniques, strongly suggest that actomyosin is found in endothelial cells. Another relationship that needs further study (in the endothelium) is that between the filamentous structures and microtubules; the latter have been considered to be contractile, supportive, or conductive.[51] The distribution of microtubules in various endothelia is variable, and occasionally they are numerous, especially at the periphery of the cell.

Although endothelial contraction goes a long way to provide a mechanism for gap formation, at least in the case of histamine, several factors remain to be clarified: If histamine acts directly on endothelial cells, why is vascular leakage selectively localized to venules, sparing arterioles and capillaries? Are the gaps induced by other injuries also caused by endothelial contraction? Review of our material on leakage induced by thermal injury,[10,13,16] bacterial toxin,[12] and delayed hypersensitivity [60] suggests that the phenomenon also plays a role in these models but that some gaps occur in the absence of obviously contracted nuclei.

PLATELETS, THROMBI, AND ENDOTHELIAL GAPS

As mentioned above, platelets can be found within an endothelial gap, on its luminal side, or within the wall, having presumably leaked through a gap. When tracer particles are also injected, one sometimes sees a platelet "plugging" a gap and preventing tracer particles (and, therefore, presumably plasma) from further leakage. One cannot escape the feeling that platelets actually plug "leaky pores." [33,49,62] Although this mechanical aspect of platelet contribution may be oversimplified, it cannot be dismissed altogether.[62]

Transient accumulations of platelets in the vicinity of the endothelial gaps do occur, but stable thrombi, with fibrin deposition, develop only infrequently in histamine edema and in all models of acute inflammation in which the stimulus is essentially subnecrotizing. Some of the platelets in the area show pseudopod formation, but extensive degranulation and membrane disruption are uncommon.

Recently, much evidence has been presented to suggest that the basement membrane of glomerulus, lens capsule, and some epithelia [17,48,54] consists of a protein with an amino acid composition akin to (but not identical with) collagen. Inasmuch as collagen is known to cause adherence and clumping of platelets *in vitro* [25,61] and *in vivo*,[19,20,29,33] this may be a mechanism by which platelets adhere in endothelial gaps. Collagen also induces platelet transformation and degranulation,[31] but degranulated platelets are uncommon except in the presence of overt endothelial degeneration or denudation. At any rate, the reaction of platelets with such nonfibroblast collagen-like proteins deserves further study, because these would be the first extracellular elements encountered by platelets when the endothelium becomes discontinuous.

DIRECT ENDOTHELIAL INJURY

Although chemical mediators are probably responsible for much of the increased vascular permeability after local injury, a variety of endothelial changes leading to vascular leakage, as well as to thrombosis, may result from direct injury to the endothelium by the injurious agent.[15,34] This is obvious after grossly necrotizing injury but also occurs after subnecrotizing or minimally necrotizing stimuli, such as those that induce delayed and prolonged vascular responses.[10,12,16] Direct injury affects capillaries, venules, and arterioles and may be manifested by (1) formation of endothelial gaps, (2) focal cytoplasmic changes, and (3) endothelial necrosis with outright sloughing and denudation.

1. Because formation of intercellular endothelial gaps is the major known morphologic manifestation of leakage induced by chemical mediators, the role of direct injury in the induction of endothelial gaps is difficult to assess. However, gaps in capillaries, which are known to be resistant to the action of most mediators, may represent the result of direct injury. Often, capillary gaps occur in the presence of obvious endothelial damage,[10,12,16] although in some models [13] evidence of endothelial damage may not be impressive. In addition, there is no *a priori* reason to deny that mere separation of endothelial cells can occur after mild injury that in itself would be insufficient to cause other signs of endothelial damage.

2. Focal cytoplasmic changes include focal dilatation of mitochondria and endoplasmic reticulum, cytoplasmic vacuolization (often localized to one endothelial cell), and formation of cytoplasmic blebs that project into the lumen.[7] These changes have also been described in the endothelium of large veins and arteries subjected to traumatic [2] and chemical injury.[24] As in larger vessels,[2] platelets do not commonly adhere to cells exhibiting these changes in the microcirculation. However, at times we have found small aggregates of platelets free in the lumen, or apposed to slightly altered or apparently intact endothelium as a result of a variety of mild stimuli, such as heat,[13] surgical trauma, and venous congestion. Such platelets are sometimes surrounded or separated from endothelium by an amorphous, somewhat electron-dense material—probably absorbed plasma protein.[11] Stehbens and Biscoe [55] and Ashford and Freiman [2] described similar aggregates and appositions to endothelium in mildly traumatized tissues; the latter authors showed that such platelet accumulations could be readily duplicated by infusion of ADP. Recently, Chiang *et al.*[7] suggested that cytoplasmic "blebs" induced in cerebral vessels by ischemia constrict small capillaries and impede blood flow. This may contribute secondarily to thrombosis in the microcirculation.

3. Platelet and platelet–fibrin thrombi are prominent in the microcirculation after stimuli that induce necrosis of tissues, including endothelium. The sequence of events has been studied after thermal injury,[10,15,34] including those by minute burns,[16] chemicals,[21,27,34] and high concentrations of bacterial toxin.[12] In such experimental models, increased vascular permeability is a consequence both of gaps and of endothelial-cell degeneration, necrosis, and outright denudation. By injecting electron-dense tracer materials before or after injury, we have found that the development and outcome of thrombosis after direct injury depends to a great extent on the size of the vessel.[16] For example, when thermal injury affects arterioles or venules 50 μ or more in

diameter, platelet and platelet–fibrin thrombi adhere to denuded endothelium at several points around the circumference of the vessel, but such thrombi do not coalesce, and the blood flow to the area is generally maintained. This can be shown directly by the presence of tracers (injected after the injury) in the lumina of affected vessels.[16] However, small venules and capillaries are often completely plugged with thrombotic material, and tracer particles do not reach the lumina of such vessels. The various hemodynamic and other mechanisms that play a role in the evolution of the thrombus in larger, as opposed to smaller, vessels are beyond the scope of this presentation and have been discussed in previous reviews [33] and elsewhere in this volume.

If one focuses on the fine structure of the endothelium after direct injury and its relation to thrombosis, one discerns a spectrum of changes: (1) In some vessels, many endothelial cells appear swollen, and show various evidence of degeneration, disorganization of organelles, dilatation of mitochondria or endoplasmic reticulum, intracytoplasmic vacuoles, and occasional myelin figures—but such cells may have an intact plasma membrane. This usually occurs at the periphery of the lesion and probably represents an intermediate form of injury. It is unusual to find thrombi overlying such degenerated (but not denuded) endothelium, except in the smallest vessels; and even in these, one cannot be certain that denuded endothelium or gaps are not present in adjacent levels of the same vessel. These findings are consistent with those seen in larger vessels.[2] (2) In some areas, relatively intact platelets replace a large part of denuded endothelium layer by adhering to the underlying subendothelial tissue. In smaller vessels, this consists essentially of basement membrane material. Some platelets have pseudopods, but severe platelet transformation does not occur. Fibrin is rare. (3) The most advanced stage occurs when masses of platelets, some of which are degranulated, and varying amounts of fibrin are seen intraluminally, within the vessel wall, as well as in interstitial tissues. The endothelium proper may be completely denuded, or fragments of cytoplasm and membrane material may be seen.

This account has dealt with the effect of injury of endothelium on the local deposition of platelets and fibrin. Recent evidence indicates that platelets and other by-products of coagulation—e.g., fibrinopeptides [9]—may in turn affect the endothelium itself. Thus, platelets are known to contain various vasoactive amines and proteolytic and hydrolytic enzymes.[33] Mustard *et al.* reported that platelets exposed to antigen–antibody complexes, collagen, and other particles release factors that increase vascular permeability in rabbits.[45] To what extent

this phenomenon plays a role in the evolution of hypersensitivity states is not clear. Electron microscopic studies on Arthus's phenomenon,[56] for example, have shown that platelet thrombi and endothelial defects are common, but whether the platelet aggregates are due primarily to interaction with complexes or to endothelial disruption itself has not been determined.[9]

SUMMARY AND CONCLUSIONS

Three forms of endothelial injury in the microcirculation have been described: (1) Endothelial cells may show cytoplasmic alterations but retain an intact plasma membrane. The cytoplasmic changes include mitochondrial and endoplasmic reticulum dilatation, endothelial vacuolization, intracytoplasmic myelin figures, and formation of cytoplasmic blebs. These changes are not associated with increased permeability, do not generally lead to the formation of stable thrombi, and may be reversible. (2) Increased vascular permeability, in virtually all models of acute inflammation induced by chemical mediators and subnecrotizing stimuli, occurs by the formation of endothelial gaps at or near intercellular junctions. Recent work indicates that such gaps are caused, at least in part, by contraction of endothelial cells. Platelet accumulations may form in these models, but stable platelet–fibrin thrombi are rare. (3) Direct necrotizing injury results in both increased vascular permeability and the formation of thrombi of variable stability. This type of injury is characterized by the presence of endothelial degeneration and denudation.

The work reported here was supported by Public Health Service grant HE 08251 from the National Heart Institute and a career development award.

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The Role of the Endothelium in the Resolution of a Thrombus

THOMAS P. ASHFORD

The juxtaposition and contact of the vascular endothelium with blood suggests that it may play a prominent role in the events that precede or follow thrombosis. Endothelial cells have two properties that might be important in the resolution of a thrombus: (1) the ability to stimulate fibrinolysis and (2) the formation of a barrier, separating the procoagulants and platelets of the blood from underlying tissue. This presentation will deal with the possible role of these properties in the resolution of thrombosis.

ENDOTHELIUM IN STIMULATION OF FIBRINOLYSIS

Mole called attention to the relationship between the endothelium and fibrinolysis by observing an inverse relationship between fibrinolytic activity and the diameter of the vessel during autopsy.[30] Earlier evidence, such as Virchow's observation that capillary blood in cadavers is always incoagulable, in contrast with the finding of occasional clots in larger vessels,[44] seemed to substantiate this relationship. It was suggested that some tissue operated through release of an agent that acted on an enzyme precursor in the blood.[10] That was later substantiated when Astrup [5] designed a fibrin-plate technique to study the interaction of cellular material with a fibrin clot, and demonstrated that tissue activator acts directly on plasminogen. Activator is increased after hypoxia, administration of histamine or vasoactive drugs,[18] or trauma [37]; and plasmin, the protease resulting from this reaction, initiates fibrinolysis. Examination of various layers of vessels [6] showed a rough correlation between the vascularization of a tissue and its fibrinolytic activity.

Todd was able to demonstrate fibrinolytic activity by an application

of histochemical methods to the fibrin-plate technique.[42] This technique was used by Kwaan,[23] who had shown that plasminogen activation is especially prominent near endothelial cells of granulation tissue [24] and also demonstrated a difference in the apparent plasminogen activation by endothelial cells in different locations. Small veins appeared to possess the most active endothelial cells. The evidence of a connection between fibrinolytic activity and a cholinergic effector mechanism [24] is difficult to interpret.[7] However, Sherry *et al.* demonstrated increased fibrinolysis after electroconvulsive shock and intravenous injection of acetylcholine,[36] suggesting that plasminogen activators might be locally induced by a neurogenic mechanism. Warren,[45] using sheets of endothelial cells in an *en face* preparation, provided further evidence that the induction of lysis is restricted to endothelial cells in vessels. Clear zones in the fibrin plate after incubation were found associated only with endothelial cells.

Fibrinolysis is a very rapid process *in vivo*. Ashford and associates [2,4] have demonstrated, in rats, marked increase in fibrin in veins subjected to 10 min of stasis after injection of homologous serum when plasminogen activation was inhibited with epsilon-aminocaproic acid (EACA). In the same work, the alterations in the amounts of fibrin and platelets produced by a fixed dose of serum were markedly increased with EACA in very small veins and capillaries, whereas thrombosis in large veins appeared complete without EACA. This evidence was interpreted as indicating more effective fibrinolysis in small vessels. Although some of the more intense activity might be attributed to increased activity of endothelial cells at these sites, as suggested by Kwaan,[23] the greater surface:volume ratio inherent in small vessels, which constitute nearly all the acres [1] of vessel surface in the body, must be taken into consideration. There was no change in the appearance of any of the endothelial cells in this study. This observation suggests that the release of plasminogen activator by the endothelial cell is a normal physiologic process. Active fibrinolysis in small vessels is valuable, in that capillaries are not easily replaced by alternate routes and are surgically inaccessible.

EACA also influences the amount of fibrin seen in vessels after trauma. The vessels of the lamina propria of the small intestine of the rat generally showed scattered foci of fibrin production 1 hr after injury produced by pinching. These fibrin formations were usually delicate and were easily related to lesions of the vascular wall (Figure 1). After EACA, such thrombi, although not definitely more frequent, displayed such intense fibrin production that the point of origin (Figure 2) could not be ascertained.

Thrombi are made up of platelets and fibrin enmeshed with other ele-

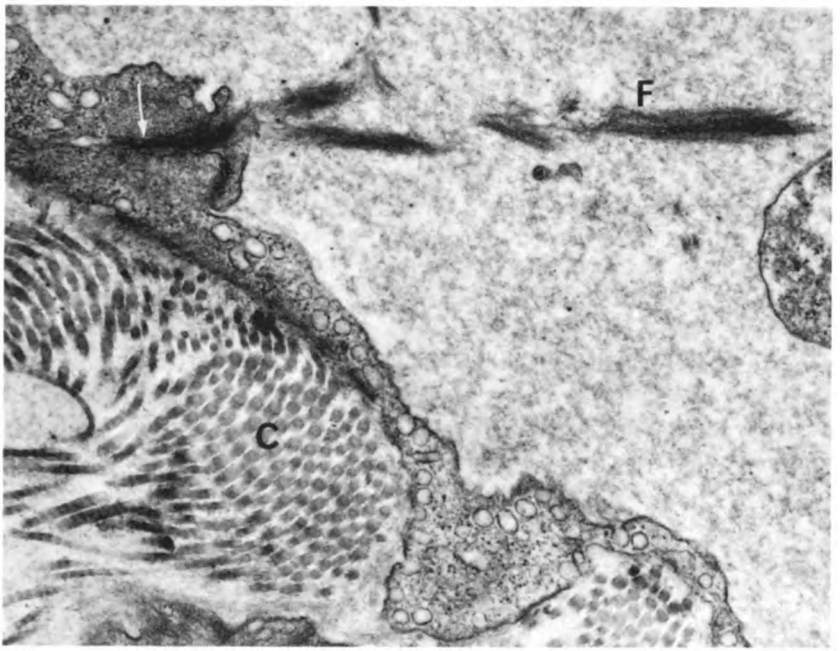

FIGURE 1 Electron micrograph of vein of rat small intestine 1 hr after mechanical injury. Fibrin (F) in lumen is associated with junction of endothelial cells and is found between them (arrow). C, collagen. (× 20,640)

ments of the blood. Injury of the vascular wall is probably the primary stimulus to thrombosis, especially in the arterial system, where the thrombus is initiated by deposition of a nidus of platelets.[38] Fibrin is a more significant component of the thrombus in veins or in local circumstances that promote stasis. Thrombi formed after heparinization are extremely fragile [3,19] and are easily swept away. Fibrinolysis can be an effective mechanism in the resolution of the thrombus simply through removal of fibrin. The endothelium is in an advantageous position to stimulate fibrinolysis, in that it surrounds the thrombus and has direct contact with it from the beginning of thrombosis; furthermore, local trauma [37] and hypoxia [18] stimulate the appearance of plasminogen activator. This mechanism of fibrinolytic activation would be particularly effective in the case of small thrombi lodged between cells. Such thrombi at intercellular gaps are easily demonstrated (Figures 1 and 3). Rapid fibrinolysis should lead to early disappearance of such gaps, probably by means of closure by contiguous cells. More extensive

thrombi, especially those obliterating the lumen, are invaded by the endothelial buds [47]; plasminogen activation might allow invasion and organization by helping to resolve the supporting fibrin framework of the clot.

Contact of blood with tissue thromboplastin results in prothrombin activation via the extrinsic pathway, whereas contact with collagen is capable of activating factor XII (Hageman factor) via the intrinsic pathway.[31] Platelets adhere to collagen,[20,48] which induces platelet degranulation.[22] There is evidence, however, that the endothelium constitutes a barrier between the blood and activating mechanisms. This was demonstrated *in vivo* by electron microscopy in the traumatized femoral vein of the rat.[3] Altered platelets and fibrin were seen only in regions denuded of endothelium and not in regions of injured but intact endothelium, despite the degree of injury. Delicate lesions can be produced in the vessels of the small bowel of the rat; 1 hr after mechanical injury,

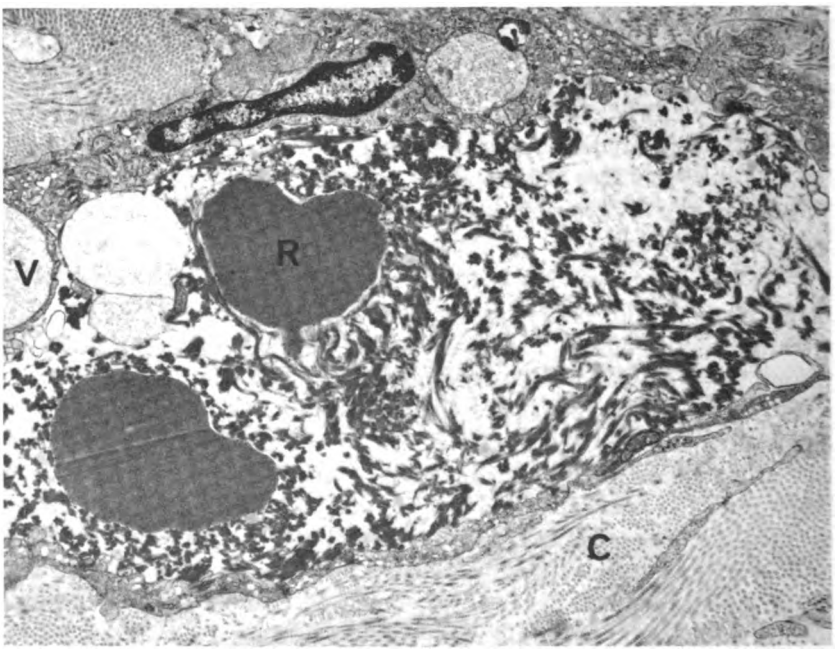

FIGURE 2 Electron micrograph of venule of rat small intestine 1 hr after mechanical injury following administration of epsilon-aminocaproic acid to inhibit fibrinolysis. Dense fibrin thrombus occludes lumen. Endothelial vacuoles (V) indicate endothelial injury, but site of collagen exposure is not identified. C, collagen; R, erythrocyte. (× 9460)

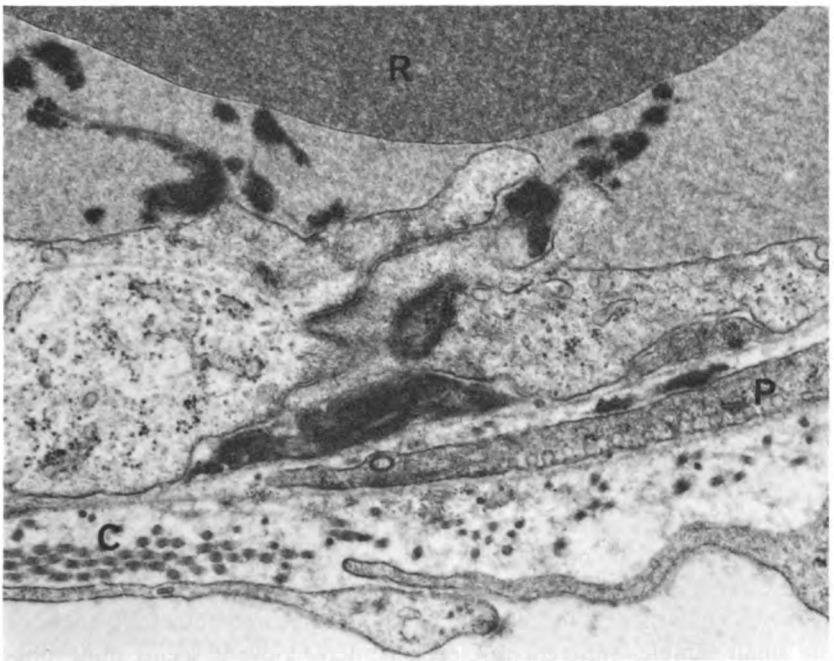

FIGURE 3 Electron micrograph of venule of rat small intestine 1 hr after mechanical injury. Fibrin is found in the lumen and subendothelially at an intercellular junction. C, collagen; P, pericyte; R, erythrocyte. (× 21,500)

fibrin could be found emanating from the interendothelial space (Figure 1). Large lesions in the intestine resembled those found in the femoral vein. Although in most cases the fibrin could not be traced to the deeper vascular layers, it was clearly identified subendothelially (Figure 3).

Platelets were infrequently seen in lesions of the small vessels, and fibrin seemed especially prominent. This might be best explained by stasis, demonstrated in the discussion of the microcirculation by Dr. Cotran in the preceding paper.

Single or loosely organized small groups of unaltered platelets that are not associated with fibrin are often found near slightly injured but intact endothelial cells. Because these aggregations resemble those induced by adenosine diphosphate (ADP) *in vivo* and *in vitro*, it is possible that they are attracted to endothelium by a similar mechanism. The presence of an active enzyme in endothelial cells capable of splitting ADP, adenosine triphosphate, and other nucleotides [28] suggests that platelets

may take an active part in this process. The observation by Jørgensen and Borchgrevink,[21] that adhesion of platelets to endothelial cells is impaired in von Willebrand's disease when platelet response to ADP is also abnormal,[32] lends further support to this hypothesis.

Several findings, however, suggest that this mechanism might not be responsible for the appearance of isolated platelets near the endothelium, but that such platelets are aggregated about minute endothelial gaps. Robertson *et al.*,[35] using *en face* preparations to study the result of minimal trauma, found platelets clustered around the junctions of endothelial cells. The considerable arborization of the platelets demonstrated by serial sections [3] in our work makes determination of the site of adhesion of these aggregations by electron microscopy difficult. In recent experiments, we repeated examination of the femoral vein following the minimal trauma of isolating the femoral canal from surrounding structures.[3] In 20 rats not treated with EACA, small groups of platelets were observed near the endothelial surface, although no fibrin or endothelial discontinuity was demonstrated (Figure 4). When EACA (90 mg/kg) was administered before dissection of the vessel, small groups of platelets near the endothelial surface were incorporated into fibrin streamers (Figure 5), although the endothelial cells appeared intact. In view of the small samples inherent in electron microscopy, it seems quite possible that minute endothelial discontinuities were overlooked and that associated fibrinolysis had removed detectable fibrin.

ENDOTHELIUM AS A BARRIER TO THROMBOSIS

A vascular surface without an endothelial layer represents an unstable balance of the several factors that determine the development of a thrombus. These include the quantity and adhesiveness of platelets, procoagulant concentrations and activation, and blood flow. For example, a new synthetic vascular graft will become occluded if flow is stopped. It is obvious that the regeneration of an endothelial surface is necessary for normal stability to be achieved after thrombosis. Endothelial contraction after physiologic stimuli may represent the simplest form of separation of endothelial cells to allow platelet deposition and fibrin formation (Figure 1). This possibility is suggested by the work of Robertson *et al.*,[35] in which *en face* preparations demonstrated transient minute lesions in response to delicate mechanical trauma. Majno and Leventhal [27] have recently demonstrated changes suggesting endothelial contraction in response to histamine. Endothelial integrity is known to be lost in venules of the rat exposed to carbon. Gaps between en-

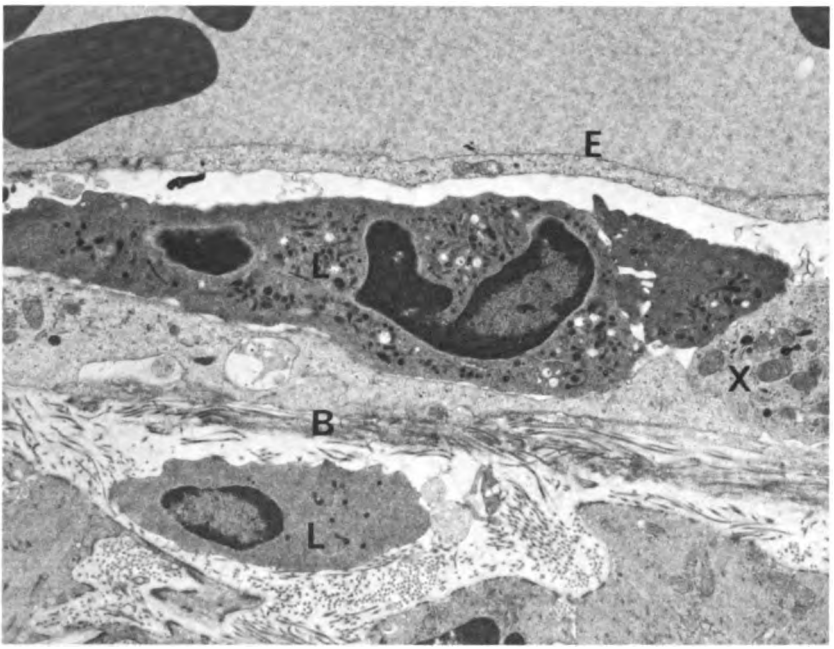

FIGURE 4 Electron micrograph of rat femoral vein 2 hr after mechanical injury. Although endothelium (E) is intact in this section, leukocytes (L) have invaded the vascular wall. A layer of cells not usually present in the intima (X) is seen on the luminal side of the subendothelial barrier (B) of collagen and elastic tissue. Origin of these cells is obscure. (× 6455)

dothelial cells are accompanied by simultaneous changes in the endothelial nuclei, and the pinched nuclear folds (Figure 6) resemble those in the nuclei of contracted smooth-muscle cells.[25] Small filaments similar to the myofilaments of smooth muscle can sometimes be found in endothelial cells (Figure 7) and might represent a contractile apparatus. More advanced thrombi are generally covered by endothelium in 5–7 days [39]; some lesions are invaded by endothelium and later recanalized. This phenomenon seems to be inversely related to intraluminal pressure, rather than related to the nature of the vessels.[8] The denuded surface resulting from endarterectomy,[34] like the surface of a vascular prosthesis,[12] is eventually covered with endothelium, although the process may require a year or more.[34] The rapidity with which regeneration occurs appears to vary with the species. Although earlier investigators failed to find an endothelium lining in human vascular grafts, probably

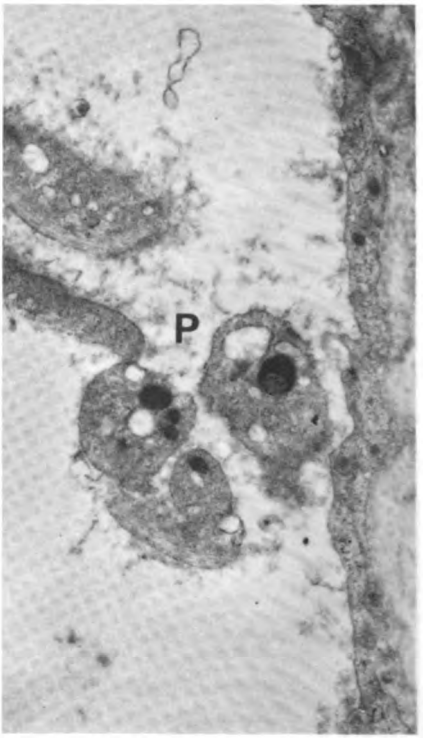

FIGURE 5 Platelets (P) in lumen of rat femoral vein 10 min after gentle separation of femoral sheath from surrounding structures. Fibrin cannot be identified. Endothelium always appears intact in this preparation. (× 10,000)

for technical reasons, later reports describe reasonably intact endothelium.[9]

SOURCE OF NEW ENDOTHELIUM

Advances in surgery and allied disciplines have resulted in increasing clinical use of endarterectomy and vascular grafting to re-establish arterial flow. These cases represent extremely large losses of the endothelial barrier. Although the mechanism of endothelial replacement is not strictly within the scope of this discussion, the importance of this lesion justifies its inclusion.

The sources of new endothelium have not yet been established, but the classical hypothesis proposes that it is formed by ingrowth of new cells from the periphery of the injured area. Many earlier reports proposed that fibroblasts of the vascular wall or graft were a source of new endothelial cells, but the use of newer methods, particularly electron microscopy, have not supported that explanation. Ts'ao [43] has recently

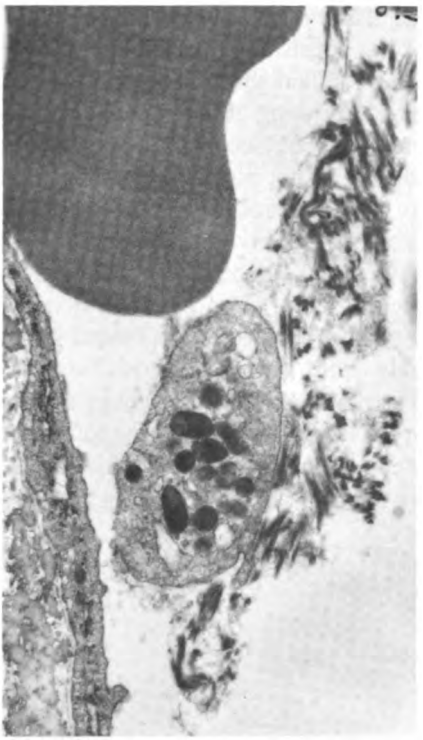

FIGURE 6 Femoral vein of rat treated in the same manner as that of Figure 5 after administration of epsilon-aminocaproic acid. Endothelium again appears intact, but fibrin streamers are associated with platelet. (× 10,000)

observed a concomitant rupture of the elastica of rat aorta and vena cava, with intrusion of smooth-muscle cells into the intima after trauma. The increase in subendothelial "myointimal" cells has led him to propose that this might represent a source of endothelial cells (Figure 8). Several investigators have suggested that a cell from circulating blood or from the thrombus, possibly the monocyte, might be the source of new endothelial cells. Dible,[11] using serial sections, could establish no continuity between the lining cells of lacunae of thrombi and the endothelial lining of vessels.

It is generally accepted that endothelial buds originating from the vessel wall or from the interstices of knitted grafts [17] might contribute to the production of an endothelial surface. Several ingenious experiments have been devised to exclude endothelial buds by suspension of prostheses out of contact with vessel walls or by use of graft material impermeable to cells. One such experiment used a Dacron hub suspended intra-arterially by polyethylene threads. Inspection of the covering of the hub by light [41] and electron microscopy [33] demonstrated that

the tissue on the hub was identical with that on the surrounding Dacron graft, consisting of endothelial cells, smooth-muscle cells, and fibroblasts. When thrombi were encapsulated in a Millipore filter, celloidin, or a dialyzing membrane, cells resembling endothelium that covered lacunae were seen only when dialyzing membrane was used, and even then were not plentiful.[40] Others using prostheses that were long enough to rule out endothelial extension from the suture line have encased the prosthesis in nylon,[15] polyethylene,[14] silicone rubber,[16] and siliconized nylon [29] in an attempt to exclude interference from ingrowing endothelial buds. Although central unconnected endothelial cells were seen by some, Ghidoni *et al.*[16] and Meijne [29] indicated that cells originated only from the anastomosis between the graft and the normal artery. These variable results would suggest that, even though subsequent investigations confirm the hypothesis that endothelial cells originate from transformed blood cells, this process probably plays a secondary role in the development of new endothelium. Dible [11] has suggested that

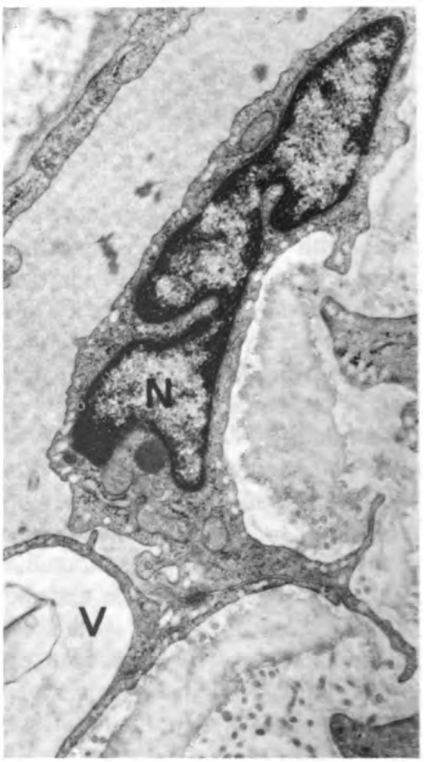

FIGURE 7 Electron micrograph of endothelial cell of rat vein 10 min after mechanical injury. Nucleus (N) displays pinched folding often associated with contraction. Adjacent endothelial cell displays vacuole (V) often associated with injury. (× 12,500)

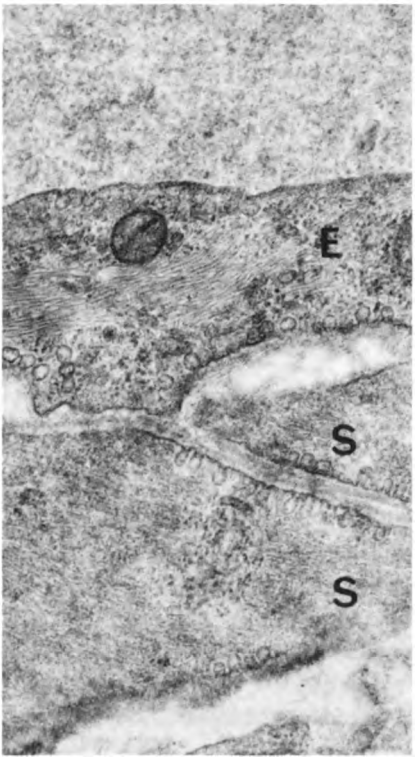

FIGURE 8 Endothelial cell (E) and smooth-muscle cells (S) of rat vein. Note similarity of filaments. (× 26,000)

endothelial cells circulating with the blood might become implanted on the thrombus.

Several workers have examined the lining of vascular prostheses [12,13,26,29] by light or electron microscopy and have demonstrated cells indistinguishable from normal endothelial cells. Poole *et al.*,[34] using *en face* preparations, demonstrated such regeneration over Dacron grafts placed in the aortas of baboons: there was progressive invasion of endothelial cells from the anastomosis. Silver staining of the intercellular junctions [13] emphasized the sheetlike arrangement of endothelial cells. Islands of endothelium could also be identified around small vascular openings after 2 weeks, and electron microscopy established the close similarity of these cells to endothelial cells. Robertson *et al.*,[35] using graded mechanical injury to the inferior vena cava of the rat and *en face* preparations with both silver and conventional stains, demonstrated the advance of regenerating endothelium from the periphery of the wound. After 48 hr, mitotic figures had appeared in the surrounding cells and actual division could be seen. Warren and Brock [46] combined

morphologic studies with tests of plasminogen activation in vascular prostheses from canine aorta. Cells structurally similar to normal endothelial cells in *en face* preparations and electron microscopy were able to induce fibrinolysis of canine fibrin at about half the rate of adjacent normal endothelial cells. Although not all areas were covered by such cells, the areas adjacent to normal endothelium were. The consistency and clarity of these reports suggest that this simplest hypothesis accounts for the formation of most new endothelium.

SUMMARY

The endothelium apparently participates in the resolution of a thrombus by stimulating fibrinolysis in adverse conditions, such as local trauma or hypoxia. This is accomplished through production of plasminogen activator. Removal of the support of fibrin allows fragmentation and embolization of the thrombus, and later vascular repair, or allows excavation of the thrombus during organization.

Intact endothelium provides a barrier between procoagulants of the blood and the activating collagen of the vessel wall. Newly formed endothelium provides stability against variables in platelet adhesion and activation of coagulation factors, minimizing the development of the underlying thrombus.

The work reported here was supported by Public Health Service grants HE 09342 and HE 06316 from the National Heart Institute.

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The Role of Stasis in Thrombosis

STANFORD WESSLER

The concept of the role of stasis in the initiation of thrombosis has a long heritage. After Harvey's experiments had dispelled the Galenian theory of the humors, and Newtonian mechanics had been accepted, a new school of medical thinking developed. Disease was explained in terms of mechanical derangements in internal viscera. Stagnation of the circulation during pregnancy was simply the result of uterine pressure on the iliac veins. Virchow's writings [21] supported the role of stasis in thrombosis; but neither he nor his students ever provided a mechanistic basis for the thrombogenic effects of retarded blood flow.

Stasis can be demonstrated to occur under physiologic conditions in normal man subjected to various degrees of immobilization or during pregnancy, and in diverse pathologic states, including polycythemia, the dysproteinemias, shock, and congestive heart failure. In many of these clinical situations, an increased incidence of venous thrombosis has been recorded. Clinical support for the relation of blood flow to thrombosis derives from the fact that thyrotoxic patients with shortened circulation times rarely, if ever, develop spontaneous phlebitis.

Although the association of stasis and thrombosis appeared valid, data from both animals and man suggest that retarded blood flow alone does not initiate thrombosis. Hewson, in 1771, demonstrated how retarded coagulation is: when he trapped flowing blood between two ligatures in canine jugular veins, the blood remained fluid for more than 3 hr.[12] Comparable findings were later recorded by Lister,[13] Glénard,[11] Baumgarten,[3] and me.[22]

Roentgenographic studies in man by Stanton *et al.*[19] and McLachlin *et al.*[15] have demonstrated that blood flow can be profoundly retarded in the deep veins of the leg without causing thrombosis. If radiopaque dye is introduced into a dorsal foot vein of a supine elderly subject, the

dye is retained in venous valve pockets, on occasion, for as long as 60 min and leaves the vein immediately when the subject's legs are raised slightly from the horizontal.

Taken together, these observations on the remarkable intravascular resistance to coagulation of a static column of blood suggest that retarded blood flow alone cannot initiate massive thrombosis. Therefore, in the presence of a normal intima, some alteration in the circulating constituents of the blood must be present to trigger intravascular coagulation.

Best has suggested that experiments might be more profitably directed to the question of why blood remains fluid within the circulation, rather than the question of why it gels.[4] One may, in fact, view the contribution of altered blood flow, not as an initiator of intravascular coagulation, but as a permissive factor that defines in part the nature of the thrombogenic trigger and of antithrombotic mechanisms. Retarded blood flow then becomes a useful means through which to recognize several of the factors involved in the control of excessive intravascular coagulation.

If, under specified conditions, one injects homologous or heterologous plasma or serum into a mammal, thrombi are not observed in the vasculature grossly or by light microscopy. In contrast, if one repeats these experiments and isolates a systemic vein immediately after the infusion, macroscopic thrombi are observed in the isolated segment after serum infusion (but not after plasma infusion).[10,23] It is also possible to recognize thrombi in isolated vein segments after infusions of either trypsin or commercial thrombin preparations in concentrations too small to induce generalized thrombosis.[20] Moreover, it is not possible in the microvasculature of the hamster's cheek-pouch to induce vascular obstruction with serum infusions and stasis, whereas massive jugular vein thrombosis in the same animal occurs regularly.[24]

Although they bypass the key question of how a thrombogenic agent can gain access to the circulation, these experiments demonstrate that retarded blood flow can play a role in the distinctions between thrombogenic and nonthrombogenic material, and they can serve as a useful bridge between *in vitro* and *in vivo* studies of clotting reactions.

In searching for trigger mechanisms in thrombosis, attention has frequently been focused on established clotting factors as causative agents. Interest in this relationship has been heightened by the finding of increased levels of clotting factors in several altered physiologic states associated with venous thrombosis.[9,16] It might reasonably be argued that a severalfold increase in a circulating clotting factor would set the stage for a higher rate of activation of coagulation enzymes, leading to

thrombosis; but no experiments in man or animals have provided evidence to support this hypothesis.

To test this concept, we recently prepared highly purified fractions of a clotting factor in both its nonactivated and its activated forms and examined the thrombogenicity of these fractions in an *in vivo* model system involving stasis. For this study, nonactivated and activated factor X was obtained from bovine plasma; each fraction was devoid of the other, as well as of other clotting moieties. The specific activities of the resulting fraction of the nonactivated and activated factor X were 20,000 and 22,000 units/mg of tyrosine, respectively.[8,30] We also used a modification of the standard model for the production of stasis thrombi.[25]

Whereas amounts of nonactivated factor X as great as 630 units failed to induce stasis thrombi, activated factor X in quantities as small as five units was fully thrombogenic. Stated differently, the infusion into a rabbit of an amount of activated factor X equivalent to the amount that can be derived from 10 ml of bovine plasma was thrombogenic, but the infusion of an amount of nonactivated factor X equivalent to the injection of more than 1 liter of bovine plasma was inert.[28]

These experiments demonstrate, at least for one form of experimental thrombosis, that although stasis may play no role in the formation of the trigger it can be used to show that hypercoagulability is not related to the total quantity of a specific clotting factor in the circulation, but rather to the state of activation of the factor. These experiments support the view of Duckert and Streuli that hypercoagulability should be defined as a state in which active products and intermediates normally absent from circulating blood are found intravascularly and may also be released from tissues.[7]

In addition to these qualitative insights, with their own implied clinical applications, this *in vivo* model permits quantitative comparisons of the thrombogenic potential of different activated clotting factors. Starting with commercial bovine thrombin, it is possible to achieve a complete separation and partial purification of both thrombin and activated factor X—each free from the other, from other clotting factors, and from tissue thromboplastin and phosphatides—by a stepwise chromatographic technique using DEAE cellulose.[30] The specific activities of thrombin and activated factor X are 10,000 NIH units/mg and 22,000 units/mg of tyrosine, respectively. The activated factor X is indistinguishable biologically from that derived from activation of highly purified nonactivated factor X by either venom or trypsin.

Whereas five units of activated factor X produce stasis thrombi, 100 NIH units of thrombin fail to initiate thrombosis. Only with 200 NIH

units of thrombin did stasis thrombi form.[27] We have also shown that purified activated factor X does not convert fibrinogen to fibrin *in vitro*. The *in vivo* results, therefore, suggest that the infused activated factor X is triggering the conversion of the recipient animal's prothrombin to thrombin and that the amount of thrombin formed *in vivo* is greater than the 100 units of thrombin infused. These data are consonant with the view of others that activated factor X is the prime activator of prothrombinase in the conversion of prothrombin to thrombin.[2] It would also appear that activated factor X is more explosive than thrombin itself in its activation of prothrombin *in vivo*.

The antithrombins, various circulating inhibitors of thrombin, have long been recognized *in vitro*. Although it has been suggested that activated factor X is cleared by the liver,[6] we have recently found that serum has a capacity to neutralize purified activated factor X comparable with its ability to inhibit the biologic activity of thrombin. When five units of purified activated factor X in 1 ml of saline are incubated with an equal volume of normal serum at 37 C, 80% of the activated factor X disappears in 2 min, and the remainder in 4 min. This is comparable with the inactivation of purified thrombin under the same experimental conditions. These data are consistent with the fact that normal serum does not contain activated factor X.

The transient effects of thrombin and of activated factor X suggest that their effects are antagonized by inhibitors that bind or alter them so rapidly that their thrombogenic effects in small doses can be recognized only if the circulation is arrested. Stasis, in contrast to free flow, favors reactions that tend to complete the coagulation sequence at the expense of reactions that would oppose the elaboration of fibrin.

A second compensatory mechanism can be recognized, if one studies the effect of activated factor XI in the stasis model. The duration of hypercoagulability induced by activated factor XI, infused in serum devoid of thrombin and activated factor X, persists for as long as 120 sec.[29]

Spaet and Kropatkin have proposed that *in vivo* blood fluidity is preserved with the aid of a clearance mechanism, whereby blood coagulation intermediates are rapidly removed from circulating blood by some cells.[18] We later demonstrated, by paired infusions of serum into the ear veins and portal veins of rabbits, that the former invariably produce systemic venous thrombosis, whereas the latter fail to achieve the same result with comparable dosages. These experiments suggested to us that a thrombogenic moiety in serum was being inactivated in the liver.[26] Subsequent experiments by Spaet,[17] by Deykin,[5] and by us,[29] using liver perfusion, confirmed the role of liver clearance in protecting against thrombosis.

Normal human serum contains activated factor XI, in addition to the heat-labile and heat-stable forms of factor IX, factor VII, and nonactivated factor X. Within 5 min of the start of the perfusion of 60 ml of human serum through a rabbit's liver, there are profound losses of activated factor XI and heat-labile factor IX, concomitant with the loss of the thrombogenic activity of serum, whereas the heat-stable forms of factor IX and nonactivated factors VII and X are not significantly altered.[29] The liver appears to have the capacity to discriminate between circulating activated clotting factors, such as factor XI, and procoagulants, such as stable factor IX, factor X, and factor VII. The latter are not cleared from the circulation by the liver.

Comparison of the stability of activated clotting factors in serum and the effects of liver clearance suggest, in a preliminary manner, that factors closer to the reaction that converts fibrinogen to fibrin are more rapidly inactivated by naturally occurring circulating inhibitors than factors that function near the onset of the intrinsic clotting mechanism and are removed by the possibly slower pathway of liver clearance.

As early as 1905, Leo Loeb speculated that intact endothelial cells may act indirectly to prevent intravascular coagulation.[14] This speculation now enjoys some experimental support. In studies undertaken jointly with Drs. David Freiman and Herbert Berman, we were able to distinguish between the thrombogenicity of serum in the large veins of hamsters and the apparent absence of thrombogenicity in the microcirculatory vessels of the cheek-pouch.[24]

In a more sophisticated study involving the technique of serum-induced stasis thrombosis in large and small vessels of the rat, Ashford and Freiman produced massive thrombi in large veins, whereas in the venules only occasional wisps of fibrin could be identified by electron microscopy. Pretreatment of their animals with epsilon-aminocaproic acid (EACA) markedly augmented the deposition of fibrin in the venules when serum and stasis were used later.[1]

Recently, in preliminary experiments involving the cheek pouches and jugular veins of fasted hamsters, we have confirmed and extended the observations of Ashford and Freiman. Five units of activated factor X produced jugular vein thrombi, but no microcirculatory obstruction in the cheek-pouch. In contrast, if cephalin was mixed with activated factor X before infusion, jugular vein thrombi resulted with only one tenth as much activated factor X as before, and microcirculatory obstruction occurred, as well.

Control experiments have demonstrated that cephalin is not itself thrombogenic, and, when mixed with nonactivated factor X before infusion, does not confer thrombogenicity on it. These observations are consistent with recently published data that indicate that activated

factor X in the presence of phospholipid, calcium, and factor V forms prothrombin activator—the moiety responsible for the conversion of prothrombin to thrombin.[2]

Aside from the fact that the combination of activated factor X and lipid was a more potent thrombogenic agent than activated factor X alone, it was clear that the mixture resulted in a jugular vein thrombus that lysed spontaneously *in vitro,* whereas that did not occur with activated factor X alone under the same experimental conditions.

Pretreatment of hamsters with EACA abolished the thrombolysis observed after infusions of mixtures of activated factor X and lipid. Of equal importance was the fact that pretreatment with EACA also potentiated the thrombogenic effects of infusions of activated factor X alone.

These preliminary data suggest that it is the quantity of plasminogen activated at the onset of coagulation that influences whether a thrombus will form, as well as determining its rate of dissolution. With a given stimulus, thrombosis occurs less readily in smaller vessels, which have greater surface:volume ratios than larger vessels. This balance can be altered in favor of thrombosis either by increasing the potency of the stimulus or by decreasing the capacity of the fibrinolytic response. These interpretations are consistent with the experience in man wherein thrombi are found frequently in the large and small veins of the extremities but rarely in the venules.

If clotting factors have a role in either the initiation or propagation of thrombi, stasis permits recognition of the particular clotting factor that is thrombogenic and provides a means of estimating its potency. Stasis also facilitates the progress of intravascular coagulation by protecting activated factors from circulating inhibitors, from liver clearance, and, in large vessels, from the dissolution of thrombi by fibrinolysis. In addition to these three compensatory mechanisms, there is a fourth means whereby retarded blood flow favors intravascular coagulation: stasis profoundly alters the physical properties of a column of blood so as to predispose to fibrin formation.

The work reported here was supported in part by Public Health Service research grant HE 09326 from the National Heart Institute, the Milton Frank Vascular Research Fund, and the Llewellyn Sale, Sr., Memorial Fund.

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Rheologic Aspects of Stasis in Thrombus Formation

ROE E. WELLS

The rheologic properties of blood are such that it thickens as flow is reduced. This is due, not to any change in the concentrations of the fluid or cellular elements, but rather to a change in the relative forces between these elements. As flow retardation approaches the point of stasis, blood viscosity increases to five to 10 times that in the normal arterial stream.[21] The principal factor responsible for the character and magnitude of these forces at zero flow is the formation of bridges of protein linking one red cell to another.[25] The largest and strongest cable in this bridge is fibrinogen.[12,23] The attractive force between cells, or the viscous integrity of the static fluid structure, is considerably less when the same cells are in serum.[13]

As a result of stasis, there is the attachment of one red cell to another, the well-known erythrocyte aggregation. This, too, is principally a function of fibrinogen: aggregation is not seen when cells and serum of freshly drawn blood from normal subjects are mixed together,[12,22] but the integrity of this cell aggregation, and thus the fluid structure of stasis, is significantly increased in disease or following trauma.[7] This increased intercellular attraction may be due to increased fibrinogen but may also occur in the presence of normal fibrinogen levels. Some other factor, presumably protein, probably accounts for the instances in which aggregation and accelerated cell settling occur in some disease states.[8]

The other factors that influence the fluid structure of blood in stasis are the number of cells,[24] their deformability,[14] and time.[2] The lower the hematocrit, the smaller is the force necessary to return the static blood to flow; the higher the hematocrit, the greater is the force necessary to reverse the "static structure." This has its clinical corollary in the increased incidence of thrombosis in polycythemia. The viscosity of the

red cell itself is a product of various factors. Conditions that rigidify the red cell, such as abnormal pH and osmolarity, also increase the viscosity of whole blood and the "static viscous structure." [14,16] Studies of the effects of time reveal that the change in velocity gradient and the duration and character of the prior shearing experience influence both viscosity and the character of a viscous body.[2,4]

Stasis, as one of the historical cornerstones of the thrombotic process, is not difficult to accept as a prime factor in venous thrombosis. Prolonged venous stasis is part of the sedentary aspect of life, but prolonged stasis in health does not appear to produce significant venous thrombosis. The combination of venous thrombosis in the setting of body immobilization and acute illness or trauma is so common as to be endemic in the hospital population.[5,18] It appears, therefore, that stress or disease adds a critical factor that converts the static column of blood into a thrombus. The concept of hypercoagulability is invoked to describe this extra factor. Hypercoagulability is not solely a function of an increased fibrinogen concentration. We have found an increased concentration of fibrinogen only rarely in immobilized patients with venous thrombosis; but virtually all these patients do have a greatly increased erythrocyte sedimentation rate. Their red cells demonstrate increased aggregation when flowing in serum, as well as in plasma.

There are, therefore, two related and unresolved aspects of the conversion of the static column of venous blood to a thrombus: (1) aggregation of cells, which is due primarily to fibrinogen in the normal state, can be seen to develop to a pronounced degree in disease in which fibrinogen concentration may or may not be increased; and (2) the hypercoagulability phenomenon, or whatever permits the static blood to coagulate *in vivo,* appears as a function of disease or stress. Although many logical and impressive arguments have been presented to explain this phenomenon, its development in the immobile ill patient has not been specifically demonstrated at the time the thrombosis develops.[27] There is little question but that stasis provides the most fertile soil for conversion of static blood into a thrombus. But the final catalytic step appears to require the still undefined hypercoagulable factor.

Platelet adhesiveness also appears to be increased in the venous thrombotic state.[9,10] The concept that the venous thrombus consists principally of red cells and fibrin, and that platelet aggregation is a secondary or subsequent development, has broad support.[15] The malignancy of a growing thrombus, however, may be magnified by increased platelet aggregation and adherence.

The rheologic concepts of stasis as applied to the arterial thrombus are less apparent than in the venous system. The thrombus of the

arterial stream arises in a high-velocity environment. Arterial thrombi originate most frequently in areas of hydraulic stress, such as bends, angles, and orifices [15,26] (Figures 1–3). However, some intimal lesion, such as a tear, ischemia, hemorrhage, or intimal hypertrophy, must precede the formation of the thrombus. Once there is an aberration in the normally smooth intimal layer, rheologic conditions for the development of a thrombus or plaque are established. The essence of this process is that if a fluid in laminar flow in a pipe or tube suddenly passes over an irregularity or protuberance at the wall, or if the tube is suddenly enlarged in diameter, the conditions for nonlaminar or turbulent flow exist.[3] It is an area of turbulence, whether on a microscopic or macroscopic scale, that appears to stimulate the accretions of platelets at these sites. The fibrin accumulation that follows increases the size of the lesion, and a vicious circle is established: an increase in wall mass increases turbulence, which increases wall mass. At the same time, however, the thrombotic material begins to emerge into the higher-velocity environment near the central stream. A set of opposing forces is then operative: i.e., there is greater turbulence, but also an increased velocity at the thrombus surface, which may represent more dispersive energy than the adherence forces of the sticky platelets. If upstream pressure mounts, and flow velocity thereby is increased, there is a greater shear at the surface of the thrombus and also greater turbulence in the eddies behind the obstruction. Currents of flow move both upstream and down-

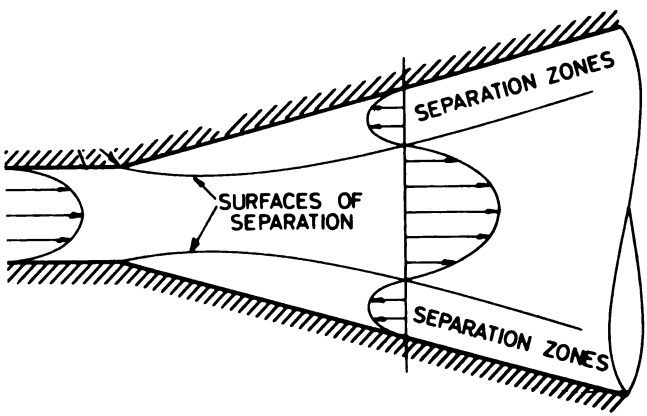

FIGURE 1 Separation zones in expanding tube. Arrows indicate mean flow direction (reversal at wall). (Reprinted with permission from Fox and Hugh.[6])

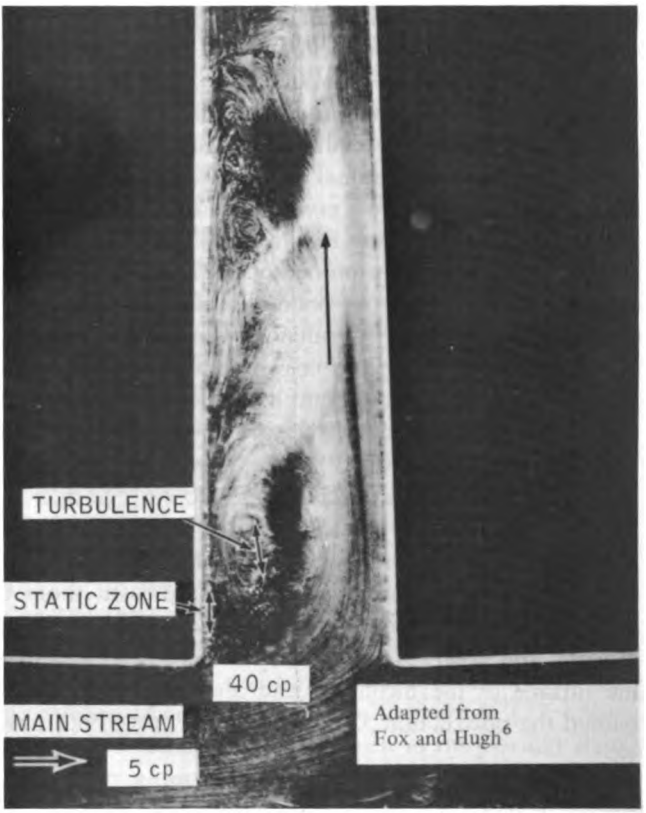

FIGURE 2 Areas of turbulence in right-angle takeoff. Blood viscosity, 40 cp at stasis, 5 cp in high-flow stream.

stream. The boundary layer between these two stream directions incorporates sites of negligible flow, or stasis.[6] Stasis is part of the character of the eddies and pools created by the thrombotic roughness on the intimal wall.

The fluid mechanics of the separation phase between laminar and nonlaminar flow of blood in the arterial stream has been examined by engineers, pathologists, and surgeons. There is essential agreement that nonlaminar flow is most easily established at the aortic arch, the right-angle artery branches off the aorta, and the aortic division into the iliac arteries, where turbulence would be most easily manifest and where in fact atherosclerotic plaques and thrombi originate with significant fre-

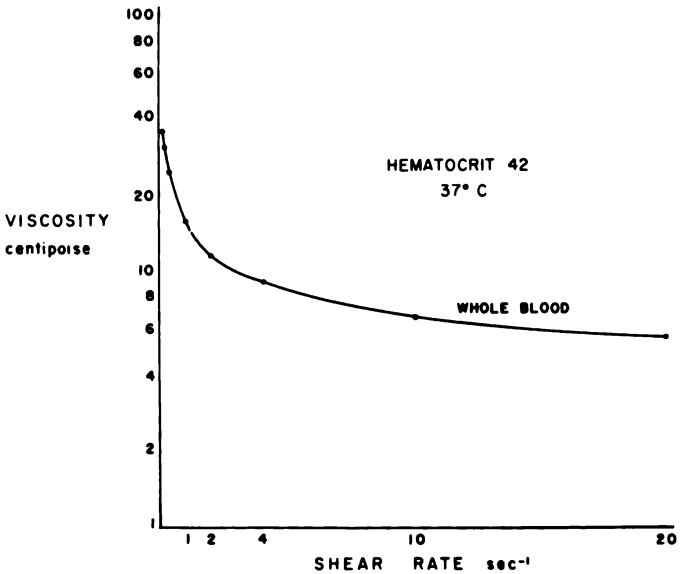

FIGURE 3 Viscosity as a function of shear rate. At stasis (shear rate, zero) viscosity is 40 cp.

quency (Figures 1–4). The coronary vessels of the heart experience the most unusual hydraulic strains and stresses. The vessels are compressed as the heart contracts and at the same time undergo a twisting contraction. The greatest degree of flexion appears at the base of the ventricle, where the coronary arteries enter the muscle and where the thrombotic process is often most marked. They are the only vessels in the body that fill or have their forward pulse during the relaxation phase of the heart cycle. The most important common denominator of these changes is not so much the hydraulics, but rather the etiology of the intimal defect, whether tear, hemorrhage, duplication, or other aberration.

Finally, the observation that anticoagulant therapy is effective in venous thrombotic disease and of variable value in arterial thrombotic disease (without venous sequelae) represents information still not fully exploited. There appears to be no practicable agent used that directly influences the arterial (platelet) thrombus. Dextran has a significant effect on platelet function and aggregation, but the dose required causes significant volume expansion,[1,19] which contraindicates its use in some patients with cardiac and renal insufficiency. Another less well-known action of dextran is its ability to form a complex with fibrinogen.[11] At

FIGURE 4 White areas indicate site of floc accumulation at bend and downstream inside of bend.

artificially high concentrations of either dextran or fibrinogen *in vitro*, a visible coagulum is produced.[20] Dextran might influence the availability of fibrinogen by this mechanism, but this has not been examined. The evidence of dextran's advantages in the therapy of thrombophlebitis is great,[17] but its use in an attack on the arterial thrombus has been so limited that conclusions cannot yet be drawn.

In summary, the rheologic properties of blood are such that its viscosity is greatly increased with stasis. In health, however, blood does not lose its fluidity, even with stasis. With disease, trauma, or stress, stasis results in an extremely high incidence of thrombus formation. With increased fibrinogen, dramatic increases in cell adhesion and in

viscosity occur. However, similar changes in the blood's viscosity may develop in disease without increased fibrinogen levels. This indicates that yet-undefined other factors, probably related to the hypercoagulability phenomenon, may determine viscosity changes.

Rheology as it influences arterial thrombus formation is a function of the boundary-layer phenomenon created by the obstruction of laminar flow at the vessel wall by the intimal lesion. There is high shear at the surface of the mass and stasis in the eddy formations just downstream from the mass. The high-shear environment "activates" platelets with adherence one to the other. In the proximate area of stasis, fibrin and red cells are incorporated in the thrombus, thus permitting the "red tail" to develop.

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Rheology of Human Blood: *In Vitro* Observations and Theoretical Considerations

EDWARD W. MERRILL

INTRODUCTION

Rheology, in its broadest sense, is the science of deformation and flow. It includes the quantitative description of the relationships between forces acting in shear within a particular medium and of the relative motion thereby produced between various parts of the medium. The objective of such description may be, in one case, solely to permit generalized prediction of what will happen in different combinations of geometric arrangements and shear forces, and in another case, to gain insight into the mechanical or molecular structure of the medium. The approach suitable to a given problem in rheology varies widely, being sometimes mathematical, sometimes experimental. The basis of one approach may be a model chosen from continuum mechanics, and the basis of another approach may be a model in which a physical or molecular description of the system is highly detailed.

The study of the rheology of human blood has proved especially interesting because of the variety of problems, of approaches, and of purposes.

Steady-state viscosity is the particular subject within the broad domain of rheology that is especially, although not exclusively, relevant to blood. "Steady-state" implies that uniform, nonaccelerated flow is under discussion; "viscosity" implies that, in addition, the flow is laminar, not turbulent. Why steady-state viscosity is relevant to blood flow in the vasculature, which is neither generally steady nor always laminar, will be considered below.

Steady-state viscosity implies a relationship between shear stress, τ, and shear rate, $\dot{\gamma}$, which may be described by the following analogy:

Suppose that the fluid whose viscosity is to be determined is like a new, clean deck of playing cards on a table. If one pushes horizontally on the top card, the cards will progressively slip over each other horizontally, each card experiencing slipping friction with the cards immediately above and below. The less the friction, the more the cards will slip horizontally within a given instant under a given horizontal push. Imagining the fluid to be like a deck of cards, we define shear stress as the horizontal pushing force divided by the area of the "card" (force/area); and we define shear rate (also called "velocity gradient") as the horizontal distance of displacement per second of a given card beyond its immediate lower neighbor, divided by its thickness, and we measure it as (centimeters/second)/(centimeters); or sec^{-1}. Finally, the viscosity (the analog of friction between playing cards), η, is defined as the shear stress divided by shear rate.

Newtonian fluids are those for which the viscosity remains the same, whatever the shear rate at which it is measured. These fluids also follow the equation derived by Poiseuille for laminar flow through straight cylindrical pipes, namely:

$$Q = (\Pi \Delta P r_w^4)/(8\eta L),$$

where

Q = flow rate, cm^3/sec,
ΔP = pressure drop along tube, $dyne/cm^2$,
r_w = radius of tube, cm,
η = viscosity, poises (1 poise = 1 dyne-sec/cm^2), and
L = length of tube, cm.

Numerous fluids of industrial, scientific, or biologic importance are non-Newtonian; i.e., their viscosity is not constant, but rather a function of shear stress or shear rate. As will be shown, human blood, and probably all normal mammalian blood, has a distinctive kind of non-Newtonian viscosity over a range of low creeping flows relevant to venular and some venous flows, whereas at high flow rates, the viscosity becomes constant, and thus "Newtonian."

A particularly distinctive feature of blood viscosity is the existence of "yield stress," a shearing stress below which no flow at all occurs. The deck of cards is a good representation of a fluid with a yield stress, in that, in order to get the deck to slip at all under a horizontal thrust, a finite thrust must be developed that is intimately related to the coefficient of static friction between the cards. The yield stress, τ_y, would be defined as the horizontal thrust (in dynes) required to start the slip process, divided by the area of the card (in square centimeters).

Given that one has a problem, in blood, of determining the variable ratio of shear stress to shear rate, and particularly of determining the yield stress at *zero* shear rate, the viscosimeter by which these relations are to be measured becomes an important consideration. The Couette or coaxial cylinder viscosimeter is particularly convenient, in that it represents the cylindric approximation of a deck of playing cards, by which one can determine the shear stress and the corresponding shear rate separately and easily.

Viewed in over-all perspective, the steady-state viscosity of whole blood involves predominantly the following mechanisms:

At *high shear rates* (arterial flow): (1) viscous shear in plasma, (2) rotation and deformation of formed elements, mainly red cells, and (3) multibody collisions of formed elements;

At *low shear rates* (near zero flow): (4) make-and-break contacts between red cells and clusters of red cells, in combination with mechanisms 1 and 2; and

At *zero shear* (stasis): (5) adhesion of adjacent red cells to create a three-dimensional random structure, (6) evolution of contiguous, adherent rouleaux from the original structure, and (7) densification of the red-cell structure and syneresis of plasma.

Mechanisms 1, 2, and 3 in the absence of the others result in Newtonian flow and, indeed, the Newtonian viscosity as a function of hematocrit is surprisingly well predicted by an equation of Vand [16] for suspension of rigid, neutrally bouyant spheres.

Mechanisms 4, 5, 6, and 7 are all manifestations of the "bridging" of red-cell surfaces by native (nonactivated) fibrinogen. Mechanisms 4 and 5 are the origin of the distinctive non-Newtonian stress–shear rate relation of blood and of the yield stress displayed when flow is abruptly terminated. The yield stress, a measure of the strength of the red-cell network, varies approximately as the cube of the hematocrit and as the square of the fibrinogen concentration.

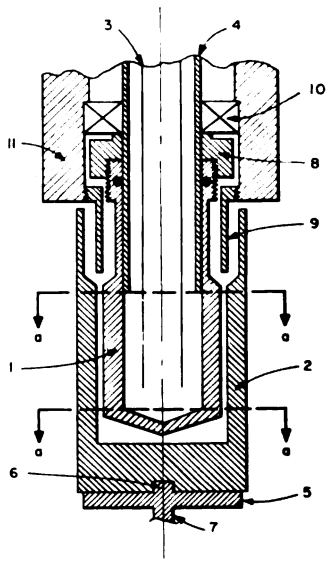
Mechanisms 6 and 7 underlie the time dependency of the measured shear stress at very low shear rates and many of the artifacts of low-shear viscosimetry of whole blood.

Of special relevance in this case are mechanisms 5, 6, and 7 and the speculation implied therefrom that, in stasis, thrombus evolution would be favored in the plasma phase within the static red-cell network because thrombin is protected against dilution to subcritical concentrations and because nascent fibrin is protected against premature dispersion.

VISCOSIMETRY WITH THE COUETTE VISCOSIMETER

WALL EFFECTS AND SURFACE EFFECTS

Despite the capability of a Couette viscosimeter [5] to impose nearly uniform shear rates on the test fluid at any desired value, and to resolve small torque levels with high precision, erratic and confusing data are usually obtained on normal samples of blood when the shear rate is below about 0.1 sec^{-1}, if smooth cylindric surfaces are used. It was discovered [4] that a thin layer of plasma collected near the outer torque-measuring cylinder (2 in Figure 1), thereby effectively "lubricating" the suspension of red cells in the annulus. Because the viscous resistance of the red-cell suspension is much greater than that of clear plasma, the effect of the wall layer of plasma is to give spuriously low readings of torque, and thus of shear stress. Definite proof of this effect was ob-



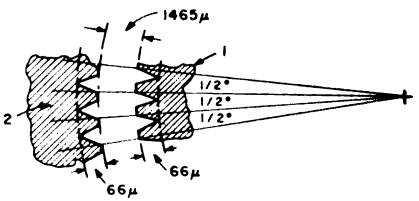


FIG. 1

FIGURE 1 Above, cross section of coaxial cylinder assembly in GDM viscosimeter. Below, detail of grooves in rotor and stator cylinders at section aa. (Reprinted with permission from Merrill *et al.*[10])

tained [4] by using blood from a patient with acute hyperlipemia. The plasma, containing 15% lipids, was milky. When blood was introduced into the Couette viscosimeter, it appeared, through the transparent plastic wall of the outer cup, homogeneous to the eye as long as no rotation occurred. As soon as slow rotation of the inner cylinder began, an unmistakable cloudy, milky layer of plasma formed next to the transparent wall. To minimize this effect, it is convenient to provide both the rotor (1) and the stator (2) of the Couette viscosimeter (Figure 1) with vertical grooves (66 μ deep). The metal rotating cylinder (1), perfused with water from a constant-temperature bath through a tube (3), was rotated on shaft 4 by means of a synchronous motor and gear train at speeds of 0.05–300 rpm. The stationary guard ring (9) had the essential function of intercepting the rigid layer of denatured material [9] that is found to form spontaneously. Because the torque-measuring table has a maximum deflection of 75 μrad, it does not "see" the rigid surface layer when the layer is immobilized by the guard ring (9). When the guard ring is absent, substantial spurious torque (in excess of that produced by true fluid shear) is detected under conditions of measurement near the yield stress.*

METHODS OF PLOTTING AND THE CASSON EQUATION [4,9–11,14]

Having eliminated or minimized wall and surface effects, one is faced with the problem of how best to represent the steady-state viscosity function of blood, i.e., the relationship of the shear stress to the shear rate. Given the fact that the viscosity of blood at low shear rates is profoundly non-Newtonian (i.e., the ratio of shear stress to shear rate approaches infinity at zero shear rate), one seeks a convenient means for conveying the essential features of the data. It is more convenient to represent stress as a function of shear rate than viscosity as a function of shear rate, when the shear rate is near zero. It is expedient to plot the square root of stress against the square root of shear rate, because of the approximate linearization of the data according to the Casson equation [2]:

$$\tau^{1/2} = a^{1/2}\dot{\gamma}^{1/2} + b^{1/2},$$

where a and b are constants, and b is, in fact, the yield stress. Experimental shear-stress–shear-rate data for normal human blood show marked curvature when plotted directly (Figure 2), but virtual linearity

* Without a guard ring, some embarrassing artifacts are certain to arise under conditions of low torque measurements (Wells, R. E., Jr. Shear rate dependence of the viscosity of whole blood and plasma. Science 133:763–764, 1961).

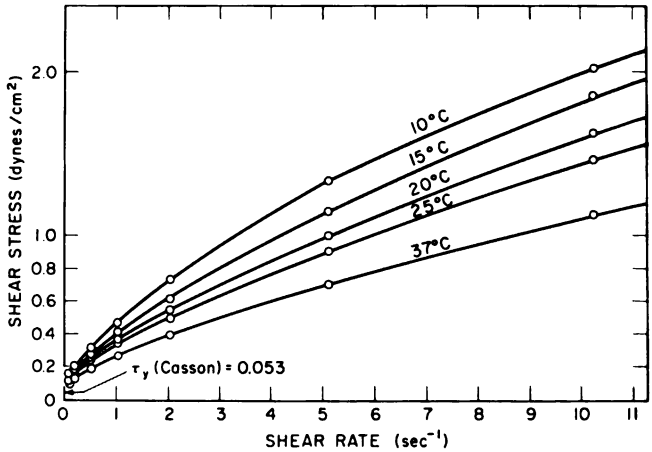


FIG. 2

FIGURE 2 Shear stress versus shear rate for samples of normal human blood at several temperatures. (Reprinted with permission from Merrill *et al.*[10])

when replotted as square root of shear stress versus square root of shear rate, up to approximately 16 sec^{-1}.

Although the physical model from which Casson derived his equation seems inapplicable to blood,[14] one nonetheless has in the Casson form of plotting a convenient means of comparing low-shear-rate data, and particularly of showing the location of the yield stress with respect to the rest of the rheologic data. (Note, for example, the confusion of data points near zero in Figure 2 and the separation of these points in Figure 3).

RED CELLS IN PLASMA COMPARED WITH RED CELLS IN SERUM

The most significant non-Newtonian characteristics of blood are produced by the interaction of red cells and fibrinogen. Figure 4 shows a typical set of curves for red cells suspended in (1) anticoagulated (ACD) plasma, (2) defibrinated serum from this plasma, and (3) a mixture of plasma and serum chosen to produce a concentration of fibrinogen half that in the original plasma.[9] Suspensions of red cells in the original plasma exhibit characteristic yield stress and linearity on the double-square-root plot. There is marked curvature and no yield stress in the curve for red cells in serum. The curve for red cells in plasma diluted by serum lies between these extremes. Inasmuch as both plasma and serum are Newtonian liquids in this range of shear rate, having

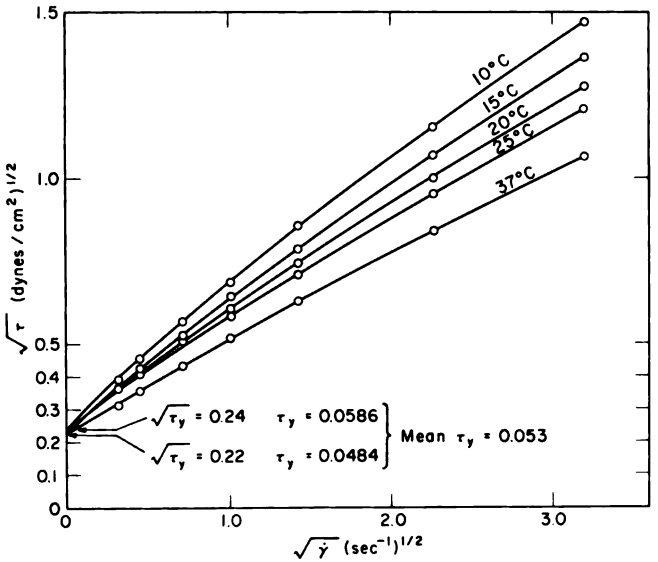


FIGURE 3 Casson plot: square root of shear stress versus square root of shear rate for the same sample, at the same temperatures as in Figure 2. (Reprinted with permission from Merrill *et al.*[10])

almost identical viscosities, the non-Newtonian characteristics of red cells suspended in plasma must result from the specific red-cell–fibrinogen interaction that leads to rouleaux.

EFFECT OF FIBRINOGEN COMPARED WITH OTHER PROTEIN FRACTIONS

Numerous studies of blood from normal human donors have established the approximately quadratic relation between yield stress and fibrinogen content as innately found in the plasma of the donor, *viz.*:

$$\tau_y = Kc_f^2,$$

where c_f = g of fibrinogen/100 ml of plasma and K is a constant.

The special influence of fibrinogen on blood rheology was further demonstrated [12] by progressive addition of dried, clinically administered fibrinogen to red cells in saline solution. The results are shown in Figure 5 for three types of red cells, all adjusted to hematocrit levels of 40% and measured at 37 C. It is clear that, with zero fibrinogen, zero yield stress is obtained, and that, as the fibrinogen concentration is increased,

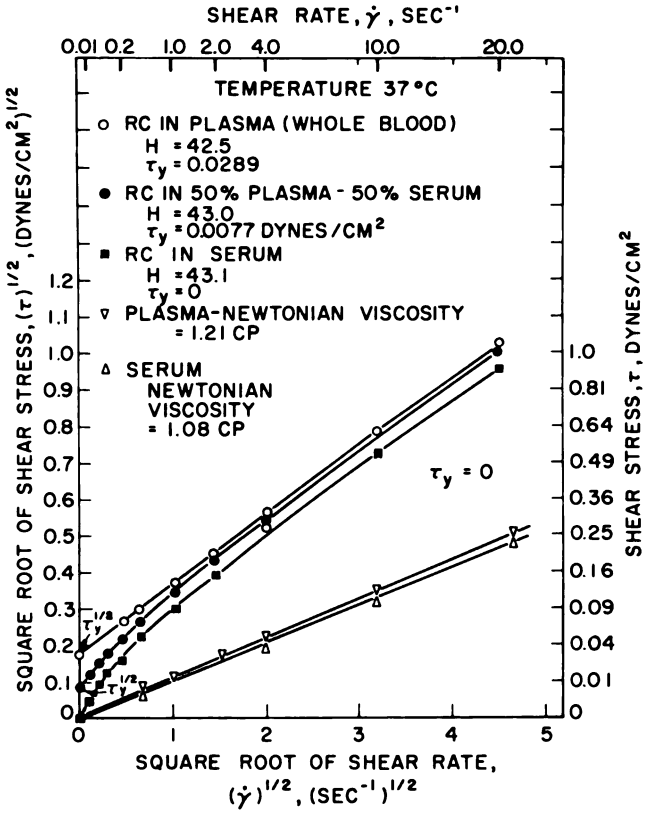


FIGURE 4 Square root of shear stress versus square root of shear rate for suspension of red cells in plasma, in completely defibrinated serum, and in a mixture of these. Curves for plain serum and plain plasma are also shown. H, hematocrit. τ_y, yield stress, dynes/cm^2. (Reprinted with permission from Merrill *et al.*[9])

the ordinate intercept, which is the square root of the yield stress, is progressively increased. It is also noted that, although the data for the O$^+$ cells are rather well linearized on the double-square-root plot, the data for the A$^+$ cells at high fibrinogen concentrations fall on distinct curves.

In a different set of experiments,[13] normal red cells (type O) were suspended in wet Cohn fractions II, III, IV-1, and IV-4. Of these, only fraction III had a significant fibrinogen concentration (0.12 g/100 ml),

the main constituent being β-lipoprotein. Fraction III was tested as received and after defibrination, i.e., after a "serum" was made from fraction III. The results are shown in Figures 6 and 7. Again, the conclusion is inescapable that fibrinogen is essential in producing the red-cell structures that, in turn, are responsible for the yield stress and special rheologic properties in the immediate region of the yield stress.

The effect of β-lipoprotein, the major constituent in fraction III (Figure 7), is not understood. By itself incapable of producing yield stress in a red-cell suspension, it appears to bring about yield stress

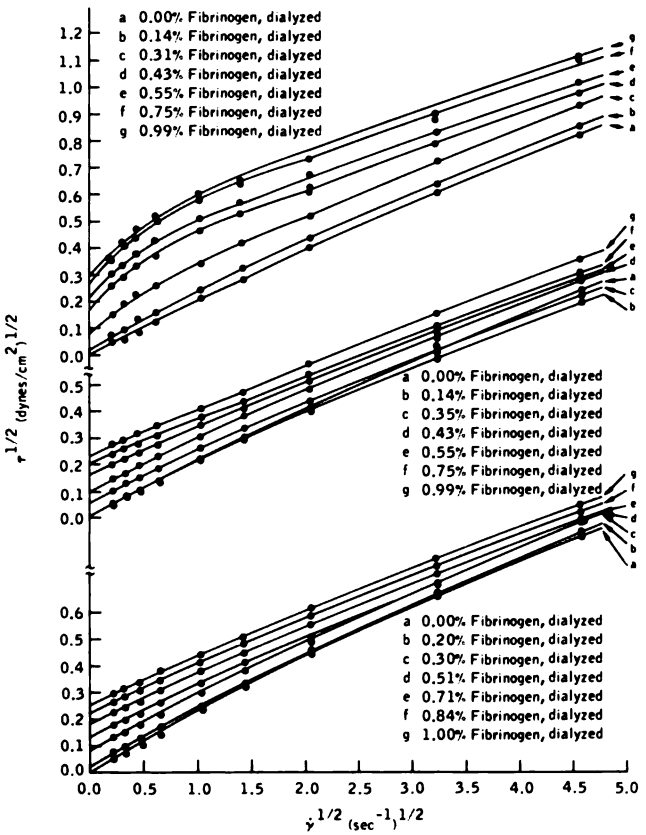


FIGURE 5 Dialyzed fibrinogen. Effect of concentration on rheology. Double-square-root plots of shear stress versus shear rate. Samples T9 (A⁺), T8 (O⁺), and T7 (O⁺) from top to bottom. Hematocrit, 40; temperature, 37 C. (Reprinted with permission from Merrill *et al.*[12])

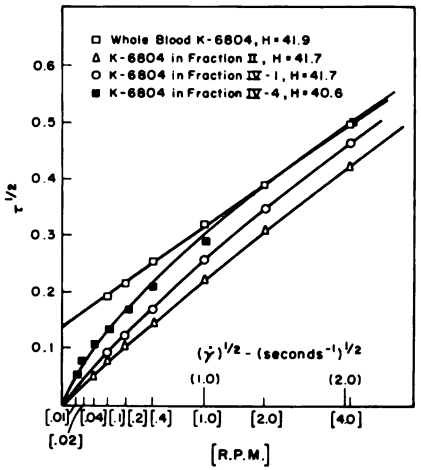


FIGURE 6 Red cells suspended in protein fractions. (Reprinted with permission from Merrill *et al.*[18])

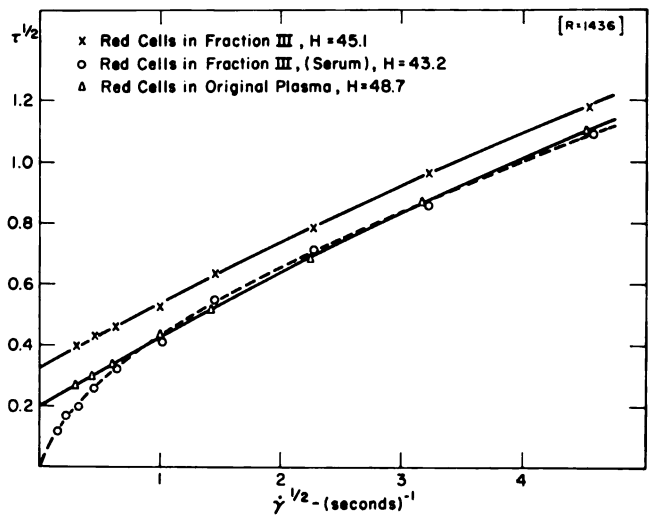


FIGURE 7 Red cells suspended in fraction III. (Reprinted with permission from Merrill *et al.*[18])

and level of viscosity near zero shear rate in the presence of fibrinogen.

Except for β-lipoprotein, the ordinary globulins * have little or no influence on rheologic properties. The rheologic characteristics of red-

* Macroglobulins and cryoglobulins produce bizarre rheologic effects not directly pertinent here.

cell suspensions in these globulin solutions are about the same as of red cells in saline. This is demonstrated in part by the curves of Figure 7, for fraction II is mostly γ-globulin, fraction IV-1 is about 90% α-globulin and 10% β-globulin, and fraction IV-4 is about 30% albumin, 40% α-globulin, and 30% β-globulin. In other experiments [3] on suspensions of red cells in isotonic solutions made from administrable γ-globulin, the same conclusions were drawn.

DEPENDENCE OF VISCOSITY PROPERTIES ON HEMATOCRIT IN NORMAL BLOOD

From numerous studies [3,11] on samples of normal blood as obtained and as remixed *in vitro* after centrifugal separation of red cells and plasma so as to produce suspensions differing in hematocrit, it was found that the yield stress depends strongly on hematocrit, as shown in Figure 8. There is approximately a 16-fold change in yield stress over the hematocrit range 20 to 50. The non-Newtonian viscosity (as measured by the

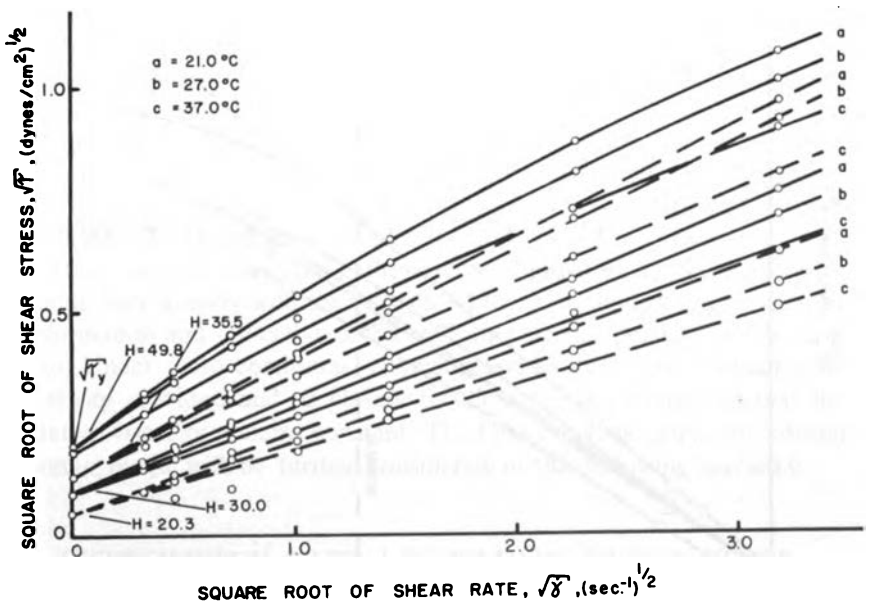


FIGURE 8 Casson plots (square root of shear stress versus square root of shear rate) for a typical normal human blood, ACD as anticoagulant, at three temperatures for each of four hematocrit levels. (Reprinted with permission from Merrill *et al.*[11])

shear stress at a fixed arbitrary shear rate) increases by about the same proportions.

By replotting the data of Figure 8 to show the cube root of yield stress versus hematocrit, as in Figure 9, it was found that, over the range of hematocrit up to at least 50, the yield stress varies as the cube of hematocrit (H), *viz.*:

$$\tau_y = \text{const}(H - H_c)^3,$$

where H_c varies from person to person but averages around 5. The separations noted between the curves for the five samples from five donors (1–5 in Figure 9) are attributable to differences in innate fibrinogen concentration (cf. Figure 5). Figures 5 through 9 taken together clearly demonstrate that interaction between red cells and fibrinogen is the origin of the special rheologic characteristics of blood near zero shear rate, including the yield stress, and call attention to the possibility of greatly elevated values of yield stress (also to greatly increased values of the non-Newtonian viscosity at, say, 1 sec^{-1}) in a patient with concomitant polycythemia and hyperfibrinogenemia.

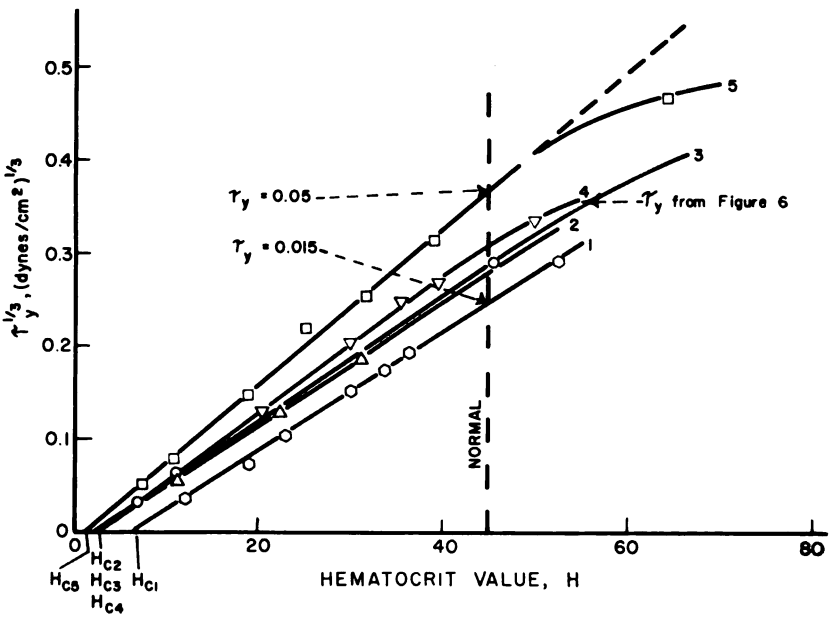


FIGURE 9 Cube root of yield stress versus hematocrit, computed from these and other samples of blood. (Reprinted with permission from Merrill *et al.*[11])

DEPENDENCE OF VISCOSITY PROPERTIES ON TEMPERATURE IN NORMAL BLOOD

The effect of temperature on normal human blood [11] can be summarized by two generalizations: (1) The yield shear stress at constant hematocrit and constant plasma composition is almost independent of temperature, as shown, for example, in Figures 3 and 8, over at least the range of 10–37 C. (2) At shear rates above about 80 sec^{-1} and over the same temperature range, the relative viscosity of blood, measured as the ratio of shear stress produced in blood to shear stress produced in water at the same shear rate, is independent of temperature.

When these observations are combined with cinephotomicroscopy of blood flow in capillary fibers at low shear rates, a reasonable hypothesis can be formulated, as follows: (1) the bonding forces between red cells induced by fibrinogen are nearly independent of temperature; (2) below the yield shear stress, red cells aggregate into a three-dimensional network, whose strength is independent of temperature; (3) at stresses slightly exceeding the yield stress, the continuous network that existed below the yield stress collapses into aggregates of various sizes separated from each other by lubricating layers of plasma; (4) the size distribution of these aggregates is controlled predominantly by the frequency and intensity of impacts with each other, these variables in turn being functions of the shear stress; (5) at low shear rates, the flow process consists mainly of disintegration and reforming of aggregates of red cells; and (6) at high shear rates, the process of flow is mainly viscous dissipation in the plasma layer between red cells, as well as rotation of individual red cells, deformation of red cells, and collisions of red cells.

Thus, at high shear rates, the viscous dissipation in the suspension should vary linearly with the viscosity of the continuous phase, if red-cell deformation and red-cell–red-cell collisions are independent of temperature. Under these conditions, changing temperature should change the viscosity of blood and of plasma by the same percentage, so that the relative viscosity remains constant. The total rheologic curve for normal human blood will be further considered in the following section.

VISCOSIMETRY WITH A CAPILLARY VISCOSIMETER: THE "COMPLETE" VISCOSITY FUNCTION OF HUMAN BLOOD

Capillary viscosimeters are less convenient than the Couette viscosimeter for the study of the non-Newtonian viscosity function of blood

because, as considered in detail elsewhere,[7] it is difficult (although not impossible) to deduce the shear rate at the capillary wall, $\dot{\gamma}_w$, corresponding to a measured wall shear stress. But capillary viscosimeters have been useful in corroborating data obtained with Couette viscosimeters.

For example,[1,8] it has been shown that the yield stress may be determined clearly by two different tests with a capillary viscosimeter and that the yield stress thus determined agrees, to within 10%, with the value determined by a Couette viscosimeter. The capillary viscosimeter may be operated, and its data interpreted, so as to yield the same information as provided by the Couette viscosimeter over the same range,[11] as in Figures 5–9.

The capillary viscosimeter makes it possible to visualize the flow while measuring it, and thus to relate the observed non-Newtonian viscosity characteristics to the flow process (discrete cells, aggregates of cells, etc.).

Finally, it has been found advantageous to use the capillary viscosimeter for flows under relatively high shear rates, to complement the data obtained from the Couette viscosimeter.

The combined data from a Couette viscosimeter experiment (continued up to a shear rate of 315 sec^{-1}, just short of secondary flow and turbulent flow) and from a capillary experiment on the same sample of normal human blood are given in Figure 10. In a sense, this demonstrates the complete steady-state viscosity function of normal human blood.

One notes three different regimes in Figure 10: (1) with a shear rate of from zero to about 20 sec^{-1}, obedience to the Casson equation, $\tau^{\frac{1}{2}} = a^{\frac{1}{2}}\dot{\gamma}^{\frac{1}{2}} + b^{\frac{1}{2}}$; (2) from around 20 sec^{-1} to around 100 sec^{-1}, a transition from the Casson equation to the final regime; and (3) the Newtonian regime, at a shear rate above about 100 sec^{-1}, wherein the data lie on a straight line extending through the origin, and therefore representing a constant ratio of shear stress to shear rate, and thus a constant (Newtonian) viscosity, which is around 3–4 cP.

Examination of data from many samples of normal blood indicate that the linear sections in regimes 1 and 2 are parallel, and that therefore the constant $a^{\frac{1}{2}}$, the slope in the Casson regime, is equal to the constant $\eta_N{}^{\frac{1}{2}}$, the slope in the Newtonian regime, where η_N is the limiting Newtonian viscosity. Thus, $a \approx \eta_N$, which suggests that, in general, it should be possible to deduce the complete viscosity function for normal human blood from only two sets of data points plotted on double-square-root coordinates: one set at 200 sec^{-1}, which determines the Newtonian slope η_N (by a line drawn through this set of data points

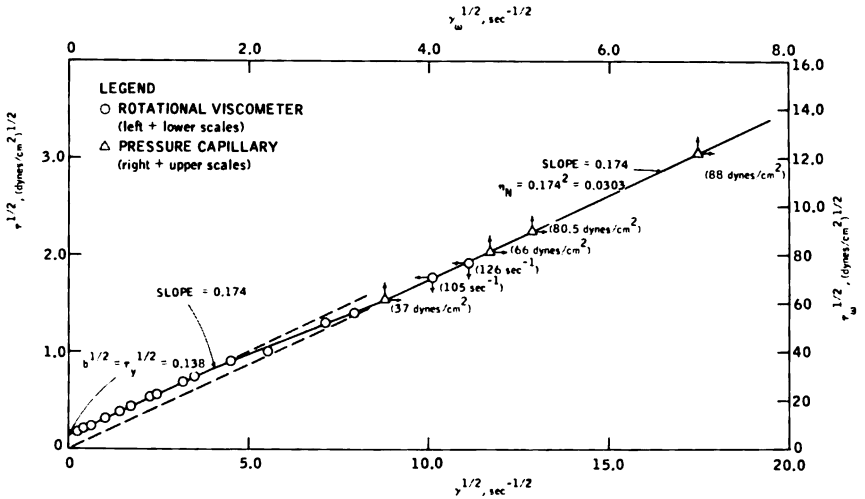


FIGURE 10 Coaxial cylinder viscosimeter and capillary viscosimeter data from blood sample 159 (hematocrit, 40; temperature, 37 C; fibrinogen concentration, 0.18 g/100 ml), plotted as square root of wall shear stress, Γ_w, versus square root of wall shear rate, $\dot{\gamma}$. Values of actual shear rates (coaxial cylinder) or wall shear stress (capillary) are indicated within parentheses against the point. (Reprinted with permission from Merrill and Pelletier.[15])

and the origin), and one set at about 10 sec^{-1}, which fixes the "Casson" line as a line parallel to the Newtonian, of which the extrapolated limit at zero shear rate is the yield stress.

In the Newtonian regime, the principal flow mechanisms involved are 1, 2, and 3, i.e., viscous shear of plasma, deformation, and collisions of red cells. The "Casson" regime is dominated by mechanism 4, make-and-break contacts between red cells and clusters of red cells. In the transition, mechanism 4 becomes increasingly small, compared with mechanisms 1, 2, and 3.

PHYSIOLOGIC AND BIOPHYSICAL PROBLEMS RELATED TO RHEOLOGIC OBSERVATIONS

From the physiologic point of view, the non-Newtonian viscosity of blood near or at zero shear rate (i.e., in the Casson regime) would appear to have relevance to irregular, slow, or stalled circulation anywhere in the vasculature, especially in the venules and veins, where stasis occurs intermittently even in normal subjects. The range of shear

rates in the Casson regime appears to be appropriate to venular and capillary flow, as observed by other workers in the cheek pouch of the normal hamster (discussed in reference 1) and to flow rates observed in the venules of the human eye.[6] In addition to normal physiologic states in which stalled or slow flow may occur, one may cite abnormal conditions, such as stoppage induced by compression of tissue (including compression by tourniquets), stoppage induced by profound vasoconstriction, and stoppage induced by exposure of tissue to cold.

Inasmuch as the experimental data on the non-Newtonian viscosity of blood clearly suggest that fibrinogen, in its native, undenatured state, is adsorbed on the red-cell membrane and acts as a reversible adhesive agent for red cells, the role of fibrinogen adsorption on red cells in thrombosis, either platelet thrombosis or fibrin thrombosis, needs reexamination.

The purely mechanical role of the rheologic effect is obvious: if a platelet or other thrombus is effective in halting or nearly halting the flow of blood, the stalled or slowly flowing blood will aid in the process of thrombosis by mechanically protecting it from being washed away by fluid blood.

A more subtle, but perhaps more important, consequence of the rheology of stalled blood lies in an area that might be called the chemical kinetics of thrombosis, namely, the activation of the successive clotting factors in the cascade that leads ultimately to thrombin, and then fibrin. The red-cell structure, of which yield stress is direct evidence, will retard dilution of the activated clotting factors by fresh plasma and will aid in the process of increasing the concentration of each of the clotting factors to the critical level for activation of the next in the cascade. In respect to the "red" fibrin thrombus,* it is possible that fibrinogen adsorbed on the red-cell surfaces has the additional function of facilitating the incorporation of red cells as "quantum units" in the red clot. On this account, the mechanical strength of the thrombus should increase faster than if the red cells were absent.

It is even conceivable, although not demonstrated, that the activated clotting factors would adsorb preferentially on the red-cell membrane adjacent to fibrinogen molecules, and that the activation of fibrinogen as adsorbed on a red-cell membrane would be more rapid than the activation of fibrinogen as it exists in plasma solution.

One can argue, therefore, that concomitant polycythemia and hyperfibrinogenemia are particularly dangerous because of the tenacity of

* This proposition is not directly relevant to the concept of thrombus in the coronary artery, in contradistinction to clot, set forth by Dr. J. R. A. Mitchell (pp. 123–124).

the red-cell structure that is established in static blood, as evidenced by greatly increased yield stress and by the increased viscosity of such blood at all shear rates. These speculations on the influence of the non-Newtonian viscosity of blood, and in particular on the value of the yield stress, suggest that physicians should carefully examine not only the hemoglobin content of blood, but also the fibrinogen level, in connection with studies of clot-induced stroke and of coronary thrombosis.

Although the main purpose of this paper has been to focus attention on the unique properties of blood near zero flow rate, and although steady-state viscosimetry reveals Newtonian flow in blood at shear rates comparable with those in arterial flow, it is not necessarily true that these are no significant rheologic effects even in the so-called Newtonian regime. One can foresee at least two different effects: First, it is well known that suspended particles at high concentration in a Newtonian continuum, e.g., polystyrene beads in water, can produce a fluid that, under steady-state viscosimetric conditions, appears to be Newtonian, but that in turbulent flow shows a drastically changed turbulence; the direction of the change is, in general, to reduce the intensity and increase the scale, or, more broadly, to decrease the effectiveness of a turbulent mixing process. Inasmuch as blood is a suspension of red cells at high concentration, this principle may be operative at junctions or sites where the flow is separated and undergoing secondary or quasi-turbulent motion, as around the aortic valve and at various positions around the aortic arch. If one could treat arterial flow as nonpulsatile flow in inelastic conduits, there would be no hesitancy to rely on the steady-state Newtonian viscosity of blood in describing its flow. Inasmuch as the flow is indeed pulsatile, and the velocity profile at various positions fluctuates drastically with time, it may be necessary in the future to take into account another subdivision of the field of rheology, namely, the viscoelastic response of blood, in which it is necessary to measure the interrelations of stress, deformation, and time over a range of frequencies similar to those in arterial flow.

Second, of the mechanisms involved in blood flow outlined earlier, it is now reasonable to inquire whether mechanism 3, collisions between individual formed elements, has some indirect role in the process of thrombogenesis. Even if the flow is laminar, and at sufficiently high shear rates that the viscosity is constant (so that the blood is in the Newtonian regime), it is possible that the impulse of a collision between a pair of red cells would be sufficiently violent to begin the process of membrane disruption; that the collision between a red cell and a leukocyte would be sufficient to change the molecular structure of the leukocyte surface, increasing its adhesiveness; and that the platelets, if

involved in energetic collisions with other formed elements, would themselves become activated toward mutual adhesiveness. Thus, even when blood is flowing sufficiently rapidly (or, more exactly, under conditions of sufficiently high shear rate), so that it would appear to be Newtonian if measured in a viscosimeter operated at steady state at the same shear rate, there may be factors at work of immediate relevance to thrombosis.

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The Fate of Thrombi

J. F. MUSTARD

The location, size, and fate of a thrombus that forms in flowing blood is determined largely by the nature of the initiating stimulus, the pattern of blood flow, the characteristics of the vessel wall, the activity of enzymes that dephosphorylate adenosine diphosphate (ADP) to adenosine monophosphate (AMP), antithrombin, and fibrinolysis.[37] There is considerable evidence that at least three mechanisms are involved in the formation of a thrombus or hemostatic plug,[17,37,44] and that they can be modified by the factors just cited. These mechanisms are: (1) the interaction of platelets with such surfaces as collagen or with particulate stimuli, such as antigen–antibody complexes, bacteria, and viruses; (2) the adherence of platelets to each other, which is thought to be caused by ADP; and (3) the generation of thrombin, which causes further platelet aggregation and fibrin formation.

INITIATING STIMULI

There appear to be two types of stimuli that can initiate the formation of thromboemboli: (1) vessel-wall stimuli and (2) intravascular stimuli. It is well established that collagen is the principal component of the normal vessel wall with which platelets can interact.[37,52] The basement membrane may also be active, but the evidence is inconclusive.[8] Platelets apparently do not interact with elastin.[52] Although it has been difficult to demonstrate that platelets can adhere to normal endothelium, in some circumstances interaction must occur, inasmuch as endothelial cells have been found to phagocytose platelets.[24] However, there is no evidence that injured or altered endothelial cells to which platelets might adhere can cause release of platelet ADP,[16,53] leading to the forma-

tion of platelet aggregates. Studies of the relationship between atherosclerotic lesions and thrombosis suggest that breaks in the endothelial lining are important in initiating the thrombi.[5] Evidence from a number of studies indicates that most thrombi initiated by stimuli in the vessel wall are the result of endothelial injury that leads to the exposure of subendothelial tissues, such as collagen and basement membrane.[8] If that is the case, then one of the important problems to be studied is the cause of endothelial injury.

A number of intravascular stimuli can induce platelet aggregation and thus cause the formation of platelet emboli. These intravascular stimuli include antigen–antibody complexes, viruses, bacteria,[35,44] long-chain saturated fatty acids,[14] and some amines, such as epinephrine and norepinephrine.[26] As well as being capable of causing platelet aggregation, epinephrine may act as a powerful potentiator of ADP.[1] It has been suggested that the release of platelet epinephrine during ADP-induced platelet aggregation may help to maintain the platelet aggregates [3]; although the amount of epinephrine released from human platelets is small, it is nevertheless sufficient to produce the effect.

In addition, other factors may influence these platelet stimuli. Our *in vitro* studies have indicated that fibrinogen can diminish the extent of release of platelet constituents induced by antigen–antibody complexes. Fatty acids bound to albumin are less active than unbound fatty acids in causing platelet aggregation.[14] It may well be that, if fatty acids are to induce an effect on platelet aggregation, there must be sufficient amounts in the bloodstream to exceed the normal plasma albumin binding capacity. Furthermore, some fatty acids are bound to albumin at a lower rate than others.[54] Therefore, the release of fatty acids into the plasma might lead to the brief presence of unbound fatty acids.

The surface of vascular prosthetic devices provides a stimulus to platelet aggregation.[6,34] This may be related either to the surface properties of the vascular prosthesis or to the plasma proteins that are absorbed by its surface. It has been found *in vitro* that nonbiologic surfaces that have been coated with immunoglobulin G (IgG) can cause the release of platelet constituents, including ADP, resulting in platelet aggregation.[35,44] In contrast, fibrinogen-coated surfaces potentiate platelet adherence to the surfaces but cause much less release of platelet constituents than IgG-coated surfaces. Surfaces coated with albumin appear to be relatively inert with respect to promoting platelet adherence and the release of platelet constituents.

It is recognized that the duration of platelet aggregation and the size of the platelet mass are related to the amount of ADP that is available and the speed with which it is dephosphorylated to AMP.[2,19,46]

BLOOD FLOW

There is considerable evidence that stasis promotes red-cell agglutination, blood coagulation, and thrombosis.[39,41] However, stasis by itself does not cause thrombosis; it has to be coupled with another factor, such as vessel injury or stimulation of the clotting mechanism. It is known that, if blood flow is retarded, the viscosity of blood increases.[49,55] The principal factor responsible for this change is thought to be fibrinogen, which links the red cells to each other.[49,55] In addition, stasis creates conditions that favor the formation of thrombin and that diminish the amount of anticoagulant material available to inhibit the clotting reaction.

Although stasis may be important in the initiation of some thrombi, it seems likely that most of them, particularly those in arteries, are formed in flowing blood. If the endothelium of the aorta is damaged in zones of both laminar and turbulent flow, the thrombus is most extensive in the area in which flow is disturbed. In the region in which flow is laminar, the deposit that forms on the surface consists of only a thin layer of platelets and leukocytes.[42] This effect of flow can also be illustrated in extracorporeal shunts containing bifurcations.[33,42] Furthermore, it seems likely that, as the thrombus forms, blood flow can disrupt part of the mass. In studies of the microcirculation, it has been shown that there is a fairly continuous formation and breaking up of the platelet mass at a point of vessel injury.[9,15] Thus, it would appear that blood flow affects the structure, size, and stability of a thrombus.

PLATELET AGGREGATES AND TISSUE INJURY

Some forms of tissue infarction attributed to vascular disease are not due to occlusive thrombi in major arteries.[21] Because it is recognized that portions of thrombi break off as showers of platelet emboli, and because platelet aggregates can be induced by intravascular stimuli, consideration has to be given to the effects of transient platelet thromboemboli on organ structure and function.

It has been shown that myocardial infarction can be produced by infusing ADP into the myocardial circulation of pigs [21] in doses sufficient to cause platelet aggregation for 5 min. The changes that occur in such experiments have been studied by examining the effects of these infusions on the mesenteric microcirculation. Within 30 sec of the start of the infusion, the venous flow is arrested and that in the arteries is slow and arrested intermittently. These changes in flow are preceded by the formation of platelet aggregates, which probably become impacted in

the lumen of vessels. This arrest of flow is probably responsible for the packing of the red blood cells. When the infusion of ADP is stopped, flow is quickly restored to normal. It seems reasonable to suppose that, in the experiments designed to examine the effect of transient platelet aggregates in causing tissue injury, flow in the microcirculation may have been interrupted for 5–10 min. In these circumstances, collateral circulation probably does not occur. It seems likely, therefore, that the ischemia in the affected areas is virtually absolute. Myocardial cells can be irreversibly injured by 6–9 min of complete ischemia.[23] The areas of infarction are probably the result of the transient ischemia due to the temporary arrest of the microcirculation. But it is possible that the initial alteration in the microcirculation produces areas of focal vasculitis that serve as sites for the formation of further platelet thromboemboli.[21] We have repeated these experiments on the renal circulation in rabbits, with similar results. An important observation in these studies was the appearance, within 24 hr of the initial injury, of fibrin masses within the lumen of the glomerular vessels.

Although the results of these studies indicate that transient platelet aggregates can produce tissue injury, two problems have to be examined: (1) The relevance of this to human disorders and (2) the possible trigger mechanisms that can induce transient platelet thromboemboli. The first is considered elsewhere in this publication. The possible intravascular trigger mechanisms include antigen–antibody complexes, viruses, bacteria, bacterial products (such as endotoxin[44]), and fatty acids. The intravenous infusion of these agents can cause a decrease in the platelet count. The results of *in vitro* studies show that antigen–antibody complexes, viruses, and bacteria can cause release of platelet nucleotides, including ADP.[44] Fatty acids have also been shown to be capable of causing platelet aggregation in *in vitro* studies.[12] In addition to platelet emboli produced by intravascular stimuli, showers of fragments from mural thrombi can produce organ dysfunction and injury.[10,27] This has been demonstrated in both the renal and cerebral circulations in man and in the renal circulation in rabbits,[28] and has been well documented in studies of the microcirculation.

PLATELETS AND VESSEL INJURY

Most of the stimuli that cause platelet aggregation induce the release from human or pig platelets of factors that increase the permeability of vessels in the skin of rabbits and guinea pigs.[38,44] The factors are active in rabbits treated with sufficient mepyramine maleate to inhibit

the action of histamine at a concentration of 10 mg/ml. Serotonin does not cause increased vascular permeability in guinea pigs or rabbits, so there appears to be a factor (or factors) in addition to histamine and serotonin that influences such permeability. The material retains its activity on being heated to 100 C for 30 min and passes through membrane filters that hold back molecules greater than 10,000 in molecular weight.[44] It may be similar to the factors released from polymorphonuclear leukocytes that can increase vascular permeability.[20,31]

When leukocytes are exposed to antigen–antibody complexes, they release a factor that causes contraction of smooth muscle (H. Z. Movat, personal communication). It is known that both histamine and serotonin can cause contraction of smooth muscle. The platelets release a factor (or factors) that causes contraction of guinea pig ileum and rat uterus.[44] Although both histamine and serotonin are partly responsible for this effect, there appears to be another factor that causes contraction of smooth muscle. This may be related to the material from the platelets that causes increased vascular permeability. The increased permeability produced by some compounds may act by causing contraction of the endothelial cells, creating gaps between them. Inasmuch as the leukocyte also contains factors that can cause increased vascular permeability and the contraction of smooth muscle, it is apparent that the accumulation of formed elements on the surface of vessels may have effects on the endothelium.

Platelets (like leukocytes) contain lysosomal granules,[25] and some of the lysosomal enzymes are released from the platelets by many of the stimuli that cause platelet aggregation.[50] The escape of these enzymes from platelets at a point of contact with the vessel wall could result in endothelial injury. Anoxia of tissues at the base of the thrombus may be an additional factor in causing tissue injury.

Hughes and Tonks [18] observed vascular injury in association with platelet aggregates. In the early stages of intravascular inflammatory reactions, it is not unusual to find platelets in contact with the vessel wall at the sites of increased permeability.[30] Often, the platelets can be seen protruding through gaps between the endothelial cells. Although platelets have not been considered important in producing the vascular changes seen in such conditions as allergic inflammation, the results of recent experiments suggest that this should be re-examined.

The formed elements may be involved in changes in the vessel wall in areas of disturbed flow. In the aorta of pigs and rabbits, albumin accumulates in the intima in zones in which disturbed flow could be expected to occur, such as around vessel branches.[45] These sites showed intimal edema and formed elements on the endothelial surface, indicat-

ing that these are areas of vessel injury. Although leukocytes and platelets would be expected to accumulate at such sites in response to injury, the formed elements may also have been involved in producing the tissue injury. Even if the platelet aggregates were transient, they might injure the vessel wall and produce a focus for the formation of more stable platelet aggregates.

STABILITY AND FATE OF THROMBI

It is apparent from the preceding discussion that many platelet aggregates are unstable. The stability of the aggregates is related to the balance between the factors promoting their formation and the factors promoting their breakup. Fibrin formation appears to be one of the important factors in stabilizing platelet aggregates.[17,22] For example, if ADP is infused into the circulation, the platelet aggregates that form break up rapidly when the infusion is stopped, and the platelets return to the circulation.[21] The effect of ADP in binding the platelets to each other is probably quickly lost as the ADP is dephosphorylated to AMP by enzymes in the plasma and on the platelet membrane.[2,19,46]

In contrast with the effects of ADP infusion, when thrombin is infused, the platelet aggregates that form have fibrin around them and 1 or 2 hr will elapse before these aggregates break up.[40,50] This delay is probably related to the time required for the lysis of the fibrin around the platelet aggregates.

More direct evidence about this has been obtained by the study of the formation of platelet aggregates in injured vessels in normal dogs and dogs with congenital deficits in coagulation factor VIII or factor IX.[17] The platelet masses that formed in the animals with congenital defects in the intrinsic pathway of coagulation were unstable, probably because of a lack of fibrin around the platelets. In experiments with rabbits, the platelet plugs at the ends of transected vessels could be made unstable by lysis of the fibrin around the periphery of the platelets.[13] The balance between fibrin formation and lysis may be as important as the amount of ADP in determining the stability of the platelet aggregates.

One little understood but important aspect is the mechanism by which coagulation is either activated or accelerated in association with platelet aggregation. Some stimuli that cause platelet aggregation—such as collagen,[43] fatty acids,[4] and antigen–antibody complexes [29] can activate the intrinsic pathway of blood coagulation. In addition, it has been shown that ADP-induced platelet aggregation leads to the availability of the platelet phospholipid, which has a profound accelerating effect on

the clotting reactions.[11,36] It is apparent that the activation and acceleration of clotting are centered around the platelet aggregates and that coagulation does not usually extend further into the vessel lumen, unless there is considerable stasis or extensive stimulation of the clotting mechanism.

The platelet masses that persist are usually transformed to deposits of fibrin within 24 hr.[22] The transformation involves the separation of the platelets, the formation of fibrin between the platelets, and their eventual disintegration or ingestion by phagocytic cells. This transformation is a well-recognized process, the resolution and organization of platelet-rich thrombi.

The reversibility or dissolution of thrombi formed in response to vessel injury can be demonstrated by studying the platelet reactions associated with kidney transplants. During the first few weeks after transplantation, renal shutdown may occur, and the glomerular capillaries of kidneys biopsied during such an episode are found to be obstructed by platelet aggregates.[48] Immunofluorescent studies show that the vessels are coated with IgG. It seems likely that either the IgG lining, injury to endothelial cells, or exposure to collagen is the trigger mechanism for the formation of the platelet thrombi. The interaction of platelets with surfaces or particles can be inhibited by a number of compounds, including hydrocortisone, phenylbutazone, sulfinpyrazone,[47] and acetylsalicylic acid.[7] It has been found that the administration of cortisone [48] or phenylbutazone [32] to patients during episodes of renal shutdown reverses the process. There is a decrease in platelet count in association with the onset of renal shutdown, with return of the platelets when the process is reversed. It seems reasonable to interpret this evidence as indicating that platelet thrombi formed as a result of vascular stimuli can be reversed by inhibiting the platelet–surface reaction. Inasmuch as many of the thrombi were probably established for at least 0.5 hr before the initiation of treatment, it appears that, even at this stage, they can be reversed by inhibiting the primary mechanism. This is probably related to the fact that the thrombi at this stage are constantly forming and breaking up.

The effects of transient thromboemboli, as well as persistent and occlusive emboli, may be important in causing the complications seen with vascular disease. Platelet aggregates can injure the vessel wall directly and can also cause disturbances in the microcirculation, which in turn can result in focal injury in organs. Recognition of the effects of transient thromboemboli may help to explain some forms of tissue injury seen with vascular disease in circumstances in which thrombi are not found in postmortem studies.

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The Role of Platelet Embolism from Crumbling Thrombi and of Platelet Aggregates Arising in Flowing Blood

LEIF JØRGENSEN

Thrombi are continually changing: They grow by deposition of platelets, blood cells, and fibrin.[16,31–33,146,150] They are transformed from an initial mass of platelets to a body of fibrin.[6,77,86,130,146] They tend to break down by fragmentation of platelet aggregates [16,39,68,75–77,91,129,132,150] and by lysis of fibrin, either through the action of the plasma fibrinolytic system [47,52,77,90,122–124,128] or by leukocytic enzymes.[12,59,78,122,147] Remaining parts of the thrombi are covered and invaded by cells derived from the blood and the vessel wall.[27,30,53,56,58,78,86,118,139,147,148] Several or all of these processes may go on simultaneously, and end only when the thrombi are completely removed or organized. The dynamics of the thrombotic process is reflected by the great variability of the structure of thrombi when observed at various points in time.[6,77,147]

Among the processes mentioned, the steady formation and subsequent fragmentation of platelet aggregates may be of particular importance. From a mural thrombus in a larger artery, platelet aggregates may embolize and lodge in the arterial bed downstream,[48,49] forming transient or more long-standing obstacles to the flow in the peripheral vessels. However, similar aggregates may arise in flowing blood independently of an upstream thrombus attached to the wall.[77] Experimentally, this has been achieved by injection of adenosine diphosphate (ADP),[54,113,115,116,132] thrombin,[95,126,145,149] suspension of collagen particles,[54] bacterial endotoxin,[25,99,138] bacteria,[23,91] viruses,[125] antigen (in sensitized animals),[70,71,109] thorotrast,[35] fatty acids,[64,65] lipids,[126,137] and snake poison.[72,126]

Furthermore, platelet aggregates not secondary to localized sessile thrombi have been produced by anoxia,[54,61,62] cooling,[54] mobilization of fatty acids by ACTH,[66] and subcutaneous injection of 2α-methyl-9α-chlorocortisol acetate combined with intragastric instillation of sodium

phosphate.[70] In man, the phenomenon has been observed after massive blood transfusion,[74,141] after major vascular surgery,[17] after injuries and burns,[34] and in association with acute hemolytic anemia.[3]

This presentation will discuss the possible significance of platelet aggregates in the microcirculation with regard to organ function and structure. The emphasis will be on platelet aggregates in localized circulatory areas. Both aggregates arising from crumbling thrombi and platelet masses assumed to have formed in flowing blood will be dealt with. The aggregates may or may not be associated with evidence of coagulation. If they are formed by ADP without any significant stimulation of coagulation, they will probably be transient and will break up.[18,19,113] It will be shown that, even in that case, the aggregates may be injurious to the organs. If thrombin is generated in association with the platelet massing, the aggregates will become stabilized and associated with fibrin.[69,79] There is probably no sharp line of demarcation between platelet aggregates formed in flowing blood and the syndrome of disseminated intravascular coagulation. However, the latter syndrome is characterized by widespread small-vessel occlusions and a particularly strong stimulation of coagulation, leading to both platelet aggregation and rapid and extensive intravascular fibrin formation. Disseminated intravascular coagulation has been thoroughly reviewed in two recent monographs [55,98] and will not be considered here.

PLATELET EMBOLISM FROM CRUMBLING THROMBI

In an autopsy study of cerebrovascular diseases, Torvik and I looked for platelet aggregates, with or without fibrin, in the vascular bed of occluded arteries supplying the brain (Figure 1).[87] All occlusions were less than 1.5 months old, and the great majority less than 14 days old. Among 29 patients with gross arterial thrombi (Table 1), a few such aggregates were found in the meningeal or intracerebral arteries in 16 cases (55.2%). Among 30 patients with grossly visible emboli from the heart or other intrathoracic source, such aggregates were found in nine cases (30%). Venous platelet aggregates were particularly prevalent among the embolic cases. Many of the platelet aggregates were probably secondary to the anoxia of the tissue. However, when we considered only the cases with platelet aggregates in the meningeal arteries, which are upstream of the infarcted cerebral tissue, a marked difference between the thrombotic and embolic cases appeared: the number of cases with platelet aggregates here was significantly higher among patients with thrombosis than among patients with embolism.

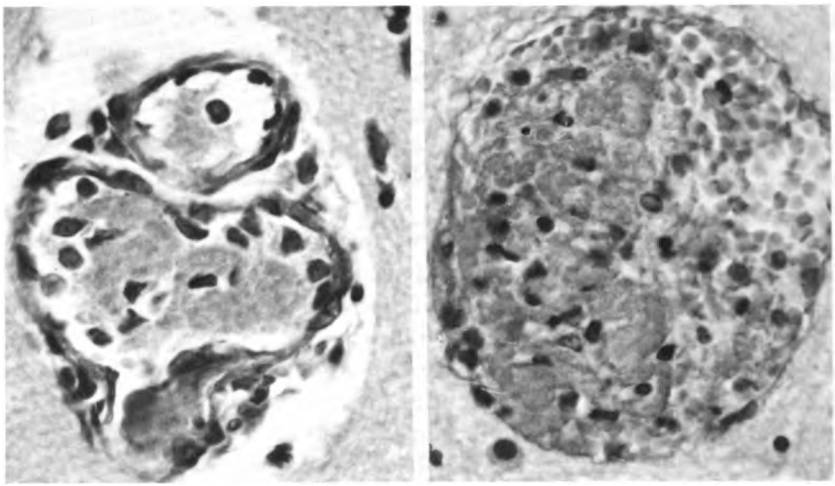

FIGURE 1 Platelet aggregates in peripheral vessels in patients with cerebral thrombosis. Left, arterial aggregates in the brain stem of a patient with thrombosis of the basilar artery. Before the final apoplectic episode, 6 days before death, he had numerous transient ischemic attacks. Right, venous aggregate in a patient with thrombosis of the internal carotid artery. She had gradually increasing symptoms, ending in coma and massive cerebral infarction 5 days before death. Hematoxylin–eosin. (× 435)

That probably indicates that the majority of the meningeal aggregates are microemboli from an upstream thrombus.

This conclusion is at variance with that of Romanul and Abramowicz,[120] who studied at autopsy 13 cases of cerebral infarcts in arterial border zones. Five had occlusion of larger neck arteries. Arterial platelet aggregates were found overlying and within the infarcts. The authors considered all of them to be sessile thrombi secondary to the anoxia, partly because the aggregates were found both with and without upstream occlusion, and partly because it was felt that cerebral infarcts due to embolism in general are not in border zones. However, they did not consider that platelet aggregates may form in flowing blood, or that the effect of microembolization of platelet aggregates is not comparable with that of an embolic occlusion in a major artery. The accumulation of aggregates in the border zones in their cases may be due partly to the sluggish flow in their areas, favoring persistence of the platelet masses, whereas in areas of more rapid flow they would tend to break up.[144] The reduction of flow may be caused primarily by the mechanisms Romanul and Abramowicz [120] proposed: occlusion of a neck artery, a decreased

TABLE 1 Platelet Aggregates in Smaller Arteries and Veins of the Brain in 64 Cases of Gross Recent Thromboembolic Occlusion of Arteries Supplying the Brain

Type of Occlusion	No. Cases Examined	Cases with Arterial Aggregates			Cases with Venous Aggregates
		Meningeal [a]	Intracerebral	Either	
Thrombi	29	13 (44.8%)	9 (31.0%)	16 (55.2%)	12 (41.4%)
Emboli	30	2 (6.7%)	7 (23.3%)	9 (30.0%)	18 (60.0%)
Undetermined	5	1 (20.0%)	0	1 (20.0%)	1 (20.0%)

[a] Difference between thrombotic and embolic cases with regard to occurrence of platelet aggregates in meningeal arteries significant at $p < 0.01$.

cardiac output, or systemic hypotension. Otherwise, it would be difficult to explain the particular localization of the infarcts.[144]

Experimentally, microembolization to the meningeal arteries has been reproduced in rabbits by Honour and Russell,[68] and possibly also in monkeys by Denny-Brown and Meyer,[26] although the latter authors only assumed the presence of an upstream thrombus. Immediately after the appearance of microemboli, they noted transient sludging of the red blood cells, stasis, and local cortical hypoxia. In another correlative study of meningeal microcirculation and cortical metabolism, Meyer and associates [101] concluded that the tissue was more severely damaged by the embolization of many small particles than by mere ligation of an artery. This was explained by the blocking of the collateral flow by multiple small occlusions.

Clinically, Millikan and Siekert [102,104] called attention to patients with repeated, short-lasting attacks of neurologic symptoms associated with basilar or carotid arterial occlusions. They suggested that microembolization from an upstream thrombus may be responsible.[105] That thrombosis plays a part was supported by the favorable effect of anticoagulant therapy on the transient ischemic attacks,[103,105] an observation that has since been confirmed in larger series.[10,11,37,131] More direct evidence of the importance of microembolization in transient symptoms was presented by Fisher [38] and others.[7,96,121] They observed passage of microemboli through the retinal arterioles during transient attacks of visual loss, and McBrien and co-workers [96] showed histologically that the microemboli were platelet aggregates.

In a thorough clinical and pathologic study of carotid occlusions, Gunning and associates [48] suggested that the transient ocular and cerebral ischemic attacks were caused by friable microemboli, whereas less friable emboli might cause permanent symptoms due to irreversible tissue damage. Our autopsy study of carotid occlusions [144] made it unlikely that microembolism is a specific feature in transient ischemic attacks. We found arterial platelet aggregates in the meninges and the brain in patients with a protracted clinical course, whereas patients with a sudden onset of symptoms showed this much less frequently. That does not mean that all forms of protracted course—e.g., stepwise or gradually increasing symptoms—are governed by microembolization. The increasing reduction of arterial flow due to the increasing size of the upstream mural arterial thrombus may be just as important. It has been shown that a few, rapidly passing emboli in the retinal arterioles need not be accompanied by clinical symptoms.[136]

Necrotic debris with cholesterol crystals derived from ruptured necrotic atherosclerotic plaques may also form microemboli that flow to the eyes [24] and the brain.[140] Hollenhorst [67] claimed that these emboli

were frequently observed in the eyegrounds of patients with occlusive disease of the neck arteries, but he did not give any evidence of their origin apart from their macroscopic appearance. However, microembolization of necrotic debris must, at least in Oslo, be much less common than that of platelet masses: Torvik and I [87] did not observe any case of microembolism of necrotic debris in our autopsy study of cerebrovascular diseases.

Compared with the fair amount of data concerning platelet microembolism in the cerebral and ocular circulation, the information about similar processes in the heart is rather meager. In an autopsy study of patients who died within 48 hr of myocardial ischemia,[80,82] including sudden deaths, we found a few platelet aggregates in downstream epicardial or intramyocardial arteries in 14 of 39 patients (36%) with thrombosis in a main coronary artery (Table 2). Seven patients (18%) had platelet aggregates in the intramyocardial arteries. Haerem [50] in our laboratory examined in a similar manner the myocardium in 215 consecutive autopsies and found arterial platelet aggregates in only six patients (3%), three of whom had had recent myocardial infarcts (Table 2). In another autopsy study of 30 cases of instantaneous death, Haerem [50] observed coronary thrombosis in 13 patients, five of whom (38%) had platelet aggregates in their intramyocardial arteries.

Thus, coronary thrombi seem to predispose to platelet aggregation in smaller cardiac arteries. As in the brain, many of them may be secondary to anoxic damage, but it is reasonable to believe that at least some of them, particularly in the epicardial arteries, are emboli from the coronary thrombi. We can only guess the clinical importance of platelet microembolism in the coronary circulation. The situation is probably not very different from that in the brain and the eyes, and it is possible that some attacks of transient ischemia of the myocardium are caused by showers of platelet microemboli.

In neither of the two autopsy studies of acute coronary disease mentioned [50,80] were there obvious emboli of atherosclerotic necrotic debris in the peripheral arterial bed, with the exception of a fairly large embolus to the sinus node artery in one patient who died suddenly.[50] Nevertheless, rupture of necrotic plaques was frequently observed. The explanation may be that the ruptured plaque often leads to rapid death or rapid development of massive platelet aggregation.

The kidney is another organ in which platelet microembolism may play an important role. In an autopsy study, Moore [106] found a very strong correlation between superficial cortical scars of the kidney and significant aortic atherosclerosis upstream of the renal arteries. Hypertension is associated with both superficial renal scars [60] and increased aortic atherosclerosis.[119] The observed correlation could well be ex-

TABLE 2 Platelet Aggregates in Smaller Arteries and Veins of the Heart in 39 Cases of Coronary Thrombosis (<48 hr Old) and in a Series of 215 Consecutive Autopsies [a]

Series	No. Cases Examined	Cases with Arterial Aggregates			Cases with Venous Aggregates
		Epicardial	Intramyocardial	Either	
Coronary thrombosis	39	8 (20.5%)	7 (17.9%)	14 (35.9%)	7 (17.9%)
Consecutive autopsies[50]	215		6[b] (2.8%)		2 (0.9%)

[a] Smaller epicardial vessels were not examined in the consecutive series.
[b] Three of these cases had recent myocardial infarct.

plained by assuming that hypertension had been a common cause of both the kidney and aortic changes. However, Moore [106] offered another, challenging hypothesis—that the kidney lesions were caused by multiple emboli of thrombotic material from small mural thrombi on the altered aortic surface. In subsequent studies in rabbits, Moore and Mersereau [100,107,108] showed that multiple, repeated platelet emboli from experimentally produced mural thrombi in the aorta upstream of the renal arteries caused cortical infarcts, small superficial scars with atrophy of the nephrons, increased granularity of juxtaglomerular cells, and hypertension. Previously, it had been demonstrated in man [5,36,41] and, experimentally, in animals [1,21,92] that kidney infarction may give rise to transient or more lasting hypertension.

PLATELET AGGREGATES IN FLOWING BLOOD INDEPENDENT OF UPSTREAM THROMBI

At autopsy, a few platelet aggregates, mostly without fibrin, may be found in vessels, even without upstream crumbling thrombi (Figure 2).

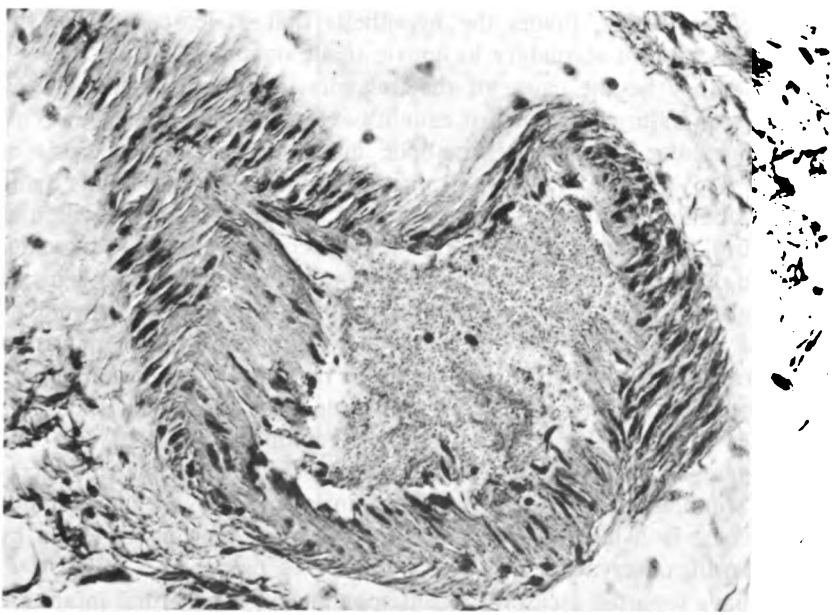

FIGURE 2 Platelet aggregate in an intramyocardial artery in a patient who died a few minutes after the clinical onset of acute coronary disease. No acute lesion was found in the main coronary arteries. Hematoxylin–eosin. (× 218)

In cases with myocardial ischemia,[80,82] platelet aggregates were encountered in six of 17 cases (35%) of rupture of or hemorrhage into necrotic atherosclerotic plaques without secondary thrombi (Table 3). In four cases (24%), aggregates were found in the downstream epicardial arteries. Most of these patients had died immediately after or soon after the clinical onset of the acute disease. Among 16 patients without any acute lesion in the main coronary arteries, five (31%) had arterial aggregates. Three (19%) had aggregates in the epicardial arteries. In Haerem's study of instantaneous death,[50] intramyocardial arterial aggregates were present in three of five cases of ruptured necrotic plaque and in two of seven cases (29%) without any acute lesion in the main coronary arteries. In addition, there were three cases of platelet aggregates in epicardial arteries without obvious mural attachment and without any acute upstream coronary lesion.

Considering the low frequency of platelet aggregates in the myocardial vessels in the consecutive-autopsy series (Table 2), these figures probably indicate that ischemia, even without upstream sessile thrombi, is associated with platelet aggregates in the microcirculation. Again, many of them may be secondary to anoxia. However, the fact that some are present in upstream arterial segments, even immediately after the onset of symptoms, favors the hypothesis that at least some of the aggregates are *not* secondary to anoxic tissue damage.

Could they be the cause of the ischemia in the cases without any acute lesion in larger arteries? It cannot be doubted that extensive occlusion of smaller vessels by thrombotic material can cause necrosis of tissue.[63,134,135,143] The argument against this concept—that patients dying of thrombotic thrombocytopenic purpura may have widespread vascular occlusions without corresponding tissue necrosis [13,45]—is not valid, because death may have occurred before obvious morphologic signs of necrosis developed. However, the few platelet aggregates present at the time of death in some of the patients with unexplained cerebral or myocardial ischemia could alone hardly be responsible for the infarction. Their appearance was generally compatible with formation under the influence of ADP.[77] If so, they would be rapidly reversible,[18,19,113] and their presence indicates only the existence *in vivo* of a platelet-aggregating stimulus, such as ADP, in the circulation. That such a stimulus may persist in a localized vascular bed for some time is suggested by the following observation:

We have reported a case of recent spontaneous myocardial infarction in an old dog that did not have any significant lesion in its main coronary arteries.[84] Platelet aggregates were present in several intramyocardial vessels, but not in other organs. Because some arterial platelet aggre-

TABLE 3 Platelet Aggregates in Smaller Arteries and Veins of the Heart in 39 Cases of Myocardial Ischemia (<48 hr) without Coronary Thrombosis

Type of Lesion	No. Cases Examined	Cases with Arterial Aggregates			Cases with Venous Aggregates
		Epicardial	Intramyocardial	Either	
Rupture of or hemorrhage into necrotic plaque without thrombus	17	4 (23.5%)	2 (11.8%)	6 (35.3%)	2 (11.8%)
Hemorrhage into fibrous plaque	6	0	1 (16.7%)	1 (16.7%)	2 (33.3%)
No acute lesion	16	3 (18.8%)	2 (12.5%)	5 (31.3%)	4 (25.0%)

gates were found in the myocardium outside the infarcted area, and because organizing thrombi were encountered in smaller myocardial arteries, it was assumed that platelet aggregation had taken place for some time independently of and before the infarction. Finally, in a local area it might have assumed an intensity severe enough to cause ischemic necrosis.

That showers of platelet aggregates in the myocardial circulation may cause ischemic necrosis was shown experimentally in rabbits by Hughes and Tonks.[70,71] They produced showers in three ways: (1) by infusing preformed aggregates into the systemic venous circulation or into a pulmonary vein; (2) by infusing an antigen into sensitized animals, which resulted in the formation of antigen–antibody complexes in flowing blood; and (3) by injecting 2α-methyl-9α-chlorocortisol acetate subcutaneously and instilling sodium phosphate intragastrically. As in our old dog,[84] many platelet aggregates were still found in the myocardial sections at autopsy, apparently in a larger number than in the human autopsy series of myocardial ischemia without acute lesion in main coronary arteries.[50,80,82]

The observations of Hughes and Tonks have been confirmed and extended in a series of experiments performed by Mustard's group.[85,114] ADP in Ringer's solution was infused into one of the coronary arteries or the left ventricle in pigs over a period of 3–5 min. This resulted in an immediate fall in the blood pressure and platelet count. Observation of the mesenteric microcirculation during the cardiac infusion revealed, first, the passing of many platelet aggregates, and, later, red-blood-cell sludging and complete arrest of flow in many vessels. Shortly after the infusion, the circulation was restored and the platelet count returned toward preinfusion levels. This resulted in myocardial infarction in most of the animals (Table 4). Infusion of adenosine monophosphate (AMP) caused no change in platelet count, a moderate fall in blood pressure, slight microcirculatory changes, and infarction in only a few cases (Table 4). Infarction did not occur after infusion of ADP into thrombocytopenic animals or into animals in which the platelets were made refractory to the action of ADP [19] by an immediately preceding infusion of ADP downstream into the aorta. Histologic examination of the myocardium during and at various intervals after the infusion of ADP into animals without preparatory treatment showed platelet aggregates in the vessels; these were numerous during the infusion (Figure 3), but few and in only some of the animals after infusion (Table 5).

These experiments show that even transient platelet aggregation in the myocardial circulation may produce permanent ischemic changes. The frequency with which platelet aggregates were found in the pig

TABLE 4 Myocardial Infarction in 60 Pigs Subjected to Adenine Nucleotide Infusion into the Coronary Circulation and Surviving More than 2 hr

Type of Infusion	No. Pigs	Pigs with Myocardial Infarct Judged by			p for Difference in Prevalence of Microscopic Infarct
		Electrocardiogram	Gross Examination	Microscopic Examination [a]	
Intracoronary					
ADP	20	17 (85.0%)	16 (80.0%)	17 (89.5%)	<0.001
AMP	17	3 (17.6%)	3 (17.6%)	4 (25.0%)	
Intraventricular					
ADP	17	11 (64.7%)	8 (47.1%)	10 (62.5%)	0.025
AMP	6	0	0	0	

[a] In each group except the intraventricular AMP group, one animal did not have sections prepared for microscopic examination.

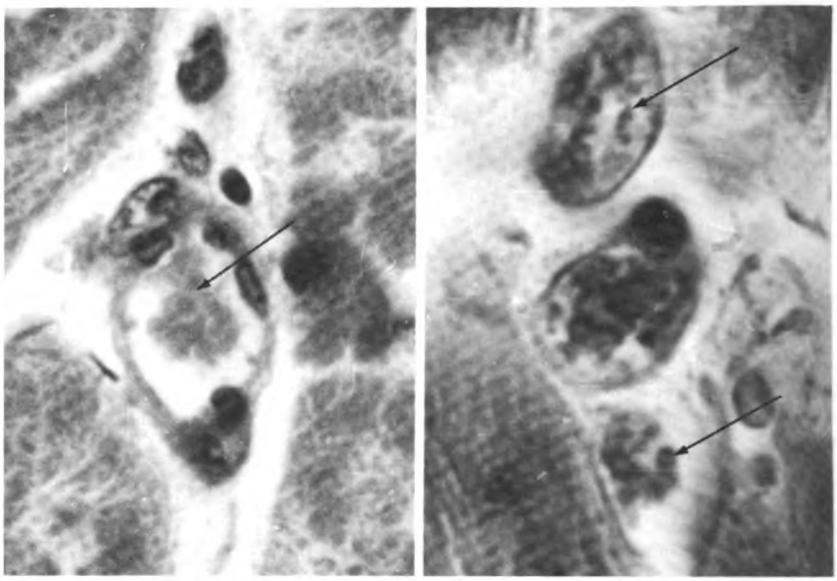

FIGURE 3 Platelet aggregates (arrows) in an arteriole (*left*) and in capillaries (*right*) in pig myocardium. The tissue was obtained during the infusion of ADP into the myocardial circulation. Hematoxylin–phloxine–saffron. (× 1507, *left*, and ×1654, *right*)

myocardium after the acute episode, represented by the infusion, was around the same as in the human cases of myocardial ischemia without acute lesion in the main coronary arteries (Table 3). That does not prove that transient platelet aggregation is the cause of the myocardial ischemia in man. However, it does mean that the finding of only a few or no platelet aggregates at autopsy does not contradict such a hypothesis.

Adenine nucleotides were also infused into the aorta of rabbits just

TABLE 5 Platelet Aggregates in Smaller Arteries and Veins of the Myocardium in 59 Pigs Subjected to Intracoronary or Intraventricular Infusion of ADP

Time of Death from Start of Infusion	No. Pigs	Pigs with Arterial Aggregates	Pigs with Venous Aggregates
0 to 5 min (infusion period)	9	9 (100.0%)	7 (77.8%)
5 min to 2 hr	15	5 (33.3%)	6 (40.0%)
2 hr to 24 hr	35	14 (40.0%)	12 (34.3%)

upstream of the renal arteries.[43,44,81] ADP caused a transient plugging of the renal vessels by platelet aggregates, and the changes in the platelet count and the mesenteric microcirculation were similar to those in the previous experiments. Renal infarct or focal tubular necrosis was obtained in six of 20 animals that received ADP and were permitted to live from 24 hr to 2 weeks after the infusion. No necrotic lesion was observed in any of the six animals that received AMP and were killed within that period. Perhaps more important was the observation that the rabbits that received ADP developed a rise in systolic blood pressure that lasted for the rest of the observation period, usually 2–4 months (Table 6). The control animals that received AMP or Ringer's solution did not show this result; nor did the animals that received ADP develop a rise in blood pressure if the infusion was given when the platelets were made refractory to its effect by a preparatory infusion into the aorta downstream of the renal arteries.

These experiments confirm the observation by Moore and Mersereau [107] that platelet aggregates in the renal circulation may cause elevation of the blood pressure. In addition, they show that a single massive shower of transient platelet aggregates formed in flowing blood is enough to produce this effect. In man, widespread persistent occlusion of the renal microcirculatory vessels may cause hypertension,[42] but so far we have no direct evidence that transient platelet aggregates may do the same.

Another important observation was made by Hughes and Tonks,[70] by Seaman and co-workers,[127] and in the heart and kidney infu-

TABLE 6 Effect on the Systolic Blood Pressure in 84 Rabbits Subjected to Infusion of Adenine Nucleotides or Ringer's Solution into the Aorta

Material Infused	No. Rabbits	Mean Systolic Blood Pressure before Experiment, mm Hg	Mean Change in Systolic Blood Pressure, mm Hg [a]
ADP	28	75.0	+16.8
AMP	17	67.8	—0.1
Ringer's solution	27	77.6	—6.6
ADP, immediately after a preparatory infusion of ADP into an extrarenal arterial bed	12	72.5	—3.8

[a] Difference in change between animals that received ADP once and each of the other groups significant at $p < 0.001$.

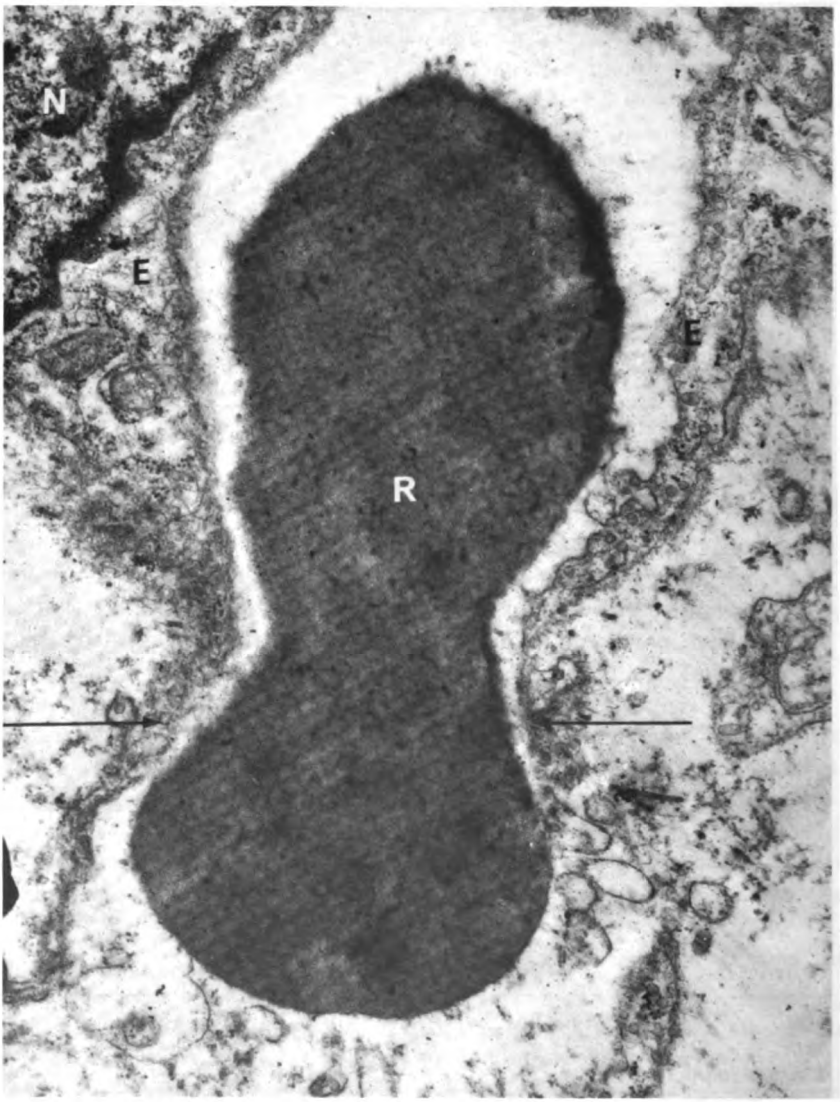

FIGURE 4 An electron micrograph of pig myocardial capillary. The tissue was obtained during ADP infusion. A red blood cell (R) is on its way out through a gap in the endothelial lining (E), between the arrows. The nucleus of the endothelial cell (N) is in the upper left corner. (×15,000)

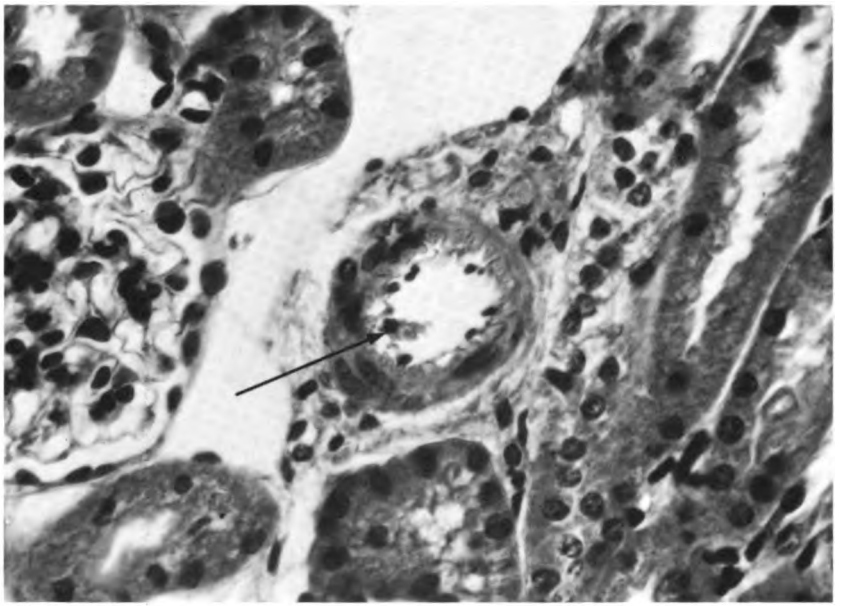

FIGURE 5 A small mural platelet thrombus (arrow) attached to the endothelium of an artery in a rabbit kidney. The tissue was obtained at the end of the infusion of ADP into the aorta. Hematoxylin–phloxine–saffron. (× 535)

sion experiments just described [83,85]: platelet aggregates may cause injury to the vessels. Even during the infusion of ADP, focal gaps between endothelial cells were observed, resulting in extravasation of blood cells and platelets (Figure 4). After the infusion, some of the observed platelet aggregates seemed to be attached to the vessel wall as small sessile mural thrombi (Figure 5). Initially, the endothelial cells were present underneath the thrombi, but 4–5 hr later, they were not recognizable anymore, and the underlying vessel wall exhibited edema and leukocytic accumulation (Figure 6). In some vessels, inflammatory lesions were observed, even without the presence at autopsy of an overlying platelet aggregate. Most of the vascular lesions appeared to heal without sequelae, but a few mural thrombi appeared to undergo transformation to fibrin thrombi [77,86] and became organized, resulting in cushion- or polyp-like intimal thickenings.

From these observations two important points may be derived:

1. Platelet aggregates formed in flowing blood may give rise to sessile mural thrombi; i.e., platelet aggregation may be the primary event, and adherence to the vessel wall a later event.

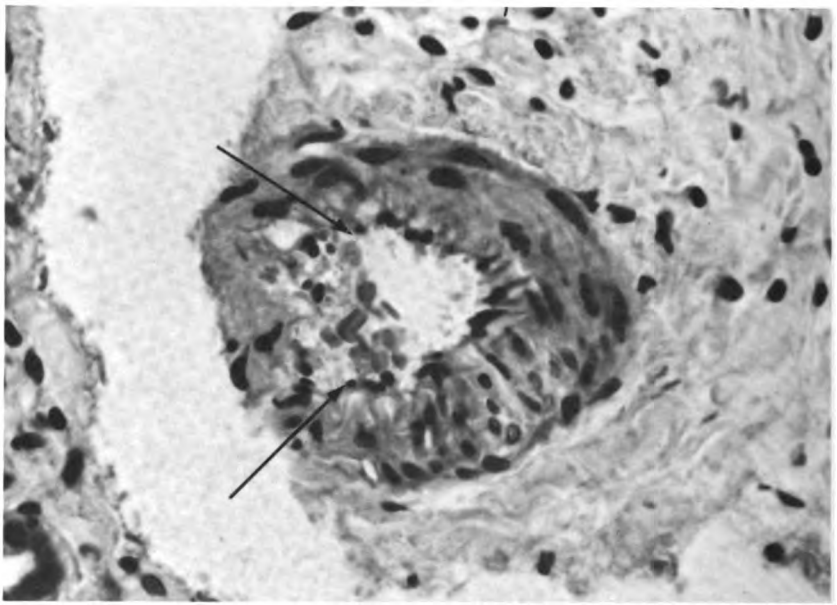

FIGURE 6 A localized inflammation in a sector of the wall of an artery (between the arrows) in a rabbit kidney 5 hr after ADP infusion. A small mural thrombus of platelets and red blood cells appears adherent to the wall. In the underlying part of the vessel wall, there is increased space between the structures, suggestive of edema, and a few polymorphonuclear leukocytes are present. The endothelial cells are partly unrecognizable in the affected sector. Hematoxylin–phloxine–saffron. (× 535)

2. Transient platelet aggregates may cause immediate damage to the vessel wall, with leakage of blood elements followed by focal vasculitis. This means that it cannot be inferred from the presence of a defect in the endothelial lining at the site of a thrombus that the defect is a prerequisite for thrombus formation, as has been claimed by authors who have studied initiation of thrombi.[8,40] In some instances, the defect might result from intraluminal platelet aggregation, rather than cause it.

A mechanism whereby platelet aggregates may induce vascular lesions could be the release of factors that cause increased permeability,[112,117] such as serotonin [46] and lysosomal enzymes.[14,89,94] Thus, it has been shown that serotonin may cause gaps between endothelial cells,[93] and that the lysosomes of leukocytes may be mediators of inflammatory reactions.[20,73,110] Local anoxia in the vessel wall underlying the mural thrombus may be an aggravating factor, at least in the later stages.

In the rabbit kidneys, the vascular injury also had the form of a focal, mostly segmental glomerulitis, affecting only a few glomeruli (Figure 7).[44,81] There were collapse of the glomerular loops, swelling and proliferation of glomerular cells, accumulation of leukocytes, and later adhesions between the loops and the Bowman's capsule. Focal glomerulitis was found in 32 of 41 rabbits that received ADP (78%) but significantly less often in the controls (Table 7). This observation forms a bridge between two apparently opposing concepts of the pathogenesis of focal glomerulonephritis; it is claimed on one side that the lesions are embolic,[9] and on the other side that they are allergic.[2] However, in this discussion it has not been recognized that complexing of antigens and antibodies in flowing blood leads to platelet aggregation.[70,71,109] The effect on the glomeruli may be similar, whether the platelet masses are derived from an endocardial thrombus or are formed in flowing blood.

In man, focal glomerulonephritis may be associated with hypertension.[60] However, focal glomerulitic lesions have been reported in many cases of primary hypertension.[15,88,97] The experiments with adenine nu-

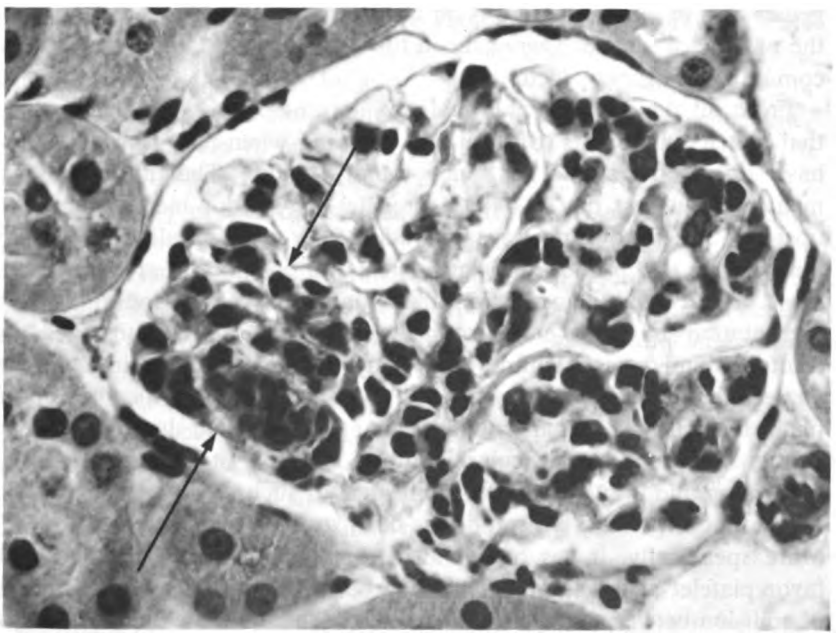

FIGURE 7 Focal glomerulitis 48 hr after ADP infusion. Between the arrows, the loops are collapsed and appear more cellular, partly because of leukocytic infiltration. Hematoxylin–phloxine–saffron. (× 745)

TABLE 7 Focal Glomerulitis in 80 Rabbits Killed 24 hr to 6 months after Infusion of Adenine Nucleotides or Ringer's Solution into the Aorta

Material Infused	No. Rabbits	Rabbits with Focal Glomerulitis [a]
ADP	41	32 (78.0%)
AMP	17	1 (5.9%)
Ringer's solution	16	5 (31.3%)
ADP, immediately after a preparatory infusion of ADP into an extrarenal arterial bed	6	0

[a] Difference in prevalence of glomerulitis between animals that received ADP once and animals that received AMP or ADP twice significant at $p < 0.001$; difference in prevalence of glomerulitis between animals that received ADP once and animals that received Ringer's solution significant at $p < 0.01$.

cleotide infusions into the aorta of rabbits [80,81] show that the two phenomena, elevation of blood pressure and focal glomerulitis, may be pathogenetically related. That these two phenomena often occur together also in human disease is an indirect indication that, even in man, the passing of platelet aggregates in the renal microcirculation may be a common pathogenetic factor.

From all the experimental evidence presented, it should be obvious that platelet aggregates in flowing blood, even when only transient, may have serious effects on organ function and structure. The autopsy studies mentioned suggest that platelet aggregates may occur independently of upstream sessile thrombi, even in man. One can speculate about the mechanisms that produce the aggregates. The possibilities are numerous; most of the experimental stimuli mentioned at the beginning of this presentation may also be operating spontaneously. Such substances as serotonin and catecholamines may play a role by enhancing platelet aggregation caused by other stimuli.[4,133,142] A preferential localization of platelet aggregates to particular vascular beds may be due to hemodynamic disturbances, particularly eddy formation at sites of stenosis, branching, and curvature. The significance of such flow disturbances for the formation of sessile thrombi has been stressed repeatedly.[28,31,33,77,111] More specifically, it has been shown that high velocity gradients will favor platelet aggregation.[29] This may be related to the greater chance of collision between the formed elements of the blood in such an irregular flow. Not only will this bring the formed elements close together, but they may be mechanically damaged. Because of their large volume and their fragility,[22] the red blood cells may be the most important elements

in this connection. Mechanically damaged erythrocytes release ADP,[57] which then aggregates the platelets. These mechanisms may not always lead to the formation of a sessile thrombus, but perhaps, or mainly, to platelet aggregation in flowing blood. However, at present these considerations can be only speculations, or at best a hypothesis for further work.

SUMMARY

Thrombosis is a dynamic process, as reflected by the great variability in thrombus structure. At the site of a growing thrombus, platelet aggregation and subsequent fragmentation of platelet aggregates take place. Platelet masses from an arterial thrombus are embolized into the peripheral arterial bed. Clinical and postmortem studies indicate that this occurs frequently in man and may be of importance for ischemic lesions in the brain, eyes, heart, and kidneys. It is probable that attacks of transient symptoms can be caused by microembolization from an upstream crumbling thrombus.

Platelet aggregates may also arise in flowing blood independently of an upstream thrombus. The possible stimuli for such aggregation are numerous. If the platelet aggregates are formed without significant stimulation of coagulation—e.g., by ADP—they may be reversible and break up rapidly. Autopsy studies in man suggest that platelet aggregates independent of upstream thrombi may form in local circulatory areas in association with ischemic disease of the heart. Experimentally, it has been shown in pigs and rabbits that even transient showers of platelet aggregates in the heart and kidney may cause infarction and damage to the vessels, resulting in mural thrombi and focal vasculitis. The latter observation indicates that intraluminal platelet aggregation may be the initial event in thrombosis, and adherence to the vessel wall a subsequent stage.

Platelet aggregates in the renal microcirculation, even when transient, may result in focal glomerulitis in rabbits and cause a rise in blood pressure.

The evidence presented shows that platelet aggregates in the microcirculation, even when transient, may have serious effects on organ function and structure.

The work reported here was supported by the Norwegian Research Council for Science and the Humanities, the Norwegian Council on Cardiovascular Diseases, grants MT–1309 and MA–1312 from the Medical Research Council of Canada, the Canadian Heart Foundation, the Ontario Heart Foundation, grants A–4964 and H–6912 from the National Institutes of Health, and Geigy Pharmaceuticals.

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Studies of Thromboembolism and Related Phenomena in the Microvascular System

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Investigations of thromboembolism in the microvascular systems of living subjects with simultaneous microscopic observation permit sequential and integrated study of the morphologic, biochemical, and physical components related directly or indirectly to this complex reaction. The complexity of thromboembolism in the total environment, however, constitutes a serious limitation. Difficulties are caused, for instance, by our inability to segregate the individual phases and quantify responses adequately. Nevertheless, such an easily observed microvascular system as that in the hamster cheek-pouch serves as an instructive, natural, *in vivo* test tube, as well as an experimental bridge linking *in vitro* work on blood clotting with that on thromboembolism in the intact organism. Furthermore, many different "living laboratories" are available for this type of approach, and the potential is considerable. The purpose of this report is to review the status of present research on various aspects of thromboembolism in the microvascular system. This will be done by presenting specific examples in each of the three major divisions noted above, taken, with the exception of that on the lung, from our own studies.

MORPHOLOGIC COMPONENTS

A simple anatomic–functional relationship to explore is that between the vascular pattern of a tissue or organ and the comparative freedom of its parenchymal cells from interruption of the local blood supply by thromboembolism. For example, the pattern in the hamster cheek-pouch consists of three peripheral networks: arterial, capillary, and venous, each apparently continuous within itself and connected to the next network by many vessels in parallel.[2] The arrangement ensures

an appreciable local collateral blood flow and potential delivery of blood to the proximity of the capillary bed at a relatively uniform high pressure. This type of pattern strongly suggests that, in the cheek-pouch and in other tissues with similar circuitry, including some with arteriovenous anastomoses, the vascular pattern is adapted to absorb a limited but as yet unknown number of microemboli and thrombi with minimal effect to the tissue and that the severity of the functional disturbance observed during thromboembolism is related to the degree of blockage of the larger vessels that supply the peripheral bed and to the metabolic requirements of the parenchymal cells. Similar vascular patterns (but, in most cases, with capillary bed bypasses) are also present in the peripheral vasculature of the mesentery, skeletal muscle, skin, musculature of gut, submucosa, and bulbar conjunctiva.[37] Quantitative information is sparse on vascular pattern and density, endothelial surface area, minute flow volume, and the metabolic requirements for integrated function of the tissue and survival of the cells in different tissues and organs.

In the lung, the vascular structure is of special interest, because it serves, not only as the primary filter for systemic venous blood, but also as part of the essential tonometer of the body. The distensibility of its vessels and the vast reserve of lung tissue provide a unique adaptation for the screening and sequestration of small emboli from the circulating blood. Nevertheless, unexpected, intense, sometimes fatal, and often unexplained crises can be evoked by comparatively few emboli. For this reason, detailed knowledge of the pulmonary arterial bed in man may be particularly relevant.

The pulmonary arterial vessels maintain a comparatively large diameter practically to the capillary bed. Lymph spaces around the arteries, but not arterioles, allow slippage between artery and parenchymal tissue.[16] In man (but not in some other species), the vascular smooth-muscle coat becomes discontinuous in distributing arterioles with outer diameters less than 70 μ and sparse or absent in vessels less than 40 μ in diameter.[33] Precapillary arterioles are sinusoid-like, arise at approximately right angles from the parent vessels, and may have diameters of 60 μ or more.[16,22,30] Smooth muscle is present only at the origin of the precapillary arteriole and may constitute a vascular sphincter.[16,21,22] Sphincter-like bands are also present at the origin of some arterioles from their small parent arteries (and at the confluences of some of the larger venules).[16] These sphincters and the contractility of small arteries are probably involved in the regulation of blood flow.[16] The abundance and distribution of the vasomotor innervation parallel that of the smooth muscle. The wide precapillary vessels lie between alveolar ducts and give rise to short branches about half the length of an alveolus and up

to three times the diameter of a pulmonary capillary. These branches divide into 12–20 afferent capillaries, 5–15 μ in diameter, that join the continuous alveolar capillary network. In man, collateral arterial circulation is not considered capable of compensating for acute blockage of the larger vessels.[16,33] However, the terminal arteriolar and collateral capillary circulation should be able to compensate for blockage of terminal arterioles. Arteriovenous anastomoses are present in the region of the bronchi and pleura [16,23,33] and may also be widely distributed throughout the peripheral bed [9] (V. E. Krahl, personal communication).

Microemboli tend to pass easily down the broad distributing arteries and arterioles and lodge in the blunt or rapidly tapering arteriolar ends called "catch-traps" by Knisely and Knisely.[20] In these bottlenecks, the emboli are mechanically buffeted and washed by the heavy flow of blood and subjected potentially to the chemical action of the components of the blood and vascular wall. This extreme localization of microemboli concomitant with good collateral circulation and redundancy of pulmonary tissue should minimize interference with the oxygenation of blood and the nutrition of parenchymal tissue, while providing a large safety factor in the form of a depository for circulating emboli. Nevertheless, this safety factor can be overwhelmed by great but widely varying numbers of small emboli and fewer large ones.[11,26,29,31]

Pathologic and experimental observations indicate that many embolizations that do not interfere directly with appreciable amounts of pulmonary tissue still may cause severe or fatal crises, in spite of the anatomic adaptations and vast reserve of the lungs. The explanation may involve the intense vasomotor response that sometimes accompanies embolization. Four categories of relevant experimental observations have been reported: (1) reflex vasoconstriction following impaction of emboli [11,25,27]; (2) reflex local vasoconstriction induced by hypoxia and unaffected by vagal section or excision of the stellate ganglion [15,33] but considered (by Stroud and Rahn [32]) to act, at least in part, through the sympathetic nervous system; (3) closure of muscular pulmonary arteriolar sphincters upon stimulation of the distal stump of the severed cervical vagus nerve,[21,22] with possible shunting of blood through local arteriovenous anastomoses (V. E. Krahl, personal communication), thereby bypassing the capillary bed; and (4) sphincter-like action of some capillary endothelial cell nuclei that protrude into the capillary lumen to various degrees (E. H. Bloch, personal communication). Reflex airway constriction has also been implicated.[25,26] The evoking stimuli and regulatory mechanisms of these reactions are still poorly understood. In the first two cases, the supporting evidence for the reactions is indirect, based on increased pulmonary arterial pressure, with the specific

sites of vasoconstriction undetermined. Vasoactive substances may also be liberated from emboli or the vessel wall or be formed locally in blood. Vasoconstriction and hyaline embolization have been observed in pulmonary vessels of living anesthetized mammals during anaphylaxis by Burrage and Irwin [9] and others.[10,18,19] Survival was associated with vascular dilatation, re-establishment of flow, and disappearance of hyaline emboli and red-cell masses. The presence of an antigen–antibody reaction and complexes, however, complicates the situation and does not permit unquestionable implication of any of the above potentiating mechanisms in other states of embolization. Detailed knowledge and control of the potentiating mechanism(s) could increase the efficiency of the lung as a filter by enhancing its ability to withstand pulmonary embolization and thereby to maintain adequate circulation and oxygenation of blood.

BIOCHEMICAL COMPONENTS

Studies of the interactions of the components of the microvascular system and blood in transilluminated living membranes have helped to clarify the relationship between platelet thrombosis and fibrin clot formation *in vivo*. In vessels that have been slightly injured (for example, by infusion of some plasma expanders, infection, necrotic tumors, administration of dicumarol or similar drugs, or "gentle" physical manipulation), white blood cells may adhere to, roll in excessive numbers along, or coat the endothelium, predominantly in venous vessels. This phenomenon is further favored by slow flow. More intense injury of the wall, by any means, precipitates the formation of a thrombus (consisting primarily of platelets) at the site of injury. Topical application of thrombin, Russell's viper venom, and ADP to uninjured vessels with flowing blood or exposure of the circulating blood to "collagenous" tissue also induces local, easily observable platelet thrombosis and not fibrin clot formation. These platelet thrombi establish conditions that favor fibrin clot formation by drastically curtailing flow or causing stasis. Fibrin clots may then form in the area of stasis. A wide margin of safety, however, exists between the formation of platelet thrombi and fibrin clots. Furthermore, if fibrin clots do form in stagnant blood in very small, intact vessels (as in the hamster cheek-pouch), their consistency is relatively loose. An area of thrombosis usually enlarges by the formation of additional platelet thrombi at junctions where stationary columns contact circulating blood. If the precipitating conditions are sufficiently intense, stoppage of flow with possible direct precipitation of a fibrin clot or denaturation of protein may occur without prior formation of occluding platelet thrombi.

In the microcirculation, venules are the type of blood vessels most susceptible to thrombosis; arterioles are next, and the capillaries appear to be the least susceptible.[5,6] High shear rates of flow may protect the arterioles and the capillaries, but the capillaries seem to have an additional protective factor. Recent findings reaffirm and supplement similar observations made before the turn of the century: blood normally remains fluid as long as it is in contact with an uninjured blood-vessel wall; injury to the wall, slow flow, and hypercoagulability (Virchow's triad) favor thromboembolism; and platelet thrombosis precedes fibrin clot formation *in vivo* and occurs during flow at points of vascular injury.[8,14,34] One should add to these generalizations the recent finding of Wessler [35,36] and Deykin [12] that some activated clotting factors are rapidly neutralized or removed from the circulation, and that stasis or near-stasis is needed to permit the local catalysis essential for the formation of a fibrin clot in larger vessels.

Elementary biochemical studies of thromboembolism may be made in membrane preparations. One approach is to titrate the reaction, using the platelet thrombus as an endpoint. In this manner, it is possible to evaluate quantitatively the effectiveness of various drugs as inhibitors of platelet thrombosis for the precipitating conditions. Two types of approach, other than bleeding times, have been developed: local application in increasing concentrations of a solution of a thrombotic agent, such as thrombin or ADP; and local injury of the vessel wall in a controlled and graded manner by means of a microelectrode or other agent.[1,6] In both, the conditions are carefully standardized. Anticoagulants or potential inhibitors of fibrin clot formation or platelet aggregation may be administered topically, systemically, or simultaneously by both routes. Such experiments have shown that present anticoagulant therapy can apparently provide adequate protection against fibrin clot formation and thrombosis but not against platelet thrombosis. Exorbitant concentrations of heparin (e.g., 1000 units/ml applied topically to the cheek-pouch of the hamster, or 500 or more units/100 g of body weight given intravenously) were required to alter the conditions significantly from normal for initiation of platelet thrombosis. More heparin was required to prevent or reduce thrombosis following electric injury of the vessel wall than thrombosis following topically applied thrombin. Although dicumarol and Sintrom, administered intraperitoneally for 5–12 days, extended the prothrombin time of the hamster, it did not alter the platelet thrombus threshold induced by electric stimulation.[1] Inhibitors of platelet aggregation induced by adenosine diphosphate (ADP), such as adenosine, adenosine monophosphate (AMP), and arginine methyl ester, can raise the electrical threshold required to produce thrombosis,

but they were not potent, even in pharmacologic concentrations.[3] Similar studies with fibrinolysins in the hamster indicated that little change occurs in the threshold of platelet thrombosis until the clotting factors are measurably depleted.[7]

Notable variations can be observed with the *in vivo* testing procedures. Some of these changes appear to be time-dependent. Figure 1 shows the effect on the electrode-induced platelet thrombus endpoint of varying the interval before testing after topical application of the test substance to the hamster cheek-pouch. (In these tests, a 1-msec square-wave pulse was delivered to the wall of venules in the hamster cheek-pouch with a microelectrode. After each stimulus, the electrode was moved upstream and the intensity of the pulse appropriately altered. The procedure was continued until a platelet thrombus could just be detected at a magnification of 120. The current of this threshold stimulus was taken as the endpoint.) Topical application of 1000 USP units of heparin (Panheparin, Abbott Laboratories) to carefully exposed blood vessels in the hamster cheek-pouch produced a progressive increase in the microelectrode threshold until an equilibrium state or plateau was evidently attained

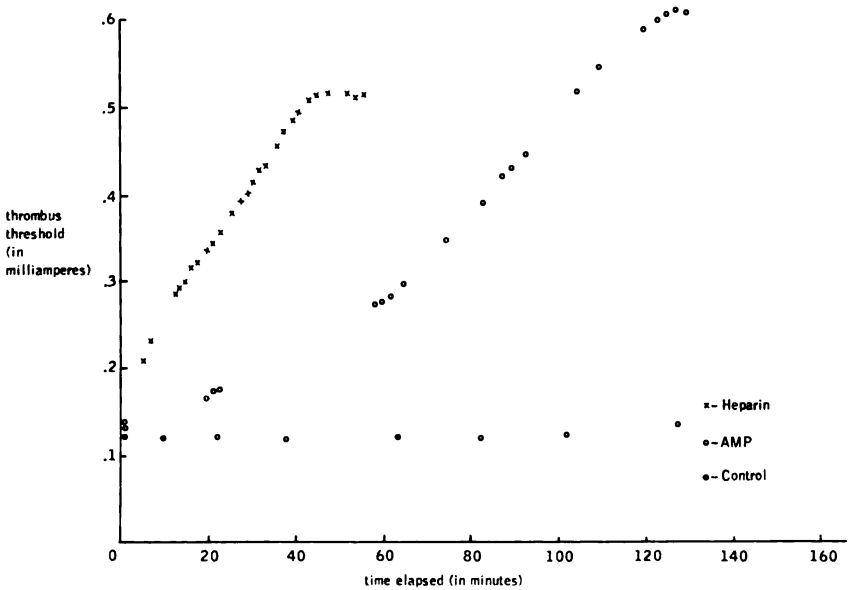

FIGURE 1 Changes with time in the minimal electric stimulus required for threshold thrombus formation after topical application of heparin (1000 units/ml) or AMP (0.1 M) to exposed vessels in the hamster cheek-pouch. Control, 0.9% saline.

after some 40–60 min. Such change probably depends in part on the penetration of the test substance into the dense connective tissue and cells surrounding the blood-vessel lumen, establishment of an effective concentration of the test substance at the local reactive sites, the specific initiating event, and the state of blood flow. Similar findings have also been observed after topical application of AMP (0.1 M, Sigma Chemical Company). However, here the increase in the threshold was more delayed and slower than in the case of heparin, in spite of the significantly lower molecular weight of AMP (347, in contrast with ~6000 for heparin). This slower increase could be due to the mode of inhibition and the higher concentration of AMP than heparin needed to suppress the reaction.

Variations in the testing procedure may also affect the experimental findings. When a square-wave pulse was applied for 30 msec and the endpoint was taken as a mural thrombus occupying about one fourth of the lumen of a venule, no change in the threshold current from normal was observed after topical application of heparin.[17] Here, the endpoint current intensity averaged 0.089 mA,[17] compared with 0.13 mA with the 1-msec pulse and smaller thrombus. The larger endpoint thrombus may be initiated by and follow a reaction (e.g., exposure to collagen) not operative (or much less effectively operative) under the originally described conditions.

The present *in vivo* findings reveal the inadequacy of known inhibitory agents for the prevention and treatment of platelet thrombosis *in vivo*. Clinically used anticoagulants have been found to be effective against fibrin clot formation but not platelet thrombosis. Several agents that inhibit ADP-induced platelet aggregation *in vitro* provide some protective effect *in vivo*, but again the protection is not appreciable and is noted only when high concentrations of the substances are present.

PHYSICAL COMPONENTS

Direct quantitative measurements of blood flow in individual vessels of the microvascular system are few. Except for broad generalizations (e.g., Virchow's triad), specific correlations of susceptibility to thrombosis with rate of blood flow have not been made. In general, accurate measurements of other physical characteristics of small blood vessels and their relationship to thromboembolism are even less available or nonexistent. For example, methods for measuring blood viscosity in small vessels *in vivo* are nonexistent, and procedures for assessing blood pres-

sure in arterial vessels less than ~25 μ in diameter in animals with rapid heart rates are questionable.[28,38]

An improved design [4] of a particle velocity meter [24] permits the direct determination of the velocity profile of red blood cells in small vessels 6–150 μ in diameter (without use and analysis of film). In transilluminated membranes (such as in the hamster cheek-pouch), profiles of blood flow have been determined in different types of vessels. From these data, the mean linear velocity ($\bar{V}$) of flow, flow volume (Q), and shear rates ($\dot{\gamma}_w$) can be calculated. If a viscosity (η) for blood is assumed, the shear stresses at the wall (τ_w) and the Reynolds number, $\mathrm{Re} = (\bar{V} \cdot D \cdot \rho)/\eta$, can also be calculated (Table 1). These data give a relatively close approximation of the rheologic characteristics of blood flow in the different types of vessels under conditions of anesthesia, except for very-small-diameter precapillary arterioles, capillaries, and postcapillary venules, in which sharp departures from Newtonian flow would be expected.

The particle velocity meter measures the linear velocity of streak-image. The technique is based on the fact that a fast particle, as it traverses the microscopic field under observation, appears as a streak to the observer. A prism placed in the optical axis of the microscope and rotated at right angles to the direction of flow deflects the original streak at an angle which represents the resultant vector of the rate of rotation of the prism and the linear velocity of the red cells. By adjusting the rate of rotation of the prism, the observer can bring the streak lines parallel to a fixed reference angle superimposed in the optical axis. The angular velocity of the prism at this point is directly proportional to the red-cell velocity. The system is calibrated by moving partially exposed, granular black-and-white film, while in sharp focus, at several known rates across the microscopic field at the same magnification used in the experimental work. The velocity profile is obtained by determining the flow in a number of narrow segments across the diameter of the blood vessel. The visual particle velocity meter (VPVM) consists of an eight-sided prism positioned between the field and eye lenses of an ocular. Two motors are attached to the prism. The number of revolutions per minute of a dc motor is adjusted by a variable dc power supply; this motor drives the prism. The second (unpowered ac) motor is used as a tachometer. The current generated by the rotation of the system, a function of revolutions per minute, is read on a microammeter, which, after suitable calibration, gives a measurement of corpuscular velocity.

With this system, Fuhro and I [4] measured the mean linear velocities in the different types of blood vessels in the cheek-pouch of the anesthetized hamster (Table 1). On the arterial side of the capillary net, the

TABLE 1 Rheologic Characteristics of Blood Flow in Different Types of Vessels in the Hamster Cheek-Pouch (Arithmetic Means)

Type of Vessel	No. Experiments	Diameter (D), μ	Mean Linear Velocity ($\bar{V}$), mm/sec	Flow Volume (Q), $mm^3 \times 10^{-3}$/sec	Shear Rate at Wall ($\dot{\gamma}_w$), sec^{-1}	Shear Stress at Wall (τ_w), dynes/cm^2 [a]	Reynolds Number [b]
Arterial	32	65	5.63	18.7×10^{-3}	693	8.3	1.8×10^{-1}
	60	39	4.38	5.2×10^{-3}	898	10.8	8.5×10^{-2}
	34	16	3.12	0.63×10^{-3}	1560	18.7	2.5×10^{-2}
Venous	37	40	1.59	2.0×10^{-3}	318	3.8	3.2×10^{-2}
	27	64	2.09	6.7×10^{-3}	261	3.1	6.7×10^{-2}

[a] Viscosity assumed to be 1.2 cP.
[b] Viscosity assumed to be 2.0 cP.

velocities averaged 5.6, 4.4, and 3.1 mm/sec in vessels with average diameters of 65 μ, 39 μ, and 16 μ, respectively. On the venous side, the mean linear velocities were 1.6 and 2.1 mm/sec in vessels with average diameters of 40 μ and 64 μ. The flow volumes calculated by $Q = \pi r^2 \overline{V}$ for these arterial vessels averaged 18.7, 5.2, and 0.63×10^{-3} mm^3/sec, respectively, and 2.0 and 6.7×10^{-3} mm^3/sec for the venous vessels. The shear rates at the wall, estimated from the relationship $\dot{\gamma}_w = 8\ \overline{V}/D$, assuming Newtonian flow, averaged 693, 898, and 1560 sec^{-1} in the arterial vessels, and 318 and 261 sec^{-1} in the venous vessels. The highest shear rates were observed in the smallest arterioles, indicating that these vessels provide the greatest average resistance to flow per unit length. At an assumed viscosity of blood at the vessel wall of 1.2 cP, essentially that of plasma, the comparable shear stresses at the wall were 8.3, 10.8, and 18.7 dynes/cm^2 in the arterial vessels and 3.8 and 3.1 dynes/cm^2 in the venous vessels. Assuming a viscosity of 2.0 cP, a similar calculation of the Reynolds number (Re), which is commonly used to characterize flow, gave values of 1.8×10^{-1}, 8.5×10^{-2}, 2.5×10^{-2}, 3.2×10^{-2}, and 6.7×10^{-2}, respectively. (A viscosity of 1.2 cP was assumed in the calculations of shear stress because, if a plasma layer exists along the wall, its viscosity may closely approach that of plasma measured *in vitro*. A viscosity of 2.0 cP was assumed for the calculations of the Reynolds numbers because the viscosity in this case would probably reflect that of the "bulk" phase. However, the viscosity of blood should vary widely in these vessels.) The shapes of the velocity profiles varied with shear rates, being flat at low values and tending toward a paraboloid as the shear rate in these blood vessels increased.

From these data obtained for blood flow in vessels in the cheek-pouch of normal hamsters anesthetized with Nembutal sodium, it may be concluded that blood flow in small vessels is boundary flow characterized by a low Reynolds number and by shearing rates or stresses along the arterial wall about 2.8 times as great as those in venous vessels of comparable diameter.

The *in vitro* findings of Dintenfass [13] made in viscosimeters may be relevant here with respect to the importance of vigorous blood flow and high shearing forces at the wall. At zero or very low shear rates, a solid fibrin clot formed with a viscosity, or firmness, of 100 P or more. As the clotting blood was subjected to greater and greater shearing forces or velocity gradients, the viscosity of the ensuing clot decreased. Finally, at shear rates of 400 sec^{-1}, even though fibrin formed, no visible change occurred in the viscosity of the blood, thus showing that high rates of shear will prevent the formation of firm fibrin clots *in vitro*. Based on *in vitro* work, Dintenfass also reported that an increase in the rate of

shear favored platelet aggregation, and therefore, potentially, platelet thrombosis. As previously noted, we observed that topical application of thrombin, an incomplete thromboplastin (Russell's viper venom), and other types of initiating stimuli produced platelet thrombosis under conditions of blood flow sufficient to occlude the vessel and predispose to fibrin clot formation in the columns of stagnant blood.[6] Great difficulty was encountered in the production of firm fibrin clots in the microvascular system, whereas platelet thromboses were induced with comparative ease.

The work reported here was supported by Public Health Service grant HE 00902 from the National Heart Institute and contract DA-49-193-MD-2696 from the Office of the Surgeon General, Department of the Army.

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Appraisal

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Excellent summaries of current work and ideas have been given on the many factors that determine the initiation and growth of a thrombus. The growth phase of the thrombotic process appears best understood. This phase is a dynamic one, with a rich interplay among several elements—the altered blood vessel, the altered plasmatic and cellular elements of the blood, and the altered circulatory dynamics. The thrombotic process is obviously more complicated than any one of the elements contributing to it. And each of the elements—the nature of cellular injury and related tissue changes, the sequences in the fibrin-clotting mechanism, the basic mechanisms of the adhesion and aggregation of platelets and other blood cells, and the rheology of the blood—is complex in its own right. The term, "converging complexities," is apt.

At this stage of development of knowledge, it does not appear possible to propound a well-founded statement of the order or the exact nature of the cellular and biochemical mechanisms that provide the seedbed in which a thrombus will be started. The pathophysiologic processes leading to thrombosis appear to be much the same throughout the circulatory system, whether in the veins, in the heart, in the arteries, or in the microcirculation—or even, for that matter, on prosthetic surfaces, whether vascular, valvular, or cardiac. There may be quantitative but not significant qualitative differences at these various sites. An example of the former would be the exaggerated role of the fibrinolytic process in the microcirculation. If all these various assumptions are correct, more detailed study of the different blood and tissue systems that contribute to thrombosis is urgent, if a fuller understanding is to be attained and if a rational control program in recognition, management, and prevention of thrombosis is to be developed.

Of the various systems concerned in the thrombotic process, knowl-

edge of blood coagulation or fibrin formation appears most advanced. The vicissitudes of fate favored the development of better understanding of clotting with the creation of the several genetic defects—the bleeder states—that helped to unravel many of the component enzyme systems. Further progress in validating the hypotheses now proposed, such as the waterfall or cascade theory of clotting, appears more complicated; but, if one can consider the fibrinogen story, with the elegant work on its structure and degradation, as a model that can be emulated, one can anticipate more definitive work on the nature of the plasma procoagulants and their action. The fact that the plasma procoagulants, unlike fibrinogen, are trace proteins will probably not be helpful. In direct relation to thrombosis, there are families with thrombophilia, some of whose members are prone to have recurrent thrombotic and embolic episodes that may be fatal. The detailed study of such families may throw light on the relation of procoagulant excesses, as well as inhibitor deficits, to the pathogenesis of thrombosis.

Cellular clotting or platelet aggregation appears to be even more important than fibrin clotting in the initiation of thrombosis. In the development of thrombi, platelets appear before fibrin. In cardiac and arterial thrombi, platelets may contribute significantly to the growth and total mass of the thrombus. In lower animals, cellular clotting is the main or only mechanism by which hemostasis is attained. But once a clottable protein is present in plasma, cellular clotting appears to be inextricably tied up with fibrin clotting, both biochemically by furnishing lipoproteins and perhaps activated enzymes, and physically by furnishing surfaces and structures to which the fibrin fibrils are attached. The platelet is being studied intensively as a cell. Much the same as with other cells, there are problems in cell respiration and energy metabolism, in the nature of cellular membranes, and in contractility, as well as in intercellular organization with adherence to other platelets, other cells, and various surfaces.

A new order of complexity, cell biology, has been reached. New technologies, such as cell electrophoresis and the study of histochemical ultrastructure, are being applied to the study of the platelet. The ultralocalization of platelet constituents, such as lipids, proenzymes, and thrombosthenin, is a current topic of study. The mechanism of the control of alterations in the cell membrane may be the most important aspect of platelet change, in that this may be the attribute that permits platelets to be actively incorporated into the growing thrombus. All these things undoubtedly need to be well understood if all the steps and pathways, both chemical and structural, in the initiation and growth of a thrombus are to be well enough appreciated to allow effective control of the platelet component of thrombosis.

Injury to the vessel wall, with the concomitant pathologic alterations, may initiate the first of a series of events that culminate in thrombosis. One is struck with that wonderful lining material of the vascular system, the endothelium, and the possibility that endothelial alterations may trigger the thrombotic process. The endothelium normally permits such a nice relationship between the blood and the tissues, allowing the blood to remain fluid—whether rapidly flowing, as in the arteries, or almost stagnant, as in the sinusoids of the spleen. Is it possible to ascertain and duplicate the qualities of the vascular lining that make it nonthrombogenic? If so, our search for nonthrombogenic vascular prostheses may soon be over. Opposed to the nonthrombogenic character of the endothelium is the thrombosis-promoting character of the deeper structures of the vessel wall, particularly the basement membrane and the collagenous and possibly other tissue components. What are the qualities of these tissue elements that make them thrombogenic? Apparently one is faced with a still higher order of complexity, dealing basically with tissue organization and functional changes with injury. More advanced technologies probably need to be applied, if one is to delineate the physiologic and pathologic characteristics of the constituents of the vessel wall that promote one or another of the thrombotic sequences.

The contribution of changes in blood flow to thrombosis is real, and they are becoming increasingly measurable. Physicists have enunciated various principles of rheology and the special characteristics of the blood with its non-Newtonian flow properties and the related changes in blood viscosity. It would appear that altered rheology abets the thrombotic process mainly in two ways: by amplifying the enzymatic and cellular reactions important in thrombogenesis and by producing further injury to the vessel wall. In regard to amplification, stasis appears to act in part by preventing dilution of thrombin and other procoagulant enzymes that contribute to cellular and fibrin clotting. The increase in viscosity that occurs as flow rate decreases may partially counterbalance this effect if it reduces molecular and cellular collisions in the blood. Turbulence and the related loss of laminar flow could likewise result in amplification of some reactions; this is perhaps analogous to accelerating a chemical reaction in a test tube by stirring vigorously with a glass rod. Cellular injury to the vessel wall may be extended by rheologic alterations associated with thrombi. Thus, with focal narrowing of a vessel lumen by a thrombus, the Venturi effect and jet streams may result in further damage to the vessel wall. Reduced flow may directly cause further vascular damage, owing to inadequate local circulation. Extension of the cellular injury, in effect, may create a vicious circle, providing new foci for thrombus formation on the adjacent vascular surfaces.

Thus, many mechanisms appear to contribute to the development of a

thrombus. But which mechanism is primary, which are secondary? What are the sequences and interrelationships of the different mechanisms? The few models currently proposed appear to need modification, and better models need to be developed. Faced with the ubiquity of thrombosis and its importance in innumerable disease processes, can we do anything, in addition to continuing to contribute as individual scientists to the normal evolution of knowledge and new applications of old knowledge? The need for more complete delineation of each element of the thrombotic process at the basic level is self-evident. Are any facets of the process far enough advanced that a programmed approach would be profitable? In 1952, M. C. Winternitz, then Chairman of the Division of Medical Sciences of the National Research Council, asked a small group of coagulationists (W. H. Seegers, B. Alexander, and myself) to explore the possibility of a programmed approach to the question: "Can a blood test be devised that would allow a clinician to determine whether or not a thrombus exists in the body?" Serious attention was given to this question for many years, and much was learned. But the answer to the question after much effort, as is well known, was at best equivocal. Only recently has a shortened partial thromboplastin time been emphasized as being related to hypercoagulability and possibly to thrombosis. Much has happened since 1952, and it may well be timely to ask ourselves once again whether a planned approach to some segments of the thrombotic process would be more profitable than in the past. It does appear that, if the questions are wisely chosen and relatively specific, some aspects of the dilemma of thrombosis could be resolved relatively promptly, thereby providing earlier availability of information required to control this pathologic process. It is hoped that both programmed research in applied and developmental problems and individual and group research to acquire much-needed basic information will be fully supported in the coming years. This appears to be the only way to advance the field fully in the immediate future.

V
HYPERCOAGULABILITY AND FIBRINOLYSIS

Blood States That Predispose to Thrombosis

GEORGE D. PENICK

Of the three long-accepted basic disturbances that may lead to thrombosis—stasis, vascular disease, and alterations of the circulating blood—changes in the blood seem to have been regarded with the least favor. A large and often conflicting body of investigative data on blood changes has accumulated, in part because of the ease with which the bloodstream can be tapped for sampling or modified by injection. Furthermore, in this day of laboratory medicine, there is an almost compulsive clamor to discover a "blood test" that might warn a patient and his physician of impending thrombosis.

The available techniques have largely dictated which blood component would be studied in relation to thrombotic tendencies (Table 1). Light microscopy made it possible to see and count the cellular elements. Polycythemia vera, characterized by an increased number of circulating erythrocytes, has long been known to be associated with an increased incidence of thrombosis.[54] Also, the bizarre shapes assumed by red cells in sickle-cell disease alter the ability of the cells to flow over surfaces and over one another. The resulting blood-flow disturbances facilitate thrombosis and the infarction of many tissues.[85]

Hemolysis of blood cells may be associated with intravascular thrombosis. Some of the earliest studies of the effects of lysed blood revealed that intravascular clotting need not be invariably associated with focal thrombosis but can be manifested as a more diffuse type of reaction.[86] Any fibrin that is formed is promptly removed by such defense mechanisms as phagocytosis [6,24] and lysis.[50] It is necessary in our consideration of blood states that predispose to thrombosis to include the development of diffuse intravascular coagulation as an equivalent end-result of the fundamental blood disturbances. Originally, fibrinogen depletion was considered the hallmark of this reaction, and the term "defibrination"

TABLE 1 Blood States That Predispose to Thrombosis

Disturbances of cellular components
Platelets
Thrombocytosis
Enhanced adhesiveness
Leukocytes
Erythrocytes
Polycythemia
Hemolytic reactions
Disturbances of plasma components ("hypercoagulability")
Elevated levels of procoagulants
Depressed levels of inhibitors

has been widely adopted.[74] In the 1950's, it was realized that other factors were also consumed during intravascular coagulation. For instance, platelets, prothrombin, and factor VIII could also be depressed in the blood of experimental animals by injecting tissue thromboplastin.[65] Other consumable factors were also shown to be inactivated in conditions in which intravascular coagulation may occur.[10,40] A wide variety of diseases has now been associated with intravascular coagulation.[30,49,63,75] Whether thrombi result depends largely on a combination of local factors, such as stasis and endothelial damage. It was recognized recently, for example, that malaria can be accompanied by massive hemolysis and manifested by diffuse intravascular clotting or thrombosis. Such patients show a significant depletion of factors V, VIII, and occasionally X, and respond favorably to heparin therapy.[14]

Thrombosis may also complicate an increase in cellular elements other than erythrocytes. Thrombocytosis may follow splenectomy [34,71] and has been seen, with multiple arterial thromboses, in congenital absence of the spleen.[27] Primary thrombocytosis is even said to have been causally related to myocardial infarction in an 8-year-old child.[78]

In the last couple of decades, various techniques have been devised to measure the tendency of platelets to adhere to surfaces or to one another. Recently, it has been recognized that platelet aggregation within the microcirculation can produce lethal cellular damage, even if the aggregation is reversible and the interruption of blood flow transitory.[28] Increased platelet adhesiveness has been observed in many different diseases and is considered in detail in other portions of this volume.

An increase in the number of white cells may also be associated with thrombosis or with acquired hypofibrinogenemia. This relationship may be seen in a variety of cases of leukemia,[33] but especially in acute pro-

myelocytic leukemia.[59,72] In some instances, heparin therapy may eliminate the apparent intravascular coagulation.[5]

A more debatable category of blood disturbances that may predispose to thrombosis includes alterations of the plasma components of the blood coagulation system. Theoretically, just as insufficient quantities of blood-clotting factors may establish a hemorrhagic tendency, an opposite disturbance may enhance a tendency toward intravascular coagulation. The latter condition has been repeatedly referred to as "hypercoagulability." This term has become popular during a period of active experimental investigations of *in vivo* coagulation, as well as during widespread studies of clinical conditions associated with thrombosis or "disseminated intravascular coagulation." [30,49,75] As a consequence, the term "hypercoagulability" has acquired such a diversity of connotations that the original concept has become clouded and its significance disparaged. Specifically, the term has been applied to: (1) changes in the concentrations of clotting factors and/or fibrinolytic factors such that *in vitro* clotting tests are accelerated [36,77,79]; (2) a stage of intravascular coagulation during which clotting factors are activated [8,52]; and (3) the experimental initiation of intravascular coagulation by injecting thromboplastin,[39,55,65,67] thrombin,[42,48,66,81] activated clotting factors,[3,15] etc. It is only in the first sense that the term will be used in this paper (i.e., to mean altered levels of procoagulant or inhibitory factors), and only in relation to clinical entities in which an increased incidence of thrombosis has been observed. It is emphasized that the plasma concentration disturbances are thought to *predispose* to intravascular coagulation under appropriate *initiating* circumstances. The resultant intravascular coagulation can be manifested in either (1) the production of visible clots or thrombi or (2) systemic coagulation and defibrination.

Increased concentrations of many of the plasma procoagulants have been incriminated in thrombophilic states. Elevation of fibrinogen appears to be a basic reaction of the body to inflammatory states [29,47] and may predispose to thrombosis largely by increasing the viscosity of the blood.[22] As with a number of the procoagulants, it is frequently difficult to tell whether an infarctive lesion is the result or the cause of the increased concentration. It has recently been suggested that fibrinogen may at times be qualitatively altered so as to increase its sensitivity to thrombin and thereby facilitate the development of thrombi.[18]

One of the earlier attempts to correlate clotting-factor changes with thrombotic disease demonstrated that about 88% of patients with thromboembolic disorders had elevated levels of factor V (called "Ac-globulin" at the time).[56] Since then, elevated levels of factor V have been observed during the puerperium [11] and in experimental intestinal obstruction.[31]

Recently, a high incidence of thrombosis has been described in members of a family whose plasma contained high levels of factor V.[26]

Factors IX,[73,82] VII, [2,7,35,58,73] and X [35,58,73] have all been measured in greater than normal amounts in a number of conditions related to clinical thromboembolism.

The acceleration of clotting and cellular agglutination by plasma lipids must be included in a survey of blood changes that predispose to thrombosis, but the effects of lipemia are presented in other sections of this volume. Similarly, a detailed discussion of increased inhibition of the fibrinolytic reactions that might enhance thrombogenesis is not presented here. Increased antiplasmin activity [53] and increased inhibition of tissue activator [9] have been so incriminated, as has the administration of exogenous fibrinolytic inhibitors, such as epsilon-aminocaproic acid.[51]

Increases in the plasma levels of the procoagulant factor VIII have perhaps attracted as much attention and debate as any of the blood disturbances thought to be associated with hypercoagulability. Of the many diseases with increased factor VIII activity, some appear to be accompanied by tissue necrosis, fever, or both. Levels up to 1600% have been reported in patients with massive liver necrosis.[80] Renal disease, which in its terminal uremic stage is more classically associated with a hemorrhagic tendency, may at times be accompanied by high levels of factor VIII activity and thrombotic predisposition.[16,61,64]

A second group of conditions associated with elevated factor VIII levels have endocrine imbalance as a common denominator, such as diabetes,[19] hyperthyroidism,[20] Cushing's syndrome,[76] and pregnancy.[43] A body of experimental data is accumulating that suggests that levels of factor VIII within the circulating blood may be in part under hormonal control. This subject has been thoroughly investigated and reviewed by Ingram.[37] There is evidence that oral contraceptives raise the levels of circulating factor VIII.[21] Factor VIII is usually increased in the bloodstream after infusions of epinephrine,[38] unless the spleen has been previously removed.[46] In recent organ-perfusion experiments, there was suggestive evidence of a relationship between epinephrine and release of factor VIII from the isolated normal canine spleen [84] (Figure 1). Similar response to epinephrine has been observed after transplantation of normal canine spleens to hemophilic dogs.[83]

It is in relation to factor VIII elevations, however, that an extra note of caution must be sounded about the interpretation of the increased activity observed in *in vitro* assays. Most current methods of measurement are based on biologic activity in clotting systems, and it is well known that factor VIII can be activated by trace amounts of thrombin [57,60,68] and thereby appear to be present at high levels. Blood samples

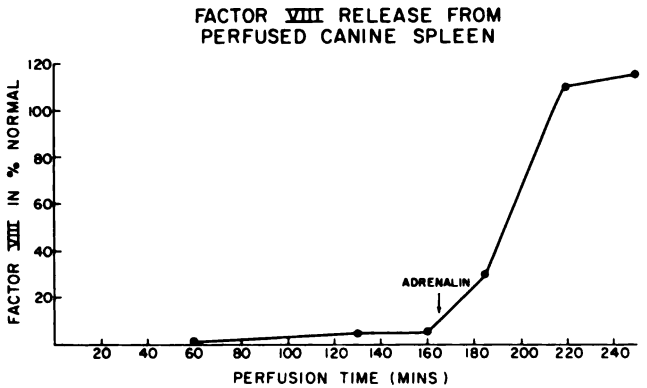

FIGURE 1 Isolated normal canine spleen perfused with oxygenated hemophilic canine blood. Factor VIII assayed [45] and expressed in terms of percent of normal canine plasma activity.

removed from patients during a stage of active defibrination may appear to have very high levels of factor VIII activity before inactivation occurs.[52] However, there is considerable evidence that the high levels of factor VIII that appear after infusion of epinephrine, and that are seen in patients with renal disease and in members of families with familial thrombophilia, are true increases of factor VIII.[61,62] For example, plasma from one of these patients with high levels of factor VIII has a proportionally increased effectiveness when transfused into a patient with classic hemophilia.[64]

One of the most outstanding associations of a thrombotic tendency and a disturbance of plasma components is that seen in familial antithrombin deficiency.[17,41] In a case recently observed by Dejanov *et al.*,[13] a 29-year-old woman developed bilateral thrombophlebitis, pulmonary embolism, and retinal infarction. Many members of her family had also experienced thromboembolic episodes. Her nonidentical twin sister died at the age of 28 years, after a pregnancy that was accompanied by many thromboembolic complications. Their brother developed spontaneous thrombophlebitis at the age of only 17 years, and died from massive pulmonary embolism. The patient's father, paternal grandfather, and great grandfather also suffered from thromboembolic disease.

The results of assays of the patient's blood-clotting factors are seen in Figure 2. All values were normal, except those of antithrombin. Antithrombin III, progressive antithrombin, was measured on two occasions as 44%. Antithrombin II, heparin cofactor, was measured at 25%. When her plasma was filtered through Sephadex G-200 (Fig-

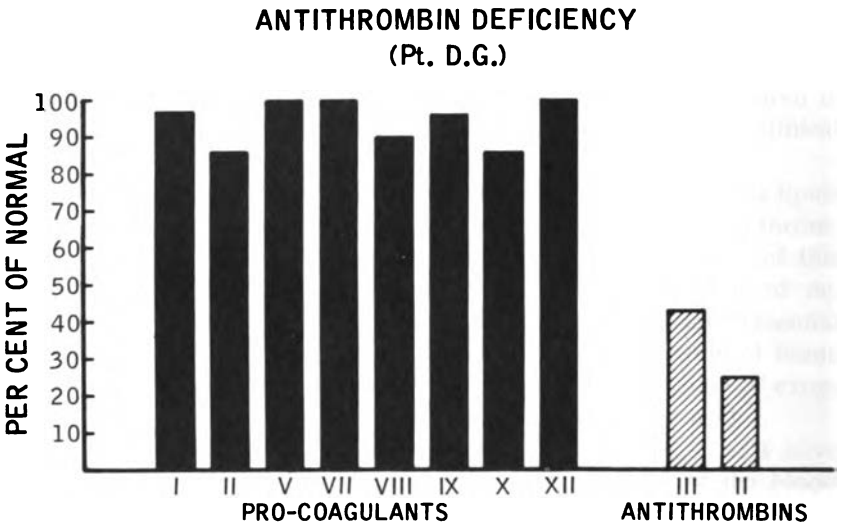

FIGURE 2 Coagulation factors in patient's plasma (see text). Procoagulants were measured by previously described methods.[70] Antithrombin II was determined by the method of Egeberg,[17] and antithrombin III by the methods of Astrup and Darling [4] and Hensen and Loeliger.[32]

ure 3A), two distinct peaks of progressive antithrombic activity were separated: the activity in the first peak was comparable with that of normal plasma (Figure 3B); the second peak of progressive antithrombin activity was lower than that of control plasma. The antithrombin II activity of the patient's plasma (Figure 4A) was shown to be considerably less than that of control plasma (Figure 4B).

The deficient antithrombic activity revealed by these functional assays was confirmed by demonstrating diminished material capable of reacting with a specific antiserum to antithrombin. The specific antiserum was prepared by injecting into rabbits a purified protein that had both antithrombin II and antithrombin III activity.[12] By simple immunodiffusion, antithrombin II and III were both found to be about 25% of the levels in normal plasma.

It is known that several proteinase inhibitors of plasma, including α_2-macroglobulin and α_1-antitrypsin, may contribute to the progressive inhibition of thrombin.[1,44,69] Using immunoelectrophoresis, the patient's plasma was shown to contain a normal quantity of α_1-antitrypsin, and, conversely, the plasma of a patient with α_1-antitrypsin deficiency contained normal amounts of antithrombin II and III. Inasmuch as these other antithrombins do not exhibit heparin cofactor activity, the mea-

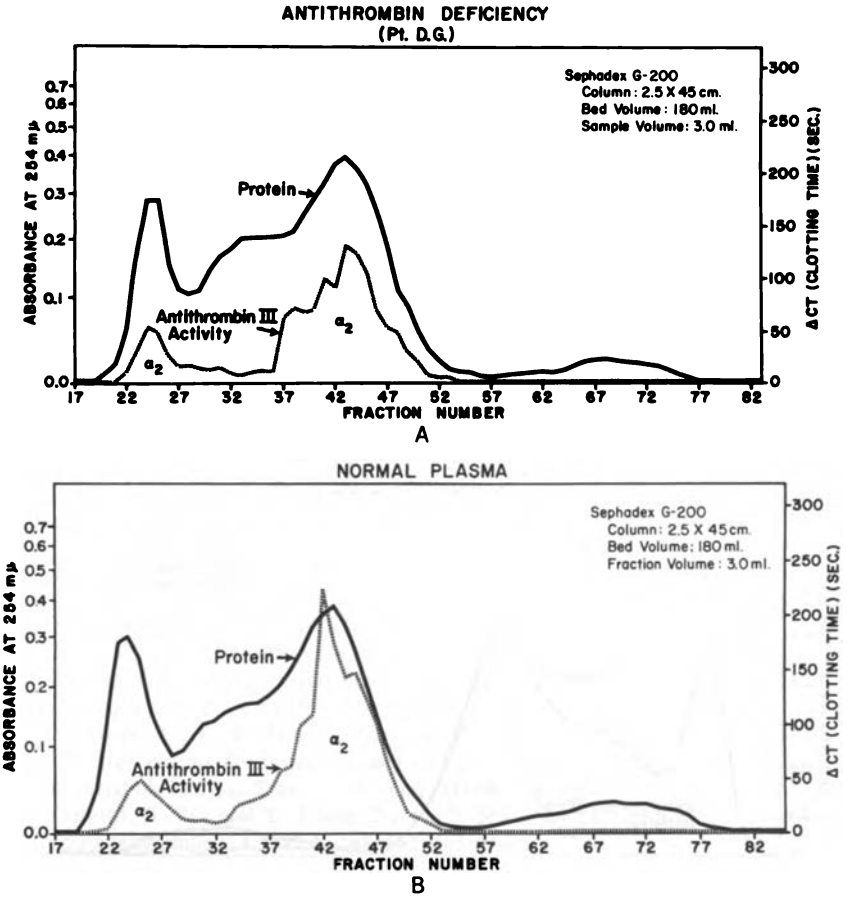

FIGURE 3 Separation of antithrombin III activity by gelfiltration. (See text.)

surements of antithrombin II in this patient appeared to be the more accurate index of her plasma disturbance.

These results suggest that the higher levels of antithrombin III originally detected in this patient's plasma by functional tests may be accounted for by other antithrombic substances. Furthermore, the *in vivo* effectiveness of antithrombin in the prevention of thrombosis may be related almost entirely to the relatively small fraction of the antithrombin complex measured by these specific techniques. This is dramatically indicated by the pronounced thrombophilia in this patient and her family, in contrast with the apparent absence of thrombosis in patients with deficiency of α_1-antitrypsin.[23,25]

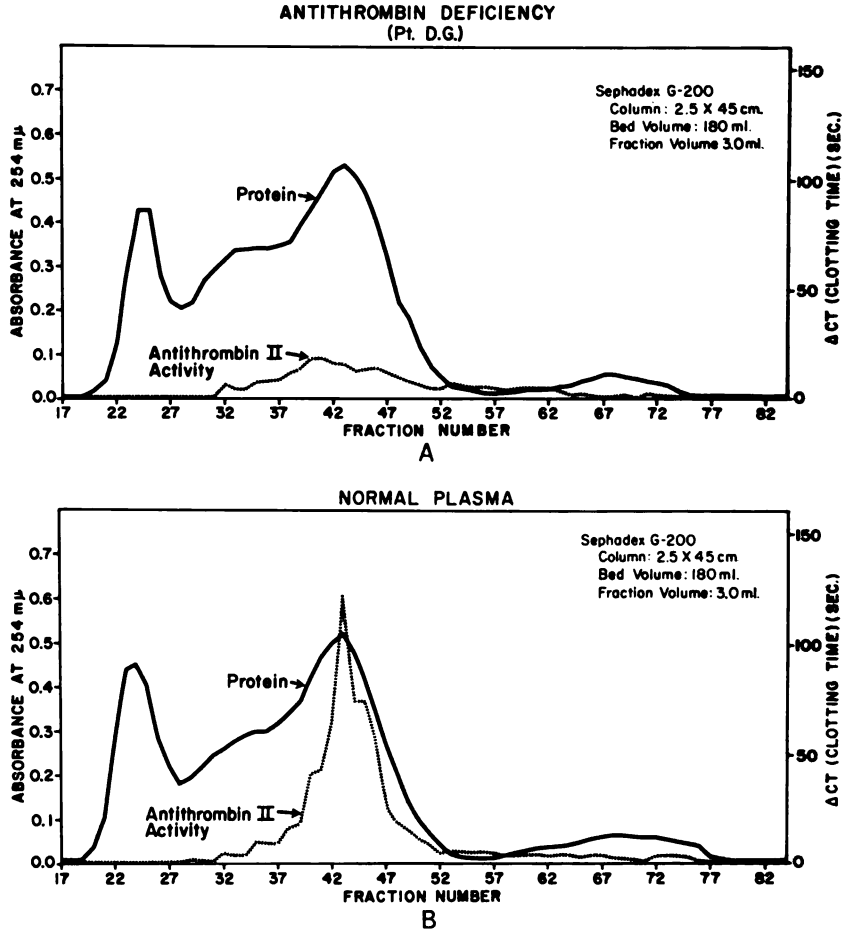

FIGURE 4 Separation of antithrombin II activity by gelfiltration. (See text.)

In summary, the disturbances in the circulating blood that may be associated with an increased tendency to thrombosis are diverse and may be reflected in a wide variety of test procedures. Nevertheless, the large number of conditions in which there seems to be enhanced cellular aggregation or accelerated fibrin formation, occurring in association with clinical thrombosis, makes it difficult to ignore hypercoagulability as a significant factor in thromboembolic disease.

The work reported here was supported in part by Public Health Service grants HE 06350 and HE 01648 from the National Heart Institute and FR-46 from the

General Clinical Research Centers Branch of the Division of Research Facilities and Resources.

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Discrimination between Normals and Patients with Venous Thrombosis Based on Blood Tests

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Diagnosis in thromboembolism is inaccurate. This handicaps clinical management, the development of new therapy, and the accumulation of valid statistics. More accurate diagnosis is possible with phlebograms, but they are not well suited to screening a large number of patients. Blood tests that correlate well with venous thrombosis would represent a more convenient screening method. In 1966 [3] and 1967,[5] we reported that discrimination between patients with and without venous thrombosis was possible on the basis of some blood tests. Multivariate analysis of the blood-test variables is presented in this report. Inasmuch as no one who had previously had thromboembolism or any condition frequently associated with it was included in the control group, the control group was not representative of the entire population at risk. A second series of patients was studied; the control group included patients with many types of acute and chronic illness, so that the risk of thrombosis was comparable with that encountered generally in hospitalized patients. The findings in the second series are presented here, and the use of blood tests as an aid to diagnosis in thromboembolism is discussed.

METHODS

Twenty-nine patients with a hospital-admission diagnosis of venous thrombosis were included in the study with 29 controls admitted for elective surgery or diagnosis. Blood samples were taken on admission and before specific treatment for venous thrombosis. The methods of collecting the blood and performing the tests have been described previously.[3,5] The following tests were performed:

1. fibrinogen (Fg), in milligrams %;
2. maximum amplitude (MA) of the thromboelastogram, in millimeters;
3. direct platelet count (PC), in thousands, by phase microscopy;
4. hematocrit (Hct), in %;
5. partial thromboplastin time (PTT), in seconds, using cephalin–celite reagent and plasma diluted 1:8 with veronal buffer;
6. platelet entrapment in glass spheres (PEGS),[2] as % of platelets that remain in a column containing 1 g of glass spheres through which 1 ml of native blood flows by gravity, by a method adapted from that of Hellem and of Salzman; and
7. transit time (Flow), as time, in seconds, for 1 ml of blood to flow through the column.

RESULTS

Table 1 presents the means, standard deviations (S.D.), standard errors (S.E.), and coefficients of variation (C.V.) of seven blood tests. Except for Flow, all mean values were significantly different in the two groups.

On the one hand, for laboratory efficiency and economy, a large prospective study should include as few tests as possible. On the other hand, one is inclined to consider as many tests as possible, in order not to overlook any characteristic useful for discrimination. A compromise is to select a subset of tests that includes all those which contribute significantly to the improvement of discrimination.

Stepwise regression analysis provides such a subset of variables.

TABLE 1 Summary of Results of Blood Tests

	Control (29 Cases)				Thrombosis (29 Cases)				
Variable	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.	t [a]
PEGS	38.7	4.4	0.8	0.11	45.7	3.1	0.6	0.07	−7.13
Hct	41.9	3.5	0.6	0.08	38.9	4.6	0.8	0.12	2.82
PTT	110.4	11.1	2.0	0.10	99.1	12.5	2.3	0.13	3.63
PC	173.9	43.3	8.0	0.25	198.3	43.3	8.0	0.22	−2.14
Flow	47.4	8.3	1.5	0.17	44.7	9.0	1.7	0.20	1.20
MA	51.9	5.3	1.0	0.10	56.7	7.2	1.3	0.13	−2.84
Fg	343.1	69.4	12.9	0.20	392.1	100.5	18.7	0.26	−2.15

[a] The means between groups are significantly different at the 5% level of significance if $|t_{0.975(56)}| > 2.004$, where t is the test statistic that follows a Student t distribution.

Table 2 presents the results of such analysis. The program for stepwise regression was taken from BMDO2R (UCLA Health Sciences Computing Facility, 1965). The first three tests—PEGS, Hct, and PTT—account for 68% of the variation. The other four tests can account for only an additional 2% of the total variation.

The first three variables selected by stepwise regression are used to obtain the discriminant function. The purpose in using a discriminant analysis is to find a set of coefficients for the set of variables, such that the resultant index, Y, obtained has the maximum discriminating power between the two groups. It is achieved if the ratio of (1) the difference between the mean indices for the two groups to (2) the pooled standard deviation of the indices is maximized. The program for such a purpose is available at the Yale Computer Center (Memorandum 44S, 1965). The result is as follows:

$$Y = -1.4703 \text{ PEGS} + 0.2112 \text{ PTT} + 1.000 \text{ Hct}.$$

The value of Y for any person is obtained by substituting the set of values of PEGS, PTT, and Hct in the above equation. The critical value of Y to classify a person in this series into the control or thrombosis group is 0.4817. In Table 3, the two groups are arranged according to the values of the discriminant function, and misclassified cases are noted. Only six cases (10.3%) were misclassified. If all seven blood tests were used, the number of misclassified cases would be reduced to five—a negligible improvement.

The steps of selection of variables and discriminant analysis can be performed in a combined operation if the stepwise discriminant analysis was carried out. The stepwise discriminant analysis is the same as the

TABLE 2 Results from Stepwise Regression Analysis

Variable	Regression Coefficient	Multiple Corr. Coeff., R	R^2	Increase in R^2	F Value to Enter
PEGS	−0.0647 *	0.6892	0.4750	0.4750	50.6658 *
Hct	0.0405 *	0.7936	0.6297	0.1548	22.9882 *
PTT	0.0090 *	0.8269	0.6838	0.0540	9.2275 *
PC	−0.0014	0.8341	0.6957	0.0119	2.0681
Flow	0.0053	0.8377	0.7018	0.0061	1.0648
MA	0.0073	0.8397	0.7051	0.0033	0.5689
Fg	−0.0003	0.8407	0.7067	0.0016	0.2810
Intercept	0.3885				

* Significant at the 5% level.

discriminant analysis, but the selection of variables included in the discriminant function is carried out in stepwise fashion. The results obtained from this procedure are the same as before. The computer program for the stepwise discriminant analysis is BMDO7M, which is available at the UCLA Health Sciences Computing Facility.

In an effort to make the control group more representative of all hospitalized patients, a second series was collected in which a variety of acute and chronic illnesses were present among the 37 control cases. Twenty-two patients with venous thrombosis were examined concurrently. The same three blood tests were of significance for discrimination according to the stepwise regression analysis. By calculating discriminant function, we misclassified 16 cases in this series (27%).

Items in the medical history were reviewed. Age, sex, obesity, tobacco consumption, family history of thrombosis, pregnancy, drugs taken, trauma or operation, arterial occlusion, heart disease, cancer, diabetes, and gout were considered, using quantitative scoring, when possible, and semiquantitative scoring for the qualitative variables. No significant difference was noted between the groups with regard to these items. But significant differences were observed in regard to two variables in the history: previous thromboembolism and immobilization. Taking these two additional variables with the laboratory data and calculating a new discriminant function, we misclassified only 10 cases (17%).

DISCUSSION

The results of the first series suggest that a factor (or factors) can be measured in the blood to permit successful discrimination between venous thrombosis and low-risk controls. The use of such blood tests to predict the risk of venous thrombosis in ambulatory patients using anovulatory hormones has been proposed.[4]

However, when the population studied contains high-risk patients, such as those hospitalized for acute illness, the discrimination is not as good, as shown in the second series. Further improvement in discrimination is needed. Other blood tests may be considered. In these two series and in previous work, the following tests did *not* improve discrimination: euglobulin clot lysis time, erythrocyte sedimentation rate, prothrombin time of plasma or plasma diluted 1:8 in buffer, comparison of clotting time in glass and plastic containers, adhesion of platelets to glass after ADP was added to platelet-rich plasma, and platelet adhesion to glass when blood was collected by suction through a column of glass beads. Other blood tests are under consideration.

Breneman has predicted the risk of postoperative venous thrombosis,

TABLE 3 Values of Discriminant Function (Y) for 29 Patients Having Venous Thrombosis and 29 Control Patients

Subject	Control (29 Cases)	Thrombosis (29 Cases)
1	19.0990	
2	17.8320	
3	17.2423	
4	17.1765	
5	14.5697	
6	14.3267	
7	14.1096	
8	11.2263	
9	9.8854	
10	9.6435	
11	9.1667	
12	8.8695	
13	8.7984	
14	8.3949	
15	8.1140	
16	7.6227	
17	7.5038	
18	7.3801	
19	6.8843	
20	5.1701	
21	5.0833	
22	4.5909	
23	4.4068	
24		3.8608*
25	2.9898	
26	2.2756	
27	2.2068	
28		0.9711*
29		0.4868*
30	0.1498*	
31		0.0291
32	−0.7218*	
33		−1.7829
34	−1.9434*	
35		−3.1632
36		−4.2136
37		−4.2289
38		−4.2756
39		−5.0935
40		−5.7471
41		−7.5954
42		−7.6373
43		−7.9628
44		−8.1028
45		−8.5918

TABLE 3 Continued

Subject	Control (29 Cases)	Thrombosis (29 Cases)
46		—8.5977
47		—8.9334
48		—9.2346
49		—9.5545
50		—9.6490
51		—9.6645
52		—10.5768
53		—11.2873
54		—11.5871
55		—11.9912
56		—14.3244
57		—16.0471
58		—19.6218
MEAN	8.3467	—7.3833

* Denotes the cases that are misclassified according to the following criterion: classify the subject as "control" if $Y \geqslant 0.4817$, and classify the subject as "thrombosis" if $Y < 0.4817$.

using a discriminant function based on items in the medical history.[1] The use of such items in the present series did improve discrimination, but it may not be valid to use them in this context. The decision by which patients were categorized as thrombosis or control (the dependent variable) occasionally may have been based on such items from the history. If so, they cannot validly be used also as independent variables. If other criteria for the diagnosis are used, these items could be used. Previous reports [2,6–8] suggest that phlebography, radioactive localization of propagating thrombus, necropsy results, or a combination of these can be offered as alternative criteria.

It is reasonable to suppose that such studies in their ultimate refinement will permit correct identification of patients with a high risk of thrombosis. This information will do much to simplify diagnosis, decisions on therapy (e.g., prophylactic anticoagulants, anovulatory hormones), and clinical investigation of new drugs. Because the detection of risk involves only clinical examination and collection of blood samples, it can be used as a screening method. Some of the alternate criteria mentioned previously may nevertheless be necessary for conclusive diagnosis.

Any relationship between pathogenesis of venous thrombosis and the observed blood-test changes is still conjectural. The patients were studied only once, and the venous thrombosis cases were seen only *after* the diagnosis became manifest; therefore, the characteristic findings in the

blood could as well be secondary to thrombosis as related etiologically. Prospective study of high-risk groups of patients is needed to clarify this point.

The work reported here was supported in part by Public Health Service grants HE 09095 and HE 05140 from the National Heart Institute.

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Factor VIII and TGA Types of Hypercoagulability

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If "hypercoagulability" means an increased concentration of a normal or abnormal procoagulant or a deficiency of a normal anticoagulant or acceleration of some coagulation reaction, then hypercoagulability is probably very common. One can only speculate as to whether hypercoagulability, alone or in conjunction with stasis and changes in vessel walls, is etiologically important in the development of thromboembolic phenomena. The association of some hypercoagulable states with thromboembolism suggests that the relationship may be more than coincidental. This report documents the association of increased levels of factor VIII and of the somewhat similar factor that we have described—thromboplastin generation accelerator (TGA)—in several physiologic and pathologic situations often associated with thrombosis. Factor VIII, apparently a globulin (its older name is "antihemophilic globulin"), is closely associated with fibrinogen in plasma and can be separated from it only with difficulty.[40] It is relatively labile, both *in vitro* [6] and *in vivo*.[5]

PHYSIOLOGIC STATES

EXERCISE

The level of factor VIII in the plasma is clearly increased by exercise.[4,22,25,27,36,41,71] We asked five of our associates to run up and down two steps until they were fatigued. Immediately afterward, blood was drawn for factor VIII assay. The factor VIII level increased by an average of 56% (range, 7–100%) over the pre-exercise levels.

Perhaps exertion stimulates the release of epinephrine, which in turn is responsible for the hypercoagulability. At any rate, infusions of epi-

nephrine also increase factor VIII.[18,42] The response to exercise and to epinephrine is so prompt that it appears to represent release of preformed factor VIII, perhaps from the reticuloendothelial system [15,51,90] or the spleen,[8,67,89,91,92] rather than synthesis of the factor *de novo*. The possibility that the suddenly released factor VIII comes from stores appears to be supported by the diminishing release as exercise or epinephrine injection is repeated.[18,27] Only limited advantage has been taken of the exercise–epinephrine phenomenon in efforts to obtain enriched plasma. Egeberg [18,27] found that "exercise-activated plasma" was superior to normal plasma in the treatment of patients with von Willebrand's disease.

SEX

There is reported to be little difference between men and women in factor VIII activity,[66,70] although Preston and Barr [69] found higher levels in men.

AGE

Cooperberg and Teitelbaum [10] and Pitney and associates [66] observed a progressive increase in factor VIII levels with increasing age. No such increase was found by Preston and Barr [69] or by us.[2,45]

PREGNANCY

There have been a number of reports of increased factor VIII levels during pregnancy in normal women and in women with von Willebrand's disease.[43,52,59,73,81,82,85] We [84] found that an occasional pregnant woman may have a factor VIII level several times normal, but this is the exception. However, pregnant women with increased levels of factor VIII tend to remain in a hypercoagulable state for the last few months of pregnancy. In none of our patients was thromboembolism detected, so the relationship, if any, between increased factor VIII level and thrombosis in this state could not be assessed.

Because the coagulation changes accompanying the continued use of oral contraceptive hormones mimic those of pregnancy, but to a milder degree, the effect of these agents on factor VIII has been studied. Egeberg and Owren [32] found the factor VIII level to be startlingly increased in one patient, but only modestly in four others. We could detect no significant change in factor VIII in our series,[55] although factor VII was consistently increased. Penick [60] suggested that the increase in factor VIII in these patients may be related to thromboembolism, inasmuch as he and his associates [61] were able to shorten the time required for a thrombus

to form in a segment of blood vessel in an animal that received a transfusion of factor VIII.

RACE

The level of factor VIII is apparently a function of race, being higher among the Bantu,[48] the Australian aborigine,[65] and some Caucasians.[70] In none of these races has an increased incidence of thromboembolism been found; in fact, it may be lower in these and some other races.[33,50] Perhaps counterbalancing the hypercoagulability, members of these races also tend to have exaggerated fibrinolytic activity.

PATHOLOGIC STATES

MALIGNANCY

We found [2] that metastatic malignancies or cancers more than 3 cm in diameter are regularly associated with increased factor VIII activity, sometimes to as much as five times normal. It seemed to make little difference where the malignancy arose (lung, pancreas, prostate, breast, stomach, tongue, or biliary tract) or whether the neoplasm was carcinomatous or sarcomatous. In contrast, patients with small, localized tumors usually had no factor VIII abnormality. Godal and associates [35] reported a factor VIII level of 300% in a patient with vulvar carcinoma and thrombophlebitis, and Fletcher [9] found accelerated generation of thromboplastin in a patient with hepatic carcinoma and thrombophlebitis.

That the incidence of venous thromboembolism is increased in patients with malignant disease has been reported on many occasions.[14,38,44,46,64,80]

POSTOPERATIVE STATE

In contrast with the sparsely investigated malignancies, there have been numerous coagulation studies of postoperative thromboembolism. We reported [2] that factor VIII level was consistently increased in all of 25 patients, whether the operation was a herniorrhaphy or a pulmonary resection. Patients who exhibited hypercoagulability preoperatively often showed a further increase in factor VIII postoperatively. The onset of the change in factor VIII may be as early as the 1st day after operation, but often it is not clearly evident for a few days. In the few patients we have followed, the return of factor VIII to normal required weeks to months. Others have also reported postoperative increases in factor VIII.[19,88]

Changes in factor VIII during and after cardiac bypass surgery perhaps need separate consideration. We studied a series of these patients during the operation and for the next 24 hr.[93] The level of factor VIII increased early during operation, to an average of 164% of the preoperative level. Little further change occurred during the operative procedure, but 2 hr after the patient had received protamine (to neutralize heparin), the factor VIII level was slightly more than double the preoperative level. The factor VIII level steadily subsided; 24 hr later, it was back to normal. The studies were not pursued for a longer period, so we cannot state whether the usual delayed postoperative increase in factor VIII occurred. These changes during cardiac surgery are comparable with those reported to occur during obstetric deliveries.[12,68]

CHRONIC ULCERATIVE COLITIS

For many years, it has been known that patients with chronic ulcerative colitis are unusually likely to have thromboembolic complications.[3,37] This is particularly significant, in that younger patients are often involved, and pathologic findings in the vessel walls are relatively mild, compared with the seriousness of the disease.

Our preliminary findings[78] of accelerated generation of thromboplastin suggested the presence of a hypercoagulable state in these patients. We have recently studied two groups. Factor VIII levels were significantly increased in seven of nine patients with active colitis but in only one of seven with quiescent disease.[45]

SYSTEMIC LUPUS ERYTHEMATOSUS

One occasionally finds the curious anomaly of hypercoagulability and hypocoagulability at the same time in these patients.[7] The patient may have either or both of two classes of anticoagulants, one that coats the platelets and renders them thrombopathic and one that interferes with the prothrombin-time test. Concurrently, there may be a remarkably accelerated rate of thromboplastin generation, which is attributable to increases in factor VIII or TGA. When the patient is in a state of hypercoagulability, thromboembolic phenomena are likely, whether the circulating anticoagulants are present or not.

OTHER CONDITIONS

One common denominator in malignancy or chronic ulcerative colitis, and during the postoperative period, is tissue destruction. One could speculate that such destruction is responsible for the factor VIII type of

hypercoagulability. The association of increased factor VIII level with acute pancreatitis,[72] whole-body irradiation,[75] hypermetabolism,[23,39,74] and fever [29] might support the concept.

Increases of factor VIII have also been reported in renal failure,[17] sickle cell anemia,[1] paroxysmal nocturnal hemoglobinuria,[16] coronary artery disease,[11,20,47] diabetes mellitus,[23] and severe hemorrhage [86,87]; during diuresis in patients with congestive heart failure,[24] after trauma and childbirth [12]; and during corticosteroid therapy.[57]

In rabbits treated with warfarin, Sise and associates [76] found an increase in factor VIII, perhaps as the result of reduced utilization of the factor. Egeberg [31] was able to find such factor VIII hypercoagulability only in patients with advanced atherosclerosis when they were treated with phenindione.

Other experimental procedures have been found to be associated with increased factor VIII activity. Rats fed an atherogenic diet became hyperlipemic, and their factor VIII levels increased.[49] When a tourniquet was placed on the upper arm of a man and constricted to a pressure of 90 mm Hg, within 10 min the forearm blood exhibited marked increases in factor VIII.[30] Factor VIII levels increased in humans 1–2 days after the intravenous injection of serum [28] or the intramuscular administration of whole blood.[26]

HYPERCOAGULABILITY IN PATIENTS WITH THROMBOEMBOLISM

When a patient is undergoing an acute thrombotic episode, the altered blood coagulation may not necessarily be due to a pre-existing thrombosing tendency; the formation of a thrombus itself could cause the observed coagulation changes. However, if the hypercoagulability persists for months or years after the thrombotic episode, one might be more inclined to ascribe the thrombosis to the hypercoagulable state.

In 1961, Owen and Thompson [54] observed a significant acceleration of thromboplastin generation from the plasma of some patients with acute thrombotic disease and suggested that excess factor VIII might be responsible. Subsequent study of a number of patients with arterial and venous thrombi confirmed the suggestion. In addition, it was shown that in some patients the accelerated generation of thromboplastin was accompanied by an increase in some coagulation activity other than factor VIII. Because this "new"activity did not seem to conform to the criteria for any of the established coagulation factors, it was given the name "thromboplastin generation accelerator" (TGA).[58]

TGA was found to have some properties similar to those of factor VIII.

It is present in oxalated plasma after adsorption with barium sulfate, and an excess of it causes acceleration in the thromboplastin generation test. TGA differs from factor VIII, however. TGA is considerably more heat-stable, its electrophoretic mobility and ammonium sulfate salting-out characteristics are different, and (most important from the standpoint of identification) it is readily removed from plasma by aluminum hydroxide, whereas factor VIII is poorly adsorbed by this substance.

Spittell and colleagues [77] studied thromboplastin generation in 41 patients with chronic arteriosclerosis obliterans and found acceleration in only six (15%). In another group of 64 patients with the same disease process, but with the additional complication of acute thrombotic occlusion, acceleration was found in 45 (70%). Ten of this latter group of "accelerators" were studied for up to 3 years after the acute thrombotic episode had subsided. In three of the 10, the thromboplastin generation test had reverted to normal within 3 months; in the other seven, the hypercoagulability remained unchanged as long as the patients were studied (2½ months to 3½ years), despite the clinical absence of further thromboses. Although the majority of these patients with accelerated thromboplastin generation were found to have increased levels of factor VIII, some had increased TGA, as measured by our "retarded" test.[56,83]

In our first study of patients with venous thrombosis,[53] we found seven of 17 such patients to have significant acceleration of thromboplastin generation. We now have tested more than 100 patients with idiopathic recurrent thrombophlebitis; about half exhibited the accelerated clotting pattern. Just as in the patients with arterial disease, the majority had increased levels of factor VIII,[79] as others have also found [13]; a few had the TGA type of hypercoagulability. Also, the abnormal clotting pattern has persisted for more than a year without further evidence of thrombotic complications in some of these patients. In one, the hypercoagulability did diminish and oral anticoagulation therapy was discontinued, whereupon the generation of thromboplastin again became very rapid and an acute thrombotic episode soon followed. It is difficult to ascribe sustained hypercoagulability to an old thrombotic process, particularly in patients who have recurrent idiopathic thrombophlebitis but in whom no local or generalized disease, other than thromboembolism, can be found over a period of years.

CONCLUSIONS

A coagulation activity, indistinguishable from that of normal factor VIII,[62,63] can increase severalfold in the plasma of patients undergoing

a variety of physiologic stresses. It is also regularly increased in patients with tissue destruction (such as caused by malignancy, chronic ulcerative colitis, or an operation). Because these conditions are notoriously associated with thromboembolic complications, one is inclined to suspect a relationship between the hypercoagulability and the thrombotic events.

In patients with arteriosclerosis obliterans and hypercoagulability, one might attribute the altered coagulation to activation of some clotting factors as they pass the atherosclerotic areas,[34] which could account for chronic hypercoagulability without thrombosis in these patients. However, idiopathic recurrent thrombophlebitis and chronic ulcerative colitis, which are often accompanied by chronic hypercoagulability of the factor VIII type and occasionally of the TGA type, are characteristically found in younger patients without evidence of vascular or other disease at the site of the thrombosis. Here it seems as logical to attribute thrombosis to hypercoagulability as to blame hemorrhage on hypocoagulability.

The work reported here was supported in part by Public Health Service research grants HE 5008 and HE 9863 from the National Heart Institute.

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Fibrinolysis and Thrombosis

SOL SHERRY

The resolution of fibrin (whose deposition is common in health and disease) is a fundamental biologic repair mechanism known as fibrinolysis. Fibrinolysis bears an obvious relation to thrombosis: the end product of thrombosis, the thrombus, depends on fibrin for its tensile strength and structural integrity. However, the relationship between the two processes is considerably more intimate, in that it is evident that a thrombus is not static, but rather provides an interface at which the dynamic events of thrombosis and fibrinolysis continuously overlap. As a consequence, it is increasingly apparent that the study of thrombosis must include fibrinolysis as an integral part.

Less than a quarter-century has passed since a scientific base for the study of fibrinolysis was developed with the observation that such phenomena are controlled and regulated by a proteolytic enzyme system termed the "plasminogen–plasmin system." This enzyme system occurs naturally as an inactive precursor, plasminogen, which is a plasma globulin. Activators (or kinases) convert plasminogen to plasmin, a proteolytic enzyme that is active at neutral pH and capable of digesting fibrin into several soluble fragments. Potent inhibitors in the plasma keep the enzyme system in check. It has since become apparent that the ramifications of this system, in terms of its biochemistry, physiology, pathology, and applications to therapy, are as extensive as those of the clotting mechanism.

This general review has been planned as an interpretative one, stressing areas of major current interest and citing recent studies only if they bear on the points made in the review. The reader is urged to consult previous general reviews [10,90,102,115] for earlier work, and his attention is drawn to an excellent monthly bibliography, with an annual compilation, devoted to fibrinolysis and allied subjects.[113]

COMPONENTS OF THE FIBRINOLYTIC ENZYME SYSTEM

PLASMINOGEN

Plasminogen is widely distributed throughout the body; it is present in all body fluids and secretions. Its concentration in plasma may be estimated at 0.1–0.2 mg/ml. Plasminogen has a tendency to coprecipitate and, whenever fibrin is laid down, significant quantities of plasminogen are incorporated into such deposits.

The site (or sites) of plasminogen production is still obscure. It has been suggested, on the basis of immunofluorescent studies,[14] that plasminogen is synthesized and stored in the bone marrow eosinophil and then transported to the circulation and tissues when needed. This concept is difficult to reconcile with studies on plasminogen turnover, and many still suspect that the liver plays the major role in the production and maintenance of circulating plasminogen levels.

Major advances have been made in the isolation, purification, and characterization of human plasminogen. Most of the earlier work was carried out with the acid extraction of Cohn fraction III, but those preparations were only slightly soluble at neutral pH. More recently, on the basis of chromatographic procedures, a number of investigators [3,50,92] have reported independently on preparations of comparable or higher specific activity that are soluble at neutral pH, approximating the native state. Although the molecular characteristics of these preparations have varied with the techniques of purification, a finding explained by Alkjaersig [3] as due to the plasticity of the plasminogen molecule, recent studies on highly purified preparations [92,93] suggest that plasminogen is a monomeric protein with a molecular weight of approximately 89,000. Simple techniques are now available for the preparation of high-purity soluble plasminogen.[50]

PLASMIN

Plasmin is a proteolytic enzyme of the endopeptidase type with many features similar to but not identical with those of trypsin; it possesses the ability to hydrolyze susceptible arginine and lysine bonds in proteins, and it splits arginine and lysine esters with great avidity.[92,101] Plasmin digests fibrinogen at a rate similar to its action on fibrin, and the details of such action are under extensive investigation. It also hydrolyzes a number of plasma proteins, particularly coagulation factors V and VIII, and digests a great variety of protein substrates commonly used in the laboratory. For laboratory assay, α-casein, which is more soluble and sensitive than casein, is the substrate of choice.

The activation of plasminogen to plasmin occurs enzymatically and, with urokinase, appears to involve the splitting of a single arginine–valine bond within the plasminogen molecule, which results in two peptide chains held together by a single disulfide bond.[93]

Techniques are available for the preparation of plasmin, of a purity comparable with that achieved for plasminogen [55,92]; this has required the use of stabilizing agents, like glycerol or lysine, in the activation mixture. Recent studies by Robbins *et al.*[93] suggest that plasmin has the same molecular weight as plasminogen, but the molecule becomes more compact as a result of the opening of an arginine–valine bond.

PLASMINOGEN ACTIVATORS

Because plasmin is normally represented by the inactive precursor, plasminogen, much attention has been given to the naturally occurring biologic activators of plasminogen. These are proteolytic enzymes, highly specific for the activation of plasminogen, and can be found (1) in trace amounts in all body fluids (its level in plasma rises sharply whenever there is increased fibrinolytic activity in the blood); (2) in urine, where the activator is termed "urokinase"; and (3) in most body tissues. Tissue activators are probably concentrated in two sites: in the lysosomal granules, as suggested by Lack,[63] and in the endothelial cells of blood vessels.[110,116] In the second site, they are in a soluble and readily diffusible form (presumably, the form released in response to vasoactive stimuli); in the first site, they are difficult to extract and do not lend themselves well to purification. Nevertheless, progress has been made in purifying and characterizing the plasminogen activator from pig heart [12] and rabbit kidney.[1]

Urokinase, because of its potential as a thrombolytic agent, has been extensively studied and has been crystallized by Lesuk *et al.*[68] It is a single polypeptide chain with a molecular weight of 54,000 and has an active enzyme center capable of hydrolyzing arginine and lysine bonds. For a time, there was controversy as to whether multimolecular forms of urokinase may exist,[117] but recent work [69] suggests that urokinase is a single species. The confusion probably arose because limited proteolytic digestion of native urokinase, as may occur during the purification of urokinase, results in smaller active fragments (e.g., a molecular weight of 36,000), which retain some ability to activate plasminogen.

The question of whether urokinase is a renal product or results from the plasma clearance of circulating plasminogen activator has been reinvestigated by Kucinski *et al.*,[60] who used a potent antiserum capable of neutralizing the biochemical action of both purified human urokinase

and native urokinase in human urine. *In vitro* studies showed a lack of immunologic identity between human urokinase and human milk activator or human adrenal tissue activator but identity between urokinase and activator from human kidney tissue-culture supernate. *In vivo* studies in man failed to show detectable urokinase in either peripheral human venous plasma or renal venous plasma in a variety of clinical states and when plasminogen activator levels were increased. These observations are consistent with the view that urokinase is produced by the kidneys and confirm observations [17,47,118] that urokinase excretion is independent of circulating plasma activator levels.

There is little evidence of a proactivator in plasma as a separate and distinct entity. Originally invoked to explain the two-step activation of plasminogen by the bacterial substance, streptokinase, the "proactivator" in this instance is plasminogen and/or plasmin itself; the "proactivator" reacts with streptokinase in a 1:1 molar ratio to form a streptokinase–plasmin complex that is a potent activator for plasminogen.[26,30,54,56]

INHIBITORS

Plasma contains large amounts of antiplasmin activity, sufficient to inactivate 10 times all the available plasmin in plasma.[99] The activity is of two types, immediate and slow, and previously has been attributable to two separate plasma α-globulin components. However, a recent reinvestigation of this subject has resulted in the isolation of a single α_1-globulin component (molecular weight, 47,000), probably identical with the α_1-antitrypsin, which exhibits both slow and immediate antiplasmin activity [91,98] and may account for the total antiplasmin activity of plasma.

Platelets also contain antiplasmin activity.[2,45] The platelet inhibitor has not been sufficiently purified for characterization, but its action appears to be immediate.[2]

Evidence is accumulating of the existence of plasma constituents capable of inhibiting the activation of plasminogen [21,51]; such naturally occurring substances are not yet well characterized, nor has their separate identity from the naturally occurring antiplasmin activity been established.

METHODS FOR ASSAY OF COMPONENTS

The *in vivo* investigation of the activity of the plasminogen–plasmin system has posed difficult problems. It has been hampered particularly by deficiencies in methodology (for description of methods frequently used see Tocantins and Kazal [109]); many of the methods are more qualitative than quantitative, and their specificity is limited. Consequently, the Sub-

committee on Standardization of the National Heart Institute's Committee on Thrombolytic Agents (CTA), using standardized reagents, has developed a series of assays (fibrinolytic, caseinolytic, and esterolytic) for the measurement of plasminogen, plasmin, and urokinase in purified systems. These assays are reproducible from laboratory to laboratory and are being recommended for general use.[49] CTA standards are also available for human plasmin, human plasminogen, and urokinase,* and international standards are being developed by the World Health Organization. Collaborative studies of this type represent an important beginning toward the solution of previous inadequacies.

THE NORMAL FIBRINOLYTIC MECHANISM

For many years, the mechanism by which the organism utilized plasmin, a nonspecific enzyme, to lyse fibrin selectively without destroying other susceptible plasma proteins of biologic significance posed problems of considerable importance, particularly inasmuch as (1) hyperplasminemic states were associated with severe coagulation defects and a sometimes catastrophic hemorrhagic diathesis; and (2) no plasmin could be demonstrated in the circulation normally or following a physiologic stress (e.g., exercise), even though small to large amounts of circulating fibrinolytic activity were present.

Rapid strides were made in understanding how this enzyme system worked when it became apparent (1) that the fibrinolytic activity of plasma was directly related to its plasminogen activator content (in fact, was a measure of it); (2) that plasminogen was deposited in significant amounts in fibrin whenever fibrin was deposited; and (3) that plasminogen activation in the immediate proximity of fibrin (e.g., in the interstices of a clot) was the most sensitive mechanism for fibrinolysis. This has led to the development of a hypothesis [102] that appears to account for the facts: *in vivo,* plasminogen exists as a two-phase system, a soluble phase in the body fluids (plasma plasminogen) and a gel phase in thrombi and fibrinous deposits. The effect of activators in the two phases and the consequences of plasminogen activation in the two sites are dissimilar. Minor or slow activation of plasma plasminogen, because of the presence of inhibitors, will not result in detectable signs of plasma proteolysis, because the enzyme is effectively inhibited on its formation; but rapid activation of plasma plasminogen produces excessive plasma proteolysis, resulting in the rapid degradation of fibrino-

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gen, the most abundant available substrate. However, activation of gel phase or clot plasminogen, resulting from diffusion or incorporation of the activator into the clot, produces fibrinolysis, for here the enzyme is activated in the presence of fibrin, which is the only substrate available; and the reaction appears, at least initially, to be independent of inhibitors in the body fluids.

These considerations attach great specificity to fibrinolysis, in that, under physiologic circumstances, fibrinolytic phenomena appear to be regulated by the concentration of plasminogen activator. Following an appropriate stimulus, activator is released transiently into the circulation and directly raises the clot-dissolving activity of the plasma by its ability to activate gel phase plasminogen, but without invoking the consequences of increased plasma proteolysis. The mechanism is particularly effective if significant quantities of activator are present when fibrin formation occurs; under these circumstances, the activator is incorporated into the clot while it is forming, and the subsequent widespread activation of clot plasminogen leads to very rapid fibrinolysis. Dissent has been expressed about this view,[6,27,42] but data supporting other concepts are deficient.

The fibrinolytic mechanism *in vivo* appears to be continuously active, and it is dynamic in response to stimuli. As noted previously, the plasma of healthy adults normally contains significant (but small) amounts of plasminogen activator activity, whose level rises sharply whenever there is increased circulating fibrinolytic activity. Enhanced fibrinolytic activity is observed frequently in some diseases—e.g., hematologic malignancies, cirrhosis of the liver, and various infections—but more striking changes are produced by a great variety of physiologic and pharmacologic stimuli, e.g., electroshock, pneumoencephalography, hypoglycemia, ischemia, anoxia, intense exercise, and parenteral injections of epinephrine, acetylcholine, nicotinic acid, or pyrogen. Fibrinolysis is viewed best as a local phenomenon, and there is abundant evidence that plasminogen activator is released into the circulation at the site of ischemia or other acute vascular change, of either a vasoconstrictive or a vasodilatory nature.[44]

The source of the small amounts of the normally circulating plasma activator, as well as the increased levels found in enhanced fibrinolytic states, has not yet been established; most likely, it is derived from small blood vessels, particularly the capillary bed,[11,16,61] where most of the endothelial cells of the body are concentrated. Because plasminogen activator is rapidly cleared from the circulation, probably by the liver,[35,48,104] a significant basal state of activity presupposes an almost continuous release of activator from small blood vessels into the cir-

culation. If so, then significant arteriovenous differences in plasminogen activator levels should exist. Such differences have been demonstrated repeatedly for the renal circulation [20,23,46] but less impressively for the brachial circulation.[24] The observation of a significant renal arteriovenous difference in fibrinolytic activity, coupled with observations that plasma activator concentration in animals fell during mercuric chloride intoxication [86,119] and clamping of the renal vessels,[48] suggests that the kidney may be a significant source of plasma activator; immunochemical studies [60] indicate, however, that the activator released from the kidney into the circulation is not urokinase.

At present, the evidence suggests that this renal source of plasma activator is not unique. It assumes quantitative significance from the fact that this organ receives, per unit of weight, a greater volume of blood than any other major region supplied by the left ventricle, and vasoactive responses occur frequently because of the low vascular resistance of the organ. Such considerations emphasize the need for studies of the dynamics of the fibrinolytic response in local circulations, inasmuch as organ differences in fibrinolytic responsiveness probably exist and may well account for variations among organs in their disposition of fibrin deposits and thrombi.

The presence of small but measurable amounts of normally circulating plasminogen activator also raises questions as to the significance of this finding in the natural disposition of fibrinous and thrombotic deposits. Such deposits arising within the vascular system, regardless of their ability to stimulate the local release of new increments of activity, will incorporate some activator during the formation of fibrin, which will enhance spontaneous resolution. This may represent an important mechanism for keeping the capillary bed free of small fibrinous deposits, and may explain, in part, the high rates of spontaneous resolution recently documented for pulmonary emboli [36,111]; in connection with the latter, one is reminded that sterile clotted blood slowly undergoes spontaneous dissolution to complete liquefaction.

Undoubtedly, a number of factors influence the normal fibrinolytic process. One of these is the age of the fibrin. Early observations demonstrated that, after 5–7 days *in situ*, experimental clots in animals usually became quite resistant to lysis by thrombolytic agents; in general, this has been the experience of most clinical investigators who have used such agents in man. The mechanism for this increased resistance has not been entirely clarified. A major consideration, as demonstrated in the recent experiments of Gormsen *et al.*,[37] is the fibrin stabilization reaction, in which thrombin-activated factor XIII (fibrin-stabilizing factor) catalyzes a transamidation between fibrin polymers,[73,74] bonding

them together so as to provide a stronger structural integrity for the clot, and, in so doing, rendering the clot more resistant to lysis.

Considering that the basic mechanisms involved in fibrinolysis are just beginning to be understood, the dearth of valid information on other factors (circulating, hormonal, nutritional, etc.), which may well have important effects on fibrinolytic phenomena, is not surprising. Recent observations suggest that sex hormones may suppress plasminogen activator activity both in the endometrium [11] and in blood,[18,19,100] and the influence of lipids has been the subject of a previous review.[82] Other pertinent considerations are discussed in three recent symposia.[28,103,108]

FIBRINOLYSIS IN DISEASE

IMPAIRED FIBRINOLYSIS

The dynamic state of fibrinolysis *in vivo* (continuously active and readily stimulated) presupposes an important role for this process in limiting the extent of fibrin deposition and thus influencing the clinical and pathologic features of thromboembolic disorders. Once fibrin is deposited, its course must depend on the balance achieved between the rates of new fibrin formation and fibrin resolution; in this context, thrombosis is viewed as a continuing and changing process in which the thrombus undergoes modification either in growth or in resolution, depending on the interplay of two opposing reactions. Much evidence supporting such a concept has been obtained over the years by direct visualization of the course of experimental thrombi produced either in the microcirculation, in small blood vessels, or in shunts.[83] Consequently, fibrinolysis becomes a most important factor in the study of the dynamics of thrombosis *in vivo,* and the contribution of the fibrinolytic process should not be underestimated. Nevertheless, it is also subject to misinterpretation, unless information is available on the rates of new fibrin formation and fibrin resolution. For example, if new fibrin deposition continues to exceed or balance the rate of resolution, the observations may be considered as evidence of an impaired fibrinolytic mechanism. Conversely, if new fibrin formation becomes much slower or ceases, the observations may suggest accelerated resolution. These considerations probably are pertinent to the high spontaneous resolution rates of pulmonary emboli,[36,111] in that the observations are being made in patients on anticoagulant therapy, in whom the equilibrium between new fibrin formation and fibrin resolution at the site of the embolus may be significantly different from the untreated state.

Regardless of this *in vivo* complexity, one may suppose that, following an initiating thrombotic event, impaired fibrinolysis significantly favors the growth and development of fibrin deposition. Recent observations [9,75] clearly support this prediction; the induction of serum-induced thrombosis in rats pretreated with epsilon-aminocaproic acid, a powerful inhibitor of plasminogen activator, led to extensive fibrin deposition both in the microcirculation and in the small veins of the mesentery, whereas control animals were usually spared this complication. These animal experiments extend clinical studies [78,85] in which the administration of epsilon-aminocaproic acid to patients with severe hemorrhage secondary to disseminated intravascular coagulation resulted in the rapid development of multiple thrombi.

Unfortunately, current methodology for assaying circulating fibrinolytic activity is capable of discriminating only between enhanced and normal levels of fibrinolytic activity; it is not yet sufficiently sensitive to distinguish between the small amounts of normal activity and abnormally low levels. Such a distinction should be an important goal for future investigation, because it may provide a better understanding of the predisposition to and course of thromboembolic disease in a variety of clinical conditions. Particularly pertinent is the problem of venous thrombosis in states of immobilization, in which the perfusion of inactive muscles may not provide sufficient activator to halt the development of significant thrombi in the deep veins; in congestive heart failure, in which the resolution of pulmonary emboli appears to be delayed [111]; and in malignant disease, in which there is some evidence of impairment in the fibrinolytic mechanism.[35,62]

There may be several mechanisms for reduced fibrinolytic activity (e.g., inadequate production and release of plasminogen activator, accelerated clearance from the circulation, severe deficiency of plasminogen, and excessive amounts of inhibitor), but the literature of clinical investigation contains little concerning patients with hereditary or acquired abnormalities in these mechanisms. Nilsson *et al.*[88] described a young man with extensive and progressive thrombosis of the vena caval system in whom the coagulation mechanism was normal but in whom there was an excessive amount of a fibrinolysis inhibitor (believed to be primarily an activator inhibitor); Naeye [84] called attention to abnormally high inhibitor levels in two other patients with a thrombotic disorder; and Brakman *et al.*[22] recently described four patients with an increased thrombotic tendency who exhibited a selective increase in inhibition of tissue plasminogen activator. This aspect of the subject needs to be extensively studied with sophisticated methodology, and with special care to delineate the significance of the high inhibitor level, particularly inas-

much as there is a need to distinguish between the factors that initiate thrombosis and those which may influence its course. In this regard, the administration of epsilon-aminocaproic acid to animals has not initiated clotting, nor has its use in man been associated with a significant incidence of thrombosis [25,87,105]—although, as previously noted, it may influence the course of an underlying thrombotic tendency.[78,85]

A deficiency of plasminogen activator activity has been described in hyaline membrane disease of the newborn, and a pathogenetic role ascribed to it,[71] but further investigation has shown that the problem is much more complex [70]; it is not clear whether there is a true deficiency [94] or, if there is, whether it is hereditary, developmental, or acquired. However, the presence of fibrin in the alveolar membrane has stimulated interest in its lysis with fibrinolytic agents; the observations reported by Ambrus *et al.*[7] suggest that such therapy may influence the mortality rate in this disorder.

Severe deficiencies of plasminogen have been described only during intense thrombolytic states induced by the administration of plasminogen activators. When rethrombosis occurs, under these circumstances, fibrin resolution is difficult to achieve.[52]

Fibrinolysis has been inhibited in man for therapeutic purposes, both for systemic states of excessive fibrinolysis and to control local fibrinolytic phenomena; this has been accomplished by the administration of antifibrinolytic agents, e.g., epsilon-aminocaproic acid,[25,87,105] trasylol, which is derived from ox lung,[96] *p*-aminomethylcyclohexanecarboxylic acid,[8] and *p*-aminomethylbenzoic acid.[76] These compounds enjoy their greatest usefulness in the management of postprostatectomy bleeding, in which inhibition of urokinase activity significantly improves hemostasis in the urinary tract; several controlled studies, the latest of which is by Schmutzler and Fürstenberg,[96] attest to their value.

EXCESSIVE FIBRINOLYSIS

Physiologically, fibrinolysis is accomplished without a significant increase in plasma proteolysis. But sometimes there is excessive digestion of fibrin or fibrinogen. This state is associated with an acute or chronic coagulation disorder, and, when the onset is sudden, a serious hemorrhagic diathesis may develop rapidly with severe bleeding, usually beginning at the site of underlying disease or previous surgery or trauma, but later spreading to other areas. Although the blood of patients suffering from this disorder frequently demonstrates multiple coagulation defects, the most striking finding is poor and slow blood clotting (if any), even after the addition of thrombin; and, if a clot forms, it is loose and

friable. The clot may undergo spontaneous dissolution in a matter of minutes or hours, and that makes the syndrome recognizable; it is referred to as "pathologic fibrinolysis" or "fibrinolytic bleeding." The severity of this disorder can be readily attributed to the particularly ineffective form of hemostasis present; clotting is slow and inadequate, and the clot may later dissolve.

The understanding of this hemorrhagic diathesis has come from studies that demonstrated that the products of the proteolytic digestion of fibrinogen and fibrin interfere with blood clotting, and that the addition of such proteolysis products to normal blood reproduces, *in vitro,* the abnormal blood clotting seen in the fibrinolytic disorders [30,31,58,112]; this subject has been reviewed recently.[32]

Study of the problem has revealed that fibrinogen (molecular weight, 320,000) is degraded by plasmin sequentially in a series of complex reactions.[33] Although a variety of derivatives are formed, the degradation occurs in a defined sequence. In the first stage, approximately 10% of the fibrinogen is cleaved off in the form of low-molecular-weight fragments, leaving a derivative with a molecular weight of 265,000 that is still clottable by thrombin and indistinguishable from normal fibrinogen by conventional plasma assays; but this moiety clots slowly and abnormally, owing to its grossly altered configuration. There is also evidence to suggest that the early fragment interferes with some platelet functions, namely, aggregation, adhesiveness, and viscous metamorphosis,[43,59,64] but whether it directly inhibits the action of thrombin itself, as has been claimed,[64,66] is not resolved.

Following release of a further 5–10% of peptide material, partially clottable intermediate derivatives with a molecular weight of 100,000–200,000 are formed; these, too, interfere with the normal clotting reaction. Finally, these intermediates are split into two fragments with molecular weights of 88,000 and 30,000, which resist further degradation by plasmin. These two final fragments have different antigenic determinants (D and E determinants, both of which are present in fibrinogen and the early intermediates), which can be distinguished readily by immunodiffusion. The 88,000-molecular-weight fragment carrying the D determinant is a polymerization inhibitor; i.e., it inhibits the interconversion of fibrinogen to fibrin, not by blocking the action of thrombin, but by inhibiting the subsequent steps (the spontaneous polymerization of fibrin monomer, the subunit of the long insoluble fibrin polymers of the normal clot). For this reason, this fragment and its precursors are referred to as "polymerization inhibitors" [4,67]; when present in large amounts, they delay normal polymerization and cause the formation of abnormal polymers that weaken and distort the final clot.[13,43] This

anomaly of polymerization has been termed "defective fibrin polymerization," and the aberration, the slow clottability of the early fragments, and their effects on platelet function are now recognized as the major factors in the coagulation defect underlying the hemorrhagic diathesis seen in clinically encountered fibrinolytic disorders. The proteolysis of fibrin, although not as well studied, yields fragments with similar biologic effects and immunologic identity. So far, it has not been possible to distinguish between fibrinogen and fibrin breakdown products *in vivo*. The appearance in plasma of breakdown products during states of excessive fibrinogen or fibrin proteolysis can be demonstrated most readily by immunologic techniques,[81] and the fragments can be characterized as to type by immunoelectrophoresis.[29] However, under clinical conditions, the coagulation disorder can be screened for most simply by measuring the thrombin clotting time. In the presence of excess thrombin, the thrombin clotting time is virtually a measure of the polymerization time; as a result, the thrombin clotting time, although lacking specificity, is useful. The delay in clotting is also reflected in a prolongation of the one-stage prothrombin time, which, even though less specific, also correlates well with the coagulation disorder.

Thus, the immediate problem in the fibrinolytic disorders is related not to the level of fibrinolytic activity or plasmin in the circulation but to the presence of abnormal amounts of fibrinogen or fibrin breakdown products, which arise as a result of the excessive digestion of fibrinogen or fibrin. These breakdown products, in high concentrations, seriously interfere with the normal clotting mechanism and precipitate a severe hemorrhagic diathesis. Pathogenetically, two major types of fibrinolytic disorders exist: the primary and the secondary (or mixed) forms. In the primary fibrinolytic disorders, the state is induced directly by a sustained increase in plasma proteolytic activity (hyperplasminemia) sufficient to digest large amounts of circulating fibrinogen (fibrinogenolysis) [34]; in the secondary or mixed forms, the state appears either as a response to or in association with intravascular clotting or defibrination,[53] when the coexistence of clotting and fibrinolysis in large portions of the vascular bed results in the appearance of excessive amounts of fibrin (rather than fibrinogen) breakdown products. It is noteworthy that the blood of patients with the secondary fibrinolytic disorders is much less likely to demonstrate an increase in circulating fibrinolytic activity,[53,80] inasmuch as the extensive dissolution of fibrin is due to localized fibrinolytic phenomena, rather than to heightened systemic activity of the fibrinolytic system, as seen in the primary disorders; animal experiments support this observation.[57]

PRIMARY FIBRINOLYTIC DISORDERS

There are three mechanisms for the production of the primary disorders (excessive plasma proteolytic activity with fibrinogenolysis): (1) Inordinate amounts of plasminogen activator may be administered for therapeutic purposes, or released endogenously from activator-rich neoplastic tissue (e.g., in metastatic prostatic carcinoma) or in response to profound stimuli (e.g., in severe anoxia or shock or following extensive surgical procedures, particularly on the lung or during protracted cardiac bypass); this temporarily overwhelms normal plasma inhibitory mechanisms, sustaining free levels of plasmin in the circulation for long periods. (2) Impaired inhibitory mechanisms may exist among patients with disease (e.g., in cirrhosis of the liver, in which the ability to clear plasminogen activator from the circulation is deficient [35]). Under these circumstances, and particularly during portacaval shunting procedures,[38] the body cannot cope with plasminogen activator released in amounts that ordinarily would not produce significant hyperplasminemia. (3) Proteolytic enzymes, other than plasmin but also capable of degrading fibrinogen, may appear in the circulation and produce a similar state; there is reason to suspect that this may occur in the late stages of some leukemias [34] when cellular turnover is markedly increased and intracellular proteases are liberated in excessive quantities.

Examination of the blood of patients with sustained increases in circulating proteolytic activity has demonstrated the following: (1) delayed clotting and abnormal appearance of the blood clot (shaggy and fragile); (2) prolonged thrombin clotting time; (3) prolonged prothrombin time (two-stage prothrombin time usually normal); (4) moderately reduced fibrinogen level; (5) excessive amounts of immunologically identifiable fibrinogen breakdown products; (6) moderate reduction of factors V and VIII; (7) severely reduced plasminogen level; and (8) greatly increased fibrinolytic activity (rapid whole-blood or euglobulin-clot lysis). Of these, the first five abnormalities can be traced to the effects of fibrinogen proteolysis; the others represent the proteolytic effects on other susceptible clotting components (factors V and VIII) and evidence of a marked activation of the fibrinolytic enzyme system.

Fortunately, the polymerization inhibitors formed during fibrinogen or fibrin proteolysis are spontaneously cleared from the circulation; the half-life of the late plasmin-resistant fragment is approximately 9 hr,[34] but the earlier higher-molecular-weight moieties are cleared more slowly.[29] Thus, control of the underlying mechanism responsible for

the fibrinolytic disorder is followed by spontaneous recovery; such recovery is usually rapid, because the severity of the disorder is critically dependent on the concentration of the breakdown products in the circulation. Control of the primary fibrinolytic disorders can be accomplished by administering the synthetic amino acid, epsilon-aminocaproic acid. Shortly after the oral or intravenous administration of this agent in appropriate dosage (loading dose of 5 g, followed by 20–30 g/day [79]), plasma plasminogen activation ceases and the hemorrhagic diathesis usually subsides spontaneously (frequently dramatically). Inasmuch as the underlying pathogenetic mechanisms are usually transient, 2–3 days of treatment have been sufficient; however, in the fibrinolytic disorder occasionally encountered in metastatic carcinoma of the prostate, much longer periods of treatment appear desirable. Trasylol and *p*-aminomethylcyclohexanecarboxylic acid have also been claimed to be effective in the management of the primary fibrinolytic disorders.[5,8]

SECONDARY OR MIXED FIBRINOLYTIC DISORDERS

The secondary fibrinolytic disorders are seen in association with the disseminated intravascular coagulation syndrome (also referred to as "diffuse intravascular clotting," "acute defibrination syndrome," and "consumption coagulopathy"). These disorders are more common than previously suspected and are being recognized with increasing frequency. Present evidence indicates that most of the acutely acquired hypofibrinogenemic disorders encountered clinically (i.e., those in which plasma fibrinogen is strikingly reduced or absent) are due to disseminated intravascular coagulation. Fibrinolysis contributes significantly to this disorder, usually as a secondary local response [57] but sometimes because of the simultaneous release of tissue thromboplastin and tissue activator into the circulation.

Interest in the disseminated intravascular coagulation syndrome was aroused first by the observation that slow infusions of thromboplastin or thrombin into animals cause an intravascular defibrination with progressive consumption of many blood coagulation factors and, eventually, precipitate a severe hemorrhagic diathesis. This mechanism was shown to be operative in the obstetric catastrophes following abruptio placentae and retained dead fetus; thromboplastic substances derived from the placenta were indicted as being liberated into the maternal circulation and producing extensive intravascular defibrination, followed by a serious hemorrhagic diathesis. The same mechanism was shown to operate in the severe bleeding following incompatible blood transfusions; the intravascular lysis of red cells presumably liberates their

stromal thromboplastin and triggers the defibrination. Since these early observations were made, the subject has grown rapidly and has been the basis of recent symposia,[106,107] reviews,[95,114] and books.[40,77,97]

The characteristic profile in patients with an acute consumption coagulopathy is a severe hypofibrinogenemia or afibrinogenemia and sharply reduced levels of prothrombin, platelets, and other coagulation factors, most notably factors V and VIII (labile substances known to be consumed in the process of clotting). Usually, the plasma contains a cold-insoluble fibrinogen (cryofibrinogen) and, on occasion, acceleration of thromboplastin generation can be demonstrated in the laboratory; these constitute suggestive evidence of a thrombosing state. In addition, there is the the associated evidence of fibrinolysis (fibrin breakdown products) resulting from the dissolution of the extensive fibrin deposits, and the contribution of those products to the hemorrhagic diathesis and to the laboratory findings varies from case to case. Because of the virtual disappearance of fibrinogen, platelets, and clotting factors, the presence of a cryofibrinogen, and the tendency of the blood to be completely incoagulable or for the clot to shrivel up into a small ball (because of inadequate fibrinogen) that usually does not undergo lysis, the fully developed stage of this syndrome is not too difficult to recognize. From a practical standpoint, the sudden appearance of a severe hypofibrinogenemia in a patient should immediately suggest acute disseminated intravascular coagulation. This will almost invariably prove to be present if afibrinogenemia is demonstrable.

Fortunately, afibrinogenemic episodes are usually short-lived and, by the time the patient is seen, the acute stage of defibrination is usually over; thus, if the patient can be adequately supported by common measures for hemorrhage and shock, the coagulation disturbance will disappear spontaneously over the next 24–48 hr. The use of fibrinogen intravenously (4–6 g repeated as necessary) is usually restricted to cases in which excessive or uncontrolled bleeding continues or surgery is contemplated; otherwise, the patient is exposed to a 20% risk of hepatitis and, occasionally, to further intravascular clotting.

A similar type of clinical state, although less dramatic and frequently more protracted and slower in evolution (subacute form), in which multiple thrombi in vessels of various sizes are often associated with extensive hemorrhage, is now being described in an ever-increasing number of clinical situations.[65,95,114] Unfortunately, there is a considerable variation in the laboratory findings and these are not so striking as in the acute, more devastating syndrome; the most consistent finding has been the presence of large amounts of circulating breakdown products.[80] As a result, the diagnosis is more difficult to establish. When the findings

are not so typical or the state so protracted, and disseminated intravascular coagulation is suspected on clinical grounds, the judicious administration of heparin is useful as a diagnostic test (provided the patient is not bleeding actively), to determine whether the coagulation disorder is reversed (an expected result if the disorder is due to a consumption coagulopathy).

Well-documented pathologic reports have appeared on the occurrence of disseminated intravascular coagulation in purpura fulminans, Waterhouse-Friderichsen syndrome, carcinomatosis, lymphoma, acute promyelocytic leukemia, thrombotic thrombocytopenic purpura, the Kasabach-Merritt syndrome, viper snake bites, and overwhelming infections, particularly sepsis with gram-negative organisms. It has also been observed as a complication of extracorporeal circulation and has appeared following extensive surgery, particularly on the lung or heart, and following protracted hemodynamic shock of various types. Obviously, these represent a heterogeneous group of cases, and it is quite likely that the mechanisms responsible for disseminated intravascular coagulation may be somewhat different in each. But they have provided clinical counterparts for a variety of animal observations that have been made in recent years.[40,97,106] Included among these are: (1) the defibrination syndromes that have been produced by the systemic administration of various thromboplastic materials or other activators of the coagulation mechanism (e.g., snake venom); (2) the demonstration of the occurrence of intravascular clotting following severe forms of hemodynamic shock, antigen–antibody reactions, and a variety of bacterial toxins; and (3) the importance of intravascular coagulation in the pathogenesis of the Shwartzman phenomenon, of both local and generalized types.

In contrast with the recommended treatment for the primary fibrinolytic disorders, different therapeutic principles have governed the management of the protracted forms of disseminated intravascular coagulation, and heparin has been the agent of choice. Experience with the use of rapid anticoagulation in this disorder is still limited. However, since the report of the successful use of heparin in the management of purpura fulminans,[72] this agent has been used in a number of hypofibrinogenemic states associated with a hemorrhagic diathesis occurring in a variety of diseases and fulfilling the criteria for the disseminated intravascular coagulation syndrome. This aspect of the literature has been reviewed recently by McKay and Müller-Berghaus,[78] Rodríguez-Erdmann,[95] and Verstraete and associates.[114] Despite the established virtue of heparin therapy in the treatment of purpura fulminans and in the management of the hypofibrinogenemic disorder seen with malignant diseases and other forms of protracted disseminated intravascular coagu-

lation, its value in the management of the more acute episodes (e.g., those seen in overwhelming infections and the Waterhouse-Friderichsen syndrome and as a complication of shock and surgery) remains to be established, particularly in the face of the hazards involved.

Other agents like dextran [89] and thrombolytically active substances like streptokinase [65] are also being investigated as potential therapeutic measures in the management of widespread intravascular coagulation, but experience with these agents for this purpose is too limited to recommend them. Antifibrinolytic agents have not been recommended, because, despite the important secondary fibrinolytic aspects of the disorder, their administration in the absence of appropriate anticoagulation may prove hazardous, owing to the aggravation of the underlying thrombosing tendency. However, there are instances in these states in which large amounts of tissue plasminogen activator are in the circulation (as shown by rapid whole-blood or euglobulin-clot lysis times, etc.). This excessive systemic fibrinolytic activity provides a mechanism for fibrinogen degradation as well and maintains the bleeding problem; under these circumstances, epsilon-aminocaproic acid and other antifibrinolytic agents have been used successfully in conjunction with anticoagulant (heparin) therapy.[15]

Because of overlapping findings and the lack of a specific diagnostic test, the differentiation between protracted intravascular clotting complicated by secondary fibrinolysis (fibrin degradation) and a primary fibrinolytic disorder (fibrinogen degradation) may be extremely difficult, if not impossible. Nevertheless, recognition of the problem should lead to the development of new laboratory methods capable of making this distinction accurately, so as to provide a more scientific and effective therapeutic approach. At present, reliance on good clinical judgment and careful laboratory study (of the amount of increased circulating fibrinolytic activity, the severity and nature of the coagulation deficiencies, decreased platelet numbers, the presence of cryofibrinogenemia, etc.) will usually help to resolve this difficulty. When such considerations still cannot provide a distinction, patients may be treated successfully with heparin and epsilon-aminocaproic acid in combination.

CONCLUSION

This brief review has been designed to place fibrinolysis in perspective and to stress areas in which it is related to thrombosis. It has not been possible to cover adequately all phases of the topic, but, fortunately, the more intimate relations of fibrinolysis to fibrin formation will be dis-

cussed in the following presentation by Astrup, and the developments in thrombolytic therapy will be reviewed later by Fletcher. It should also be noted that, although the chief emphasis has been placed on intravascular fibrin deposition, the ramifications of fibrinolysis extend to the resolution of extravascular fibrin deposits as well. (In this connection, fibrinolysis undoubtedly plays a significant role in inflammation, but this aspect remains to be elucidated.)

It should be apparent that the study of fibrinolysis will assume an increasingly important role in our understanding of the mechanism of many common clinicopathologic events; but the rate of accumulation of useful information is still hampered both by deficiencies in methodology and by a lack of understanding of all the factors that regulate and control fibrinolytic phenomena *in vivo*. These inadequacies prevent the investigator from providing definitive answers to the questions being posed. Nevertheless, identification of the problems involved, many of which have been commented on in this review, should serve as an impetus for their solution and, in turn, lead to further rapid advances in knowledge.

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Relation between Fibrin Formation and Fibrinolysis

TAGE ASTRUP

Fibrin formation is not normally the final stage of blood coagulation, in that clot formation is an unphysiologic endpoint. The true physiologic endpoint of blood coagulation is clot lysis. The conceptual background of this idea was formulated early in this century by Leo Loeb and Pierre Nolf. Fibrin formation, whether intravascular or extravascular, is part of a normal repair process after tissue injury or cell death. However, the retention of fibrin is unphysiologic and indicates that the natural processes of fibrin resolution are insufficient. Intravascularly, fibrin deposition may lead to clot development and thrombus formation, thereby changing a natural repair process into a sequence of events leading to serious pathologic changes, ultimately implicating life and death. That is why the clinician is preoccupied with the problems of blood coagulation and fibrinolysis, and it explains his growing interest in the data provided by the basic scientist who is trying to elucidate the mechanisms, biochemistry, and physiology of clot formation and clot resolution.

THE FIBRINOLYTIC SYSTEM

The removal of fibrin involves two physiologic mechanisms, one thought to consist of invasion of the site of injury by leukocytes and the other of an activation of the fibrinolytic system in the body.

Activation of the fibrinolytic system (Figure 1) entails the transformation of plasminogen (a precursor) into plasmin (a trypsin-like enzyme). This transformation may be brought about by activators present in or developed in the circulating blood or by activators present in some tissues, chiefly in vascular endothelial cells. Inhibitors in the blood exert a regulating influence on fibrinolysis, either by preventing

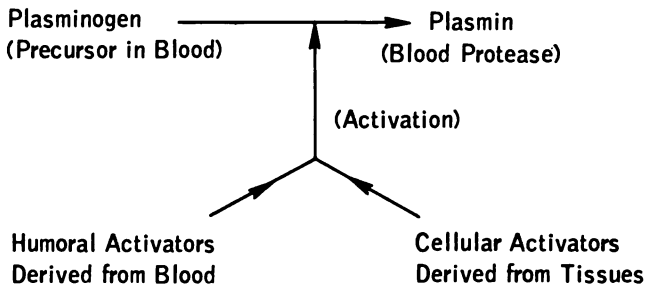

FIGURE 1 The fibrinolytic system. Activation and effect of plasmin are regulated by inhibitors.

activation of plasminogen or by neutralizing plasmin. The distribution and properties of the tissue activator, with which we will be mostly concerned here, have been reviewed recently.[15]

In disagreement with some reports (although in agreement with others), we have been unable to demonstrate plasminogen activator in normal leukocytes.[22] Hence, we believe that the fibrinolytic activity of leukocytes is caused by their content of proteases (leukoproteases) and not by an activation of the fibrinolytic system. Compared with fibrinolysis caused by the migration of capillary endothelial cells that contain activator into an area of tissue repair,[72] the activity caused by the leukocytes is weak; such lysis, under identical conditions, requires 1–2 hr, compared with 5–10 min. Therefore, leukocytic resolution of fibrin is negligible under normal physiologic conditions. It probably plays a role only in tissue repair, when large numbers of leukocytes are chemotactically attracted to the site of injury, producing an inflammatory reaction.

It seems permissible, then, to limit our considerations to fibrinolysis that works through the activation of plasminogen to plasmin, i.e., the fibrinolytic system proper.

THE ROLE OF FIBRIN

During clotting, fibrinolytic agents in blood become adsorbed on fibrin,[49,81,89,106] with two results (Figure 2): (1) removal of the active agents from their combination with inhibitors in plasma and (2) con-

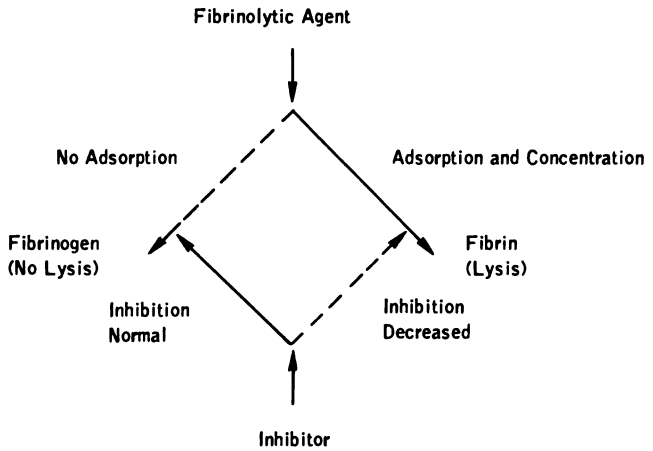

FIGURE 2 Role of fibrin formation and the effect of inhibitory compounds. Modified after Astrup.[11]

centration of the active agents on the fibrin, thereby enhancing their effects.

The physiologic consequences of this adsorption have been considered elsewhere.[11] It suffices here to mention that it is now possible to explain why fibrinogen remains unaffected in plasma samples in which even considerable fibrinolytic activity can be demonstrated by a euglobulin precipitation technique, and how fibrinolysis occurs in such samples after clotting. The absence of fibrinogenolysis in fibrinolytically active blood samples had been a puzzling phenomenon before the adsorption of fibrinolytic agents to fibrin was taken into consideration. Because the fibrinolytic agents are taken up and removed by fibrin, a determination of concentrations of fibrinolytic agents or inhibitors in blood can tell us little about what is going on at the local site of fibrin deposition. In this respect, it is significant that most of the inhibitors in blood are weak inhibitors, so they serve as a buffer system able to release fibrinolytic agents whenever fibrin is formed. The application of this principle, in the form of a plasmin–antiplasmin complex, to induce fibrinolysis for therapeutic purposes has been suggested.[3,4]

FIBRIN DEPOSITION AND THROMBUS FORMATION

The fate of a layer of fibrin deposited on a vessel wall following tissue injury depends on local conditions. It is probably not correct to consider

every parietal fibrin deposit as a mural thrombus. The initial deposition of fibrin is part of a normal tissue repair process. Whether the deposit is soon resolved after having fulfilled its purpose as a matrix for wound healing and tissue repair or remains for a longer period and thereby forms the basis for more extensive repair processes or develops into a fully established mural thrombus depends on the set of conditions prevailing at the site of the initial deposit and on conditions in the circulating blood.

If fibrin is formed in the presence of fibrinolytic agents, these will be taken up and concentrated on the fibrin, as described above, and the resulting fibrin clot will redissolve more quickly. This has been demonstrated experimentally by several investigators. If the circulating blood contains fibrinolytic agents at the moment when fibrin is formed at the site of injury, the active agents will be incorporated into the fibrin and enhance its resolution. Such a process of primary lysis of a fibrin clot is probably of major importance in the resolution of the minute deposits of fibrin that occur daily after tissue injury. If fibrin resolution is delayed and a true mural thrombus forms, accumulation of fibrinolytic agents from the circulating blood on the surface of the fibrin clot will enhance ultimate resolution. The rate of this secondary thrombolysis depends on the availability of fibrinolytic agents in the circulating blood, as well as on the surface area of the clot that is exposed to the blood. If more extensive thrombus formation occurs, the volume of fibrin remaining unexposed to this surface action is proportionally increased, and thrombolysis will be further retarded. If, ultimately, thrombus formation proceeds to complete occlusion of the vessel, the situation is acute, because now only the free surfaces at the ends of the thrombus are exposed to the fibrinolytic agents in the blood. Accessibility is further decreased by the blood stasis prevailing under such conditions, limiting the exchange of fibrinolytic components.

Hence, there are two situations to consider: (1) the formation and deposition of fibrin in the presence or absence of fibrinolytic agents, which determines the incorporation of fibrinolytic agents into the fibrin clot (primary fibrinolysis); and (2) the supply of fibrinolytic agents to the surface of a fibrin clot from the circulating blood (secondary fibrinolysis).

The first of these situations is related to the prevention of fibrin deposition, or rather the retention of a fibrin deposit beyond the period required to complete its function as a matrix in tissue repair. This is a problem of the prevention of a thrombus, rather than of its resolution, and could appropriately be described by the term "thromboprophylaxis." [10]

In contrast, the second situation concerns the removal of a thrombus, or "thrombotherapy." It is apparent that if a thrombus has developed in the absence of significant amounts of fibrinolytic agents in the blood, its ultimate resolution will depend on fibrinolytic agents brought to it from the outside. Because lysis is a relatively slow process, compared with clot formation, and local conditions determine the accessibility to the thrombus of lytic agents introduced into the bloodstream, it is apparent that there are serious obstacles to the successful, practical implementation of thrombolysis in many groups of patients.

PROPERTIES OF THE WALLS OF THE VESSELS

Assuming that fibrin is deposited on the vessel wall initially as a response to tissue injury, the properties of the walls are important. To anticipate the fate of a fibrin deposit, it is important to know whether injured tissue at the luminal surfaces tends to initiate or delay clot formation, and whether lysis of an already formed layer of fibrin is being enhanced or hindered.

Leo Loeb,[76] in 1903, divided the role of blood coagulation in thrombosis into two groups of causes: The first causes are those determined by the constituents in the blood itself. The second causes constitute the influence exercised by the surrounding tissue on coagulable fluids. He briefly mentioned the observation of coagulation-promoting activity in the blood-vessel wall, and debated whether the vascular endothelium could contain compounds that inhibit coagulation.[77]

Next, in 1910, Bernheim [28] presented observations on the relation of the blood-vessel wall to coagulation of blood. He prepared saline extracts of vessels from the dog, chicken, and pig and studied their effects on homologous blood. The pig extract was also tested with rabbit blood. In each case, clot formation was enhanced, and he remarked:

> In the blood-vessel wall itself there is a substance which, once the vessel is injured, probably aids in the formation of a clot. And if this is true, those engaged in the field of vascular surgery have still another problem to consider in connection with the already very difficult and trying technic.

Many years later, Campbell [38] prepared saline extracts of the layers of the bovine aorta and found clot-promoting activity (assayed on bovine plasma) in each.

Returning to the question of whether vascular endothelium is able to prevent coagulation, Zehnder,[135] studying the incoagulability of blood in double-ligated vessels, suggested that the lack of clot formation could

be due to a process of fibrinolysis. Mole,[87] studying postmortem blood, suggested that fibrinolysis could be caused by the endothelial lining of the vessels. This suggestion was supported by McLetchie,[85] who considered an involvement of tissue thromboplastin, released from an injured vessel wall, in the propagation of a thrombus, as well as a possible thrombolytic effect of the endothelium.

Recently, systematic studies have been instigated of the thromboplastic and fibrinolytic properties of the vessel wall and their possible relations to fibrin deposition and thrombus formation.

It was found that the human arterial wall contains relatively high concentrations of thromboplastin in its intimal and medial layers but less in the adventitia.[17,20,69,132] In contrast, the fibrinolytic activity was high in the arterial adventitia and low in the intima.[17,20,75] This would suggest that there is a tendency for the luminal arterial surface of man to deposit and retain fibrin after tissue injury. In animal arteries, the pattern is different [17–19] in that, in some animals, the intima contains little or no thromboplastic activity (monkey and dog) and sometimes even an inhibitor of coagulation (rat), or there is fibrinolytic activity (monkey and dog). From the vessels of some animal species, heparin and mucopolysaccharides with anticoagulant activity have been isolated.[27,55,66,68,130] It is believed that such differences among animal species could explain, in part, differences observed in fibrin deposition and tissue repair in response to tissue injury.[19] Such a concept was substantiated when it was found that the intima of the large human veins is often less thromboplastic than the arterial intima.[39,132] The high concentrations of thromboplastin in the human arterial intima were confirmed by several other investigators.[79,99,108,110] The fibrinolytic activity in human vessels and their extracts was also confirmed.[30,31,75,79,99,109] In veins of dog, resolution of fibrin exposed to the intima was observed.[86]

With a histochemical fibrin slide technique developed by Todd, a relation of tissue plasminogen activator to the vascular system was established. Plasminogen activator was found in endothelial cells of veins and venules.[119–121] This observation has repeatedly been confirmed.[74,124,125] Endothelial cells of newly formed capillaries are particularly active,[72] and by means of them, fibrinolytic activity is brought into an area of tissue repair.[72,73] Similarly, venous thrombi are invaded by fibrinolytically active venous endothelial cells.[71,119,121]

Close observation of the venous wall shows that fibrinolytically active sites are not evenly distributed. No fibrinolytic activity could be demonstrated at sites of attachment of a thrombus to the venous wall.[121] Application of a sclerosing agent to the venous wall destroyed the fibrinolyti-

cally active endothelial cells, and at such places a thrombus formed and became attached to the vessel wall.[71]

The lacking or low fibrinolytic activity of the luminal layers of the human arteries observed in the assays mentioned above was confirmed histochemically,[120] although Todd observed marked fibrinolytic activity of an artery in an ischemic limb.[121] In animals, Warren noticed generally a much lower activity in the arteries than in veins, although the arterial endothelium was also active in some species.[125] The endothelial cells lining an aortic graft in dogs were found similar to the normal endothelial cells.[126] They were also fibrinolytically active.

We have confirmed this common pattern of distribution in our own studies,[74] but several exceptions are now known. Thus, we found that small arteries within the eyeball were fibrinolytically active, although other small arteries on the outside were inactive.[74] Particularly high activity was observed in the retinal artery.[94] In the developing rat eye, it was possible to follow the appearance of fibrinolytically active vascular structures and their subsequent decrease in activity or complete disappearance.[95] The presence of fibrinolytically active vessels was clearly related to the differentiation of the tissues of the eye and their later functional capacity. Similarly, whereas Todd [120] had observed fibrinolytic activity in the human myometrium related only to veins, uterine arteries were found active in the rat.[70] However, vessels in the human liver,[120] human placenta,[2,26] and umbilicus [25] were inactive.

In our early studies,[17] we had observed an increase in fibrinolytic activity of the arterial adventitia with age, but we had not found significant differences in thromboplastic and fibrinolytic activity between samples from normal persons and apparently normal, or only slightly pathologic, samples from patients with various degrees of arteriosclerosis; grossly abnormal samples were not included in that work. Perlick [99] has reported a lower than normal thromboplastic activity of arteriosclerotic samples of human arteries, with an increase in antithromboplastin and in fibrinolytic activity.[100] The samples contained only the intima and media. The adventitia had been removed and was not studied. Kirk, likewise, has reported a lower content of tissue thromboplastin in the atherosclerotic intima than in the normal intima.[69] This appeared to contradict the observation reported by Stevenson *et al.*[114] of an enhanced platelet-like activity in sclerotic arteries. However, Stevenson *et al.* studied the presence of material substituting for platelets in the thromboplastin generation test. Hence, they studied the ultimate formation of plasma thromboplastin as affected by material in the vessel wall and representing the activation of the intrinsic blood coagulation

system. (The interrelation between the intrinsic system and the extrinsic system, when tissue thromboplastin is the coagulation-initiating agent, will be dealt with later.) Previously, O'Brien [92] had noted "platelet lipoid" activity, as well as "brain-like" activity, in endothelial material from the normal human aorta.

In our own studies of samples from the arteriosclerotic human aorta,[21] we observed a considerably lower intimal thromboplastin concentration in the arteriosclerotic parts than in the more normal parts of the aorta from the same patient. The concentration of thromboplastin in the media was usually lower in the pathologic samples. As before, the thromboplastic activity was low in the normal, as well as in the pathologic, adventitia. We concluded that products released from the atherosclerotic vessel walls do not induce extensive clot formation, as is generally assumed, but that clot formation is a local process that occurs at an injured, but otherwise normal, intima. We proposed that, inasmuch as the process is locally restricted, it does not necessarily lead to hypercoagulability of the circulating blood, as has been suggested.

Other reports confirm those findings. The thrombogenic properties, studied in Chandler's apparatus, were lower in atheromatous material than in normal aortic tissue, indicating that material released from an atherosclerotic plaque probably has little influence on the occurrence of luminal thrombosis.[37] The coagulant and thrombogenic properties of human atheromatous material were also studied by Lyford *et al.*[80] Unfortunately, in an otherwise careful study, their only control was a solution of sodium chloride, and comparisons were not made with material from the luminal layers of normal arteries. It is therefore difficult to evaluate the enhancements they reported and to compare them with results obtained by other investigators, such as Prentice *et al.*[103,104]

In our study,[21] fibrinolytic activity was observed in some samples of pathologic media, and there was a considerable increase in fibrinolytic activity in the adventitia of the pathologic samples. A particularly high plasminogen activator concentration was found in the adventitia behind a lesion with medial calcification. This finding probably reflects the increased vascularization of the pathologic samples. Other studies of the fibrinolytic activity of the intima of atheromatous arteries have been reported.[64]

There are also reports of a higher fibrinolytic activity in the human abdominal aorta than in samples from the ascending aorta and the thoracic descending aorta.[122] In veins from human extremities, fibrinolytic activity was found chiefly localized in the vasa vasorum, and there was little or no activity of the venous intima.[96] This observation is

contrary to other reports on human peripheral veins.[121] A higher activity was found in the adventitia of arm veins than of leg veins. As mentioned above, the lack of fibrinolytic activity in the venous intima in rat after destruction of the active endothelial cells leads to attachment of the thrombus.[71] Apparently, after treatment of veins with sclerosing agents, the local balance between fibrin deposition and fibrin resolution approaches that prevailing in human arterial intima. It is of interest, in view of the species differences previously reported,[19] that the rat has been found to respond to a lesser extent than the rabbit to intra-aortic thrombus formation.[50] A similar species difference could probably explain the observations on platelet thrombi reported by Born and Philp.[35]

In a discussion about the role of the thromboplastic activity of the normal intima in the formation of fibrin deposits, it is worth recalling that Eberth and Schimmelbusch as early as 1888 reported the regular absence of thrombus formation at arteriosclerotic parts of the vessel wall.[46] Later, Winternitz, who was a careful observer, and his co-workers found thrombosis to develop in relation to the normal intima and not at atherosclerotic parts.[131] They asked the question: "Is it possible that changes in the cellular elements of the atheroma have caused them to lose their coagulation factor, or to have developed agents which inhibit clot formation?" From the data presented above, it appears that this question can now be partially answered.

It is also apparent from this presentation that fibrin deposition on the intimal surface of vessels is basically a normal tissue repair process (Figure 3). Hence, to avoid confusing the issue, early parietal fibrin deposits should probably not be considered as constituting a mural thrombus.[9] Growth into a thrombus would require initiation of the intrinsic blood coagulation system at the surface of the existing fibrin deposit or activation by means of material released from platelets accumulating at the site of tissue injury. For a normal physiologic balance between fibrin deposition and fibrin resolution, it is of course important for resolution to begin as quickly as possible. As we have seen, in some species, like man, there is a tendency for enhanced fibrin deposition and retention caused by the particular local equilibrium between thromboplastic and fibrinolytic agents. In addition, at an injury whose surface is exposed to the circulating blood, there is a constant supply of coagulation factors, including fibrinogen, from the blood. In such a situation, much therefore depends on the tendency of clot formation in the circulating blood and on its content of fibrinolytic agents.

As explained before,[13] the preferential sites of fibrin deposition at the vessel wall are therefore those at which cellular injury occurs most

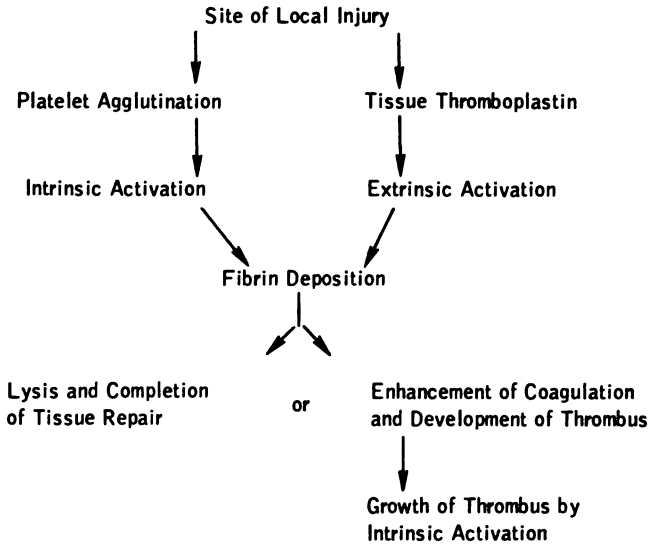

FIGURE 3 Sequence of events after injury to luminal layer of vessel wall.

frequently. Because it would be difficult to imagine that injury should not normally occur at the vessel wall, the sequences of a normal tissue repair process, including fibrin deposition, would be anticipated to occur at vulnerable areas.[12] In fact, there are now several reports on the presence of fibrin in early arterial lesions.[41,45,60,88,133,134] It has also been reported that fibrin formation and platelet destruction occur where the endothelial barrier is interrupted and the underlying connective tissues of the vessel wall exposed.[6] These studies were performed on rats.

In this context, it should be recalled that Anitschkow[5] found that cholesterol was incorporated in the vessel wall only where injuries occurred. He wrote:

> . . . Lesions of the arterial wall which are associated with hyperplasia of the intima create a local predisposition to the formation of lipoidal deposits . . . provided that they are synchronous with hypercholesterolemia.

The hyperplasia mentioned by Anitschkow indicates a response to tissue injury and a localization to sites at which cellular necrosis, followed by proliferation and growth, occurs. A similar conclusion was drawn by Waters,[127] who found that "lipids localize selectively in areas of concomitant arterial injury." Interestingly, Spaet and Lejnieks[113] have recently reported studies on the mitotic activity of rabbit blood

vessels; they found that incorporation of isotope material in the vessel wall depended on a coexistent tissue injury. It has also been reported that tagged dextran adheres to the vessel wall (in dogs) preferably at sites of injury.[33] In agreement with this, it has been found that thrombosis occurs in atheromatous arteries where the atheromatous plaques were disrupted, and that the thrombus was attached to the underlying vessel wall in fissures of the plaque.[40] Also, platelet aggregation took place at the edge of an endothelial ulceration.[51] It is reasonable to assume that, at these sites of injury, normal intimal tissue, with its high content of tissue thromboplastin, was exposed to the circulating blood, thereby inducing fibrin formation and deposition, which were ultimately followed by thrombus formation.

The reason for going into such detail in describing the formation of a fibrin deposit on the vessel wall and its subsequent transformation into a mural thrombus, if resolution is delayed, is to strengthen the concept that such fibrin deposition is primarily a response to tissue injury, and that the localization of such deposits therefore is determined by the occurrence of injuries. There have occurred in the literature many mistaken interpretations related to this point, although the interrelation was clearly explained in the early publications dealing with the equilibrium between fibrin formation and fibrin resolution as a factor regulating reparative connective-tissue formation.[12,13]

THE ROLE OF LOCAL AND HUMORAL FACTORS

It is necessary, also, to discriminate between the thrombus that develops by an extension of fibrin formation during the process of tissue repair, and that becomes attached to the vessel wall at the site of the original injury, and the thrombus that develops in relation to vascular stasis. The mechanism of stasis thrombus formation has been elucidated in particular by Wessler and his group in their many well-known studies.[128,129] The finding that, in areas of stasis-induced thrombi, the endothelium remained unaltered and appeared to play no role in the thrombus formation is important.[6] It agrees with our own observations in ligated segments of rat veins in which thrombosis was introduced by thrombin or serum. In these, the endothelium mostly remained fibrinolytically active; the thrombi were not fully attached to the wall of the vessel, and they underwent rapid resolution by fibrinolysis. This was in strict contrast with the morrhuate thrombi, which were firmly attached to the vessel wall where the fibrinolytically active endothelial lining had been destroyed.[71]

It is apparent that, in the healing of injuries at surfaces exposed to the bloodstream, the factors determining the formation and deposition of fibrin are not solely those of local, cellular origin, but that humoral factors from the intrinsic blood coagulation system ultimately become involved in clot formation.

When the relatively high thromboplastic activity of the human arterial intima had been observed,[20] the conclusion was drawn that intimal injury in the arteries of hemophilic patients, in whom the intrinsic blood coagulation system is defective, would still be able to produce fibrin because of the local release of tissue thromboplastin, and, hence, that parietal fibrin deposits could also be anticipated in such patients.[7] This deduction immediately posed the question of how to explain the fibrosis of joints so characteristic of hemophilia. A lack of tissue thromboplastin in the joint tissues, in the presence of sufficient fibrinolytic activity to dispose of the fibrin normally deposited but not the augmented amounts ultimately accumulating after the excessive extravasation of blood resulting from the delayed coagulation, would seem to provide the necessary conditions and the needed explanation. Therefore, we initiated a study of the thromboplastic and fibrinolytic activities of tissues from normal human joints.[23] The results confirmed the assumption. There was little or no thromboplastic activity in the joint tissues, and the fibrinolytic activity was in the low range. Although several authors have reported on the normal content of tissue thromboplastin in tissues from hemophilic persons, there also are exceptions to this observation, as indicated by the finding of an anticoagulant in extracts of the joint tissues of a seriously ill patient with AHF-deficiency who died at the age of 4 years.[111]

We have also found that conditions in the human heart favor the intrinsic coagulation system and cause it to play a decisive role in thrombus formation, even at the local level. Compared with other human organs, the concentration of plasminogen activator in the myocardium is low.[1] It is even lower in the heart valves,[52] and there is no marked difference between valves on the left and right sides of the heart. In view of the differences reported for the larger arteries and veins, this observation was surprising. The concentrations of tissue thromboplastin are also low, so the situation in the heart valves is reminiscent of that in the joint tissues.[23] Hence, after tissue injury there is little tendency to deposit fibrin on the valvular surfaces through an activation of the extrinsic blood coagulation system. Platelet aggregation and disintegration, followed by the local activation of components of the intrinsic blood coagulation system, probably initiate fibrin deposition. Subsequent resolution depends on the humoral fibrinolytic system

because of the low tissue plasminogen activator concentration. The prevalence of valvular disease in the left side of the human heart can therefore not be explained on the basis of differences in the hemostatic mechanism after local tissue injury. The results support the concept that there are hemodynamic differences, which cause a higher frequency of lesions in the left side than in the right side.[102] Fibrinolytic sites were observed chiefly in relation to vascular structures in the proximal parts of the atrioventricular valves and in the parts of the chordae tendineae close to their attachments to the papillary muscles. There were also a few sites of fibrinolytic activity related to the endocardial lining in the distal parts of the atrioventricular valves. These observations are related to the much-debated problem of whether there are vessels in the normal human heart valves.[58]

From our observations, it is not immediately apparent how fibrinous deposits form on the endocardium.[24] Clearly, the low content of tissue thromboplastin indicates that an activation of the intrinsic coagulation system must be involved. It is quite possible that, after focal injury of the endothelium, with loss of its protective lining, the interstitial space is infiltrated by plasma proteins, including fibrinogen and other blood-clotting components. When clot formation eventually becomes complete, wound healing is probably delayed and the re-establishment of the endothelium retarded. The lack of significant fibrinolytic activity would tend to delay resolution of the fibrin deposit, ultimately converting it to fully organized connective tissue.[83,97] Minute valvular injuries, followed by fibrin formation, growth of the fibrin deposit, and migration of fibroblasts, could be the mechanism by which excrescences and filiform connective-tissue structures are formed on the heart valves.[82] Similar structures are known to arise from the surface of the epicardium at atrial appendages.[107] Vascularized excrescences with papilliform growth were experimentally produced in dogs, and their development was related to sites of friction.[67] Interestingly, McLetchie [85] noticed, in his experiments on experimental thrombosis, the formation of filiform thrombi, which were attached to the intima of some large vessels and became rapidly organized.

The results of studies of the linings of the heart also support the concept that a focal injury will cause cell necrosis and disruption of the endothelial lining, thereby initiating a repair process in which fibrin deposition participates, and that the localization and frequency of injuries are determined to a great extent by hemodynamic factors.

In injuries not exposed to the bloodstream, the local factors gain in importance. However, also in such cases the humoral factors become involved, supplied either by the interstitial fluid or by an exudative

process after tissue injury. Fibrin formation helps to confine the injury and limit the repair process, and in this respect the situation is different from that existing on a luminal side of a vessel wall, which is freely exposed to the circulating blood. In organs supplied by fibrinolytically active vessels, such as the myometrium, tissue repair is probably rapid and little scar tissue is formed. In those lacking fibrinolytic activity, such as the liver, tissue repair is delayed, fibrosis ultimately becoming extensive. Because this aspect of the biologic role of fibrinolysis is not appropriate here, I will not discuss it further.

THE HUMORAL SYSTEM

Thrombus propagation probably depends solely on the intrinsic blood coagulation system, whereas both local factors and humoral factors play a role in local fibrin deposition. Thus, we have seen how fibrin deposition on the vessel wall can occur in hemophilic patients because of the presence of tissue thromboplastin, whereas this mechanism is at fault in the joints in which there is a local lack of tissue thromboplastin.

It is known that tissue repair progresses normally in patients treated with drugs of the prothrombinopenic type (dicumarol and similarly acting compounds) to the extent that surgery can be safely performed,[90] even on the heart or the vessels.[115,116] This prophylactic treatment, although it does not interfere with normal wound healing, decreases the frequency of postoperative thrombosis. Apparently, the amount of fibrin formed by the local release of tissue thromboplastin is sufficient to induce normal wound healing even when the prothrombin–proconvertin concentration is as low as 20% of normal. Because the decrease in concentration caused by dicumarol and similar compounds applies to factors of the intrinsic as well as the extrinsic system, it seems reasonable to assume that the primary deposition of the layer of fibrin needed in tissue repair is influenced to a lesser extent than the propagation of the thrombus, which is caused chiefly by an activation of the intrinsic blood coagulation system.[78] One should view the tissue thromboplastin as remaining bound to cellular structures at the site of injury. It remains as particulate matter and, if introduced into the bloodstream, is rapidly removed by the lung.[16] Plasma thromboplastin, formed by activation of the intrinsic system, is removed by the reticuloendothelial system.[112] Hence, it appears that the body has mechanisms by which it can rapidly rid itself of active coagulation products, thereby avoiding harmful effects. When introduced into the venous system, these active compounds produce widespread thrombosis in the lungs and the right side

of the heart, whereas the systemic arteries usually have been found free of clots.[101]

Introduction of active coagulants into the bloodstream, or activation of the intrinsic blood coagulation system, can tell us little about the mechanism of local fibrin deposition initiated by focal tissue injury. It may, however, teach us something about the conditions for a transformation of the primary fibrin deposit into a genuine thrombus and its further propagation and spreading, in that they depend on the intrinsic system. Fibrin formation at the local level is triggered by the local release of coagulation factors, but the subsequent fibrinolysis depends on local and humoral factors. It is not implicit in this concept that fibrin is deposited on a normal, uninjured endothelium.[14] There is no immediate reason to assume this to be the case, and observations tend to negate it.[61,62] Experiments aimed at the demonstration of such a deposition have been inconclusive, for a variety of reasons. Most of them have used an intravenous injection of the active coagulation agents. Inasmuch as the human venous wall and the pulmonary artery contain a fibrinolytically active endothelium, it is doubtful how far conclusions can be drawn from such experiments.[118] As far as deposition on the arterial side goes, as mentioned above, little activity escapes into the systemic circulation after the intravenous injection of blood thromboplastin [101] or tissue thromboplastin.[16] It is of interest that McLetchie,[85] using a mixture of viper venom and rabbit brain thromboplastin, could produce thin coatings of fibrin that covered large areas of the endothelial surface of pulmonary artery branches.

The hemorrhagic phenomena that occur during anticoagulant treatment concomitant with various forms of stress [65] might be due to a local effect of fibrinolytic agents acting in the presence of a delayed coagulation and a probable enhancement of the blood fibrinolytic system. In contrast, an enhancement of thrombosis has been observed when inhibitors of the fibrinolytic system were present. Some of these were mentioned by Dr. Sherry in the preceding presentation, and it is not necessary to deal further with the problem here, except to draw this conclusion: such cases demonstrate the significance of the fibrinolytic system in the development and resolution of thrombi and, in particular, prove the involvement of the humoral system in the hemostatic balance. There is also the opposite phenomenon of patients who have a lowered content of antithrombin and undergo recurrent thrombotic episodes.[47,48]

The requirement of preceding vascular injury as a basis for fibrin deposition on the vessel wall makes it simple to understand the occurrence of thrombotic phenomena also in patients with various deficiencies of the blood clotting system, e.g., in hemophilia. Several additional

examples are now known.[34,36,53,63] The complexity of the problem of thrombosis in its interrelation with the hemostatic balance is borne out by a case of myocardial infarction that occurred in the presence of an anticoagulant against factor VIII.[50] The effect of the inhibitor could be overcome by an extract from the vessel wall, probably identical with tissue thromboplastin, suggesting that tissue injury was involved in originating the thrombotic condition.

In the local formation of fibrin after tissue injury, factor VII (proconvertin) is particularly important, in that it is needed to initiate clotting by the extrinsic system. This should not lead one to assume that local hemostasis would not occur at all without factor VII; the intrinsic system could still become activated through platelet aggregation and disintegration. In fact, activation of the intrinsic system is required whenever tissue thromboplastin is absent or in too low a concentration, as was found in the human joint tissues.[23] Hence, Nature has provided a double safeguard to secure sufficient local fibrin formation for normal tissue repair. In unfortunate circumstances, both mechanisms of coagulation, the extrinsic and the intrinsic systems, can become impaired and hemorrhage develops, as when excessive doses of dicumarol and similar drugs are given.

However, because factor VII is required only in the activation of the extrinsic system, some differences between its effects and those of the components of the intrinsic system could be anticipated, and there are indications of such differences. The normal absence of hemorrhage into a joint cavity, even though the joint tissues contain little or no tissue thromboplastin, is an indication that the intrinsic blood coagulation system is extremely powerful also in producing hemostasis at the local level. It could be anticipated that patients lacking factor VII would not be particularly prone to develop hemorrhagic episodes, and that seems to be the case.[84,117] It could perhaps be significant that in many such patients death has been caused by cerebral hemorrhage.[42,57,84,93,105] It could be reasoned that hemostasis in the brain depends chiefly on its high content of tissue thromboplastin and that this hemostatic mechanism becomes deficient in the absence of factor VII. Among the female factor VII-deficient patients, a surprisingly large proportion has had uterine bleeding requiring hysterectomy.[84,117] It seems appropriate to relate this phenomenon to the high fibrinolytic activity of the myometrium, or of the endometrium in the secretory stage,[15] and to suggest that lysis here occurs too rapidly to secure hemostasis, in view of the delayed coagulation. In contrast, hemorrhage during surgery is a usual and serious complication in hemophilia (factor VIII deficiency), but hemorrhage affecting the central nervous system is reportedly rare.[43]

However, during the long periods of remission in patients with hemophilia, hemostasis presumably is effected chiefly by the extrinsic system, and the vulnerable sites would be ones that are deficient in tissue thromboplastin, such as the joints. The low frequency of cerebral hemorrhage in hemophilic patients is interesting, in view of the high fibrinolytic activity of the meninges.[15] However, deaths have occurred after epidural hemorrhage.[111] It is more difficult to understand why bleeding into the joints has been reported frequently in factor VII-deficient patients,[29,59,84,117] although the damage is not so severe as in factor VIII-deficient patients.

Of particular interest, in the context of this volume, are reports on the occurrence of venous thrombosis in factor VII-deficient patients.[54,59] These reports constitute a proof that venous thrombosis and pulmonary embolism are caused chiefly by an activation of the intrinsic system. In view of the fibrinolytic activity of the venous endothelium, attachment of thrombi to the vessel wall can be expected only where an exudative process has destroyed the fibrinolytically active endothelial cells, such as in thrombophlebitis. Two of the patients described demonstrate this phenomenon.[54,59] It is probably also significant that Hall *et al.*[59] noticed only minimal atherosclerosis of the coronary arteries and abdominal aorta in their factor VII-deficient patient. In contrast, several instances of arteriosclerotic lesions have now been reported in hemophiliacs, suggesting that release of tissue thromboplastin from the arterial wall has been followed by activation of the extrinsic blood coagulation system. This concept is supported by the absence of reports of progressive venous thrombosis in hemophilic patients.[78] Much information could probably be gained by a detailed study of the pathology of vascular lesions in relation to the various types of blood coagulation deficiencies. Tables 1 and 2 summarize the available data on hemophilic patients and patients deficient in factor VII.

Arterial thrombosis and recurrent venous thrombosis have been described also in patients with hypofibrinogenemia.[91] Such cases are difficult to evaluate, in view of the possible involvement of the so-called consumption coagulopathies, which were recently reviewed by Verstraete *et al.*[123] It would be significant if patients with proved hereditary hypofibrinogenemia could be shown to accumulate enough fibrin to produce vascular disease. This could be expected to occur if fibrin resolution happened to be simultaneously delayed by the presence of an inhibitor of fibrinolysis.

The ultimate proof of the role of fibrin in normal tissue repair is provided by patients with hemorrhagic episodes and defective wound healing caused by the lack of "fibrin stabilizing factor" (factor XIII).[44]

TABLE 1 Hemostatic Balance as Affected in Patients with Hemophilia (Deficient in Factor VIII)—Summary of Available Data

DEFECTIVE INTRINSIC SYSTEM
NORMAL EXTRINSIC SYSTEM
Hemostasis depends on the extrinsic system
Tissue thromboplastin present:
Primary hemostasis normal
Secondary fibrin formation impaired
Tissue thromboplastin absent:
Hemostasis delayed
Extensive extravasation of blood
Fibrosis
(Example: joints)
Arteries:
Lysis delayed
Organization of fibrin deposits and incorporation in vessel wall
Veins:
Lysis normal
Hemostasis impaired
Propagation of clot formation impaired
Hemostasis after surgery greatly impaired
Joints severely affected
Cerebral thrombosis rare
Arterial disease observed
Myocardial infarction described (fibrinolysis weak)
Venous thrombosis reported as rare

TABLE 2 Hemostatic Balance as Affected in Patients Deficient in Factor VII—Summary of Available Data

DEFECTIVE EXTRINSIC SYSTEM
NORMAL INTRINSIC SYSTEM
Hemostasis depends on the intrinsic system
Hemorrhagic episodes rare
Venous thrombosis described
Arterial thrombosis probably infrequent
Hemorrhage in central nervous system frequent
Uterine bleedings frequent (fibrinolysis high)
Hemostasis after surgery only slightly impaired
Joints affected, but not severely

THE METABOLISM OF FIBRINOGEN

The ubiquity of minute injuries in the organism suggests a continuous formation of fibrin followed by fibrinolysis. The sequence of these two reactions forms the so-called dynamic hemostatic balance.[12–14] It is

unknown how much of the total turnover of fibrinogen is caused by this mechanism and how much fibrinogen is catabolized by other means. Hjort and Hasselback [61,62] found little evidence of the involvement of blood coagulation in the normal metabolism of fibrinogen, although they agree on the role of the hemostatic balance in local hemostasis and tissue repair. They found no valid evidence of a continuous fibrin deposition (in the form of a fibrin film) on the *normal* endothelium of vessels. As mentioned above, deposition of fibrin on the normal vascular endothelium is not implicated in the original concept of a dynamic hemostatic balance, which was based solely on a concept of tissue repair.[13,14] However, it should be recalled that no other mechanisms for the catabolism of fibrinogen, with the capacity of the system of coagulation followed by fibrinolysis, have as yet been proposed.[8]

The normal concentrations of fibrinogen (and also of other coagulation factors) show large individual variations, and it is not possible to relate them to metabolic rates. The existence of the consumption coagulopathies makes the evaluation even more difficult. The level in blood will always be determined as a balance between synthesis and catabolism, each of which can be influenced in various ways. Normal levels may result if synthesis is increased and catabolism simultaneously enhanced, or if synthesis is decreased and catabolism impaired. If the metabolic pathway is assumed to pass through the stage of fibrin formation, the variations in the potency of the clotting system, influenced as it is by activating and inhibiting agents, can easily lead to a variety of conditions and situations. It is apparent that the concentration of a coagulation factor, including fibrinogen, is not an expression of the latent state of the coagulation system, and that an increase in concentration of coagulation factors does not necessarily mean an increase in the tendency to form a clot. Penick *et al.*[98] have concluded that a consumption of clotting factors is the basic mechanism leading to a bleeding tendency. They remarked that such a hypothesis would explain the paradox of a hemorrhagic tendency's being prevented by the prior establishment of a hypocoagulable state. This suggestion has since been amply confirmed and such conditions studied in detail.[123] An extreme example is provided by patients with giant hemangioma and fibrinogenopenia in whom fibrinogen levels are brought back to normal by anticoagulant treatment, indicating that conversion of fibrinogen into fibrin is so rapid that it exceeds the rate of synthesis.[32] In consequence, it must be assumed that fibrinolysis is also rapid and is capable of removing the fibrin, in that otherwise a process of repair leading to fibrosis would ensue. In fact, this is often a concomitant feature, and it would be interesting to study such patients in more detail.

This last example illustrates the high metabolic rate of coagulation factors and emphasizes the need for detailed and complete studies of the turnover mechanisms of coagulation factors. Certainly, specific mechanisms have to be proposed to explain the unusually rapid turnover of all the coagulation factors, compared with other plasma proteins, but this problem shall not be dealt with here.

SUMMARY

It is the purpose of this communication to discuss the interrelations between fibrin formation and fibrinolysis in the body. Factors of cellular origin, as well as humoral factors, are involved in these interrelations. Locally, cellular factors gain in influence, particularly in extravascular fibrin formation and resolution. The interrelations are closely associated with tissue injury. Fibrin formation and deposition are parts of a normal tissue repair process. Ultimately, to restore normal conditions, the fibrin must be redissolved. The presence in the organism of activating agents, precursors of active agents, as well as of various inhibiting agents, acting on blood coagulation and on the fibrinolytic system, gives rise to multiple patterns of behavior and interrelations, which depend more on conditions at the site of injury than on the level of coagulation components. Inhibitory agents and anticoagulants present in the humoral system are able to exert a pronounced influence on local processes of fibrin deposition and fibrinolysis.

Some differences between the arterial system and the venous system in the development of thrombotic processes have been pointed out. A parietal fibrin deposit in an artery is believed to be primarily a tissue repair process initiated by local release of tissue thromboplastin. Transformation into a true mural thrombus, probably caused by activation of the intrinsic coagulation system, occurs when fibrinolysis is unable to dissolve the original fibrin deposit. Venous thrombi are believed to be chiefly stasis thrombi produced by activation of the intrinsic system and attached to the venous wall only where the fibrinolytically active lining is destroyed. The physiologic mechanisms of thrombosis and thrombolysis are therefore believed to be different in arteries and veins.

The work reported here was supported by Public Health Service grant HE 05020 from the National Heart Institute.

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VI
THERAPEUTIC CONSIDERATIONS

Anticoagulant Prophylaxis and Treatment of Venous Thromboembolic Disease

PAUL M. AGGELER AND MARTIN KOSMIN

Anticoagulant drugs have been widely used for many years in the treatment of established deep-vein thrombosis and pulmonary embolism. However, the use of these drugs for prophylaxis in patients with no evidence of thromboembolism has never been popular. The practicing physician may be shocked by the tragedy of unexpected death from massive pulmonary embolism in a person whose long-term prognosis would otherwise have been good; yet he seems generally unwilling to risk the bleeding complications of anticoagulant therapy in return for the uncertain protection from venous thromboembolism that such treatment may afford.

We have undertaken an extensive review of the literature to determine what objective basis exists for present general attitudes regarding anticoagulant prophylaxis against and treatment of venous thromboembolism and to determine, if possible, what needs to be done to increase the effectiveness of such therapy.

The underlying causes of venous thrombosis (vascular injury, circulating procoagulants, and stasis of blood within the vessel) and prediction and diagnosis of thromboembolic disease are discussed elsewhere in this volume. In general, factors that contribute to development of a thrombotic state include our sedentary way of life,[68] immobilization of patients for medical disorders,[14,40,68,161] advanced age,[29,32,123,161] and associated diseases.[8,14,29,96,103,114] At present, no single laboratory test clearly differentiates patients with a high risk of developing thromboembolic disease. However, methods to predict thrombosis in a significant proportion of patients have been investigated.[19,23,59,68,84–86,116,168,182,185] Clinical diagnosis of deep-vein thrombosis or pulmonary embolism is both difficult and unreliable. Radiologic examinations and laboratory tests are helpful [14,18,37,48,77,95]; however, postmortem studies show that

the diagnosis is frequently missed [34] and that approximately half the patients with fatal pulmonary embolism had no premonitory clinical signs or symptoms.[158,162] It is obvious that estimates of the efficacy of anticoagulant treatment based on the clinical diagnosis of pulmonary embolism are much less reliable than autopsy studies.

CLINICAL MANAGEMENT OF ANTICOAGULANT THERAPY

Anticoagulant therapy is contraindicated in the presence of a hemorrhagic disorder, subacute bacterial endocarditis, bleeding gastrointestinal lesions, or major hepatic or renal damage; if there is a history of recent peptic ulcer, or surgery of the eye, brain, spinal cord, joints, or urinary tract; or if large unhealed areas are present, either external or internal, as a result of surgery or other conditions. Surgery, preferably ligation or plication of the inferior vena cava, is definitely indicated in patients with recurrent pulmonary embolism during adequate anticoagulant therapy and in those with septic pelvic thrombophlebitis with multiple pulmonary emboli. Surgery may be preferable to anticoagulant therapy in bedridden, complexly ill, surgical patients and in those with chronic congestive heart failure and recurrent thromboembolism.[40,41] It is of utmost importance that contraindications be precisely defined and rigidly observed; not only are some patients more likely to bleed if treated with anticoagulants, but they are more prone to thromboembolism than the average.[50]

Many wound hematomas in surgical and accident cases are unavoidable if anticoagulant therapy is pursued vigorously enough to prevent thromboembolism. Askey [9] found, however, that 90% of the crippling or fatal hemorrhages that occur during long-term therapy are in either the gastrointestinal tract or the brain. He noted that most of the gastrointestinal hemorrhages were caused by underlying lesions, and that only a small proportion of the lesions were diagnosed before therapy. In most of the patients with demonstrable underlying gastrointestinal lesions, hemorrhage occurred when the prothrombin activity was at a "safe" level. This is also true during short-term therapy.[151,198] Askey found a greater incidence of intracranial hemorrhage in hypertensive patients that was not affected by anticoagulant therapy; however, once bleeding occurred, anticoagulants increased the risk of death or disability.

There is a difference of opinion on when prophylactic anticoagulant therapy should begin in postoperative and injured patients. Most cases of fatal emboli occur during the early postoperative period, which indicates that postoperative thromboses develop before or during surgical

intervention; effective anticoagulation can be maintained before, during, and after major surgical operations without increasing hemorrhage or other complications if treatment is carefully controlled.[120,149,151,175,179] Some believe that therapy should be interrupted in patients undergoing surgical procedures because the incidence of fatal and nonfatal complications after hemorrhage can be 40% [167]; they recommend resumption of anticoagulants from the day on which stitches are removed to 7 days after operation. The earlier the treatment, the greater the probable protection against thromboembolism, but also the greater the risk of hemorrhage. Minimum age for anticoagulation of the immobilized patient is also subject to dispute, ranging from 40 to 65 years,[58,160] unless there are special indications.

Despite early lengthening of the prothrombin time to the "therapeutic range," oral anticoagulant drugs may fail to exert antithrombotic, as opposed to anticoagulant, effects for 5 days or more.[49] Early prophylactic treatment in postoperative patients is recommended when oral drugs are used. A period of overlap of 5 days or more may be necessary in shifting from heparin to an oral drug. When both drugs are given together, careful attention should be given to the added influence of heparin on the prothrombin time and of the oral drugs on the whole-blood clotting time.[119] It is important to determine the prothrombin time when the whole-blood clotting time is near normal.

Expert management of oral anticoagulant therapy demands thorough familiarity with all factors that can influence the patient's response to the drug. Variations in response may be either genetic [128] or acquired and may take the form of increased sensitivity or resistance to the action of the drug. They may be caused by differences in the capacity of the liver to produce vitamin K-dependent clotting factors, in the body stores of vitamin K, in the metabolism of the drug,[102] or in the affinity of receptor sites in the liver for the drug and for vitamin K.

Acquired conditions that cause increased prothrombinopenic responses to oral anticoagulants are mostly associated with vitamin K deficiency, impaired liver function, or increased turnover of plasma proteins. In man, vitamin K is obtained partly from the diet (vitamin K_1) and partly from bacterial action in the intestinal tract (vitamin K_2). The requirement for adults is only about 0.03 μg/kg of body weight per day.[61] Most foods contain a small amount of vitamin K; leafy vegetables have a high content, whereas animal tissue contains little or none. The amount of vitamin K_2 absorbed from the intestine is unknown. Natural vitamins K_1 and K_2 are fat-soluble and are not absorbed in the absence of bile salts. Thus, vitamin K deficiency develops when the flow of bile salts into the gastrointestinal tract is blocked, as in obstructive jaundice

or biliary fistula; when intestinal absorption is impaired, as in sprue, steatorrhea, gastrocolic fistula, and ulcerative colitis; and after extensive surgical resection of the intestinal tract. In man, major vitamin K deficiency cannot be produced by either dietary deprivation or sterilization of the gastrointestinal tract,[61] but either one can definitely potentiate the prothrombinopenic effect of oral anticoagulants.[107]

All forms of liver disease—such as Laennec's cirrhosis, cancer, and viral, postnecrotic, or toxic hepatitis [105]—may be associated with decreased ability to produce vitamin K-dependent clotting factors. These diseases therefore cause increased sensitivity to the oral anticoagulants. Postoperative patients and patients with congestion of the liver due to heart failure may also be unusually sensitive because of impaired liver function. Restriction of protein intake and increased metabolism of protein, as in hyperthyroidism [189] and febrile states,[145] can accentuate the drug-induced reduction in circulating clotting factors.

Concomitant administration of some drugs can increase the prothrombinopenic response to oral anticoagulants by any of several mechanisms.[57] Cinchophen [88] and other drugs may cause liver damage, and phenothiazines may induce cholestasis and consequently diminish vitamin K absorption. Phenylbutazone strikingly increases sensitivity to coumarin drugs by displacing warfarin from its binding to plasma albumin, thus making large amounts of free warfarin available to receptor sites in the liver.[3] Phenyramidol potentiates anticoagulation by inhibiting hepatic microsomal enzymes responsible for metabolism of the coumarin drugs.[171] Use of hormones in treating hypothyroidism or in lowering serum lipids may lead to increased sensitivity to coumarin drugs because of increased turnover of blood-clotting factors.[131,156] The mechanism whereby some drugs—such as benziodarone,[141] clofibrate,[156] and the C-17-alkylated anabolic steroids [142,156]—potentiate the action of coumarin drugs is not known.

Occasionally, a patient may deliberately take repeated large doses of an oral anticoagulant to produce factitious hemorrhagic disease and appear to have acquired an unusual sensitivity to the drug.[129]

Increased resistance to the prothrombinopenic action of oral anticoagulants occurs in several conditions: hyperthyroidism treated with propylthiouracils,[189] congestive heart failure relieved by operative procedures or medications, and liver disease treated medically. Pregnant patients require large doses of anticoagulants because they normally have increased levels of vitamin K-dependent clotting factors.

Increased resistance to oral anticoagulants can also result from simultaneous administration of other therapeutic agents, including vitamin K, the natural antidote. Barbital compounds,[35,45,67] glutethimide,[35]

chloral hydrate,[43] and griseofulvin [44] appear to stimulate hepatic microsomal enzymes responsible for the metabolism of coumarin drugs. Barbital preparations exert a greater effect on bishydroxycoumarin than on warfarin, perhaps because fecal excretion of bishydroxycoumarin, but not of warfarin, is increased. Any change in physical condition or introduction or withdrawal of other drugs warrants frequent laboratory examination of the anticoagulant status and appropriate adjustment of dosage.

Errors during anticoagulant therapy often result from insufficient training and experience of physicians and from lack of properly organized anticoagulant units.[111] These lead to inadequate anticoagulation in 15–25% of hospitalized patients and in 20–50% of patients on long-term therapy. Failure to recognize temporary changes in blood coagulation and consequent failure to adjust the dose properly have frequently caused inadequate anticoagulation. Overzealous use of blood transfusions and vitamin K and discontinuation of anticoagulants in patients with moderate bleeding lead to recurrence of thrombosis and death in many cases. Mayer [111] advocates establishment of hospital anticoagulant units with well-trained, full-time specialists to supervise management of anticoagulant therapy; however, Salzman *et al.*[151] believe that dosages prescribed by residents-in-training are adequate.

LABORATORY CONTROL OF ANTICOAGULANT THERAPY

The importance of precise laboratory control for prevention of hemorrhagic complications of anticoagulant therapy has been stressed for many years. No one test is better than another; but the relationships of results of different tests must be considered. A large proportion of serious hemorrhagic complications occur in patients in whom excessive hypocoagulability has been induced. Anticoagulant therapy is controlled mainly by the Quick one-stage prothrombin test that reflects the activity of factors II, VII, and X—which are reduced by the coumarin drugs—and factor V, which is not. The test is not sensitive to factor IX, which is reduced by these drugs. Generally, the prothrombin time should be kept between 1.7 and no more than 3 times the normal value. Usually, this corresponds to between 15% and 30% of normal when determined from prothrombin times of saline dilutions of normal plasma. When dilutions for the standard curve are made with barium sulfate-adsorbed plasma, the prothrombin times correspond to 5–15% of normal activity.

The prothrombin–proconvertin test [133] was developed originally to reflect the activity of prothrombin (factor II) and proconvertin (factor

VII) and was later found to reflect the factor X (Stuart) concentration. It does not measure proaccelerin (factor V) activity. Percentage values in this test are lower than values in the Quick test: 10–20% usually corresponds to 20–30% in the Quick test.

The thrombotest [132] is sensitive to factors II, VII, IX, and X but not to factor V. Thrombotest activity of 5–10% corresponds to 15–24% by the Quick method.[163]

A few investigators claim that anticoagulant therapy is better controlled by using a test that is sensitive only to prothrombin (factor II). In patients receiving long-term therapy, Moschos *et al.*[118] found that protection from thromboembolism and absence of hemorrhage were best in patients kept at factor II levels of 30–50% of normal, which corresponded in his laboratory to one-stage prothrombin times of 15–20 sec (1.6–2.2 times normal), thrombotest values of 8–20% of normal, and P and P test values of 20–50% of normal.

Sevitt and Innes [163] found that prolongation of the one-stage prothrombin time up to 1.5 times normal did not prevent thrombosis; prolongation to 1.5–2 times normal offered protection in some cases but not in others. When the prothrombin time was kept at more than twice the normal value, thrombosis was always preventable; prolongation to more than 3 times normal offered no further advantage. The relationships between the level of hypoprothrombinemia sought, the results attained, and the hemorrhagic complications that occurred were as follows (Table 1): when they aimed for prolongation of the prothrombin time to 2–3 times normal, only two thirds of the patients achieved a level above 2 times normal 70% of the time, but one third had prothrombin times exceeding

TABLE 1 Relationship between Level of Hypoprothrombinemia Sought, Level Attained, and Hemorrhagic Complications [a]

Treatment Aim	Over 70% of Prothrombin-Time Tests Greater Than 2 Times Normal, % of patients	Over 20% of Prothrombin-Time Tests greater Than 3 Times Normal, % of patients	Major Bleeding Episodes, % of patients
Prothrombin time 2–2½ times normal	15	13	6.6
Prothrombin time 2–3 times normal	65	32	13.1

[a] Adapted from Sevitt and Innes.[163]

3 times normal 20% of the time, and about one eighth had a hemorrhage serious enough to require blood transfusion.

Morbidity and mortality from bleeding during prophylaxis with oral anticoagulants can be decreased only by restricting treatment to patients who are relatively high thromboembolic risks, by withholding the drug from those who are most likely to bleed, by frequent and accurate laboratory determinations of the anticoagulant effect achieved, and by skillful adjustment of dosage when required by circumstances.

There are few well-controlled studies relating the dosage and route of administration of heparin to the incidence of hemorrhagic complications. Gurewich *et al.*[70] noted that most serious hemorrhagic complications of heparin are associated with subcutaneous or intramuscular administration. With these routes, in contrast with intravenous administration, absorption of heparin and hence its effect on blood clotting are less predictable; and if neutralization becomes necessary, determination of the proper dosage of antidote is more difficult. Most investigators recommend the use of heparin in sufficient dosage to prevent the clotting time from ever becoming shorter than 2–3 times normal during treatment. Average-sized adults generally require 7500–15,000 units every 8–12 hr subcutaneously, 5000–10,000 units every 3–4 hr by intermittent intravenous injection, and 1000–2000 units/hr by continuous intravenous drip. At these dosage ranges, the risk of hemorrhage is relatively low. Swedish investigators, who report a low rate of hemorrhagic complications, frequently dispense with controlling the dosage by laboratory means [14] and arbitrarily administer 10,000–15,000 units intravenously four times every 24 hr, injecting infusions every 5–6 hr during the daytime and allowing 7–8 hr between injections at night.

CLINICAL TRIALS OF ANTICOAGULANT THERAPY IN ESTABLISHED THROMBOEMBOLIC DISEASE

Although anticoagulant drugs are widely used in the treatment of established deep-vein thrombosis and pulmonary embolism, there have been few properly controlled studies that clearly demonstrated the efficacy of these drugs. Table 2 summarizes data contained in representative reports published over the last 20 years. This list is confined, for the most part, to the English literature, and is by no means complete. All but one of the studies listed are inadequate because of faults in the design of the clinical trial: either the criteria for the selection of the cases were not precise, the method of allocation of patients to treatment

TABLE 2 Occurrence, Recurrence, or Progression of Venous Thrombosis; Recurrence of Pulmonary Embolism; and Incidence of Hemorrhagic Complications in Patients with Thromboembolism Treated with Anticoagulant Drugs

Author	Year	No. Patients	Type of Patients	Type of anticoagulant			Venous Thrombosis, %	Pulmonary Embolism, %		Thromboembolism,[a] %	Hemorrhagic Complications, %			
				Heparin	Oral	Oral and Heparin		Total	Fatal		Mild	Moderate or Severe	Fatal	Total
Patients Treated for Venous Thrombosis														
Barker *et al.*[12]	1945	138	Surgical		X		1.4	0	0	1.4	3.6	0.7	0	4.3
Allen *et al.*[5]	1947	352	Surgical		X[b]				0	1.4	3.4	1.8	0.1	5.2[c]
Cosgriff *et al.*[26]	1948	96	Mixed			X	7.3	3.1	0	10.4				
Coon *et al.*[24]	1958	359	Mixed		X[d]	X[e]	1.7	2.5	0.8	4.2	12.4	2.8	0	15.2[c]
Byrne [27]	1960	118	Mixed	X	X	X		>18.6	18.6	>18.6				
Patients Treated for Pulmonary Embolism														
Barker *et al.*[12]	1945	180	Mixed		X		0	0	0	0	2.8	1.1	0	3.9
Allen *et al.*[5]	1947	44	Medical		X		0	2.3	0	2.3	0.66	1.0	0	1.66
Allen *et al.*[5]	1947	329	Surgical			X			0	0.9	3.4	1.8	0.1	5.2[c]
Cosgriff *et al.*[26]	1948	107	Mixed			X	2.8	2.8	0	5.6				

Ochsner *et al.*[135]	1951	60	Surgical			X		>11.7	11.7	>11.7		3.7	0	>3.7
Coon *et al.*[34]	1958	152	Mixed		X[d]	X[e]	0	7.9	2.6	7.9	12.4	2.8	0	15.2[e]
Barritt and Jordan [13]	1960	54	Mixed			X		1.9	0	5.6			1.9	7.5
Schatz and Lang [154]	1966	443	Mixed		X			1.3	0.9	>1.3		2.2	0.7	
Patients Treated for Thromboembolism														
Bruzelius [24]	1945	113	Surgical		X		2.6	4.4	0	7.1	5.3	7.1	0.9	12.4
Marks *et al.*[109]	1954	1135	Mixed			X	0	0	0	0				
Crane [40]	1957	391	Surgical	X			6.1	5.1	1.0	11.2			0.5	3.3
Fuller *et al.*[62]	1960	394	Mixed	X		X		10.2	2.3	>10.2	4.8	0.2	0	5.0
Donaldson *et al.*[58]	1961	473	Mixed	X	X	X			1.1	24.9				
Bauer [14]	1964	937	Surgical	X			2.6[f]	0.9[f]	0.7[f]	3.5[f]	1.5			1.5
Aaro *et al.*[1]	1966	47	Obstetric	X		X	0	0	0	0				
Husni *et al.*[97]	1967	15	Obstetric			X	0	6.6	0	6.6				

[a] Some authors gave results only when anticoagulant therapy was adequate.
[b] "In most instances."
[c] Statistics regarding hemorrhagic complications for venous thrombosis and pulmonary embolism were combined.
[d] 35% of patients.
[e] 65% of patients.
[f] Some occurred after cessation of treatment.

and control groups was not random, the anticoagulant treatment was not standardized, the diagnosis of thrombophlebitis and pulmonary embolism was not assessed on the basis of uniform objective diagnostic criteria, or the grounds for exclusion from the study were not defined. In many reports, it is impossible to determine what level of anticoagulation was actually achieved. In most instances, the data were analyzed retrospectively, and the results of anticoagulant treatment were compared with either (1) a contemporaneous but not randomly selected series of untreated patients in the same institutions, (2) an untreated series of patients previously observed in the same institution, (3) a series of patients observed in some other institution, or (4) simply the generally accepted norms given in the literature. A randomly selected concomitant control group was available for comparison in only one series—that of Barritt and Jordan.[13] In some studies, patients were treated with heparin alone, with oral anticoagulants alone, or with heparin and then an oral anticoagulant. It was difficult to analyze some series, in that all three methods were used, but the results were not differentiated.

Analysis of the results of these uncontrolled studies showed that recurrence of progression of venous thrombosis ranged from zero to 7.3%, fatal pulmonary embolism from zero to 18.6%, total thromboembolic complications from zero to 24.9%, and severe hemorrhagic complications from 0.2% to 7.1%. In some series, anticoagulant treatment apparently afforded virtually complete protection against the development of further thromboembolic complications with few hemorrhagic complications; in others, it resulted in almost total failure, with the added hazard of hemorrhage produced by treatment itself. However, these authors, with few exceptions,[25] considered that their data indicated a favorable response to anticoagulant therapy—even those who had a high incidence of complications or mortality.[27,125]

The one study [13] that was properly conducted gives thoroughly convincing evidence in favor of anticoagulant treatment. In a prospective study of 73 patients with pulmonary embolism, 10,000 units of heparin were administered intravenously every 6 hr for 36 hr, and oral nicoumalone concurrently for 14 days. After 35 patients were admitted to the study and assigned to treatment and nontreatment groups by random selection, the investigation was discontinued because five of 19 patients not receiving anticoagulants died of recurrent pulmonary embolism and five others had nonfatal recurrences. Only one death occurred in the treated group, and it was from pneumonia complicated by a bleeding duodenal ulcer. An additional 38 patients were taken into the treatment group, and there were no further deaths from pulmonary embolism and only one nonfatal recurrence.

ANTICOAGULANT TREATMENT

SUPERFICIAL THROMBOPHLEBITIS OF THE LEGS

Despite the paucity of well-controlled clinical trials, valuable information regarding the role of anticoagulant treatment of thromboembolic disorders has been accumulated. There is now reasonably good agreement among physicians concerning the respective roles of conservative treatment, anticoagulant therapy, and surgery in the management of thrombophlebitis and pulmonary embolism. When confined to the superficial venous system, phlebitis offers no threat to life. Only when it involves the deep veins does the possibility of pulmonary embolism arise. It is commonly held (perhaps incorrectly) that superficial thrombophlebitis is a self-limited benign disorder that resolves spontaneously and practically never leads to embolism. Thus, many physicians favor conservative treatment of an initial attack of superficial thrombophlebitis in an otherwise healthy person.[153] Patients are not put to bed unless systemic response to the inflammation requires it. Ambulatory patients are treated with an elastic support and are carefully observed. Patients confined to bed are treated with elevation of the leg, active exercises, breathing exercises, warm packs, and perhaps an anti-inflammatory drug, such as phenylbutazone; antibiotics are not used. Ambulation, with elastic support of the leg, is encouraged once the inflammatory process begins to subside. Lack of resolution within 2 days or evidence of progression requires more definitive treatment. Most physicians favor anticoagulation, but some prefer surgical intervention (ligation with or without stripping of the vein).[71]

How frequently superficial thrombophlebitis remains unassociated with involvement of the deep venous system is unknown. Zollinger *et al.*[197] noted that pulmonary embolism is not uncommon in patients with acute superficial thrombophlebitis and speculated that most of the emboli actually occur from thrombi in the deep veins that extend through communicating branches from the superficial veins. Haffner *et al.*[71] found involvement of the deep venous system in 17% of 133 patients during surgery for superficial thrombophlebitis. Thomas [178] felt that acute thrombosis at any site should be treated with anticoagulants.

It is generally agreed that immediate treatment of superficial thrombophlebitis with anticoagulants is advisable in some high-risk patients: patients with acute myocardial infarction or congestive heart failure or a history of thromboembolic disease or varicose veins; patients immobilized with a fractured hip or other traumatic disorder; and postoperative patients.

DEEP-VEIN THROMBOPHLEBITIS OF THE LEGS

Patients with established deep-vein thrombosis should be kept in bed with the affected leg in warm packs and with the foot of the bed raised to promote drainage until edema has subsided and the limb is back to normal size. Treatment with heparin should be initiated immediately at the first suspicion of the diagnosis and not delayed in the hope that signs and symptoms will either become more definite or subside. Heparin is the drug of choice because its anticoagulant effect is immediate and because its anti-inflammatory effect may relieve symptoms in the legs. A single episode of acute phlebitis can be treated with heparin alone. Treatment should be continued for 2 or 3 days after complete subsidence of active phlebitis and full ambulation, which requires at least 10 days, and then discontinued in an otherwise healthy person. In patients with recurrent thrombophlebitis, anticoagulant therapy should be continued for longer than 3 weeks; most physicians prefer to shift to an oral anticoagulant after several weeks of treatment with heparin. Duration of treatment for recurrent thrombophlebitis must be decided on the merits of the case, but durations of 3 months to 1 year have been suggested.[190] Treatment can be terminated abruptly without the occurrence of "rebound" phenomena, unless the patient has hemorrhaged [160,166]; tapering the dose is of no benefit. The importance of adequate dosage and duration of treatment with heparin in pulmonary embolism has been stressed.[173] In a study of records gathered from the world literature of some 500 patients who died of embolism during heparin treatment, in no instance was the drug given in a dose of 40,000 units/day for a minimum of 3 weeks.[190]

Anticoagulant therapy is indicated for pregnant and postpartum patients who have deep-vein thrombosis or pulmonary embolism,[1,172,194] Heparin alone is recommended for short-term treatment, and the oral drugs for extended use. Heparin has the advantage of not crossing the placental barrier. Although oral anticoagulants pass through the placenta,[150] the danger to the fetus is minimal when therapy is properly controlled; therapy should be continued until labor begins or is induced. It may be necessary to administer vitamin K before delivery to minimize the danger of hemorrhage for both mother and infant.

Aftercare of patients who have had thrombophlebitis is extremely important.[194] During the acute phase, the valves of the involved vein often become inflamed and deformed or destroyed. Absence of the valves results in chronic venous insufficiency that develops slowly over the course of months or years. The condition is characterized by varicose vein

formation, congestion of dependent tissues (especially around the ankle), hemosiderin deposits in the skin, stasis dermatitis, and ulcerations of the skin and subcutaneous tissues. If neglected, chronic ulcers may make an invalid of the patient. Although it is believed that morbidity from late sequelae is reduced by anticoagulants,[7,14,183] few data are available.[20]

PULMONARY EMBOLISM

Anticoagulant treatment of pulmonary embolism with or without apparent venous thrombosis is the same as that for deep venous thrombosis alone; however, the optimal duration of treatment in this situation is uncertain. Treatment should commence with heparin, not only for its antithrombotic and anti-inflammatory effects, but also because it may relieve bronchial spasm by preventing the release of vasoactive amines from platelets in the thrombus.[178] Acute pulmonary embolism should be treated with large doses of heparin (10,000–15,000 units) intravenously every 4 hr for the first 24–48 hr. Anticoagulant treatment should be continued for at least 21 days. This is probably sufficient in patients whose embolism has followed an accident, an operation, or childbirth. However, when pulmonary embolism occurs spontaneously, many physicians recommend continuing therapy for 3–6 months if it is the first episode and longer if it is a recurrence.[66,70,178]

Patients with congestive heart failure or recent myocardial infarction tolerate pulmonary embolism poorly, and treatment with either anticoagulants or surgical interruption is often unsuccessful. Byrne [27] studied a series of 979 cases of thrombophlebitis at the Boston City Hospital and found that 86% of 196 patients who died had cardiac disease. The mortality rate in patients treated with anticoagulants was 32% in those with heart disease and 4% in those without. Because pulmonary embolism in the cardiac patient is often a recurrent and continuing problem and because anticoagulant therapy is frequently unsuccessful, early surgical intervention should be considered.[41,140]

PULMONARY MICROEMBOLISM

At least two types of clinically important microemboli arise in the venous circulation.[55,140] Microemboli of one type, found in pulmonary arteries 0.3–2.0 mm in diameter, resemble large venous emboli. They originate under conditions of venous stasis, become detached, and are carried to the lungs. They may occur in the patient who has a large pulmonary embolus or in the absence of or for a long period after a subsidence of

signs of deep venous thrombosis in the legs. Repeated showers of microemboli may lead to obliteration of the pulmonary vascular bed, pulmonary hypertension, and cor pulmonale.[66,130] If the diagnosis is suspected early and anticoagulant therapy is prompt and prolonged, the process may be arrested.[178]

Microemboli of the other important type are found in vessels 15–200 μ in diameter. They are composed of aggregates of platelets or of compressed fibrin mixed with platelets. They arise in the venous circulation during disseminated intravascular coagulation and are carried to the lung. What triggers this condition is often not clear. This type of microembolism is a common cause of morbidity and death after emergency operations for arterial occlusive disease or ruptured abdominal aneurysm [17]; treatment with heparin is recommended. The incidence of both types of microemboli is high in patients with fatal injuries or burns.[55]

MASSIVE ILIOFEMORAL THROMBOPHLEBITIS

There have been no well-controlled studies that compared the effects of anticoagulants and surgical interruption in patients with thromboembolic disease, particularly in patients with massive iliofemoral thrombophlebitis. Anticoagulant therapy is recommended by some [20] and used with caution by others.[31,60] Catchpole [31] believes that anticoagulants increase the liability to or the extension of gangrene because hemorrhage into the leg in the initial stages of phlegmasia cerulea dolens may cause further circulatory embarrassment. He advises their use after venous channels begin to open up, to prevent recurrence of thrombosis. Iliofemoral thrombectomy is advocated by several authors [47,60,72,93,104] for both phlegmasia cerulea dolens and phlegmasia alba dolens, to relieve pain and swelling, to prevent gangrene, and to preserve deep-vein valve competence. Whether this operation is more successful than anticoagulant therapy in preventing postphlebitic complications is not certain. Postoperatively, thrombosis tends to recur and the incidence of embolization is great, particularly when anticoagulants are not used.[52] Karp and Wylie [95] noted that all patients improved clinically after iliofemoral thrombectomy, but that objective evidence of postoperative anatomic patency of the surgically treated vessels was lacking. They performed iliofemoral venous thrombectomy in 10 cases; heparin was administered during surgery and throughout the hospital stay. Postoperative phlebograms showed evidence of occlusion of the previously patent segment of the femoral vein that usually extended cephalad to involve the iliac vein. They concluded that relief of symptoms by iliofemoral thrombectomy

could not be explained by continued patency of the surgically treated segment of the femoral vein.

SUPRACRURAL VENOUS THROMBOSIS

Venous thrombosis in sites above the inguinal ligaments is uncommon. The importance of the lesion depends on the hemodynamic effects, the potential for embolization or extension, and the underlying disease process that it often heralds. Clinical manifestations depend on the anatomic location and the completeness and rapidity of formation of the venous obstruction.

The incidence of venous thrombosis and the potential for embolization of venous clots above the inguinal ligaments are probably underestimated, because the venous system is not usually completely dissected in routine autopsy, even when a pulmonary embolus is present. Although anticoagulation is believed effective, there is a dearth of prospective well-controlled studies. Most authors, whether they praise or damn anticoagulant drugs, fail to indicate dosage, adequacy of control, and other data.

Thrombophlebitis Migrans [46,63] Thrombophlebitis migrans is characterized by remissions and exacerbations involving both peripheral and visceral veins at random. Its etiology is unknown, but it may herald a deep-seated malignancy or be prodromic to Buerger's disease.

Thrombophlebitis migrans is believed responsible for 4.5–27% of all cases of thrombophlebitis. Morbidity and mortality depend on the site of thrombosis and on whether pulmonary embolism ensues. Mortality may be as high as 20% and is highest in patients with visceral vein involvement. The incidence of pulmonary embolism is no greater in this than in other forms of deep venous thrombosis. There is no conclusive evidence that any form of therapy, including long-term anticoagulation, is effective.

Thrombosis of Inferior Vena Cava and Its Tributaries [11,54,74,78,100,101,113,115,117,134,137,138,155] Obstruction of the inferior vena cava must be viewed segmentally, because collateral circulation, clinical manifestations, and etiology differ markedly, depending on the level of the block.

The lower segment of the inferior vena cava extends from the iliac veins to the renal vein ostia, and carries 25–33% of the resting cardiac output. Obstruction is usually due to a thrombus extending from the iliofemoral system. External compression and tumor invasion are less likely responsible for obstruction at this level. Collateral flow is via the

renal venous system; it develops in about 1 week but does not reach maximum efficiency for 3 months. Renal venous return, which constitutes 25% of the resting cardiac output normally and increases when the lower segment is thrombosed, prevents proximal extension of the clot. Pulmonary embolism appears to be unusual once thrombosis is complete. Short-term anticoagulation is advocated in the early stages. Long-term anticoagulation seems justified to prevent extension to the middle segment or to the renal veins in patients subject to fluctuations in renal blood flow.

The middle segment of the inferior vena cava extends from the renal veins to the hepatic vein ostia. Obstruction causes varying degrees of renal damage and is twice as likely to result from neoplasm as from thrombosis. Collateral flow is from the inferior vena cava to the portal vein. Obstruction to renal vein drainage in adults more frequently results from caval pathology, although profound alterations in renal blood flow due to renal disease may produce thrombosis in the renal vein. Primary thrombosis of renal veins occurs more frequently in debilitated infants, especially those with diabetic mothers. Regardless of the cause or site of obstruction, the kidney goes through a congestive phase, which may result in rupture or hemorrhagic infarct, and collateral flow develops and allows some degree of renal repair. Gradual obstruction is more commonly the case in adults and may result in nephrotic syndrome. Mortality is high in both adults and children with renal complications: 63% of the adults die within 2 months of onset of renal failure and an additional 15% die within 2 years. The incidence of pulmonary embolism in adults with renal vein obstruction is high. Therapy differs for children and adults. In the child, one case of dramatic improvement with massive anticoagulant therapy has been reported, but this form of treatment is not generally advocated; indeed, some authorities consider anticoagulation contraindicated. In the adult, one definite indication for anticoagulant therapy is sudden development of oliguria and uremia in patients with renal amyloidosis. Improvement of the nephrotic syndrome occurs in some patients; in one patient, complete reversal was obtained following early diagnosis and treatment with heparin.

Obstruction of the superior segment of the inferior vena cava, which receives 25% of the total venous return from the hepatic veins, is the least common. Of patients with idiopathic occlusion of the hepatic veins, 20–30% have associated thrombosis of the inferior vena cava. Symptoms are generally those of rapidly developing hepatic enlargement and ascites, whether the superior caval segment or the hepatic veins *per se* are occluded. Collateral flow is from the unobstructed portion of the inferior vena cava and from the portal vein to the superior vena cava.

Thrombosis of the portal vein occurs in 20% of patients with hepatic vein obstruction. Pulmonary embolism does not commonly accompany thrombosis of the hepatic veins or superior caval segment; but the prognosis is poor; 45% die within 3 months of the onset of hepatic enlargement and ascites, and 75% die within 1 year. Anticoagulants generally are not used, because of associated liver disease and the presence of or potential for variceal bleeding. In patients with thrombosis of hepatic veins, a case could be made for anticoagulation after portal decompression, to ensure patency of the superior caval segment.

Mesenteric Venous Occlusion [28,63,89,112,123] Venous occlusion constitutes 15–45% of all mesenteric vascular accidents, and the superior mesenteric vein is the most commonly involved vein. Of cases of mesenteric venous occlusion, 25–55% are primary and the remainder are associated with a variety of diseases, usually intraperitoneal infection. Mesenteric veins are the most frequent visceral sites of thrombophlebitis migrans. Most venous occlusions occur without infarction, and spontaneous recovery can occur if the circulation to less than 6 in. of small bowel is affected. Nevertheless, mortality is 50–80%, and recurrence is not uncommon.

Most authors do not believe that venous and arterial occlusion can be differentiated clinically; but the few who do advocate anticoagulant treatment. The use of anticoagulants is tenuous, because bleeding is often present. However, many surgeons administer anticoagulants postoperatively.

Pelvic Thrombophlebitis [90,98,106,126,147,152,157] Thrombosis of pelvic veins during puerperium or following gynecologic surgery is an established but unusual clinical event. Many consider the presence of both fever and nonspecific pelvic findings refractory to antibiotic therapy to be presumptive evidence of pelvic thrombophlebitis. In 1911, Michaelis recognized the relationship between clinical findings and pulmonary emboli. Rapid improvement with heparin therapy appears both diagnostic and curative. Occasionally, clinical manifestations suggest a severe abdominal emergency, and the diagnosis is made at laparotomy. Under these conditions, either caval or venous ligation or anticoagulant therapy has been recommended.

Pelvic thrombophlebitis has not been observed in men; however, pulmonary embolism is a cause of death following prostatic surgery, accounting for 1–6% of fatalities. Because of the risk of postoperative bleeding, anticoagulants are not widely used. In Sweden, concomitant use of antifibrinolytic therapy (EACA) with heparin has reduced the inci-

dence of postprostatectomy thromboembolism without increasing the incidence of bleeding.

Priapism [15,65,127,170] Regardless of etiology, the ultimate anatomic problem in priapism is venous thrombosis. Early administration of anticoagulants, although not always curative, is used in conjunction with a variety of procedures aimed at early detumescence.

Superior Vena Caval Syndrome [33,94,144,188] Since 1949, 734 cases of obstruction of the superior vena cava have been reported in the world literature; 70% were secondary to thoracic tumors that were primary and malignant in the majority of patients. Obstruction itself is compatible with long life; it is the etiologic factor that alters the prognosis.

Drainage is via the azygos vein if the obstruction is proximal to its junction with the superior vena cava. If the obstruction is distal to that level, collateral flow is through vessels in the thorax and abdomen to the inferior vena cava. Pulmonary embolism is not usually associated with this condition.

Anticoagulants are not generally used, and in most instances in which they were tried the results were disappointing. Improvement can occur with long-term anticoagulation, particularly with heparin in nonmalignant cases; it may be that in those cases anticoagulants promote patency of collateral vessels.

Jugular Vein Thrombosis [188,192] The jugular vein is an exceedingly rare site of thrombosis. Most cases are associated with regional infections in the neck or prolonged, severe congestive heart failure. The incidence of association with pulmonary embolism is high; thus, anticoagulant therapy is indicated.

Axillary Vein Thrombosis [2,10,42,58,83,108,174,188] The axillary veins are the most common sites of thrombosis of all the superior caval tributaries and constitute at least 1% of all cases of deep-vein thrombophlebitis. Idiopathic thrombosis of the axillary vein is at least as common as thrombosis secondary to an underlying disease and is characteristic enough to warrant an eponym (Paget-von Schroetter syndrome). The incidence of pulmonary embolism may be as high as 12%, but death rarely results. Early administration of heparin is recommended, both as prophylaxis against pulmonary embolism and as specific therapy. When pain is the primary complaint, the response to heparin is dramatic and rapid, with minimal residua; when swelling is the primary symptom, the response is less impressive, probably because the symptoms represent a more chronic process.

Chest-Wall Thrombophlebitis (Mondor's Disease) [80,148,169] Mondor's disease is rare and is important only because it must be differentiated from malignancy of the breast. It is self-limited and mild in its manifestations, except for its disquieting effect on the patient. Recurrence, emboli, and systemic effects have not been reported. All varieties of therapy, including anticoagulants, have been tried, but none seems to alter the disease course. In one of the few male patients reported, the condition developed during oral anticoagulant therapy for a myocardial infarct.

Cerebral Vein Thrombosis [21,30,139] Cerebral venous thrombosis usually results from infection in neighboring structures or from direct injury. It may also be primary in severely debilitated patients who are either very young (especially infants) or old. It is also associated with pregnancy. The incidence is about one or two cases per 3000 pregnancies; in India, it is considerably higher. Mortality during pregnancy and puerperium is about 33%, and the incidence of residual abnormalities is 20%. It may recur within a single pregnancy and in subsequent pregnancies. The incidence of pulmonary embolism is not known, but it does occur—especially when thrombosis of the transverse sinus has extended to the jugular vein.

Anticoagulant therapy did not appear to improve morbidity or mortality in the small number of patients reported. Grossly bloody spinal fluid, which occurs in fewer than 10%, is the only contraindication to anticoagulant therapy. Evidence of thrombosis elsewhere and evidence of extension of the thrombus in the cerebral vein have been cited as indications for anticoagulant therapy.

Retinal Vein Occlusion [99,186,187] Retinal vein occlusion is uncommon in the general population but not excessively rare in ophthalmologic practice. Anticoagulant therapy for this disorder was first introduced in 1938; it improves vision and prevents complications in some categories of patients. The prognosis for substantial improvement in visual acuity once the central vein is occluded is extremely poor, regardless of therapy.

Prophylactic anticoagulation is more promising, because the vessels can be seen directly. Signs and symptoms of imminent occlusion include intermittent or slight constant visual depression, venous congestion, neovascularization, and papilledema; unilateral papilledema is the most obvious of the early signs.

Infusion Thrombophlebitis [56,76] Infusion thrombophlebitis occurs in approximately 25% of patients who receive intravenous therapy over

long periods. Additional factors that increase the incidence of this condition are the use of small-caliber veins, the presence of a prolonged circulation time, sex (female), and the pH of the infusate. Pulmonary embolism is a potential threat, but the immediate problem is equally important. The place of anticoagulant therapy in preventing infusion thrombophlebitis is not clear; however, buffering infusates to a neutral pH decreases the incidence of infusion thrombophlebitis.

Portal Vein Thrombosis [82,176,180] The high incidence of bleeding of esophageal varices in patients with thrombosis of the portal vein precludes any thought of anticoagulant therapy in this condition.

ANTICOAGULANT PROPHYLAXIS

Anticoagulants are not as widely used to prevent venous thrombosis and pulmonary embolism as they are to prevent recurrence or extension in patients in whom these complications have already occurred. The principal arguments against prophylactic anticoagulation are the labor, expense, and added hazard of hemorrhagic complications. If anticoagulant therapy is pursued vigorously enough to prevent thromboembolism, it will inevitably increase the risk of hemorrhage.[161] The strongest argument for prophylaxis is that at least half the deaths from pulmonary embolism occur suddenly in patients with no previous evidence of deep-vein thrombosis. These patients cannot be saved if treatment is restricted to those with clinical evidence of thromboembolism.

A small number of deaths from pulmonary embolism occur even in the general "healthy" population.[22] Higher incidences of thromboembolism are associated with a variety of conditions mentioned previously. Obviously, it is impossible to administer prophylactic anticoagulants to the entire population. Prophylaxis has usually been confined to patients at high risk, principally postoperative and accident cases, and to those with cardiac disease. Statistics on the efficacy of prophylactic anticoagulants are confined to those categories.

SURGICAL AND ACCIDENT PATIENTS

The incidence of thromboembolism after general surgery is not very high, ranging from 0.5% to 2%. However, after some operations, particularly for lung cancer and mitral stenosis, thromboembolic complications occur in 5–10% of patients.[175] It is difficult, therefore, to compare results of treatment in one series with those in another, or to know whether

anticoagulant therapy was effective, without a concomitant randomly selected control series for comparison.

Clinical Trials of Heparin Heparin was developed for clinical use by Jorpes [92] in Stockholm and Murray and Best [122] in Toronto; it was first used for prophylaxis by Crafoord in 1937.[38] In the next few years, considerable evidence was presented [24,39,91,121,191,196] to show that the incidence of postoperative thrombosis and pulmonary embolism could be reduced when the drug was used in adequate doses over a sufficient period; none of these studies was well controlled.

Because of the expense and trouble of heparin therapy, its prophylactic use was largely abandoned when oral anticoagulants became available in the early 1940's. The recent report by Sharnoff,[164] however, merits attention because of his unorthodox dosage schedule. He felt that the object of treatment should be to maintain normal coagulation at all times, especially during periods of hypercoagulability. He gave 10,000 units of heparin subcutaneously about midnight on the day before operation to 140 patients deemed to have a high risk of developing thromboembolic complications. On completion of the operation, 2500–5000 units of heparin were administered subcutaneously every 6 hr until discharge. A second group consisted of 92 patients with the same degree of high risk who were treated in the same manner postoperatively but were not given heparin preoperatively. The author concluded that the high incidence of proved pulmonary embolism in the patients who did not receive heparin preoperatively, compared with those who did, showed that normocoagulation during the operation was essential to prevent thromboembolism. Unfortunately, the numbers of deaths and autopsies in his series were too small to allow definitive conclusions. A more extensive trial of this method of prophylaxis is indicated.

Clinical Trials of Oral Anticoagulants without Controls The first clinical reports of the prophylactic use of bishydroxycoumarin were published in 1942.[4,26,195] There have since been additional studies of the use of this drug and other oral anticoagulants for prophylaxis against thromboembolism. The results of a representative group of postoperative and accident studies in which adequate control series were not available for comparison are given in Table 3. Analysis of the data shows an incidence of venous thrombosis of zero to 3.1%; of pulmonary embolism, zero to 3.0%; of fatal pulmonary embolism, zero to 0.3%; of total thromboembolic complications, 0.1–4.3%; of serious hemorrhagic complications, less than 0.1–3.1%; and of total hemorrhagic complications, 1.0–

TABLE 3 The Incidence of Thromboembolic and Hemorrhagic Complications Postoperatively in Patients Who Received Oral Anticoagulants Prophylactically

Author	Year	No. Patients	Type of Surgery	Venous Thrombosis, %	Pulmonary embolism, %		Thromboembolism, %	Hemorrhagic Complications, %			
					Total	Fatal		Mild	Moderate or Marked	Fatal	Total
Bruzelius [24]	1945	1391	General	0.8	0.6	0.07	1.4	2.2	2.4	0.22	4.6
Barker *et al.*[12]	1945	682	General and gynecologic	0	0.6		0.6	4.2	3.1		7.3
Allen *et al.*[5]	1947	832	Gynecologic—cesarean section	0.36	0	0	0.36				
Wise *et al.*[198]	1949	3304	General and gynecologic		0.06		0.18	2.0	<0.1		2.1
Thies [177]	1961	8338	General and orthopedic	0.1	0.01		0.11				1.0
Greep [68]	1961	3113	General			0.2	3.0	1.2	0.8	0.13	2.0
Bottomley *et al.*[19]	1964	3771	Gynecologic	3.1	1.2	0.3	4.3	3.4	0.7	0.03	4.2
Fagan [59]	1964	99	Orthopedic—frac. femur	1.0	3.0		4.0				4.0
Belding [16]	1965	1414	General	0.26	0.07		0.33	0.2			
Shepard *et al.*[165]	1966	2371	General	0.25	0.17	0.13	0.42				

7.3%. Additional details regarding the individual studies are given in the following paragraphs.

Bruzelius [24] administered bishydroxycoumarin 1–5 days after operation, keeping the prothrombin-time index * between 40% and 60% of normal. Barker *et al.*[12] treated 682 surgical patients; 438 underwent abdominal hysterectomy and 61 had a history of thromboembolic disease. They attempted to keep the one-stage prothrombin concentration between 10% and 30% of normal. Allen *et al.*[5] confined their study to women who had abdominal hysterectomy and cesarean section, and prophylactic treatment was with bishydroxycoumarin. Wise *et al.*[193] also used this drug in the prophylactic treatment of gynecologic or major surgical patients. Patients under 20 years old and thoracic surgery and mastectomy patients were excluded; most of the remaining patients on the clinic service who had no obvious reason to bleed were included. Bishydroxycoumarin (200 mg) was started on the second postoperative day; the aim of treatment was to keep the prothrombin concentration between 40% and 50% of normal until discharge. The average total dose ranged from 600 to 800 mg.

Thies [177] treated patients over 30 years old with oral anticoagulants, chiefly Marcoumar. The drug was started on the third or fourth day after hemostasis was established after surgery, accident, fracture, or contusion, and on the fourteenth day after brain injury. Treatment was continued for 8 days or until the patient was ambulatory. The incidence of venous thromboembolism was 0.11%; on the same service before use of anticoagulants, it was 4%. Greep [68] reviewed the effects of prophylactic treatment in postoperative patients between 1957 and 1961; 23.3% of high-risk patients admitted to the service were treated, usually with Sintrom. Between 1952 and 1956, in a similar but untreated group, the incidence of thromboembolic complications was 4.0%; of fatal emboli, 0.4%; of major and minor hemorrhagic complications, 0.1% each; and of fatalities from bleeding, 0.008%.

Bottomley *et al.*[19] treated the following selected postoperative gynecologic patients in the United Cambridge Hospitals from 1954 through 1963 inclusive: all patients with (1) cesarean section, (2) surgery for malignancy of the genital tract, (3) major gynecologic or obstetric surgery and a history of thrombophlebitis, or (4) major gynecologic surgery and age above 40 years. Phenindione was administered on the third postoperative day and was continued to keep prothrombin concentration at 15–30% of normal, by the one-stage method, until the tenth postoperative day or ambulation. In 1961, the thrombotest was

* Prothrombin-time index $(\%) = \frac{\text{normal prothrombin time}}{\text{patient's prothrombin time}} \times 100.$

substituted and the level was kept between 10% and 20% of normal. Results were considerably better than those obtained between 1949 and 1953, when anticoagulants were used only after occurrence of signs and symptoms of thromboembolism. During this period, the incidence of venous thrombosis was 31.8%; of nonfatal pulmonary embolism, 4.2%; of fatal pulmonary embolism, 1.8%; and of total thromboembolic complications, 37.8%. This report [19] stimulated a lively correspondence in *The Lancet*. Rhodes [143] pointed out that the combined statistics for the Grosvenor, Lambeth, St. Thomas, and Lying-In Hospitals, where anticoagulant prophylaxis was not practiced, showed a lower incidence of fatal pulmonary embolism than that reported by the Cambridge group. Patterson [135] noted that, at the Samaritan Hospital for Women, where anticoagulant therapy was given only for established deep-vein thrombosis or pulmonary embolism, the incidence of nonfatal pulmonary embolism among 8555 patients who underwent major vaginal or abdominal surgery was about twice that of the Cambridge group (5.7 versus 2.3 per 1000), but the incidence of fatal pulmonary embolism was only slightly higher (1.117 versus 0.794 per 1000). He questioned whether these differences justified the much higher incidence of hemorrhagic complications reported by Bottomley *et al.* Sevitt [159] criticized the Cambridge study for being essentially uncontrolled and pleaded for properly conducted clinical trials. However, he defended the study as being more likely to approximate the truth than the analyses of Rhodes and Patterson because it was prospective, whereas the statistics reported by the latter investigators were obtained retrospectively.

Fagan [59] studied two consecutive groups of elderly women with traumatic fracture of the femur. The 63 patients in the first group were operated on within 24 hr of admission (McLaughlin pins and plates or Smith-Petersen nails), and were seated out of bed and given active exercises on the day after operation. Dindevan was administered only to patients who had already developed thromboembolism or showed a special risk of doing so, but the number of patients treated was not reported. In the second group, 99 patients, Smith-Petersen pins were inserted only for midcervical fractures. Replacement arthroplasty was performed for subcapital fractures, except in 14% of the patients, who had impaction or severe concurrent illness and were therefore usually treated with McLaughlin pins and plates. These patients were treated with dindevan in the immediate postoperative period; the therapeutic aim was to keep the one-stage prothrombin time at 1.6–2.2 times normal. Autopsy and dissection of deep veins of the legs, according to the method of Sevitt and Gallagher,[161] were carried out in 89% of the

deaths. Fagan stressed the importance of early ambulation. No deaths resulted from thromboembolism in patients who were mobilized soon after operation and continued to walk, whether they were treated with anticoagulants or not. Patients who were not mobilized soon after operation and those who failed to continue to walk or could not walk had a high death rate (48.8%) and a high incidence of thromboembolism (34.9%) if anticoagulants were not administered. When anticoagulants were given to this group, both the death rate and the incidence of thromboembolism declined (to 20.3% and 5.4%, respectively).

Belding [16] used anticoagulants routinely after all operations, except when contraindicated or when the patient was under 20 years old. Bishydroxycoumarin was used from 1952 to 1956, and warfarin from 1956 to 1964. Therapy was initiated on the second or third postoperative day in all cases, except when colonic or intestinal surgery required nasogastric suction for longer periods. Prothrombin level was kept at 20–30% of normal until discharge. Retrospective study of thromboembolic complications following 3163 consecutive operations by other surgeons at the same institution who did not use anticoagulants routinely during the same period showed higher incidence of thrombophlebitis and pulmonary embolism (0.97% and 0.4%, respectively).

Shepard *et al.*[165] used anticoagulant therapy in 82% of all operations. It was given from the first to tenth postoperative days, unless the patient was discharged sooner. Reasons for withholding treatment were oversight, age under 40 years, lowered prothrombin time as a natural occurrence, death, short hospital stay, active bleeding, and other miscellaneous factors. Bishydroxycoumarin was used from 1947 to 1959, and warfarin from 1959 to 1965. Only 51% of the patients had a prothrombin time below 30% at some time during treatment. The average of the lowest prothrombin time achieved by all patients was only 31.63%. Of the patients receiving anticoagulants, 83 died, 3.6% of them because of pulmonary embolism. The author stated that, inasmuch as nearly all investigators report that at least 6–8% of postoperative deaths are due to pulmonary embolism, their use of anticoagulants at least halved the expected death rate. This conclusion is not valid in the absence of a controlled series in their own institution.

Hjelmstedt and Sundström [79] carried out phlebographic examinations in 39 patients with 42 fractures of the shaft of the tibia. They found thrombosis of both the lower leg and the femoral veins in 10%, extensive thrombosis of veins of the lower leg in 15%, limited thrombosis of no clinical significance in 53%, and no thrombosis in 22%. In 90%, the thrombus formed within 7–14 days of injury. Although the authors

TABLE 4 Incidence of Postoperative Thromboembolic and Hemorrhagic Complications in High-Risk Patients Who Received Oral Anticoagulant Prophylaxis (P) and in Low-Risk Patients Who Did Not (NP)

Author	Year	No. Patients		Type of Surgery	Venous Thrombosis, %		Pulmonary Embolism, %				Total, Thromboembolism, %		Hemorrhagic Complications, %							
							Total		Fatal				Mild		Moderate or Marked		Fatal		Total	
		P	NP		P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP
Turnbull [198]	1960	370	465	Gynecologic—pelvic floor repair	0.3					0.2	0.3	1.5	14	3	3	0	0	0	17	3
Milch *et al.*[116]	1961	2176	2887	General	0.14	0.1	0	0	0	0	0.14	0.1	0.37		0.29		0		0.66	
Tubiana and Duparc [194]	1961	194	165	Orthopedic and leg injuries							1.5	7.8	2				0		2	
Ring [146]	1963	41	28	Orthopedic—frac. femur					2.4	7.1	>2.4	>7.1					3.6			
Persson [186,a]	1964	113	122	Orthopedic—frac. femur	1.8	9.0	0	4.1	0	4.1	1.8	13.1			2.6		1.8		4.4	
Timonen *et al.*[192]	1966	475	562	Gynecologic					0.2	0	0.84	2.85								
Skinner and Salzman [165]	1967	336	807	General	0.9	0.12	0.9	0.37	0.3[b]	0.25	1.8[c]	1.0					0.9[d]		8.6	1.4

[a] Results of treatment based entirely on autopsy findings, which included complete dissection of leg veins.
[b] Not adequately anticoagulated.
[c] Would be 0.9 if two cases of pulmonary embolism and one case of thrombophlebitis that were not adequately anticoagulated were excluded.
[d] Anticoagulation probably did not contribute to fatal outcome in two of the three cases.

did not use anticoagulants, they warned against anticoagulant prophylaxis in the first 6 days after injury, because of the chances of bleeding with resulting large hematomas at the fracture site. They noted that such patients often have other injuries and that the danger of intracranial, intrathoracic, or intra-abdominal hemorrhage had to be considered. They were not averse to anticoagulant therapy after the sixth day following injury.

Clinical Trials of Oral Anticoagulants with Treated Patients at Higher Risk Than Controls Within the last decade, there have been several series in which patients who were considered high thromboembolic risks were treated with anticoagulants and the remainder served as control subjects. These reports are summarized in Table 4. In the treated groups, the incidence of venous thrombosis was 0.3–1.8%; of fatal pulmonary embolism, zero to 2.4%; and of total thromboembolic complications, from 0.14% to greater than 2.4%. In the control group, the incidence of venous thrombosis was 0.1–9.0%; of fatal pulmonary embolism, from zero to 7.1%; and of total thromboembolic complications, 0.1–13.1%. It is difficult to compare hemorrhagic complications in the two groups, because few data were supplied for the control groups. Further details regarding the individual studies are given in the following paragraphs.

Turnbull [185] treated women undergoing pelvic floor repair who had varicose veins or a history of thromboembolism, or were 45 years old or older. Phenindione was administered from the first to the twelfth postoperative day, with an attempt to keep the one-stage prothrombin time between 25% and 35% of normal. Milch *et al.*[116] studied private general surgical patients. Bishydroxycoumarin was administered on the morning of surgery and continued until the patients were fully ambulatory or for a maximum of 10 days. An attempt was made to keep the prothrombin time between 2 and 3 times normal. High-risk patients were treated, and lower-risk patients constituted the control group. Because the results in the two groups were similar, it was concluded that anticoagulant treatment had afforded considerable protection against thromboembolism in the high-risk group. Tubiana and Duparc [184] reported a similar study of orthopedic patients admitted for injuries or for operations including repair of fractures of the femoral neck, femur shaft, or leg, hip nailing, and arthroplasty for osteoarthritis. High-risk patients were assigned to the treatment group, and the remaining patients, to the control group. Most were treated with Tromexan (some with phenindione), usually starting on the third day after operation or injury. Heparin was also administered to high-risk patients before and for a

short period after operation. Duration of treatment varied and did not necessarily depend on ambulation. Dosage was controlled by the one-stage prothrombin test and a heparin tolerance test.

Ring [146] urged early surgery in elderly patients with trochanteric fracture of the femur, believing that all cases could be secured by internal fixation. In managing these patients, he gave prophylaxis with phenindione to some patients and not to others and did not state his basis for assignment to the two groups. Hemorrhagic complications delayed the progress toward early ambulation in patients whose fractures were well secured. The author considered the dangers of anticoagulant treatment in the elderly formidable and doubted that the value in preventing thrombosis and embolism outweighed the dangers. Persson [136] used anticoagulant therapy in high-risk elderly patients treated surgically for fractured femur. The lower-risk patients constituted the control group. Bishydroxycoumarin was started on the first to the fourth postoperative day and was continued until ambulation or discharge from the hospital. An effort was made to keep the prothrombin-time index between 10% and 40% of normal. There were nine deaths in the treatment group and 21 deaths in the control group. Autopsy and complete leg dissection were performed in all cases.

Timonen *et al.*[182] treated a group of high-risk patients with warfarin sodium, starting on the third postoperative day and continuing for 7–10 days or until the patient was fully ambulatory or discharged from the hospital; the control group contained lower-risk patients. Bias in this study is suggested by the fact that four of 16 patients in the control group who developed thromboembolic complications were patients who, according to the protocol, should have been in the treatment group but had contraindications to anticoagulant treatment and were therefore put into the control group.

Skinner and Salzman [168] reviewed the effects of prophylactic warfarin in a selected group of postoperative patients who were high thromboembolic risks. Patients admitted with the diagnosis of acute thromboembolism were not included. Of the 1223 surgical patients admitted during 1965, 416 met the high-risk criteria, but 80 of them were excluded from the study because of well-recognized contraindications; the remaining patients constituted the untreated group. In the treatment group, 167 were men and 169 were women; the average age was 64 years. The desired therapeutic range was set at 1.7–2.5 times prolongation of the prothrombin time in the one-stage prothrombin test. Barium sulfate-adsorbed plasma, rather than normal saline, was used to dilute normal plasma for construction of the standardization curve. They stated that the therapeutic prothrombin-time range using this curve

corresponded to 15–30% of normal. In most cases, the loading dose of warfarin was administered immediately after operation; but in a few, it was started on the day of preoperative confinement. No warfarin was given on the day after the loading dose. A daily maintenance dose of 1–10 mg was instituted on the next day and adjusted as necessary to maintain the prothrombin time in the desired range. When secondary surgery was necessary, the prothrombin time was adjusted to 25% or higher by administration of a small dose of vitamin K_1 or by omission of the maintenance dose of warfarin. Treatment was continued until the patient was fully ambulatory or until discharge from the hospital. Retrospective examination of six of the eight patients with thromboembolic complications in the untreated group showed that indications for prophylactic anticoagulation existed, and a more strict interpretation of the criteria for anticoagulation would have removed them from the "low-risk" category. There were 22 deaths and 16 autopsies in the treated group and 46 deaths and 35 autopsies in the untreated group. In the treated group, almost half the bleeding complications occurred in patients whose prothrombin time was below 10% of normal.

The results of these studies suggest that the incidence of postoperative thromboembolism in high-risk patients treated prophylactically with oral anticoagulants was no greater, and in most cases considerably less, than in concomitantly observed low-risk patients not treated with anticoagulant drugs.

Clinical Trials of Oral Anticoagulants with Treated Patients and Controls at Equal Risk Recently, studies of oral anticoagulant prophylaxis have been carried out in which the thromboembolic risk was presumably equal in the treated and the control groups of postoperative patients. In most instances, patients were assigned to treatment and control groups by alternate entries to the hospital, by alternate dates of entry, by odd and even chart numbers, by odd and even years of birth, or by true random sampling. All but the last of these methods may contain bias in selection. The incidences in the treated and control groups (Table 5), respectively, were as follows: venous thrombosis, zero to 8.8% versus 1.4–56.0%; pulmonary embolism, zero to 1.3% versus 0.2–11.9%; fatal pulmonary embolism, zero to 1.2% versus zero to 8.0%; total thromboembolic complications, zero to 8.8% versus 2.82–56.0%; and serious hemorrhagic complications, zero to 7% versus zero to 5%. Further details regarding the individual studies are given in the following paragraphs.

Kistner and Smith [97] studied surgical patients; the method of segregating patients into control and treatment groups was not reported. Bishy-

TABLE 5 Incidence of Postoperative Thromboembolic and Hemorrhagic Complications in Patients Who Received Anticoagulant Prophylaxis (P) and in Control Patients Who Were Equal Risks But Not so Treated (NP)

Author	Year	No. Patients P	No. Patients NP	Type of Surgery	Venous Thrombosis, % P	Venous Thrombosis, % NP	Pulmonary Embolism, % Total P	Pulmonary Embolism, % Total NP	Pulmonary Embolism, % Fatal P	Pulmonary Embolism, % Fatal NP	Total, Thromboembolism, % P	Total, Thromboembolism, % NP	Hemorrhagic Complications, % Mild P	Mild NP	Moderate or Marked P	Moderate or Marked NP	Fatal P	Fatal NP	Total P	Total NP
Kistner and Smith [97]	1954	4044	4044	General and gynecologic			0	0.2	0	0.2										
Storm [175]	1958	99	103	Thoracic	0	9.0	1.0[a]	5.0	1.0[a]	4.0	1.0[a]	14.0			3.0	2.0	2.0	0	[b]	[b]
Storm [175]	1958	57	62	Cardiac	0	4.9	0	9.7	0	1.6	0[c]	14.7[d]	0	0	0	0	0	0	0[b]	0[b]
Sevitt and Gallagher [161,e]	1959	150	150	Orthopedic—frac. femur	2.0[a]	19.4	1.3[a]	10.0				20.0	12.0		3.3	1.3	0	0	23.3	13.3
Dick *et al.*[50]	1959	3190	3336	General	0.1	1.5	0.2	1.3	0.06	0.51	0.35	2.82			0.33	0.1	0.1	0.1	0.97	0.27
Turnbull [185]	1960	201	222	Gynecologic	1.0	1.4	0	1.8	0	0	1.0	3.2	21.5	11.2	1.5	0.8	0	0	22.3	12.0
Neu *et al.*[124]	1965	50	50	Orthopedic—frac. pelvis and leg	0	10.0	0	10.0	0	2.0	0	8.0	12.0	6.0	6.0	2.0	0	0	18.0	8.0
Borgström *et al.*[16,f]	1965	25	23	Orthopedic—frac. femur	8.7	56.0	0	8.0[g]	0	8.0[g]	8.7	56.0	0	0	4.0	0	0	0	4.0	0
Eskeland *et al.*[56,g]	1966	100	100	Orthopedic—frac. femur	8.0	16.0	1.0	7.0	1.0	7.0	8.0	16.0	5.0	1.0	1.0	2.0	0	0	6.0	3.0
Salzman *et al.*[151]	1966	83	83	Orthopedic—frac. femur	7.2[g]	21.7	1.2[h]	7.2	1.2[h]	4.8	8.4	26.5			7.0	5.0	0	0		
Harris *et al.*[78]	1967	57	59	Orthopedic—hip mold arth.	8.8	30.5	0	11.9	0	1.7	8.8	35.6			3.5					0

[a] All instances in inadequately treated patients or those not under current treatment.
[b] No difference in surgical blood loss between treated and nontreated groups.
[c] Plus one fatal case of cerebral embolism.
[d] Plus four fatal and four nonfatal cases of cerebral embolism.
[e] Autopsy diagnoses only.
[f] Diagnoses made by phlebography or autopsy.
[g] Only one third of patients were adequately anticoagulated.
[h] Would be 0 if one patient whose anticoagulation was discontinued 1 month previously were excluded.

droxycoumarin (100–200 mg) was administered over a 4-day period according to a set schedule, starting on the evening before operation and continuing for the entire period of hospitalization. Prothrombin times were not monitored. Storm [175] studied two series of postoperative patients: one series had thoracic surgery (pneumonectomy, lobectomy, exploratory thoracotomy), and the other had cardiac surgery for mitral stenosis. Bishydroxycoumarin was started as early as possible (5–8 days) before surgery and continued through the operation and until the patient was ambulatory (usually 2 or 3 weeks). The dosage was controlled with the prothrombin–proconvertin test of Owren and Aas.[133] An attempt was made to keep the level at 10–30% of normal, except during surgery, when 20% was preferable. Patients born in even years were assigned to the treatment group, and those born in uneven years constituted the control group. In the thoracic series, 66% of the treatment group and 73% of the controls had cancer of the lung. Pulmonary embolism, which was fatal, occurred in one patient in the treatment group, but he was not adequately anticoagulated. One nonfatal and four fatal cases of pulmonary embolism occurred in the control group. There was no significant difference between the treated and untreated groups in the amount of blood lost during surgery in spite of the fact that most patients were adequately anticoagulated at the time of surgery. In the cardiac patients, there was no significant difference between the two groups in the amount of blood transfused.

Sevitt and Gallagher [161] studied patients over 55 years old with either subcapital or pertrochanteric fracture of the femur. Phenindione was given to patients admitted on even-numbered days of the month; patients admitted on odd days formed the control group. All but eight patients in the treated series and 25 in the control group were treated surgically by pinning or nailing. Most of the operations in both series were performed by the fourth day after injury. Nineteen patients were excluded from the trial for various reasons, and adjustments were made to equalize the treated and control groups. Phenindione was initiated in the first 21 patients on the day after operation and in the remaining patients on the day of admission or on the following morning, generally before surgery. The drug was continued for 2–12 weeks (mean, 5 weeks), until the patient was ambulatory. Dosage was controlled by the one-stage prothrombin time. The aim was to prolong the time to 2–3 times normal, which corresponds to 30–15% of normal prothrombin activity. A clinical diagnosis of deep-vein thrombosis was made in 28.7% of untreated patients and in 2.7% of treated patients. Death occurred in 28% in the control group and in 17% in the treated group. Autopsy and careful dissection of deep veins of the legs were

performed in 83% of the control patients who died and 84% of the treated ones; in the control group, deep-vein thrombosis was present in 83% and pulmonary embolism in 36%, and in the treated group, thrombophlebitis was found in only 14% and pulmonary embolism in 19.5%. Furthermore, all instances of significant deep-vein thrombosis or pulmonary embolism in the treated group, whether diagnosed clinically or at autopsy, occurred after anticoagulants were discontinued. Anticoagulation was definitely responsible for serious bleeding in 0.7% and possibly the cause in 2.0%.

Dick *et al.*[50] reported the effects of Marcoumar in postsurgical patients. Patients were assigned to treatment and control groups according to odd and even numbers on admission charts; patients with generally accepted contraindications were excluded. The aim of treatment was to keep the one-stage prothrombin time between 15% and 20% of normal during the period of bedrest and until regular mobilization became possible. In the control group, the incidence of thromboembolic complications was 2.82%; in the treated group, it was 0.35%. An expansion of this study was reported by Matis [110] 2 years later. In the interval, patients were assigned to treatment and control group according to year of birth, rather than chart number. The results were essentially the same as those reported earlier.

Turnbull [185] treated women after gynecologic surgery with phenindione, instituted on the third postoperative day. Alternate cases were assigned to treatment and control groups. Deep-vein thrombosis occurred in 1% of treated patients and in 1.4% of control subjects. Pulmonary embolism did not develop in the treated patients; it occurred, but was not fatal, in 1.8% of the control patients. Neu *et al.*[124] reported a study of patients with pelvic or leg fractures; 96 cases required open reduction, and the remaining four were treated solely by immobilization. The patients were 60–97 years old (average, 67 years). Group assignment was based on odd and even hospital chart numbers; patients with absolute contraindications were not encountered. Anticoagulation was started with warfarin sodium administered intramuscularly with the preoperative medications. Anticoagulation was continued until the patient was ambulatory, unless bleeding dictated otherwise. The aim of treatment was to keep the one-stage prothrombin time at twice the normal value. Operations included internal metallic fixation, prosthetic arthroplasty, and other surgery. The results in the control group of 50 nonanticoagulated patients showed thrombophlebitis of the lower extremities in 10% and definite pulmonary embolism in 10%. Pulmonary embolism was fatal in one patient (2%), appeared abruptly and simultaneously with thrombophlebitis in three patients (6%), and

occurred without any clinical evidence of thrombophlebitis in two patients (4%). Neither pulmonary embolism nor thrombophlebitis occurred in the 50 treated patients. Hemorrhagic complications developed in 18% of the treated and in 8% of the nontreated patients. Transfusion was required for severe bleeding in 6% of the anticoagulated and 2% of the nonanticoagulated patients.

Borgström *et al.*[18] reported 58 patients with fractured neck of the femur who were assigned to groups in a predetermined random binomial sequence. Bishydroxycoumarin was started immediately after entry to the hospital. The aim was to obtain and maintain a prothrombin time index of 40%, using the one-stage method, until the patient was completely mobilized. All patients were operated on no later than the day after admission. Phlebographic examination of the injured leg was performed about 3–4 weeks after surgery; the roentgenologist was not told to which group the patient belonged. Response to treatment was determined by phlebographic or postmortem detection of thrombosis only, with no reliance on clinical manifestations. Phlebography was not performed in six cases and was unsuccessful in eight, but four of these patients died and were examined at autopsy. The remaining 10 cases and the other members of the pairs to which they belonged were eliminated from the study. Each pair of consecutive patients was compared in a restricted sequential plan; if thrombosis was found in one of a pair, preference was given to the other. The analysis took cognizance only of pairs in which a difference emerged. Results of the analysis showed that in only one of the pairs was thrombosis present in the test patient and absent in the control patient. In the other 11 pairs in which a difference was found, it was in favor of anticoagulant prophylaxis. A statistically significant difference in favor of anticoagulant prophylaxis was found when the tenth preference was reached.

Eskeland *et al.*[58] reported a study of patients 56 years old or older with hip fractures. Patients presenting special indications for or contraindications against anticoagulant therapy were excluded from the trial. Patients were assigned to treatment and nontreatment groups by means of random tables. In most cases, phenindione was started on the day of operation or the next day; it was usually not later than 5 days after injury. Therapy was controlled by the prothrombin–proconvertin test or by the thrombotest: the aim was to keep the prothrombin–proconvertin value at less than 30% of normal; the desired level for the thrombotest was not stated. Data were obtained over a period of 3 months. At autopsy, the deep venous system of the legs was carefully examined according to the method of Sevitt and Gallagher.[161] In the untreated group, there were six clinical diagnoses of venous thrombosis:

four died and all four had extensive thrombosis, but none had pulmonary embolism. In the treated group, two clinical diagnoses were made: both patients died, both showing extensive thrombosis at autopsy, and one with pulmonary embolism. Twenty-four patients died and 20 were examined post mortem in the untreated group: 16 had extensive thrombosis, and in seven of these, major pulmonary embolism was the cause of death. Nineteen died and 14 autopsies were performed in the treated group: eight had extensive thrombosis, and in one of these, major pulmonary embolism was the cause of death. The difference between the incidences of major pulmonary embolism found at autopsy in the two groups was statistically significant, but the difference between the incidences of extensive venous thrombosis was not statistically significant.

In the study of Salzman *et al.*,[151] all patients 75 years old or older who were admitted to the ward with fresh fractures of the hip were included, except patients with thrombophlebitis, peptic ulcer, or other contraindications. Patients whose hospital unit numbers were even were assigned to the control group, and the remainder were assigned to the treatment group. Treatment was with crystalline warfarin. The desired therapeutic range was 15–30% of normal (1.7–2.5 times prolongation of the time in the one-stage prothrombin time test, using a barium sulfate-adsorbed plasma dilution curve). A loading dose was administered before operative reduction of fractures in almost all cases; many were well anticoagulated preoperatively because of a delay in surgery, necessitated by complicating medical illnesses. A prothrombin activity greater than 25% was considered essential for safe conduct of major surgery in these patients. Anticoagulation was continued until after the patient became fully ambulatory, or for 3 months. All patients were followed until ambulatory, or for at least 1 month after anticoagulants were discontinued. In the control group, pulmonary embolism occurred in 7.2% of patients, and thrombophlebitis in 21.7%. Thrombophlebitis developed in 7.2% of the treated group; pulmonary embolism did not develop, except in one patient after treatment was discontinued for 1 month because of low hematocrit.

Harris *et al.*[73] studied patients over 40 years old undergoing elective hip surgery. Group assignment, anticoagulant therapy, and method of dosage control were the same as in an earlier study.[151] The initial dose of warfarin was given intramuscularly on the evening after operation; none was given on the following day. Anticoagulation was continued until the patients were ambulatory and then stopped abruptly. The prothrombin time was in the therapeutic range 61% of the time. The diagnoses of thrombophlebitis and pulmonary embolism were based on

clinical manifestations. Among the 59 patients in the control group, 30.5% developed thrombophlebitis, and 11.9%, pulmonary embolism. The one fatality in the entire series resulted from a massive pulmonary embolism in a patient in this untreated group. Of the 57 patients in the treated group, 8.8% developed thrombophlebitis, and none developed pulmonary embolism.

The results of these studies clearly demonstrate that the incidence of thromboembolic complications was considerably reduced by anticoagulant prophylaxis in postoperative patients who underwent a wide variety of surgical procedures.

MEDICAL PATIENTS

Anticoagulant prophylaxis against venous thromboembolic disease in medical patients has been investigated less extensively. No well-controlled studies of groups of patients with a broad spectrum of medical diseases have been carried out.

Myocardial Infarction It is not within the scope of this review to consider results of anticoagulant treatment of acute myocardial infarction. However, much of the reputed beneficial effect of anticoagulant therapy in these patients may result from prevention of venous thromboembolism. In a postmortem study, Gilchrist and Tulloch [64] found that the incidence of pulmonary embolism was 3.4% in 88 patients treated with anticoagulants and 17% in 150 untreated control subjects. Honey and Truelove [81] examined reports of autopsies performed before anticoagulant therapy was generally available, and found that 6% of patients with myocardial infarction who survived the first 48 hr died later from pulmonary embolism. In a later treatment period, when 74.5% of the patients were given anticoagulants, only about 1% died of pulmonary embolism. The proportion of patients examined at autopsy was about 70% in both groups.

Congestive Heart Failure In 1950, Harvey and Finch [75] studied the effect of anticoagulant prophylaxis with bishydroxycoumarin in patients with congestive heart failure. The authors attempted to keep the one-stage prothrombin activity at 30% of normal; in 71% of the observations it was actually below 50%, and in 55% it was below 30%. Patients who died within the first 48 hr of entry or those with acute myocardial infarction were excluded. Patients were assigned to treatment and nontreatment groups according to even or odd days of admission.

Toward the end of the study there were fewer patients in the treatment than in the control group, and an attempt was made to rectify this by treating all patients. The series still ended with only 80 in the treatment group and 100 in the control group. Although this suggests bias in selection of patients, the incidences of dyspnea, rales, hilar congestion, hydrothorax, cardiac enlargement, hepatomegaly, and edema were the same in both groups. In the treatment group, 9% died, but only 1.25% from thromboembolism, whereas in the control group, 17% died, 8% from thromboembolism. In the treatment group, 2.5% had thrombophlebitis and 2.5% had pulmonary embolism. In the control group, thrombophlebitis was diagnosed clinically in 4% and at autopsy in 4%, and pulmonary embolism clinically in 7% and at autopsy in 8%.

Also in 1950, Anderson and Hull [c] studied patients with congestive heart failure. Patients were assigned to control and treatment groups by alternating between services or by alternate admissions; 147 were treated and 150 were not. Bishydroxycoumarin was instituted within 24 hr of admission; therapy was controlled with the one-stage prothrombin test. Reduction of prothrombin activity to 10–30% of normal was achieved in approximately 90% of the 147 patients, usually within 48–72 hr. In the treated group, 7.5% died, and in the control group, 13.3% died. Autopsy permission was obtained in only three of the 11 cases in the treated group; no thrombi, emboli, or infarcts were found. In the eight fatal cases not studied post mortem, there were no definite clinical signs of thromboembolic complications, although one patient died rather suddenly on the eighth hospital day. In the control group, autopsy permission was obtained in 10 of the 20 cases; in eight, there was evidence of thrombosis or embolism, and in seven, recent infarction of the brain, bowel, lungs, or myocardium appeared to contribute to death. Of the 10 fatal cases not studied post mortem, two patients had definite clinical signs of severe pulmonary embolism shortly before death, and in four others, death was rather sudden and unexpected.

In 1952, Griffith *et al.*[69] reported results of treatment of patients with congestive heart failure, using heparin and an oral anticoagulant drug (Tromexan or dicumarol). There were 390 patients in the treated group and 213 control subjects; the basis of assignment to the two groups was not reported. Control of anticoagulant therapy was achieved by measuring the whole-blood coagulation time when heparin was used and by the one-stage prothrombin or prothrombin–proconvertin test when oral anticoagulants were used. Treatment was considered unsatisfactory if death resulted from any cause or if nonfatal thromboembolic episodes occurred, including coronary artery occlusion with

acute myocardial infarction, peripheral artery thrombosis or embolism, thrombophlebitis, cerebral vascular occlusion, or pulmonary infarction. Treatment was considered satisfactory in 91% of the treated patients and in 75% of the control patients. Hemorrhagic complications were minor in 0.5% and serious in 2.4% of the control patients, and minor in 1.8% and serious in 1.3% of the treated patients.

In the series reported by Thorsen [181] in 1957, 122 patients with congestive heart failure were treated with bishydroxycoumarin, but no control series was observed. Patients with acute myocardial infarction or low prothrombin values were excluded. The aim of therapy was to keep the prothrombin value at 10–30% of normal, as determined by the prothrombin–proconvertin test. Clinical diagnosis of thromboembolism was made in eight cases (6.6%): peripheral arterial embolism in two, cerebral embolism in one, pulmonary embolism in one, myocardial infarction in three, and thrombophlebitis in one. The patient with pulmonary embolism and the two with myocardial infarction died, but none was examined post mortem. Fifteen (12.3%) died, but the number of autopsies was not reported. It is difficult to draw any conclusions from this study concerning the efficacy of anticoagulant treatment in patients with congestive heart failure.

In 1966, Domenet *et al.*[51] reported the effects of treatment in patients with congestive heart failure. All patients admitted under the care of one consultant physician to one ward of Queen Elizabeth Hospital, Birmingham, were included in the study. Alternate patients were given phenindione, unless it was specifically contraindicated. Unfortunately, contraindicated patients were placed in the control group; it has been shown that such patients have a higher incidence of thromboembolism than unaffected control subjects.[50] There were 76 patients in the treated group and 80 in the control group. Treatment was regulated by the one-stage prothrombin time, and the aim was to keep the time to between 2 and 2½ times the control value. Death occurred in 17% of the anticoagulated group and in 22.5% of the untreated group. No episode of venous thrombosis uncomplicated by pulmonary embolism was observed in either group. Pulmonary embolism occurred in six treated patients (8%): in four, the diagnosis was on clinical grounds and was confirmed at autopsy in three, and the other two cases were first diagnosed at autopsy. In the control group, pulmonary embolism was diagnosed in eight cases (10%): seven were clinical diagnoses and the eighth was diagnosed at autopsy. The incidence of pulmonary embolism among the autopsied patients of both groups was 29% (six patients). The differences in death rate and incidence of pulmonary embolism between the treated and the nontreated groups were not statistically significant. A

curious feature of this study was that four of the cases of pulmonary embolism in the treated group occurred within the first 5 days, whereas all eight instances in the control group occurred later. It can be argued that the pulmonary emboli in the treated group occurred before therapy had a chance to become effective; however, the absence of emboli in the untreated group during the first 5 days is difficult to explain. The only serious complication attributed to therapy was terminal hematemesis in an 80-year-old man suffering from degenerative heart disease with congestive pneumonia. Another patient died of ruptured abdominal aortic aneurysm during adequate anticoagulation.

The results of these trials of anticoagulant prophylaxis in patients with congestive heart failure are equivocal. It is possible that better results would be achieved if treatment were instituted with heparin, rather than with the slower-acting oral anticoagulant drugs. It is difficult to measure the true value of anticoagulant therapy in patients with congestive heart failure because of the difficulty in making an accurate clinical diagnosis of pulmonary embolism. Furthermore, it may be impossible to separate pulmonary embolic from primary cardiac deaths, even at autopsy. Further controlled studies in this important group of patients are indicated.

SUMMARY

Because anticoagulant treatment cannot be given to all potential victims of thromboembolism, it is important to recognize and treat the patients most likely to develop this complication. The underlying causes of venous thrombosis are vascular injury, circulating procoagulants, and stasis of blood within the vessels. Bedrest for only a few days may predispose a person to thrombosis; the risk increases particularly after trauma, burns, surgery, and childbirth, in those with infections, cancer, acute myocardial infarction, cardiac failure, dehydration, anemia, polycythemia, marked obesity, or varicosities, and in persons of advanced age. At present, no single laboratory test clearly differentiates patients with a high risk of developing thromboembolic disease from those with a low risk, but predictions based on groups of tests and other epidemiologic data may prove useful in selecting patients for anticoagulant prophylaxis.

The importance of precise laboratory control for the prevention of hemorrhagic complications of anticoagulant therapy is stressed by many authors. Many studies show that a large proportion of the serious hemorrhages occur when hypoprothrombinemia is induced. Differences in the degree of anticoagulation are produced by equating percentage

figures obtained by the one-stage prothrombin test, the prothrombin–proconvertin test, thrombotest, and specific prothrombin assay. These must be appreciated if adequate anticoagulation is to be maintained and hemorrhagic complications kept at a minimum. Some hemorrhages occur from underlying organic lesions at "safe" prothrombin levels. A more careful search for such lesions before treatment would lower the incidence of hemorrhagic complications.

Heparin should be used initially in the treatment of established deep-vein thrombosis or pulmonary embolism, and prophylaxis with oral anticoagulant drugs should begin early after operation in surgical patients. If bedrest before operation is prolonged, oral anticoagulant therapy may be given before, during, and after the operation; the prothrombin level should be kept in the upper part of the "therapeutic range." During a shift from heparin to an oral anticoagulant drug, an overlap is advisable for optimal management. When the two drugs are given together, careful attention should be given to the added influence of heparin on the prothrombin time and of the oral anticoagulant on the whole-blood clotting time.

Expert management of oral anticoagulant therapy demands thorough familiarity with all factors that can influence the patient's response to these drugs. Increased responses are found with marked dietary deprivation of vitamin K, sterilization of the gastrointestinal tract, diseases of the liver and pancreas, hyperthyroidism, fever, and the concomitant administration of some drugs, such as phenothiazines, phenylbutazone, phenyramidol, thyroid hormone, benziadrone, clofibrate, and the C-17-alkylated steroids. Increased resistance to the prothrombinopenic action of oral anticoagulants occurs in pregnancy and during the simultaneous administration of barbital compounds, glutethimide, chloral hydrate, and griseofulvin. Obviously, when any change in the physical condition of the patient occurs or when other drugs are suddenly introduced or withdrawn, frequent laboratory determinations of the anticoagulant states must be carried out and appropriate adjustments in dosage made.

Evaluation of the efficacy of anticoagulant prophylaxis and treatment of venous thrombophlebitis of the legs and pulmonary embolism is difficult, because clinical diagnosis of these disorders is unreliable. The most reliable statistics are those obtained during prospective studies in which the results found at autopsy are compared in treated and control groups of patients selected at random.

Of the numerous clinical trials of anticoagulant therapy in cases of established deep-vein thrombosis or nonfatal pulmonary embolism conducted over the last 30 years, the only one that was properly controlled (Barritt and Jordan [13]) offers convincing evidence in favor of treatment.

In the other reports, the results of therapy are generally considered favorable when compared with previous experience in the same institution or with contemporaneous but not randomly selected control groups.

In current practice, anticoagulant therapy is not necessarily used in cases of superficial thrombophlebitis, although many advise it in all cases because of the difficulty in excluding involvement of the deep veins. The drugs generally are used in high-risk cases (e.g., patients with acute myocardial infarction, congestive heart failure, history of thromboembolic disease, or varicose veins; postoperative patients; and elderly patients with fractured hips). Anticoagulant therapy is recommended in all patients with established deep-vein thrombosis or nonfatal pulmonary embolism. The importance of early and adequate treatment is stressed. The duration of therapy varies with the circumstance; in some cases, it should be continued for as long as a year. Because pulmonary embolism in the cardiac patient is often a recurrent or continuing problem, and because anticoagulant therapy is frequently unsuccessful, early surgical intervention should be considered. Surgical treatment of massive iliofemoral thrombophlebitis, in addition to heparinization, may be desirable, to relieve pain and swelling; however, claims that it prevents postphlebitic complications need further confirmation.

Venous thrombosis is rare in sites above the inguinal ligament (e.g., thrombophlebitis migrans, infusion thrombophlebitis, and thrombosis of the superior and inferior vena cavas and pelvic, periprostatic, renal, portal, hepatic, mesenteric, jugular, retinal, cerebral, axillary, and chest-wall veins). The importance of thrombosis in these sites lies in the immediate hemodynamic consequences, the potential for embolus formation or extension, and the underlying disease processes that they often represent. Clinical manifestations depend on the anatomic location and the rapidity of formation and completeness of occlusion produced by the venous clot. There are no general therapeutic guidelines, and no adequately controlled prospective studies are available for assessing the value of anticoagulants.

Anticoagulant prophylaxis against venous thromboembolic disease has never been popular. Yet, approximately half of all patients who die of pulmonary embolism have no premonitory symptoms, and one half to two thirds of those with deep-vein thrombosis in the legs discovered at autopsy have no clinical manifestations (Sevitt and Gallagher [161]). If treatment is reserved for those with established venous thromboembolic disease, those patients are missed. Hemorrhagic complications, which constitute the principal disadvantage of anticoagulant prophylaxis, can be greatly reduced by careful attention to recognized contraindications to treatment, meticulous laboratory control of drug dosage, and

special alertness during the early stages of treatment, when hemorrhage due to the underlying disease is likely to manifest itself.

Early clinical trials of prophylaxis with heparin were encouraging but were largely uncontrolled. Trials of oral anticoagulant therapy can be divided roughly into three groups: those without contemporaneous control; those in which the results of treatment of high-risk groups are compared with the results of treatment of the remaining, lower-risk patients; and those in which the control groups are selected at random. Most of the results in postoperative surgical, gynecologic, orthopedic, and accident cases show a striking reduction in the incidence of thromboembolic complications when prophylaxis is given. The incidence of hemorrhagic complications, however, is almost always higher in the treated patients. The actual incidence of thromboembolism and bleeding varies widely among different groups, depending largely on the type of patient treated.

There are no well-controlled studies of prophylaxis against venous thromboembolism in medical patients. However, results of postmortem examinations of patients who received anticoagulant therapy for acute myocardial infarction show a lower incidence of pulmonary embolism than among nontreated controls. The results of prophylaxis in patients with congestive heart failure are equivocal. Earlier studies indicated considerable protection from thromboembolism, but more recent, and perhaps better controlled, investigations are less favorable.

The work reported here was supported in part by Public Health Service training grant TI AM-5103 from the National Institute of Arthritis and Metabolic Diseases and research grant HE 02754 and research career award IK6 HE-21835 from the National Heart Institute.

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Anticoagulant Therapy in Coronary Artery Disease

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Despite interest in anticoagulant therapy for over a quarter of a century, it is impossible to be dogmatic about its current role in coronary artery disease. A detailed account and critical appraisal of the vast majority of the reports of anticoagulant therapy in coronary artery disease would serve no useful function. It would be more useful to describe the design of clinical trials that, if carried out, would yield information on the role of this therapy. Comparison of the "ideal" design with published reports will illustrate why firm conclusions cannot be drawn from most of the reports.

THE CONTROLLED CLINICAL TRIAL OF ANTICOAGULANT THERAPY IN CORONARY ARTERY DISEASE

The results of clinical trials must, in the final analysis, form the basis for estimating the value of anticoagulant therapy in coronary artery disease. The early trials of this therapy suffered from various shortcomings, because the principles of design of such trials were only becoming apparent. Today, it is relatively simple to plan a trial of sound design. But its execution usually requires many participants at many centers, who are willing and able to sink their own personalities sufficiently to allow them to take part in a cooperative study from which little or no individual credit will accrue, to accept for the trial only patients who are approved by an independent team, to conform to an agreed plan of patient care, and to submit their case records for scrutiny and criticism by a central organization. The clinician must also consider the ethics of the trial. If he believes that anticoagulant therapy cannot ethically be withheld from a group of control patients, then he cannot participate in an adequately designed trial.

Once the physicians are selected, how should the control patients be managed? It is of the greatest importance that this group be neither labeled nor thought of as "the no-treatment group." They do receive treatment, but they do not receive significant amounts of anticoagulant drugs. The planners of the trial may arrange to give a very low dose of oral anticoagulant drug—e.g., 1 mg of phenindione (the therapeutic dose is likely to be 100 mg)—and this enables the participating clinician to tell the patients in both groups that they are receiving anticoagulant drugs.

Because of the ethical issues, the design of a scientific trial may fall short of ideal. The design of a trial must allow treatment changes under specified circumstances. If a patient on high-dosage therapy develops massive gastrointestinal bleeding, then obviously the clinician concerned must be free to reverse the anticoagulant effect if he so decides. Similarly, if a patient develops deep venous thrombosis, then the clinician must be able to treat the patient with anticoagulants. Such treatment changes or withdrawals from the initial allocation regime have caused difficulties in the interpretation of results. These treatment changes frequently reflect alterations in the patient's condition and are not randomly assigned. One method of dealing with this type of difficulty is to include the result in the initial treatment allocation. For example, if a patient allocated to the high-dosage group develops a severe hemorrhagic incident that requires therapy, and later dies, the death is included in the high-dosage group, even though the patient was not on therapy at the time of death. (This is discussed in more detail below.)

Within the ethical limitations, it is still possible to include in the trial design sufficient essential criteria to obtain an answer. Much of the current controversy in assessing the value of anticoagulants can be traced to the failure to conduct trials of impeccable design before inconclusive (but suggestive) evidence became available.

As already discussed, it is usually impossible for one person to collect sufficient patients within a reasonable period to make such investigations possible; a multicenter trial on a national or international basis is required. Such trials therefore involve many clinicians who have little or no training in medical statistics; to ensure continued cooperation, the design and analysis must be simple and easily understood. It is a mistake to try to answer too many questions; if too many are asked, the trial may result in answering none of them completely or clearly.

The statistician must be concerned with the design of the anticoagulant trial from the time of its original planning. No statistician should accept the responsibility of assessing the results of a trial unless he has been responsible for the initial design. The statistician must insist that the

criteria of patient selection are precise, that the allocation of patients is strictly random, that the treatment is standard, and that the results be assessed, as far as possible, on objective criteria. The statistician should be part of a central organization responsible for coordinating the trial, safeguarding standards, and following progress; the latter is particularly important when death is the criterion of success or failure. As deaths occur in the trial, they must be reported to the coordinating center so that the trial can be stopped as soon as there is a significant difference between control and test groups. This central office is able to take a wide view of the trial and may be able to suggest matters of importance overlooked at the participating hospitals, each of which has only a small number of patients involved in the trial.

Before considering the more detailed design of an anticoagulant trial, it is important to discuss the most important single aspect—the method of constructing a group of patients to be concurrently observed, corresponding in their characteristics to the group fully on anticoagulants but not given that particular therapy. The only way to ensure this is by random allocation. Cards allocating patients to one or the other group are made out; each is placed in an individual envelope; and the envelopes are sealed and arranged in a strictly random order. The physician in charge of the case must decide whether the patient is suitable for inclusion in the trial; only then does he open the next envelop in the series. The contents of the envelope alone will decide to which group the patient is allocated. If the allocation is not made in this way, the clinician may be biased toward acceptance or rejection.

Unless this method is adopted and unless the groups are initially comparable, no conclusion can be drawn, even in terms of objective measures, such as death rate. Large numbers of patients in themselves are of no value, in this sense, unless they are comparable. The decision on allocation cannot be left to the whim of the physician, because this practice will usually result in giving the anticoagulant therapy to the patients who are most ill. Volunteers for the test group are also not acceptable. A comparison within the experience of one physician or one hospital is inadequate. Comparison with previous experience is unacceptable. Once allocation has been made, the follow-up of the two groups must be carried out with equal vigor.

Initially, the groups will be comparable, although the members of the groups clearly will not be. With large numbers, equality of characteristics in the two groups will result in the long run. The numbers eventually entered in the trial will depend on the result obtained as the progress is followed. The result obtained is, of course, the group result—i.e., on the average, patients do better (or worse) on the anticoagulant treatment or there is no difference.

The best of all anticoagulant trial designs would be that making use of the double-blind technique. With anticoagulants, however, it is not possible to apply this technique with one observer at each participating center, because the clinician in charge must know the result of the laboratory test in order to control the anticoagulant drug dosage. It is not impossible to design a trial in which one observer controls the therapy and a second, who does not know the treatment group, adjudicates the progress of the patient. In practice, this has been found very difficult to execute; to my knowledge, only one trial of this type has been successfully accomplished.[8]

DETAILS OF DESIGN

With the help of the statistician, the clinicians of the participating centers must meet in committee before the start of the trial to agree about many details, as outlined below.

OBJECTIVE

The objective of the trial should be stated clearly, and the design should be appropriate to the objective. The temptation to collect additional data should be resisted. The simpler the design to gain the stated objective, the better will be the support for the project. For example, the objective may be to study the influence of anticoagulant therapy on mortality and complications during a 42-day period following a recent myocardial infarction.

METHOD

Patients must be selected according to well-defined criteria, and allocated randomly to one of two treatment groups, as discussed above. Except for the anticoagulant drug treatment itself, the two groups must be treated similarly. Some criteria must be laid down in the treatment schedule, but it is clearly difficult to obtain identical care of all patients being treated at different hospitals. However, provided that the other management is the same in the two groups within each center, the design is still sound. The random allocation is so arranged that each center has equal numbers of patients allocated to each of the two groups.

CRITERIA FOR SELECTION OF PATIENTS

The age and sex of patients to be included in the trial should be stated. The diagnostic criteria for patients to be included in the trial should be

stipulated. In the design of a trial of long-term anticoagulant therapy in patients with angina pectoris or after myocardial infarction, there is time for detailed and fairly leisurely appraisal. The criteria for selection in such circumstances should be stated in detail. In a trial in acute myocardial infarction, when speed of entry into the trial is important, a working clinical diagnosis may have to be made on the available evidence. The eventual diagnosis of myocardial infarction may depend on sequential electrocardiography and transaminase levels. This evidence may not be available for several days; it is during these early days that mortality is greatest and the value of therapy in considerable part has to be judged. That does not, however, prevent the execution of a trial of satisfactory design. A small proportion of patients will eventually be shown not to have had a myocardial infarction, but, owing to the random allocation procedure, equal numbers of such patients will be distributed among the two treatment groups. The problem being studied is that which faces the clinician on first seeing the patient, before final proof of infarction is available—namely, whether to give anticoagulant therapy.

GROUNDS FOR EXCLUSION

Grounds for excluding patients from the trial must be defined—for example, clinical or radiologic diagnosis of peptic ulcer or of a previous cerebrovascular accident.

ALLOCATION TO TREATMENT GROUPS

The dosage of drug for the high-dosage group and the method of administration should be stated. The patients in the comparative group should receive either a placebo containing no anticoagulant drug or a very small dosage of drug—sufficiently small to be pharmacologically ineffective, such as tablets that contain 0.1 mg of warfarin sodium.

DURATION OF TRIAL

The progress of the anticoagulant trial must be followed constantly so that it can be stopped immediately if a significant difference (e.g., in death rate) is found between the two groups. However, the level of significance should be set relatively high, such as the 1% level, to try to avoid premature cessation of entry of new patients into the trial.

OTHER MANAGEMENT

Clearly, the physician in charge must be permitted to adopt standard management for any additional aspect requiring therapy, such as the

shock of acute infarction or the development of congestive failure. The details of management of each of these issues cannot be rigidly laid down in advance; the physician at each center must understand that therapeutic measures other than anticoagulant drugs should be applied equally to both groups within each center.

LABORATORY CONTROL

Because so many modifications of Quick's original one-stage prothrombin time test [33] have been introduced, controlling therapy with this technique may result in enormous variation in the mean dosage of drug being used. The dosage of drug at one center may be twice as great as at another, although both are controlling on the same clotting-time ratio, because of the use of different sources of brain thromboplastin. The only way to produce equivalence of mean dosage is to issue the test reagents centrally. The easiest practical way to do this today is to use thrombotest or a similar commercial preparation. This will result in the same mean dosage of drug at each center.

ASSESSMENT OF RESULTS

It is important to stress that the results of the trial apply only to the patients selected according to the criteria of the trial.

Deaths If random allocation has been used, the objective measure given by deaths in the two groups provides a good index of success of a particular therapeutic regimen. It is the number of deaths from all causes that is important, not the number of deaths selected in an attempt to include only those due to thromboembolism.

Nonfatal Thromboembolism These incidents include reinfarction, deep venous thrombosis and pulmonary embolism, and systemic artery embolism (particularly cerebral, limb, and mesenteric). The diagnosis of some of these complications is subject to observer bias. Venous thrombosis and pulmonary embolism, for example, can be very difficult to diagnose clinically. For this reason, their occurrence provides a much less objective index of success of a therapeutic regimen. The adjudication of this is most satisfactory if a double-blind technique with two observers is used. If that is not possible, the best available objective indices must be used. In a long-term trial in coronary artery disease, it is not sufficient to take electrocardiograms at the time of pain; tracings must be taken routinely (say, every 3 months) to provide objective evidence of change.

Other Symptoms Assessment of symptoms, such as angina and grade of dyspnea, is difficult and unreliable unless done by a double-blind technique with two observers.

Recording of Details The details should be recorded on a standard printed record form on thin cardboard. The form should contain the minimal information required and should be so prepared that appropriate items can be ringed or ticked.

WITHDRAWAL OR CHANGE IN TREATMENT

In an ideal trial, it would be desirable not to withdraw patients once entered. For ethical reasons, this is clearly not possible. For example, anticoagulants should be withdrawn from a patient who has a major hemorrhage from an unsuspected duodenal ulcer while on anticoagulants. There must be strict adherence to the criteria for withdrawal, and they must be applied equally to the two groups. Anticoagulants should be withdrawn from a patient with a duodenal ulcer that bleeds but who belongs in the placebo or comparative group. Criteria must be laid down for the management of thromboembolism, such as venous thrombosis. The physician will have to withdraw the patient from the trial so that he can be treated without the restrictions imposed by the trial. The method of random allocation leads to equality in initial selection, but particular attention must also be paid to equal application of the withdrawal rules so that withdrawal does not become selective. As mentioned above, one method of avoiding the difficulties in interpretation that arise from withdrawals is to permit treatment changes, but without withdrawal from the trial, and to assess subsequent events according to the original treatment allocation. In a long-term trial, withdrawal may become necessary because the patient leaves the area of the study or is uncooperative. This, however, is an unusual cause of withdrawal. Setting this aside, other withdrawals can be viewed as treatment changes and the patient can be maintained on follow-up and in the trial. The analysis of results can then be based on the initial allocation to the high- or low-dosage group. For example, a patient who dies after removal from the high-dosage group because of severe hemorrhage would be allocated in the analysis to the high-dosage series. The trial is designed, then, to compare the net results, in terms of mortality, of two alternative treatment intentions, without regard to later changes that may have become necessary.

In general, an anticoagulant trial must be so designed that the conclusion can be correlated with the treatment given. Scrutiny of many

published anticoagulant trials reveals that clinicians studying patients with ischemic heart disease frequently record accurately what they observe but fail to appreciate that what was observed was not necessarily related to the anticoagulant therapy given, because the design was inadequate to permit that conclusion.

It is often impossible to repeat a trial. The difference in death rate in anticoagulant trials may be below the technical level of significance that statisticians adopt, but "not significant" is not equivalent to "there is no difference." "Not significant" corresponds merely to "not proved"; that is, there is insufficient material to allow one to dismiss chance as an alternative explanation. The trial has not proved that it is of no value. In such a situation, the ethics of repeating the trial to make certain are difficult. It seems likely that anticoagulant therapy gives only a small therapeutic benefit. It is important, therefore, that trials of anticoagulant therapy not be discontinued until sufficient numbers of cases have been included to prove that any small difference between groups is real.

ANTICOAGULANT THERAPY IN ACUTE MYOCARDIAL INFARCTION

Most of the trials of anticoagulant therapy published before 1966 fell short of ideal design. The main reason for that was usually the failure to adopt an adequate allocation procedure in prospective studies. Other major difficulties arose because of retrospective exclusion or inclusion of specified groups.

Up to 1961, the available evidence appeared to constitute an overwhelming case in favor of anticoagulant therapy, as regards both lowering of death rate and reduction in thromboembolism. The mortality in those trials was apparently reduced by one half, from approximately 30% to about 15%, as a consequence of anticoagulant therapy.[5,9,12,14,17,18,22,26,27,30–32,34,36,37,39,43]

Doubts about the conclusions reached on the basis of these trials were raised later by several authors.[19,35] Honey and Truelove,[19] for example, carried out a detailed but unfortunately retrospective analysis of the experience of 543 patients admitted to one hospital in the period 1940–1954. They concluded that any small difference in favor of anticoagulant therapy could be attributed to reduction in pulmonary embolism. The possible importance of anticoagulant therapy in preventing pulmonary embolism had previously been stressed by Gilchrist and Tulloch.[13] Despite these criticisms, anticoagulant therapy was widely used in acute myocardial infarction until 1961, when Hilden and asso-

ciates [16] reported the results of a large controlled trial of this therapy in acute myocardial infarction. The trial failed to confirm the difference in mortality rate found in previous trials, although it did confirm a reduction in thromboembolic incidents.

Since 1961, there has been appreciation of the importance of design of trials of anticoagulant therapy, and two trials in acute infarction of good design have been reported. Merskey [28] reported in favor of therapy, particularly in females, in whom he found a marked reduction in mortality. Merskey's trial was still in progress at the time of that report. Wasserman and his colleagues [41] conducted a well-designed trial, but the numbers of patients included were very small—fewer than 100 in each treatment group. They found no significant difference in mortality.

The Medical Research Council in Britain has recently completed a reassessment of the role of this therapy in the treatment of patients admitted to the hospital suffering from acute myocardial infarction. This was conducted at 12 hospital centers and involved some 50 clinicians; the study ran from 1963 to 1966. There were 1427 patients allocated at random to high-dosage or low-dosage therapy with anticoagulants. The high-dosage regimen consisted of 36 hr of heparin administration and phenindione at 15% thrombotest. The mean phenindione dosage was 72 mg/day. The low-dosage regimen consisted of 1-mg tablets of phenindione. Therapy was continued for 28 days. The allocation procedure was carried out by the sealed-envelope technique, an envelope not being opened until a decision had been made to enter the patient in the trial. The main criterion of success of the therapeutic regimen was survival. In addition to the obvious, that survival is always a most important criterion of success, this choice, in the absence of a double-blind technique, ensures objectivity of ascertainment. Most other forms of assessment are open to observer bias when a double-blind technique is not used.

An important feature of the design of that trial was that the analysis of results was based on the initial allocation to high or low dosage. No matter what treatment change may have occurred, the patient was followed for 28 days, and death, if it happened, was classified according to the patient's initial allocation group. For example, if a thrombotic episode in a patient in the low-dosage group demanded a raising of the anticoagulant dosage, subsequent morbidity or death was classified as occurring in a patient in the low-dosage group; and the death of a patient after his removal from the high-dosage group because of severe hemorrhage was allocated to the high-dosage series. In other words, the trial was designed to compare the net result in terms of mortality of two alternative treatments in acute infarction without regard to later changes

that might have become necessary. The results in previous trials had been confused by withdrawals and exclusions or inclusions of a variety of groups. The success of the allocation procedure was checked. The groups were virtually identical as regards age and sex distribution, history of previous infarction, severity of illness, and progress of mobilization.

The results of this trial have not yet been published at the time of this writing, but it is expected that they will be available soon. For the present, it must be concluded that no currently published trial in acute infarction can be accepted as providing final evidence that there is or is not a reduction in the death rate as a consequence of administering anticoagulant therapy. It seems probable, on the basis of current evidence, that there is a reduction in nonfatal thromboembolism.

LONG-TERM ANTICOAGULANT THERAPY AFTER MYOCARDIAL INFARCTION

In the world literature, 12 trials [1–3,6,10,11,15,20,21,24,25,29,38] of sound design have been established to explore the value of long-term anticoagulant therapy in patients who have suffered one or more myocardial infarctions. These are listed in Table 1; they have many points of difference in design, but they all have a satisfactory method of allocation to treatment with or without high-dosage anticoagulants. Accepting this, the difference in death rate is probably the result of a valid comparison. In a compilation like this, it is important to include all trials of satisfactory design, whatever the conclusion of the authors—i.e., whether they were in favor of anticoagulant therapy or not.

It will be noted from this compilation of results that, in all the larger trials—i.e., in those with over 100 patients in each treatment group—the direction of the result is the same, namely, a small gain in favor of therapy. These results probably indicate that there is indeed a small benefit from therapy. When the numbers are small, however, by chance this result may not be evident. On over-all assessment 1000 patients have to be treated for some years to gain 40–50 lives.

As reported by Lovell,[23] an international collaborative study is being made of some of these results. The details of the study and its results have not yet been published but are awaited with much interest. It should be possible by going to the originators of all the well-designed long-term trials to arrange the collection of some minimal data. The main assessment would have to involve death rates in the two groups. This collaborative study, because of the larger number of patients in-

TABLE 1 Long-Term Trials of Anticoagulant Therapy

	Low Dosage or No Anticoagulants		High Dosage	
	Number	Deaths	Number	Deaths
Bjerkelund [6]	118	48	119	30
M.R.C.[1,2]	188	35	195	25
MacMillan *et al.*[25]	23	0	27	8
Clausen *et al.*[10]	99	13	93	15
Harvald *et al.*[15]	170	26	145	14
Aspenström and Korsan-Bengtsen [3]	113	50	118	39
Conrad *et al.*[11]	34	5	52	4
Seaman *et al.*[38]	67	3	66	2
Veterans' Administration [21]	359	97	388	87
Loeliger *et al.*[20]	122	12	122	8
Lovell *et al.*[24]	178	39	172	33
Meuwissen [29]	70	8	68	1
	1541	336	1565	266

volved, may be able to define the category of patient who will benefit from therapy. When this is published, it is likely to provide the definitive reference on long-term therapy after myocardial infarction.

LONG-TERM ANTICOAGULANT THERAPY IN ANGINA PECTORIS

The only adequately designed trial of long-term anticoagulant therapy in angina pectoris is that of Borchgrevink.[7,8] The study covered 147 patients, all with a history of angina pectoris of less than 2 years but more than 4 weeks, and all under 70 years old. One group (74 patients) received intensive anticoagulant therapy; clotting activity was reduced to about 20% (by prothrombin–proconvertin test). The other group (73 patients) received moderate treatment; the value was 50–60%. The study lasted 2½ years, with an average observation period of 16 months. In the 50% group, seven patients died and 10 had myocardial infarction. In the 20% group, one patient died and two had myocardial infarction.

The benefit has been questioned since it was postulated that inadequate anticoagulant therapy may actually be harmful. But I doubt that little treatment is worse than no treatment.

One must have reservations about the role in angina pectoris, because of the paucity of studies and because we have seen from examination of the problem of long-term therapy after myocardial infarction that trials with small numbers may give erroneous results. Because I believe that this is the same disease as studied with long-term therapy after myocardial infarction, it seems probable to me that it also would benefit from therapy.

ANTICOAGULANT THERAPY IN INGRAVESCENT ANGINA (IMPENDING MYOCARDIAL INFARCTION, ACUTE CORONARY INSUFFICIENCY, OR ACUTE CORONARY FAILURE)

These are ill-defined clinical states between exertional angina and myocardial infarction. Patients present with intensification of angina of effort and the appearance of angina after several months or several years of effort angina. These patients frequently proceed to myocardial infarction. Strong claims have been made for the value of anticoagulant therapy in this condition.[4,40,42] However, this is another area in which there is an urgent need to establish the role of this therapy by controlled clinical trial.

A VIEWPOINT CONCERNING ANTICOAGULANT THERAPY

Every clinician requires a working rule as regards his policy of management. It would seem to me that the case for benefit, albeit small, of long-term anticoagulant therapy is established. It may be possible from the international collaborative study,[23] when it is published, to define the categories of patients who gain most benefit from this therapy and to draw up priorities. The present evidence suggests that males under 55 years old derive most benefit; but this conclusion may require revision when the results of the study are available.

If we extrapolate backward from this evidence, the patient with acute infarction should have therapy, because he is going to require it for a long term in any case, and it will also reduce the number of thromboembolic incidents, if not the death rate, significantly in the acute stage.

As regards angina pectoris, this will largely be determined by the available facilities; if one adds these large numbers of patients to the treatment commitment, the workload may become immense. This material should be used in collaborative clinical trial to define the need more clearly than it has been so far.

THE NEED FOR FURTHER STUDIES

The controlled clinical trial, despite its difficulties and limitations, is still the only widely applicable method of evaluating the role of anticoagulant therapy. The essentials of design of such trials have been described. For those who would be critical of early trials, let them recall that these were executed at a time when the essentials of this form of clinical experiment were not yet well established. Even with an appreciation of the essentials of such trials, their successful execution in recent years has not been easy or straightforward. A successful trial not only must be of impeccable design, but must be pursued with sufficient tenacity so that adequate numbers of patients are included. The collection of a sufficient number of patients can be obtained only by a multicenter collaborative effort. Such effort is often tedious and personally unrewarding. The clinicians involved must subdue their own personalities to the common cause.

The following are the conditions for which there is still a need to conduct controlled clinical trials: (1) short term in acute infarction with an intense anticoagulant effect (i.e., more intense than used in the recent British Medical Research Council trial); (2) short term in "ingravescent angina"; and (3) long term in angina pectoris.

There have been no satisfactorily designed trials in acute coronary insufficiency. The existing evidence in angina pectoris needs to be supported by other trials.

If one of the main objectives of this volume is to establish areas for further pursuit of problems in thrombosis, then international collaborative effort could well solve these important therapeutic issues. I doubt that any one center will be able to conduct such trials as will allow authoritative pronouncement on these issues. There is no shortage of clinical material to permit quite ethical studies. This therapy is likely to be used for many years to come, despite flagging enthusiasm. I suggest that there is still sufficient worldwide interest in this topic to promote the effort to learn the truth.

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Comment on Anticoagulant Therapy in Coronary Artery Disease

IRVING S. WRIGHT

In recent studies designed to evaluate the effect of anticoagulant therapy in the treatment of coronary artery disease, emphasis has been properly given to the development of detailed protocols, including blind or double-blind techniques. However, it has been unfortunate that in some of these studies equivalent care has not been taken to ensure that adequate dosage has been consistently used.

It is a fundamental rule of therapy that, to determine the value of a drug, one must administer adequate but not excessive amounts of it. The use of digitalis and diuretics offers excellent examples of the acceptance of this principle. The need for following the principle in the use of oral anticoagulants was clearly established on page 353 of the 1954 Report of the American Heart Association Committee on Anticoagulants.[9] It was shown that the rate of clinical thromboembolic complications occurring at dosage levels that produced prothrombin times longer than 25 sec (23% activity or more) was twice that occurring at the lower prothrombin levels (less than 23% activity). No further gain was to be expected by higher dosage, and the risk of hemorrhage increases with markedly increased dosages (as determined by the Quick one-stage test).

Some studies have followed this principle and have reported satisfactory results with significant findings in various subgroups with regard to deaths in specific age groups, thromboembolic complications, continuing disability, and, in long-term studies, further thromboemboli, hospitalization, and expectation of death. These include the U.S. Veterans' Administration study [6] and the long-term study of the British Medical Research Council.[1] The Veterans' Administration study was one of the most successful in this regard, achieving a therapeutic level of less than 25% of prothrombin activity in 89% of the cases. Two other double-blind studies have since been reported with favorable findings in numer-

ous respects, including effectiveness in men under 55, recurrences, hospitalization, and cerebral infarctions.[2,3] The report by Lovell includes data regarding levels of prothrombin activity. In over 80% of the cases, anticoagulant therapy was effective. In contrast, the study of Hilden *et al.*,[4,5] which showed less satisfactory results, was controlled by the prothrombin–proconvertin test, and the level of prothrombin activity achieved was 40%, as determined by the Quick test and pointed out by Uddén.[8] This is inadequate therapy to demonstrate significant clinical improvement, although pathologic studies did show a significant decrease in thromboembolic findings.

In October 1966, Seaman *et al.* presented a paper before the meeting of the American Heart Association.[7] It aroused considerable interest because it was a double-blind study, and the authors concluded that "no evidence was established that long-term prophylactic anticoagulant therapy after acute myocardial infarction reduced mortality rate or complications." It should be mentioned that the total number and subgroups of patients were small, that the patients were treated during their acute attacks with anticoagulants, and that the control patients received anticoagulants when they developed thromboembolic complications. Of interest here is the fact that the treated cases were held at a level of less than 30% prothrombin activity (not 25%) only 70% of the time. In other words, on approximately 100 days of each year, these patients were not at optimal treatment levels. This inadequacy in dosage is further substantiated by the fact that, whereas the same drug (phenindione) was given in an average daily dose of 105 mg in the British series, the cases of Seaman *et al.* received an average of only 90 mg.

In the preceding paper, Dr. Douglas offers additional data of interest in this regard. In view of the somewhat encouraging results obtained by the working party of the Medical Research Council in Britain in the long-term treatment of myocardial infarction, it was decided to carry on a similar study of anticoagulant therapy in the treatment of acute myocardial infarction. This time, however, the thrombotest was used and 15% was accepted as the preferred level. We have found that this leads to underdosage and that, to obtain satisfactory results for acute thromboembolism, a level of 7–10% is desirable. It is comparable with 20–23% by the Quick test. Dr. Owren has stated that a level of 10% is desirable. Nevertheless, the 15% thrombotest level was used—and with disappointing results. That it did result in inadequate dosage is further suggested by the fact that, whereas for the long-term prophylaxis study 105 mg was the average dose, for the acute study the average dose was 72 mg. In view of the fact that the coagulation balance is usually in favor of hypercoagulation and thrombosis during acute thrombotic

episodes, the dosage logically should have been higher, rather than lower, for acute infarction.

All this work appears to have demonstrated that satisfactory results cannot be expected in any series in which inadequate levels of therapy are used. It points to the need, in all future studies, to ensure that optimal dosage is used. Conclusions based on studies that do not meet this basic criterion cannot be regarded as valid. The use of the death rate alone, although in a sense cold and final, is not adequate to determine the value of a therapy designed to decrease the incidence and seriousness of thromboembolic complications, which, as all autopsy studies have demonstrated, are more common and more devastating than even the best clinical studies can detect.

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Use of Anticoagulants in the Prevention of Embolization from Prosthetic Heart Valves

EDWARD GENTON

Conclusive data on the influence of anticoagulant therapy on thromboembolism after prosthetic-valve insertion are not presently available, for several reasons: (1) Much of the literature concerns obsolete valves, in that their design and materials have undergone repeated changes, often because they were associated with high incidences of thrombosis. This has also contributed to the lack of many large series for a particular valve design. (2) Randomized studies with satisfactory control groups have not been conducted. The duration of postoperative follow-up is often short. The incidence of thromboembolism, even for a valve of a given design, varies with the area of placement. (3) Detection *in vivo* of the presence of thrombosis or of a minor embolic episode may be impossible, and even postmortem studies may fail to disclose such events with certainty. (4) In treated groups, the adequacy of anticoagulation is often unclear.

Three groups of investigators have presented data dealing with the problem.[1-3] There appears to be general agreement that the risk of thromboembolism in the first postoperative week is not great, approximating 5% after mitral or aortic valve replacement. Because the risk of hemorrhage is significant if anticoagulants are used early, the accepted procedure is to avoid the use of heparin and initiate oral anticoagulant therapy late in the postoperative period.

Table 1 summarizes the experience reported by Duvoisin *et al.*,[1] which is representative of most reported series. During a 12–29-month follow-up, 35% of nonanticoagulated patients with aortic prostheses developed embolic episodes, compared with only 8% of the adequately anticoagulated group. Considerably less benefit was derived in patients with mitral prostheses, 32% developing emboli even while on anticoagulants. Starr *et al.*[3] have estimated a 50% reduction in embolism

TABLE 1 Embolism after Starr-Edwards Prosthesis—Effect of Anticoagulation (after 12–29 Months) [a]

	No Anticoagulation			Anticoagulation		
		Embolism			Embolism	
Valve	No. Patients	No.	%	No. Patients	No.	%
Aortic	37	13	35	37	3	8
Mitral	95	42	44	37	12	32

[a] Derived from Duvoisin *et al.*[1]

with anticoagulants after mitral valve replacement; however, the control group in that series was too small to permit meaningful analysis, and the incidence of embolism in anticoagulated patients approximated 40%.

Extremely encouraging are the recent results obtained by Starr, using an extended cloth mitral prosthesis. In 36 patients on anticoagulants, followed for up to 2 years, the incidence of embolism was only 3%.

It would appear that the routine use of anticoagulation is justified in all patients with prosthetic cardiac valves. However, it is clear that additional measures are required, especially after mitral replacement, to reduce the incidence of thromboembolism.

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Thrombolytic Agents

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Thrombolytic or fibrinolytic therapy (therapy involving the *in vivo* lysis of fibrin clots by enzymatic means) holds great promise, because potentially it offers the physician a method for medically controlling or preventing the deleterious effects of acute thromboembolic vascular disease. The practical urgency of developing such therapeutic measures is self-evident, for, despite the introduction of anticoagulant therapy and the efforts of surgeons to extend the applicability of such techniques as thrombectomy, embolectomy, endarterectomy, vein ligation, and vascular grafting, acute thromboembolic vascular disease remains the largest single cause of mortality and morbidity in the middle-aged and elderly populations of the western world.

Although the ability of the organism both to lay down and to remove fibrin was firmly established by classical pathologic studies on wound healing, inflammation, etc., during the last century, it was only around the turn of the century that Sahli's demonstration that urine would dissolve crude fibrin clots [31] and Goodpasture's observations that sterile whole-blood clots underwent spontaneous lysis [19] suggested that humoral, apart from cellular, mechanisms might play a role in the lysis of fibrin, and sustained research interest was not aroused until Tillett and Garner discovered in the 1930's a potent bacterial "fibrinolysin" (now termed "streptokinase," a plasminogen activator), which provided the key reagent for biochemical and clinical studies,[39] and Christensen and MacLeod wrote a paper in the 1940's on the enzymatic basis of fibrinolysis, in which they introduced the modern terminology (the "plasminogen–plasmin" system).[4] Since then, intensive basic, clinical, and therapeutic investigation of the plasminogen–plasmin system has occupied the attention of many investigators; the historical development and present status of the field are treated in a number of monographs and reviews.[5,9,15–17,22,32,36,43]

Many investigators in many countries have now brought the concepts and practice of thrombolytic therapy to a crucial stage of development. Highly significant advances made during the last decade include: (1) delineation of reasonably comprehensive biochemical and physiologic concepts accounting for *in vivo* thrombolytic phenomena and serving as a guide to the therapeutic approach with thrombolytic agents [2,12,21,42]; (2) the remarkable success of the pharmaceutical industry in both purifying and producing, on a large scale, two clinically satisfactory thrombolytic agents, streptokinase and urokinase [25,44]; (3) increased understanding of the biochemical mechanisms underlying the blood coagulation defect that complicates thrombolytic therapy of sufficient intensity to produce clinical thrombolysis [1,6,8,10,20,24]—an understanding that is already providing important contributions in the clinical coagulation field [7]; and (4) the striking work of clinical investigators who, besides producing by contrast angiography unequivocal evidence of the lytic actions of these drugs on both arterial and venous thrombi, have also demonstrated that the large-scale application of thrombolytic agents in acutely ill patients is entirely feasible and often clinically beneficial.

Yet there is another side of the coin: although clinical research achievement has been most encouraging, the place of thrombolytic agents in the therapeutic armamentarium remains essentially undefined. Despite the conclusive demonstration that these agents, when acting on fresh thrombi or emboli, produce *in vivo* thrombolysis, we are now faced with the problem of determining the clinical benefits of restoring blood supply to organs and tissues that have been deprived of it for an often unknown period. That we cannot yet answer these questions is scarcely surprising, in that they involve quite novel and unique pathologic problems.

THERAPEUTIC AGENTS USED TO INDUCE THROMBOLYSIS

Although previously controversial, the rationale for the use of plasminogen activators as thrombolytic agents, in preference to proteolytic enzymes or mixtures of plasminogen activators and proteolytic enzymes, now appears universally accepted.[2,9,12,15,21,22,32,42]

Two plasminogen activators have undergone clinical study and evaluation. Streptokinase, a product of hemolytic streptococcal metabolism, with which the much greater clinical experience has been obtained, is in some important respects a less satisfactory agent than urokinase (purified on a large scale from human urine), which has recently become

available. Streptokinase is relatively inexpensive and adaptable to any desired production scale, but it is antigenic to man [11] and it has a substantially lower fibrinolysis:fibrinogenolysis (gel:soluble plasminogen activation) ratio than urokinase.[33] Because human populations display various degrees of immunization against the streptokinase antigen, acquired as the result of previous covert or overt streptococcal infections, therapy carries the danger of anaphylactic reaction—a danger that, on the basis of extensive clinical experience, appears to be of more theoretical than real consequence. Moreover, dosage schedules should be determined for the individual patient after titration of plasma streptokinase antibody, if a consistent biochemical and therapeutic response is to be obtained. Streptokinase therapy immunizes the patient, so retreatment is not feasible for several months,[12] and some patients (5–10% in different patient populations) will be wholly refractory to streptokinase therapy initially, because of pre-existing very high levels of streptokinase antibody. It has been suggested [42] that many of these practical disadvantages may be overcome by using a high standard streptokinase dosage in all patients, without prior titration of plasma antibody in the individual patient. But that sacrifices precise therapeutic control and introduces into a clinical therapeutic trial the substantial disadvantage that a proportion of the "streptokinase-treated group" will receive what, on a biochemical basis, is essentially placebo treatment (which will considerably increase therapeutic trial size, owing to the effect of "sample dilution" on the trial population). Finally, because of its lower fibrinolysis:fibrinogenolysis ratio, streptokinase induces a greater degree of blood coagulation defect and a greater degree of plasma plasminogen decline (to nearly zero levels in most cases) for a desired level of plasma thrombolytic activity, and it is thus a considerably less versatile thrombolytic agent than urokinase.[13]

Urokinase, however, possesses many properties akin to those of the ideal thrombolytic agent [13,25,33,44]: it produces predictable biochemical effects when administered on a dose/body weight basis; it induces substantially less secondary coagulation defect than streptokinase for a desired level of plasma thrombolytic activity; it permits plasma plasminogen concentrations to remain relatively high (which is important for therapeutic efficacy); it requires much less serial laboratory monitoring of treatment effects; and it can be used to produce a wide variety of thrombolytic states. Unfortunately, urokinase is very expensive, and its availability as an investigative drug is correspondingly limited. However, the pharmaceutical industry has made great progress in extracting urokinase from urine and purifying it, so that, if large-scale demand were to develop, the cost would fall sharply.

Although pharmacologic control of plasma thrombolytic activity, using synthetic drugs, is still incompletely developed, some studies [43] suggest that it may eventually prove to be a valuable subsidiary approach.

CLINICAL STUDIES

Some clinical states in which controlled clinical trial of thrombolytic therapy might be deemed appropriate, has been planned, or has been attempted are: (1) "developing" stroke, (2) thrombosis of the central artery of the retina, (3) acute myocardial infarction, (4) acute pulmonary embolism, and (5) peripheral arterial and venous disease.

In examining this list, it is to be noted that, although all the disease states listed could be described as primarily "thrombotic" disorders, the degree and nature of the "thrombotic" lesions vary so widely among them that it is by no means certain that dosage and treatment procedures suited to one will be applicable to the others. Indeed, when consideration is given to the vastly different pathologic circumstances encountered in a venous lesion, such as pulmonary embolism, and those encountered in acute myocardial infarction, it seems unlikely that a treatment schedule that is optimal in the first will be optimal in the second. This becomes important when urokinase, a considerably more versatile thrombolytic agent than streptokinase, is used, for here it is possible to select from among a variety of therapeutic regimens.

Of the disease conditions listed above, acute pulmonary embolism and acute myocardial infarction are presently of predominant concern as disease entities suitable for controlled clinical trial of thrombolytic agents. A third urgent problem of similarly wide clinical significance, the use of thrombolytic agents for the treatment of cerebral thrombosis, presents various problems discussed elsewhere [15] and does not seem as suitable for clinical trial, at least until further experimental animal studies are performed.

Pulmonary embolism has become the subject of intense therapeutic investigative interest largely because of the development of reasonably objective methods, pulmonary angiography and pulmonary isotopic scanning, for diagnosis and evaluation of patient progress. Because of this reliance on objective findings, trials of therapeutic agents in pulmonary embolism may be designed with the expectation that the majority of trial patients will contribute valid data.

In contrast, until recently, satisfactory objective measures were lacking in patients with acute myocardial infarction, and reliance had to be placed on relative patient mortality as the main criterion of treatment

success. Such trials are inherently insensitive, particularly if patient mortality is low, because trial sensitivity depends, not on the size of the trial population, but solely on the number of observed deaths.

MYOCARDIAL INFARCTION

The greatest potential utility of thrombolytic therapy is in acute myocardial infarction, but designing therapeutic trials is particularly difficult in this area. Omitting consideration of numerous studies of the use of streptokinase–plasmin mixtures in acute myocardial infarction (see the references in Fletcher and Sherry [15]), in which it was never proved that a biochemically effective dose of thrombolytic agent was administered, there are three studies of early and intensive thrombolytic therapy in acute myocardial infarction.

Those three studies, using streptokinase, suggest that, if a dose of agent sufficient to produce substantial pharmacologic effect is used, the treatment is both entirely feasible and clinically beneficial. In the first study,[14,18] intense states of markedly enhanced plasma thrombolytic activity (comparable in intensity and duration with those obtainable with urokinase) were induced and maintained by streptokinase infusion in patients with early acute myocardial infarction. Nineteen patients were treated with streptokinase for the first 30 hr and then anticoagulated; all survived the acute phase of the illness, but one patient died 3 weeks later. Although the treated group fared better than controls (selected by a double-blind procedure) and better than other patients treated in the hospital during the same period, the results were not statistically significant ($p=0.2$). But the results and the follow-up studies did demonstrate that the infarcted myocardium tolerates the induced plasma thrombolytic state, and that therefore no difficulty is likely in the urokinase trial, especially inasmuch as the coagulation defect that it induces will be much milder than that induced by streptokinase.

Recently, Schmutzler *et al.*[30,34] reported a study of 297 streptokinase-treated and 261 anticoagulant-treated patients with acute myocardial infarction. The streptokinase group received 18 hr of continuous high-dose therapy followed by coumarin administration; the controls were treated wholly with anticoagulants. The mortality in the control series through the first 40 days was 21%, compared with 14.1% in the streptokinase-treated group. Excluding the first 24 hr of hospitalization, the mortality in the control series was 16.1%, compared with 8.7% for the streptokinase group. Both differences were statistically significant.

However, some aspects of experimental design and the methods of case randomization were of doubtful validity, and the study is being repeated under different investigational conditions.

Only limited data are available concerning another, yet unpublished European study of streptokinase treatment in acute myocardial infarction [41]; 10 of 75 patients died in the streptokinase-treated group, and five of 57 in the heparin-treated control group.

These studies, although inadequate to support definitive claims of the efficacy of streptokinase in acute myocardial infarction, certainly indicate that further trial of thrombolytic therapy in this disorder is needed.

PROBLEMS IN TRIAL DESIGN

Whereas there can be no doubt as to the importance of undertaking further controlled clinical trials of thrombolytic therapy in acute myocardial infarction, there must be considerable uncertainty as to the most appropriate form of clinical trial.

There are three main possibilities: (1) to undertake a conventional controlled clinical trial using appropriate randomization and taking relative patient mortality as the sole criterion of treatment success—the design used in virtually all anticoagulant trials in acute myocardial infarction over the last two decades and a design that has proved less than adequate in practice; (2) to attempt to improve trial design sensitivity, acknowledged to be low, while retaining relative patient mortality as the criterion of treatment success; and (3) to attempt to devise clinical trial designs in which criteria of drug action other than relative patient mortality are of predominant importance.

Advances in our understanding of the natural history of acute myocardial infarction, in cardiologic practice, and particularly in the development of biomedical computer instrumentation make the last possibility both feasible and attractive.

For many reasons, the definitive trial of thrombolytic agents in acute myocardial infarction will present greater difficulty than that posed by the simpler anticoagulant trials. For instance, early diagnosis of the condition will be vital, and the incidence of unconfirmed diagnosis will necessarily be higher; furthermore, on any long-term basis, trials with multiple dose levels must be contemplated.

Table 1 lists statistical probabilities applicable to the conventional controlled clinical trial, in which relative patient mortality is the sole criterion of success. The table covers three situations: (1) treatment is totally without influence on mortality, (2) treatment produces true

TABLE 1 Probability of Drawing Conclusion as to Effect of Treatment on Mortality in Acute Myocardial Infarction

After Observing	If the True Reduction in Mortality is: 0% — The Probability of Concluding (b) is:	25% — The Probability of Concluding (a) is:	50% — The Probability of Concluding (a) is:
16 deaths	0.10	0.01	0.06
32 deaths	0.15	0.02	0.14
64 deaths	0.24	0.03	0.36
128 deaths	0.37	0.06	0.75
256 deaths	0.53	0.17	0.99
512 deaths	0.67	0.46	1.00

(a) = Probability of concluding that treatment is effective in reducing mortality.
(b) = Probability of concluding that treatment is without effect on mortality.

reduction in mortality of 25%, and (3) treatment produces true reduction in mortality of 50%. It shows the probability of demonstrating, at the conventional level of statistical significance ($p=0.05$), the effectiveness or lack of effectiveness of the treatment, as a function of the number of deaths observed.

Relative to the probability values shown in Table 1, a zero probability would indicate a zero possibility of demonstrating the validity of the stated experimental hypothesis under the defined experimental conditions, and a probability of 1.00 would indicate that the hypothesis will certainly be proved, at the conventional level of statistical significance, under the defined experimental conditions. Thus, a value of $p=0.5$ in the table would indicate that, under the stated conditions, there is a 50% chance of demonstrating the validity of the tested hypothesis at the level of $p=0.05$, and similar considerations govern the interpretation of other tabulated p values.

Three illustrative examples may be quoted from the table:

1. If the tested treatment is without effect on patient mortality, there would be an approximately even chance of reaching this conclusion after about 250 patient deaths, and about two chances out of three of so concluding after about 500 deaths.

2. If the treatment reduces true mortality by 50%, there would be an approximately even chance of obtaining a sufficiently favorable result after about 100 deaths to conclude that it has a positive effect, and this conclusion could almost certainly be drawn after about 250 deaths.

3. If the treatment reduces true mortality by only 25% (by any

account, a truly valuable mortality reduction), the prospects of demonstrating this become much poorer, in that, even after some 500 patient deaths, there is only an approximately even chance of obtaining a sufficiently favorable result to permit this conclusion.

These figures presuppose the performance of a perfect study, one with impeccable randomization and with no patients whose diagnosis of acute myocardial infarction was unconfirmed—conditions impossible to achieve in practice. If a patient mortality of 25% were assumed, the observation of 512 deaths (bottom line of table) would require a study population of over 2000 patients. If the wholly reasonable assumption were made that, if myocardial infarction were to be diagnosed early in its course (2–14 hr after its occurrence), the incidence of unconfirmed diagnosis might reach 20% (a conservative figure), then the study might well require over 3000 patients. (The 20% misdiagnosis rate can be calculated by the sample-dilution formula as requiring a 55% increase in patient sample size.) Moreover, if the drug acted differently in different patient subgroups—e.g., patients with initial attacks and patients with recurrences—these numbers would have to be doubled to handle the difference on a separate basis. And if multidose or multiprocedure trials were contemplated, the theoretical numbers for each trial design would have to be multiplied by the number of stratification stages required.

Hard experience, garnered over the last 25 years of largely frustrating clinical trial of anticoagulants, has established the veracity and importance of these simple caculations and amply illustrates the many largely insurmountable problems that stem from the inadequate clinical trial designs hitherto available for the testing of potentially valuable therapeutic agents. These design and statistical difficulties have two main causes: (1) although myocardial infarction is acknowledged to occur in widely varying degrees of severity, it has generally, from the viewpoint of statistical randomization, been treated as a homogenous whole; and (2) the endpoint of patient mortality represents only a small fraction of the potential patient data (under these circumstances, trial design efficiency is related to total patient mortality rather than to size of therapeutic trial groups).

METHODS FOR IMPROVING TRIAL DESIGN AND SENSITIVITY

Several ways exist in which increased knowledge of the natural history of acute myocardial infarction may be applied to refine experimental design in this disorder.

Shown in Figure 1 are some unpublished data from the files of the last large-scale cooperative study of anticoagulant therapy in acute myocardial infarction, which was reported in 1964.[37] In this trial, the participating clinicians computed the Schnur prognostic index [35] for each patient before entry in the trial (the abscissa); accuracy of their predictions was determined later (group mortality rates on the ordinate). This remarkable prospective demonstration of the ability of physicians to predict their patients' outcome on a statistical basis with relatively crude initial criteria was, of course, not available at the start of the study. Had it been, there is little reason to doubt that the study itself would have been designed differently.

Figure 2 shows further prognostic data, this time using the Peel prognostic index,[29] but plotted to emphasize an ever simpler approach to the improvement of trial design sensitivity. The results of the Peel index are plotted on the abscissa, and mortality rates on the ordinate. The Peel data were compiled retrospectively, but data from two other studies [3,27] were compiled prospectively and, rather significantly, using patients admitted very shortly after the onset of symptoms. There is excellent correlation between the retrospective and prospective findings and between the prognostic indices and mortality. Plotted in this fashion,

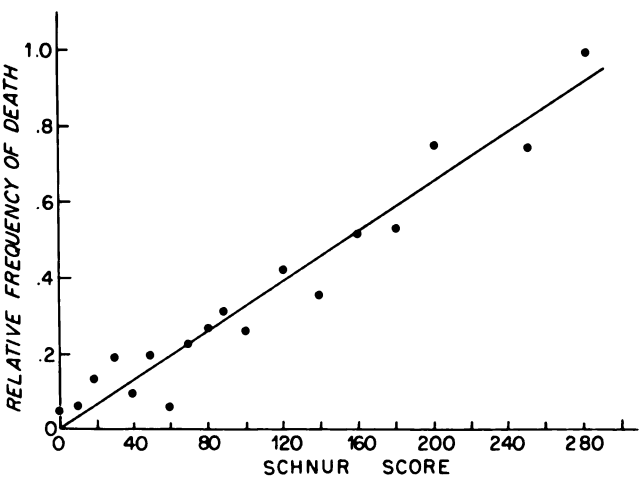

FIGURE 1 Relative frequency of death during the first month after acute myocardial infarction plotted as a function of Schnur prognostic score determined at the time of initial hospitalization. The majority of the plotted points represent groups of 20–30 patients. Data are from reference 37.

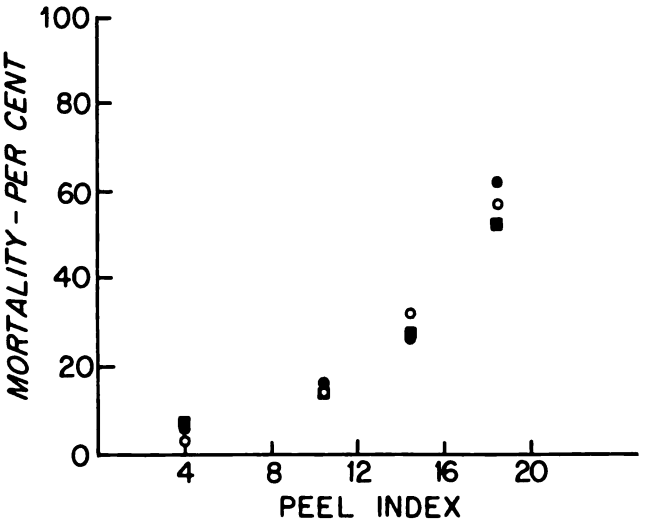

FIGURE 2 Mortality (%) during the first month after acute myocardial infarction plotted as a function of Peel prognostic index determined early during the disease. Patient groups designated ● (Peel *et al.*[29]), ○ (Balcon *et al.*[3]), and ■ (Mittra [27]). The Peel study was retrospective and the other two studies were prospective.

it is clear that patients with a Peel index of below 8 would contribute little to a mortality study, except sample dilution; furthermore, the exclusion of the approximately 40% of patients with a Peel index of below 8 from such a controlled clinical study would increase trial sensitivity by a factor of 3—a very worthwhile dividend for a very modest change in trial design.

If, by the application of moderately crude, mainly clinical prognostic criteria, we can predict disease outcome with reasonable precision, we may infer that the application of more refined serial biochemical and hemodynamic measurements also will provide objective measures of disease status and degree that will permit us to assess the effects of drug therapy in individuals or small groups of *surviving* patients. The ability to obtain valid therapeutic data from the *surviving* patient, as apart from the computation of relative patient group mortality, would revolutionize the statistical basis of clinical trial in this disorder and free it from its present limitations.

There are now many studies in the literature (the pioneering studies of the London and Edinburgh groups [23,26,28,38,40] should be mentioned)

that clearly demonstrate both the feasibility and the prognostic significance of serial determinations of cardiac output, hemodynamic measures, blood-gas determinations, acid–base studies, and additional monitorable patient characteristics. Indeed, there is now abundant evidence that severe, moderate, or even mild myocardial infarction induces significant, defined, and measurable alterations of physiologic measurements pertaining to the cardiovascular, cardiopulmonary, and probably other body systems. In several instances, it has been shown that these individual measurements (arterial-blood oxygen saturation, disturbances of acid–base balance, etc.) possess individual prognostic significance, but such data have hitherto been collected on a scattered basis and the full power of this approach remains to be developed. However, there is sufficient evidence to indicate that either reversal of these predictable physiologic anomalies or alteration in the rate of their development can be used to measure therapeutic action.

Moreover, the problem is compounded by the difficulty that so much information can be caused to flow from patient monitors, particularly electrocardiographic monitors, that data handling and analysis have now become essential limiting factors. To most definable scientific problems there are answers; here, the answer must clearly rest with the biomedical computer.

CONCLUDING COMMENT

This paper has been concerned chiefly with clinical trial problems in acute myocardial infarction because, potentially, thrombolytic agents may have their most important therapeutic role in this condition. Moreover, in this era of the intensive treatment of the patient with acute myocardial infarction and falling mortality rates in the best acute-cardiac-research units, some investigators have doubted whether it would be feasible to test single therapeutic agents in a statistically controlled manner. If relative patient mortality is accepted as the sole criterion of therapeutic success, this argument has force. However, if former conceptual and practical limitations are removed, in the manner briefly sketched earlier, the problem becomes no more difficult than that faced in other disease conditions in which the statistically controlled clinical trial has yielded high dividends. Thus, the development of thrombolytic therapy, with the consequent necessity for its adequate evaluation in acute myocardial infarction, offers a notable incentive to long-needed improvement in clinical trial methods applicable to this disease.

The concepts discussed in this manuscript have developed during conversations with Jerome Cornfield (former Chief Biometrician of the National Heart Institute and

now Chairman of the Department of Statistics at Pittsburgh University), who also kindly supplied Table 1. Data presented in Figure 1 were made available by Dr. Jacob Bearman. Their assistance is most gratefully acknowledged.

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Surgical Therapy of Venous Thrombosis

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THROMBECTOMY FOR ILIOFEMORAL THROMBOPHLEBITIS

PATHOGENESIS OF ILIOFEMORAL THROMBOPHLEBITIS

Most observers agree that the thrombus in iliofemoral thrombophlebitis (or phlebothrombosis) involves a large venous segment, whose wall was normal before the appearance of the thrombus. The thrombus may appear in a normal vein as a result of stasis, or it may originate at a small site of abnormality, commonly near a valve station,[17] and propagate into the surrounding normal portion of the vein.

Whether thrombi form initially in the calf veins and propagate upward or form initially above the femoral triangle and propagate in both directions is not known. Hampton and Castleman [13] considered, about 25 years ago, that the great majority formed in the calf, but subsequent studies by Hamilton and Angevine [12] and by McLachlin and Paterson [17] indicate that between 50% and 75% start at the femoral triangle or higher.

EFFECT ON THE VENOUS WALL

It has been observed by many, but emphasized particularly by Robertson *et al.*,[20] that the reaction of the venous wall to a coagulation thrombus in the lumen is organization with very little inflammation. Retraction and organization of the thrombus can be expected to occur. The result of recanalization from this reaction may vary from a small tortuous channel to a wide open one. In either case, the venous valves are destroyed or otherwise rendered ineffective.[8]

INDICATIONS

Because of the damage that the thrombus can do to the venous valves, it should be removed. Clinical follow-up studies of patients who had developed iliofemoral thrombophlebitis and were not treated by thrombectomy show that the majority (54%, according to Phillips [19]) developed a postthrombotic syndrome consisting of chronic edema, superficial varices, stasis ulceration, and the rest of the picture of venous stasis, which is so disabling in terms of normal occupational and social activities. The objective of the operation of thrombectomy is to prevent this complication of thrombosis. It is not to be thought of as an important measure in the prevention of pulmonary embolism. Because postphlebitic syndrome develops late, often insidiously, is not lethal, and has varying consequences, it is difficult for a surgeon faced with an acute iliofemoral thrombophlebitis to have an immediate concern for the later sequelae of the process.

However, the operation should be considered whenever there is a clear diagnosis of iliofemoral thrombophlebitis involving the common femoral vein. The level of thrombosis is easily determined by the level of swelling in the leg (e.g., if swelling is limited to the calf, the thrombus is no higher than the popliteal vein).

There is disagreement with regard to the maximum period after onset of the disease during which the operation would be worth doing. Mahorner *et al.*[16] found good results on some patients operated on as late as 3 weeks after onset. Haller [11] indicated that 10 days was the maximum. In six of 49 patients of Kaiser *et al.*,[15] the interval from onset of phlebitis to thrombectomy was 2–20 weeks. Most physicians who are treating patients routinely, and not making a special study of the problem, will not accept patients for operation more than 72 hr after onset. Hesitation in deciding to operate is rational for phlebitis in the elderly or in those very ill from other causes, in whom the potential rewards are limited with regard to the patient's total rehabilitation via prevention of postthrombotic syndrome. It is in the younger, more active patient that high rewards are expected and are being realized.

TECHNIQUE

The operation is performed through an incision in the femoral triangle. The superficial femoral vein is opened at its egress into the common femoral vein. Vigorous suctioning, milking of the legs, and, particularly, the use of Fogarty catheters (which permit clot extraction by withdrawal of an inflated balloon through the lumen of the vein) should be persisted in until all clot is removed.

Heparin administration is begun as soon as the diagnosis is made and is continued during the operation and postoperatively.

Most surgeons use general anesthesia; a few use local infiltration. The advantage of the latter is that the patient can cooperate by straining during the operation and thus extruding clot.

DISCUSSION

In 1965, Smith [23] documented 158 iliofemoral venous thrombectomies collected from the literature; 84% showed improvement. In his own series, 78% had acceptable results, half with no swelling and half with only slight swelling. He observed one possible pulmonary embolism during operation, and that was not fatal. The incidence of pulmonary embolism during operation has been extremely low. Postoperative pulmonary embolism occurred in 3.2% of the cases Smith collected from the literature, with no fatalities.

J. A. DeWeese (personal communication) considered that failures in his patients had occurred mainly because their thrombi had originated in the calf veins. His experience was that the majority of thrombi (60%) originated at the femoral triangle or higher, and his results in the cases of such thrombi were excellent. It is difficult, however, to separate the two sites of origin clinically, inasmuch as phlebography cannot be performed in the presence of the venous obstruction. If one finds at operation that one cannot clear all the clot from the distal calf, it is appropriate to clear the common femoral and iliac veins, leave the deep femoral vein draining into them, and ligate the superficial femoral vein.

In summary, the goal of this procedure is to prevent a seriously disabling, but not life-threatening, chronic condition. The lack of disease of the vein wall from which the thrombus is being removed makes thrombectomy a reasonable procedure. Clinically, the main worry—namely, the incitement of pulmonary embolism—has been of only minor importance. It is still to be determined whether inadequate removal of the thrombus or rethrombosis tends to vitiate the supposed benefits of the operation. A controlled study is badly needed here. Meanwhile, it seems reasonable to advise the operation for all patients in whom it would be likely to prevent the postthrombotic syndrome.

VENOUS INTERRUPTION OR COMPARTMENTATION FOR THE PREVENTION OF PULMONARY EMBOLISM

When surgical methods for the prevention of pulmonary embolism—namely, venous interruption or compartmentation—are used, they should

be part of an over-all plan that involves anticoagulants and other non-surgical measures.

INDICATIONS

Modern thinking does not endorse the use of venous interruption or compartmentation to prevent pulmonary embolism when no embolism has yet occurred. In view of the well-known infrequency of warning embolic phenomena before fatal massive pulmonary embolism, this may seem unrealistic. Nowadays, however, we are more often called on to attempt to save the lives of patients whose pulmonary embolism may *contribute* to death than to deal with massive embolism itself. In the former group, mostly cardiac patients, warning emboli are common.

The following is a list of indications for this procedure (adapted after Crane [5]):

1. Failure of anticoagulants
2. History of recurrent pulmonary emboli
3. Cardiac failure
4. A single massive pulmonary embolus that the patient has survived

Pregnancy is not a determining factor in the decision of whether to use this procedure.

SELECTION OF LEVEL

Crane [5] has outlined the pro's and con's of venous interruption or compartmentation at the femoral versus the inferior vena caval level in a careful review of 445 cases from the Peter Bent Brigham Hospital. His results are as follows:

Level	Further Embolism	Fatal Embolism	Clot above Region Post mortem
Femoral	10%	5%	3.5%
Inferior vena caval	1%	1%	1%

Crane found the in-hospital mortality rates of the two procedures to be relatively the same, but it must be borne in mind that the review was retrospective and that the two procedures were done in different types of patients. Most surgeons have been using inferior vena caval interruption or compartmentation, rather than femoral vein interruption.[18] Femoral vein interruption is reserved for patients in whom the clot is reasonably likely to end at the femoral triangle and who are obviously too ill for general anesthesia. And some surgeons reserve it for patients in whom heparin administration must be continued during surgery.

TECHNIQUE OF INFERIOR VENA CAVAL LIGATION OR COMPARTMENTATION

Heparin administration is stopped, and the patient's legs are raised and bandaged before preparation. Under general anesthesia, an incision is made in the flank or in the righ paramedian area, and the vena cava is exposed retroperitoneally. The vena cava is ligated or compartmented by clips or sutures as high as possible below the renal veins, preferably just below them or just below a large lumbar branch. After wound closure, the patient is watched vigilantly for leg swelling, which is dangerous. Some intravenous administration of plasma may be needed in the first 12 hr. If hypotension appears and persists for more than 12 hr despite treatment, thought should be given to removal of the clip or ligature. Because of the danger of retroperitoneal bleeding in the presence of increased venous pressure below the operative site, most surgeons agree that it is dangerous to start heparin sooner than 24 hr after the operation.

DISCUSSION

The results are typified by those of Crane, mentioned above.[5] The small proportion of failures to prevent embolism, even when the operation is performed at the level of the inferior vena cava, must be considered in making the decision to operate. The reason for failure is either formation of a clot above the ligature or the development of substantial collateral circulation through the ascending lumbar and other retroperitoneal veins around the ligature site.

Compartmentation had as its original objectives the prevention of immediate postoperative acute leg swelling with sequestration of blood and, it was hoped, the late sequelae of chronic venous stasis. The latter occurred to some degree in 24 of 25 cases reported by Shea and Robertson.[22] Most workers report the same occurrence of moderate swelling but a lower proportion of full-blown postphlebitic syndrome, with ulceration and disability. With regard to acute swelling, most extensive series of inferior vena caval ligation reveal an occurrence of 5% of immediate alarming swelling after the ligation.[5] This is almost never seen in patients in whom compartmentation is performed.[3]

Vena caval compartmentation, however, has not lived up to its promise in preventing the later leg edema. Even though Burget *et al.*[3] report only three of 17 showing late closure on cavography, others find the rate of late patency to be lower. Blazek *et al.*,[2] for example, found that, of 22 patients whose vena cavas had been compartmented with clips, 10 were observed on later phlebography to have closed vena cavas. The argument might be used, as it has been with vena caval ligation, that postcompartmentation leg swelling is a result of a phlebitis, either

nascent or occult, that existed before the operation. Blazek's finding that patency at the clip site had no correlation with symptoms in the leg tends to support the exoneration of the operation on the inferior vena cava of responsibility for late leg swelling. But, on the other side of the argument, the observations of Carmichael and Edwards [4] are of interest. They compartmented 36 vena cavas prophylactically in patients who had had operations considered to carry a high risk of postoperative phlebitis. Nine (25%) of the patients developed phlebitis of the leg, a far higher proportion than one would have expected in the absence of an operation on the inferior vena cava.

Furthermore, the hazard of postoperative embolism seems more significant in patients with compartmentation; in the series of Blazek *et al.*,[2] seven of the 22 had postoperative embolism, and six of the seven had patency at the plication site. One of Carmichael and Edwards's cases died of massive pulmonary embolism.[4]

Thus, it seems that, although vena caval compartmentation deserves extensive further trial in view of its advantage of avoiding acute sequestration of blood in the lower half of the body postoperatively, its failure to avoid late leg swelling and possibly its incitement of further pulmonary emboli must be recognized.

In summary, the place of vena caval ligation or compartmentation in the therapeutic armamentarium for pulmonary embolism seems secure. Future efforts must be directed toward decreasing the number of failures and the postoperative morbidity.

SURGICAL REMOVAL OF PULMONARY EMBOLI

INDICATIONS

At the outset of this discussion, it will be assumed that there is a place for pulmonary embolectomy, which some doubt. The controversy will be dealt with below.

The indications for pulmonary embolectomy are:

1. A clear diagnosis of embolism of the main pulmonary artery or its branches
2. Failure of vasopressors or immediate heparin therapy to support the circulation
3. Absence of an irreversible fatal associated disease

The establishment of this diagnosis may be tricky,[25] and, wherever possible, pulmonary angiography should be carried out before surgery.

PROCEDURE

Action must be rapid. The use of cardiopulmonary bypass is essential. (Gibbon [10] developed the heart–lung machine for this purpose some 30 years ago, before there was any thought of using it for intracardiac surgery.) The following steps should be followed:

1. After preparations have been made, pause and consult soberly for a few minutes before making an incision. The three indications should exist.
2. Place the patient on partial bypass (arteriovenous support), using the low-prime bubble oxygenator.
3. Make a sternal splitting incision; change to total cardiopulmonary bypass; express the clots from the lungs after pulmonary arteriotomy.
4. Ligate the inferior vena cava.

Alternate approaches are: (1) the old Trendelenburg technique,[24] consisting of approach through the third and fourth left costal cartilages, direct incision into the pulmonary artery after placing a tourniquet through the transverse sinus and suturing the pulmonary arteriotomy; (2) a similar technique, but using venous inflow occlusion; and (3) an approach through one pulmonary artery without circulatory obstruction or support.

DISCUSSION

Cross and Mowlem [6] have reported 137 cases of pulmonary embolectomy collected from a questionnaire that was sent to 40 surgeons in the United States and Canada. The immediate mortality was 57% (78 cases), and there was an additional later mortality of 9.5% (13 cases), for a total mortality of 66.5%. The causes of failure were: wrong diagnosis, small peripheral emboli, operation too late, and recurrent emboli. All nine patients in whom the diagnosis was wrong died.

This account sounds pessimistic. Should it condemn the operation? No, but it should incite us to improve diagnosis and operative technique. Those who have been interested in the field know of patients for whom hope was abandoned before the operation was done and who were then saved by it. The delineation of the indications and the perfection of the technique must await further experience. The procedure must always be an emergency, or at least urgent, and no single individual is likely to accumulate a large experience. The operation is still being done by the inexperienced. There will always be a significant mortality inherent in

the procedure itself. It is well to remember that the mortality of arterial embolectomy is still in the neighborhood of 30%; but that is due not to the operation but rather to the basic disease. In pulmonary embolism, we are striving to save life, rather than merely limb.

Donaldson *et al.*[7] have shown that 25% of patients who died of massive pulmonary embolism at the Massachusetts General Hospital survived for at least 1 hr after onset. Those who contend that pulmonary embolectomy has no place would hold that these patients could be saved by modern methods of anticoagulant or thrombolytic therapy. The success of heparin therapy in patients with massive embolism is well recognized. Barritt and Jordan [1] found that the careful and thorough use of heparin in a trial series had such great benefit that the trial had to be abandoned after 19 patients, inasmuch as it was no longer ethical to withhold heparin from the controls. Hirsh *et al.*,[14] however, recorded two carefully documented instances of massive embolism in which repeated observations were made by inlying pulmonary artery catheters through which pulmonary artery pressure readings were recorded and angiography repeated; they found in one case that heparin had no effect over a 24-hr period, but that rapid resolution of the thrombus occurred after the administration of streptokinase through the catheter.

Sautter *et al.*[21] and Fred *et al.*[9] have noted, via sequential pulmonary angiographic studies, that significant pulmonary arterial defects disappeared "spontaneously" in several cases; anticoagulants were used in most, but not all. It is to be noted that the time of the disappearance could not be well circumscribed because sequential pulmonary angiography was sometimes inadvisable. But it seemed, in most cases, that maximum disappearance required at least several days.

In summary, pulmonary embolectomy has been performed as a life-saving measure with dramatic success in a few patients, but most have not survived. The operation, in conjunction with anticoagulant and supportive therapy, has an important place when the indications outlined are observed strictly.

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