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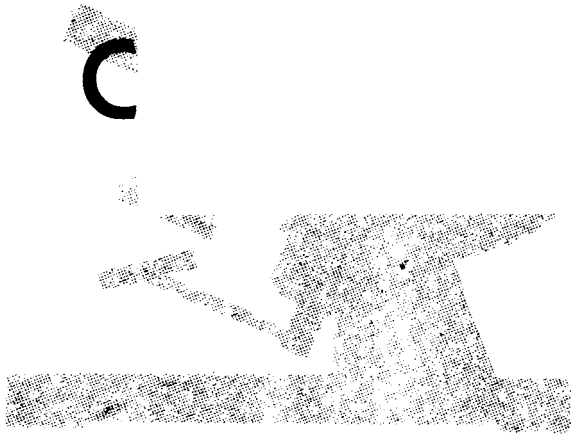

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FIRST SYMPOSIUM

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May 26-27, 1950

Sponsored by
The Chemical-Biological Coordination Center
of the
National Research Council

National Academy of Sciences-National Research Council
Washington, D. C.
1951

The Chemical-Biological Coordination Center is affiliated directly with the Executive Board of the National Research Council of which Mr. William W. Rubey is Chairman. It was established on July 1, 1946, and succeeded the O.S.R.D. Insect Control Committee. Its objectives are: (1) To assemble and organize information which describes (a) the effect of chemicals on biological systems (plants and animals, their organs, tissues, cells and cell constituents); (b) the metabolism of test chemicals within biological systems; (c) the mechanism of drug action or which provides information leading to an understanding of such action. (2) To sponsor the preliminary testing ("screening") of compounds, solicited by the Center, on a variety of plants and animals (including microorganisms) to determine the biological effects of the compounds and to make the resulting data available for use. (3) To prepare reviews of the literature on: (a) The effect of chemical structure upon various biological actions; (b) Test methods used in determining such actions. (4) To sponsor symposia concerned with the correlation of chemical structure with biological activity.

The data in the files of the Center are available to representatives of the sponsoring agencies, the screening agencies and to authorized scientists generally upon request. The sponsoring agencies are the Atomic Energy Commission, the Department of the Army, the Department of the Navy, the U.S. Public Health Service and the American Cancer Society.

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Panel Discussion on Antimetabolites, Carcinogenesis and Cancer Chemotherapy (Cont.)

WELCOME

Dr. Detlev W. Bronk
Chairman
National Research Council
Washington, D. C.

Dr. Winternitz and Members of the Conference: On behalf of the staff of the National Research Council, I express warm appreciation of the opportunity you have of aiding in the development of an undertaking of great significance.

It would be inappropriate for me to express the appreciation of the Council to you for your services to the Chemical-Biological Coordination Center, for the Council in which the Center operates is your own organization, for the fulfillment of such functions as you may desire.

I can, however, express my admiration for your unselfish participation in this cooperative effort for the furtherance of science and the advancement of human welfare.

I have expressed my conviction that this is an undertaking of significance. This I believe for several reasons.

Of first importance is the function of the Center to facilitate research in an area of fruitful progress. I need not say to you that the relations of chemical structure to the architecture and functions of biological systems is basic to advances of great importance. But what I would like to stress is the fact that the National Research Council, which has many functions relating to governmental and private agencies and activities of broad social significance, nevertheless believes that its primary function must be the furtherance of scientific research. If we ever forget that this is our primary mission, if we ever deviate from primary emphasis upon the furtherance of research, whether it be called fundamental or applied, we shall weaken the basic foundations of the Council and thereby make it less able to fulfill its other responsibilities.

I would also mention the fact that the Chemical-Biological Coordination Center exemplifies one of the most important functions, one of the most characteristic functions of the Council. I refer to the union of the sciences, without regard to the artificial boundaries of specialization and convenience. The very fact that, within this Center, there are representatives of the botanical and of the physiological, of the medical, of the chemical, of the zoological sciences is evidence of the fact that we can best fulfill our many functions by serving as a focus for the sciences which are here represented, without regard to the artificial boundaries of which I have spoken.

Because it is one of my particular enthusiasms, I might go on to say that, although the Council has long succeeded in dealing with problems without regard to the question as to whether they should be approached from the standpoint of physics or biology or chemistry or engineering or psychology or medicine or geology, we have not until recently fully recognized the necessity for eliminating the boundaries between the sciences which are called natural and the sciences which are called social, and between the sciences and the humanities.

But, during recent years, through the agency of the Conference Board of Associated Research Councils, which includes representatives not only of the National Research Council, but, also, of the American Council of Learned Societies and the Social Science Research Council and, more recently, the American Council on Education, we have, we believe, succeeded in breaking down the barriers between these various fields of learning, and have made the four councils essentially one in the attack upon common problems relating to the national welfare.

A related function of the Council, which your activity recognizes, is the function of serving as a focus for the common needs and mutual support of various agencies both governmental and private. This is of especial moment at present. The rapid growth of science makes

heavy demands upon our limited supply of scientific personnel. The National Research Council, accordingly, seeks to conserve for creative science the time and effort of scientists who are willing to satisfy the common needs of science and the needs of society by preventing the duplication in various governmental agencies and in various private agencies and in various fields of science. Because of the fact that you are thus a focus for many governmental and private agencies, we point to you with pride as exemplifying this important function of the Council.

Thoughtful scientists must regret a growing fragmentation of science, and must hope for a greater synthesis of knowledge. One of the greatest barriers to such a synthesis is the accelerated accumulation of experimentally derived facts and data. From every field of science, the National Research Council has, during the past year and a half, been urged to study means for the better dissemination and utilization of scientific knowledge. As experimenters, it has seemed to us quite right that we should encourage experimentation in the development and use of the structures of scientific knowledge. Because of this, as a result of two conferences on problems of scientific abstracting held during this past year, and of one concerned with scientific publications of a primary nature, we have developed plans for the establishment of an agency which will serve as a clearinghouse for information concerning problems and solutions of problems relating to all phases of scientific information, an agency for the stimulation of research on how the publication and dissemination and utilization of scientific information can be better achieved.

But that is something for the future. What is important is the fact that you have taken a first step in this important area of science and, because of this, have blazed new trails which, I am sure, will, in the future, not only pay great dividends in the provision of useful scientific information more readily achieved but, also, exemplify the possibility of scientists devising new means for the codification, for the presentation, for the usability of scientific information derived from scientific experimentation.

Because of your vision in participating in this significant new undertaking, I congratulate you, and express my warm appreciation.

Thank you very much.

CORRELATIONS BETWEEN THE CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY OF ARSENOSOBENZENES

by

George O. Doak, PhD. and Harry Eagle, M. D.

Syphilis Research Laboratory, USPHS, and University of North Carolina, School of Public Health, Chapel Hill, North Carolina, and the Microbiological Institute, National Institutes of Health, Bethesda, Maryland.

I. THE CHOICE OF COMPOUNDS FOR STUDY

Only three types of organic compounds containing arsenic linked directly to carbon have been used successfully in the treatment of spirochetal and protozoal diseases: the arsonic acids, the arseno compounds and the arsenoso compounds or arsenoxides (cf. Fig. 1). The simple organic radical, R, attached to arsenic is usually benzenoid, although similar compounds in which R is a heterocyclic ring, e.g. pyridine, are known to be highly active[6] against trypanosomes. Compounds in which R is aliphatic or alicyclic have also been tested, but are generally either completely devoid of activity[8] or only weakly active.

Four other types of organic arsenicals containing benzene rings are shown in Fig. 2. A few examples of these have been tested in various laboratories, but none has been found which compares in activity with the compounds illustrated in Fig. 1.[7] Accordingly, only the latter will be considered in the following analysis of the relationships between the chemical structure and biological activity of organic arsenicals.

An important consideration is whether all three types are active per se, or must first be modified in the animal host. With the arsonic acids this question is easily answered. Ehrlich, in his original studies, showed that although the arsonic acids were inactive in vitro, they were therapeutically highly active, and concluded that the arsenic was reduced to an active trivalent form by the body tissues.[28] He therefore prepared both arseno and arsenoso compounds, and found both types active in vitro. The arsenoso compounds, while extremely active, were discarded because of their high toxicity; but the arseno compounds, notably arsphenamine and neoarsphenamine, found wide therapeutic application.

Confirming and extending Ehrlich's classical studies, Voegtlin and Smith in 1920 demonstrated that with both arsonic acids and arseno compounds there was a latent period before trypanosomes disappeared from the blood of infected animals, while arsenoso compounds produced an immediate and striking decrease (Fig. 3). These results were interpreted to mean that the arsenoso compounds were directly trypanocidal, while the arsonic acids had to be reduced and the arseno compounds oxidized to the arsenoso form in the animal body before becoming active.

Other workers, however, found that the arsphenamines as such, and without further modification in the animal body, were active against trypanosomes and spirochetes.[62] The discrepancy was resolved by later in vitro studies with Treponema pallidum.[13] Although the arsphenamines were apparently quite active against this organism when tested by the usual anaerobic technic, when both the dissolution and testing of the drugs were carried out under an atmosphere of nitrogen, the activity of arsphenamines was reduced to a fraction of its previous value. The results of one experiment are given in Table I. The arsphenamines as such are clearly not directly spirocheticidal, but in the course of solution and dilution prior to testing there is sufficient oxidation to the arsenoso compound to make the solution active. The slight direct activity of arsphenamine is explainable on the basis of the known arsenoso content of this drug as marketed. In general, the arseno compounds are amorphous powders which are almost impossible to obtain in a state of chemical purity, and exist in several states of molecular aggregation. Both factors have been shown to influence their parasiticidal activity and toxicity to a marked degree.[46]

The fact that both the arsonic acids and arseno compounds must be modified in the animal body before exerting their therapeutic action makes it difficult, if not impossible, to correlate their chemical structure and biological activity unless it is assumed that all the compounds are modified to the same degree. This assumption is certainly not justified in the case of the arsonic acids, which are excreted in part unchanged. Cohen, King and Strangeways attempted to correlate the toxicity of arsonic acids with the rate of oxidation of the corresponding arsenoso compounds,[9] on the assumption that the rate of reduction of the arsonic acids in vivo might be in the same order as the reverse effect. No such correlation was found. In later studies, Eagle, Hogan, Doak and Steinman[20] also found no regular correlation between the toxicity of arsonic acids and the corresponding arsenosobenzenes, or between their trypanocidal activities in vivo (Table II).

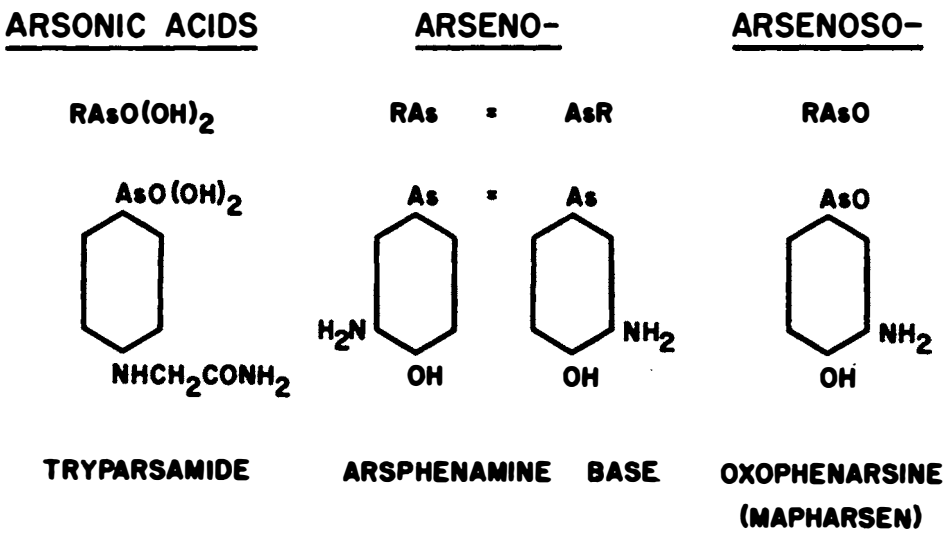


Fig. 1 Types of Aromatic Arsenicals Generally Used in Therapy

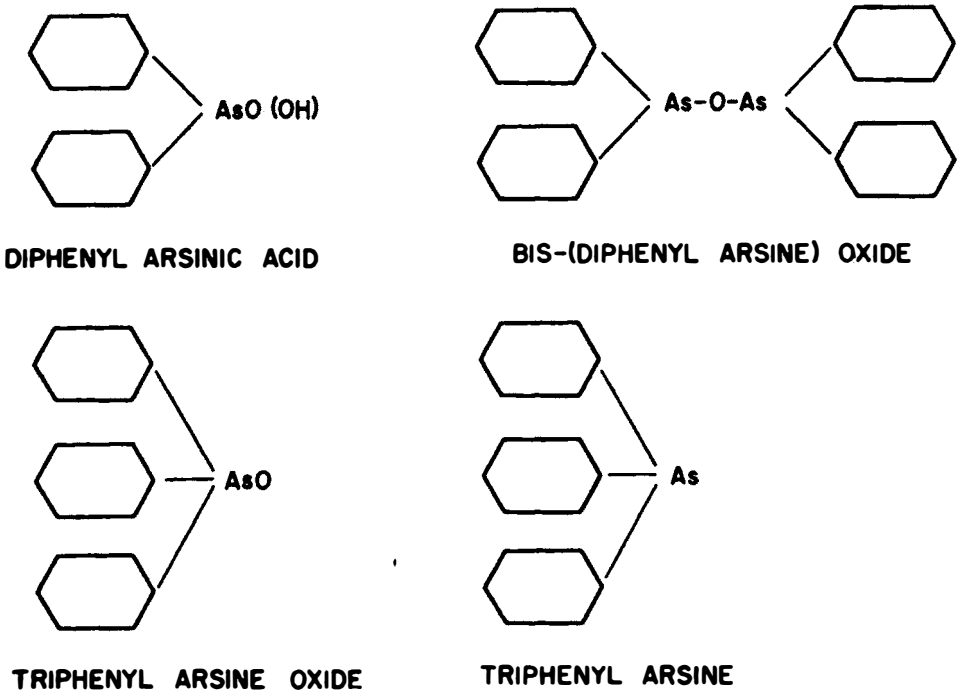


Fig. 2 Illustrative Aromatic Arsenicals Which Have Not Been Generally Used

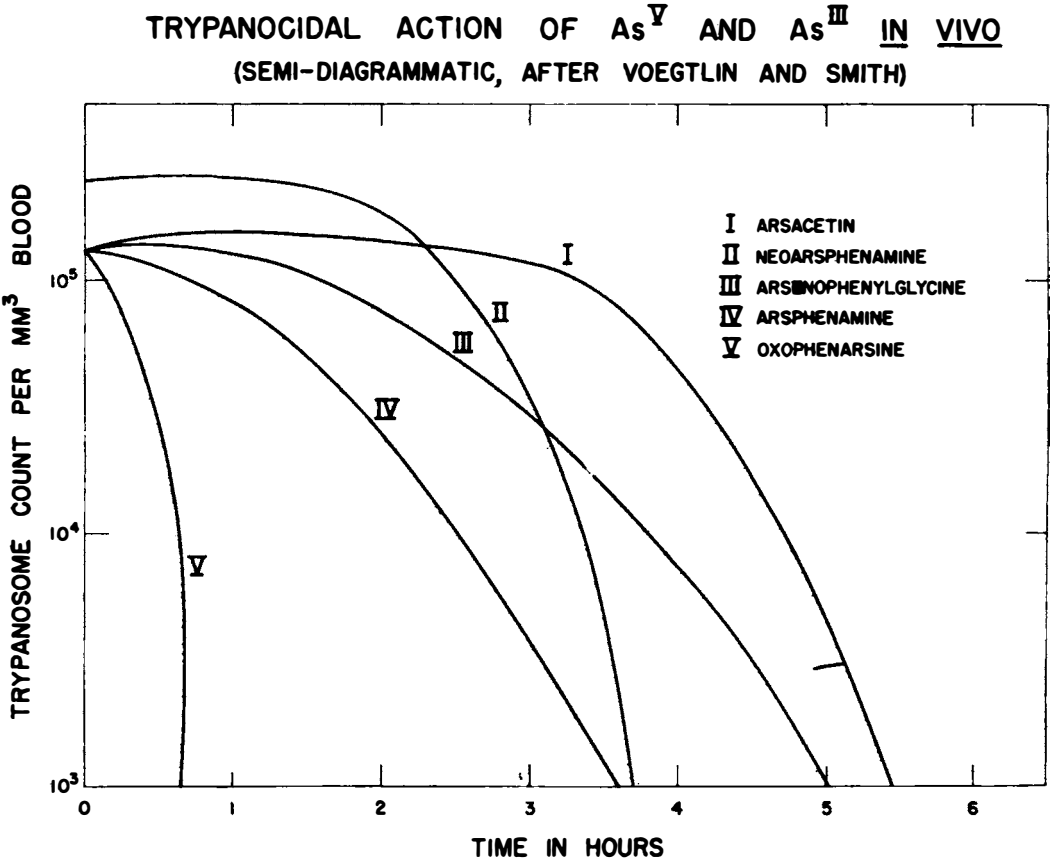


Fig. 3 The Trypanocidal Action of Pentavalent and Trivalent Arsenicals in Vivo (after Voegtlin and Smith[58])

TABLE I

THE EFFECT OF OXYGEN ON THE TREPONEMICIDAL ACTIVITY OF ARSPHENAMINES[13b]

Drug	Conditions of Test	Concentration necessary to immobilize 50% or organisms	Relative Treponemicidal Activity
Oxophenarsine	Aerobic-anaerobic[1]	1 : 180,000	100
	Anaerobic[2]	1 : 180,000	100
Neoarsphenamine	Aerobic-anaerobic	1 : 84,000	100
	Anaerobic	1 : 1,100	1
Arsphenamine	Aerobic-anaerobic	1 : 53,000	100
	Anaerobic	1 : 13,000	25
Silver-Arsphenamine	Aerobic-anaerobic	1 : 80,000	100
	Anaerobic	1 : 10,500	13

[1] Compound dissolved aerobically, and solution tested anaerobically
[2] Solution dissolved and tested under N_2 atmosphere

TABLE II

THE POOR CORRELATION BETWEEN (A) THE TOXICITIES OF ARSONIC ACID AND THE CORRESPONDING ARSENOSO COMPOUNDS (B) THE TRYPANOCIDAL ACTIVITIES IN VIVO OF ARSONIC ACIDS AND THE CORRESPONDING ARSENOSO COMPOUNDS (Summarized from 18a, b, 20 and 21b)

Substituent Group	A. Toxicity: LD_{50} in White Mice, mg./kg.			B. Trypanocidal: CD_{50} in White Mice, mg./kg.		
	As^{V}	As^{III}	Ratio of LD_{50} Doses	As^{V}	As^{III}	Ratio of CD_{50} Doses
p-$CONHCH_2CONH_2$	1750	15	115	380	1.4	270
p-$NHCH_2CONH_2$	950	19	50			
p-$NHCONH_2$	1025	11	93	305	1.16	267
p-OCH_2CONH_2	745	9.5	78	80	0.82	98
3-$NHCOCH_3$-4-OH	765	6	127	565	1.91	296
3-NH_2-4-OH	775	17	46	85	0.62	137
p-SO_2NH_2	595	18	33	170	2.0	85
p-OH	550	2	275			
"Melarsen"	260	12	217	30	0.048	625
3-NH_2-4-$CONH_2$	290	15	19			

TABLE II (Cont.)

Substituent Group	LD_{50} in White Mice, mg./kg.			CD_{50} in White Mice, mg./kg.		
	As^{V}	As^{III}	Ratio of LD_{50} Doses	As^{V}	As^{III}	Ratio of CD_{50} Doses
3-NH_2-4-$(CH_2)_3COOH$	140	12	12	>190	1.26	>150
p-NH_2	165	1.5	110			

TABLE III

REPRESENTATIVE MONOSUBSTITUTED ARSENOSOBENZENES TESTED FOR BIOLOGICAL ACTIVITY

(Summarized from 18a, b, 20, 21a, b)

AsO (X)	Single Grouping on End of Substituent Chain AsO X-R		Substitution in Single Group AsO R^1-X
o-, m-, p-NO_2	p-OCH_3	p-$CONHCH_3$	p-CH_2OH
o-, m-, p-Cl	p-$N(CH_3)_2$	p-$CON(CH_3)_2$	p-OCH_2CH_2OH
o-, m-, p-OH	p-CH_2NH_2	p-SO_2NHCH_3	p-$CONHCH_2COOH$
o-, m-, p-NH_2	p-$C_6H_4NH_2$	p-$SO_2N(CH_3)_2$	p-$CONHC_6H_4NHCOCH_3$
o-, m-, p-CH_3	m-, p-$NHCOCH_3$	p-$(CH_2)_xCOOH$	p-$CONHC_2H_4OH$
o-, m-, p-COOH	p-$OCOCH_3$	p-$(CH_2)_xCONH_2$	p-$CONHCH_2CN$
o-, p-SO_3H		p-$NHCH_2CONH_2$	
m-, p-$CONH_2$		p-CCH_2CCNH_2	
m-, p-SO_2NH_2		p-$CCNHCH_2CONH_2$	
p-F			

It is apparent that many compounds listed as X-R could just as well have been listed as R'-X, depending on which is considered the functional group.

The arsenoso compounds therefore appear to be the drugs of choice in an attempted correlation of chemical structure and biological activity. Unfortunately, most pharmacological studies on the aromatic arsenicals have dealt with arsonic acids and arseno compounds, on the assumption that the arsenoso compounds were too toxic for use. This assumption was questioned by Tatum and Cooper,[54] who demonstrated that m-amino-p-hydroxyphenylarsenoxide, which they named "mapharsen", possessed a higher therapeutic index against Treponema pallidum than the arsphenamines. Prior to the advent of penicillin, this compound was widely regarded as the drug of choice for the treatment of syphilis. Eagle, Hogan, Doak and Steinman later found a large series of arsenoso compounds to possess a higher therapeutic index than the corresponding arsonic acids against Trypanosoma equiperdum,[20] although Gough and King had previously reported no essential difference between the two classes of compounds in a much smaller series.[33]

The following sections will therefore describe the results obtained in a study of the toxicity and parasiticidal activity of a large series of arsenosobenzenes, considered in relation to the substituent groups of the phenyl ring.[18, 33]

II. THE TOXICITY, PARASITICIDAL ACTIVITY IN VITRO AND THERAPEUTIC ACTIVITY IN VIVO OF ARSENOSO COMPOUNDS, CONSIDERED IN RELATION TO THEIR STRUCTURE

A. Compounds Tested and Technics of Assay

Table III lists some of the mono-substituted derivatives of arsenosobenzene synthesized and tested in this laboratory for the purpose of this study.[11] The aryl arsenoso compounds are prepared by the reduction of the corresponding arsonic acids, using a variety of reducing agents.[27] The first column lists some of the simple substituents. The second and third columns list several representative compounds in which some of these groups were blocked by substitution (X-R), or in which the functional groups were extended on a side chain (R-X).

A number of di-substituted derivatives of arsenosobenzene were also prepared.[12] When the two substituents are the same, there are six possible isomeric compounds; when they are different, there are ten possible isomers. Some of the compounds in this group which were prepared and tested are shown in Table IV.

In addition to the derivatives of arsenosobenzene, a limited number of compounds were prepared using naphthalene, biphenyl, pyridine and similar ring structures.

The toxicity of these compounds was tested in rabbits and mice. Their parasiticidal activity was studied with Treponema pallidum and Trypanosoma equiperdum, both in vitro and in vivo; and a few representative compounds were further tested in vitro with Trypanosoma cruzi and Leishmania donovani. For the assay of treponemicidal activity in vitro, an acute testicular syphiloma was emulsified, and graded concentrations of arsenicals added to the particle-free extract. At the end of one and one-half to four hours the action of the arsenical was stopped by the addition of cysteine, and the concentration of drug which had immobilized 50 per cent of the organisms determined by microscopic observation. The activity was referred to that of a standard compound simultaneously tested, usually the unsubstituted arsenosobenzene.[15] Toxicity was determined as the LD_{50} dose on single intraperitoneal injection in white mice. In this way a "hybrid" therapeutic index was obtained, defined as the ratio of molar treponemicidal action in vitro:molar toxicity in white mice. A considerable number of compounds were also tested in syphilitic rabbits, and a therapeutic index obtained in terms of the LD_{50}/CD_{50}. The high degree of correlation between the two indices reflects the fact (1) that the toxicity of the compounds in mice paralleled their relative toxicity in rabbits (Fig. 4), and further, that the curative action in animals paralleled and was presumably determined by their direct treponemicidal action (Fig. 5).

TABLE IV

REPRESENTATIVE DISUBSTITUTED ARSENOSOBENZENES TESTED FOR BIOLOGICAL ACTIVITY[12, 18b]

	NH_2	OH	Cl	CH_3	COCH	$CONH_2$	$NHCOCH_3$
NH_2	X	X (6)[1]	X		X (10)[1]	X	
OH		X	X			X	X
Cl			X				
CH_3				X			
NO_2		X	X				
$NHCOCH_3$		X					X

[1] Number of isomeric compounds prepared

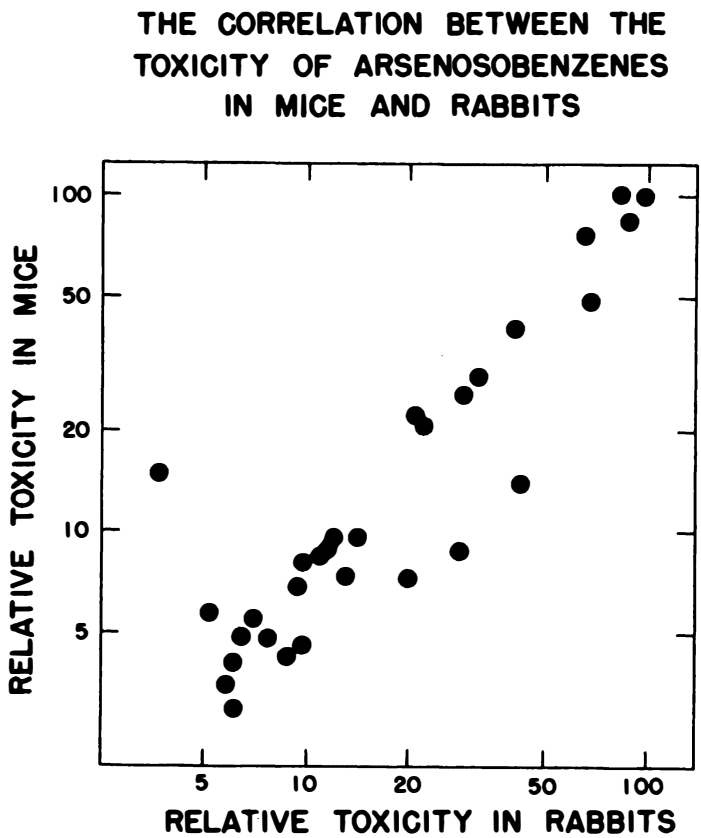


Fig. 4 The Correlation Between the Toxicity of Arsenosobenzenes in Mice and Rabbits[15, 18a, b, 21]

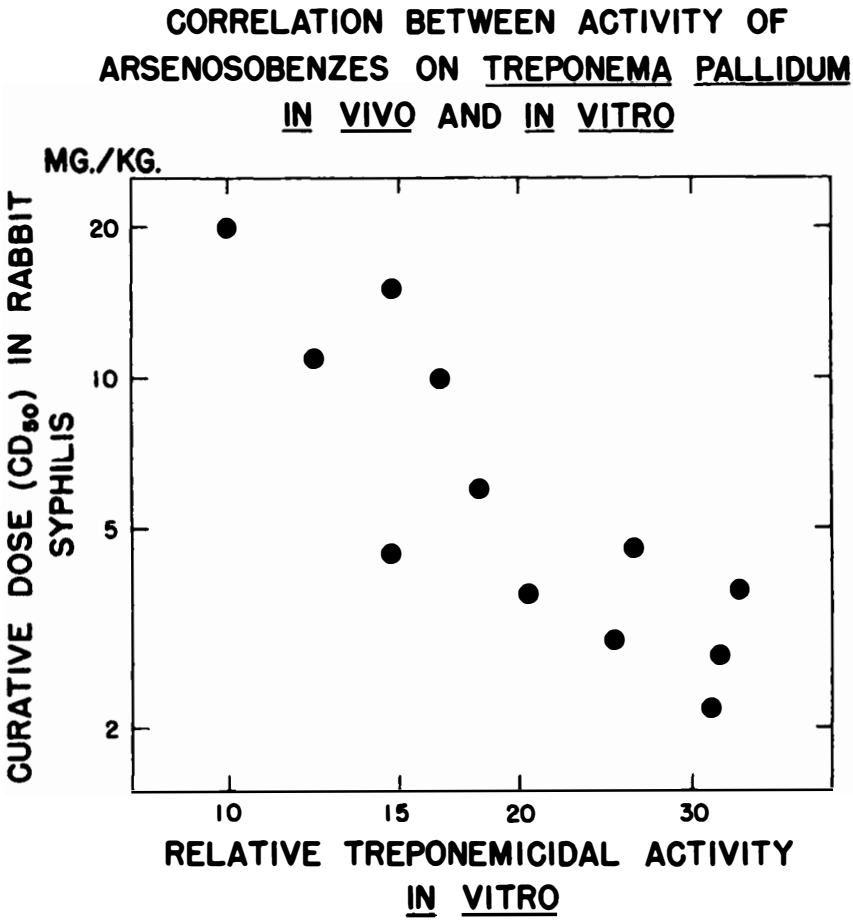


Fig. 5 Correlation Between Activity of Arsenosobenzenes on Treponema pallidum in Vivo and in Vitro [15, 18a, b, 21]

With Trypanosoma equiperdum, the in vitro assay was similar to that used in the assay against Treponoma pallidum, and the therapeutic activity was tested in both mice and rabbits.[20] Reasonably good agreement was again found between the trypanocidal activity of arsenoso compounds in vitro and in vivo, indicative of the fact that the therapeutic activity of these compounds is determined by their direct trypanocidal action. In view of the generally good correlation between the direct parasiticidal action of the arsenosobenzenes and their therapeutic action in vivo, the following discussion in the correlation between structure and biological activity is based largely on the in vitro data.

B. Biological Activity of Substituted Arsenosobenzenes

The effect of single substituents on the toxicity and treponemicidal activity of arsenosobenzene is shown in Fig. 6 and Table V. The latter shows also their direct trypanocidal activity (T. equiperdum).

Despite the wide variations encountered between closely related compounds, the data permit certain broad generalizations with respect to the correlation between chemical structure and biological activity.

1. "Inert" Substituents

There were a number of "inert" substituents (e.g. -Cl, $-NO_2$, $-NH_2$, -OH, $-CH_3$, -F), which did not significantly affect either the toxicity or parasiticidal activity of arsenosobenzene. The activity:toxicity ratios of the resulting compounds were therefore substantially the same as that of the highly active and highly toxic parent arsenosobenzene. Like the latter, these compounds are apparently general protoplasmic poisons, with no selective or specific effect on a particular parasite. The position of such substituents on the benzene ring had relatively little effect. Thus, the ortho, meta and para arsenosotoluene compounds had relative treponemicidal activities of 84, 97 and 102, respectively (referred to the parent arsenosobenzene as 100), relative toxicities of 88, 100 and 118, and activity:toxicity ratios 0.95, 0.98 and 0.83.

2. Effects of Acidic Substituents

Most acidic substituents markedly depressed both the treponemicidal and trypanocidal activity of arsenosobenzene, resulting in compounds with a low level of activity (cf. Fig. 1 and Tables V and VI). The toxicity of these compounds was also reduced; and although the individual compounds varied markedly in this respect, the toxicity was usually not reduced to the same degree as the parasiticidal activity. In consequence, the therapeutic indices of the acid-substituted compounds were even less than that of the parent compound. There were, however, a few important exceptions to this generalization, notably with the γ-(p-arsenosophenyl)-butyric acid-, β-(p arsenosophenyl)-valeric acid-, and ε-(p-arsenosophenyl)-caproic acid- substituted compounds (cf. Table VI). The first two compounds were actively trypanocidal, both in vivo and in vitro, although they had no significant activity against T. pallidum. Conversely, the last named compound had considerable treponemicidal activity, but was almost inert against T. equiperdum. These exceptions emphasize the danger of generalizing even on the basis of a large number of closely related compounds. They emphasize also the specificity of the reaction of some arsenicals with particular parasites (cf. page 33).

a. Evidence for Modification of Acid-substituted Arsenoso Compounds in Vivo. There is reason to believe that with these acidic compounds the observed toxicity is not that of the compound itself, but of a derivative formed in the body. (1) As is discussed in a following section, the toxicity of arsenicals is apparently determined by the degree to which they are bound by the host tissues.[37] There was a high degree of correlation between the rate of excretion of an arsenical and its toxicity, the least toxic compounds being excreted at the fastest rate. Further, the degree to which arsenoso compounds were bound by red cells in vitro proved a generally reliable measure of their systemic toxicity. The acid-substituted compounds were bound to only a negligible degree by erythrocytes in vitro; and correspondingly, after intravenous injection they were at first excreted rapidly. These data implied a very low toxicity, far lower than was actually the case. However, after a few hours, the rate of excretion fell off abruptly, suggesting that the arsenical had been modified to a more toxic form, which was then retained in the body. (2) After lethal doses of acid-substituted compounds, death was usually delayed as compared with similarly toxic doses of other arsenoso compounds, not containing acidic groups (Fig. 7).

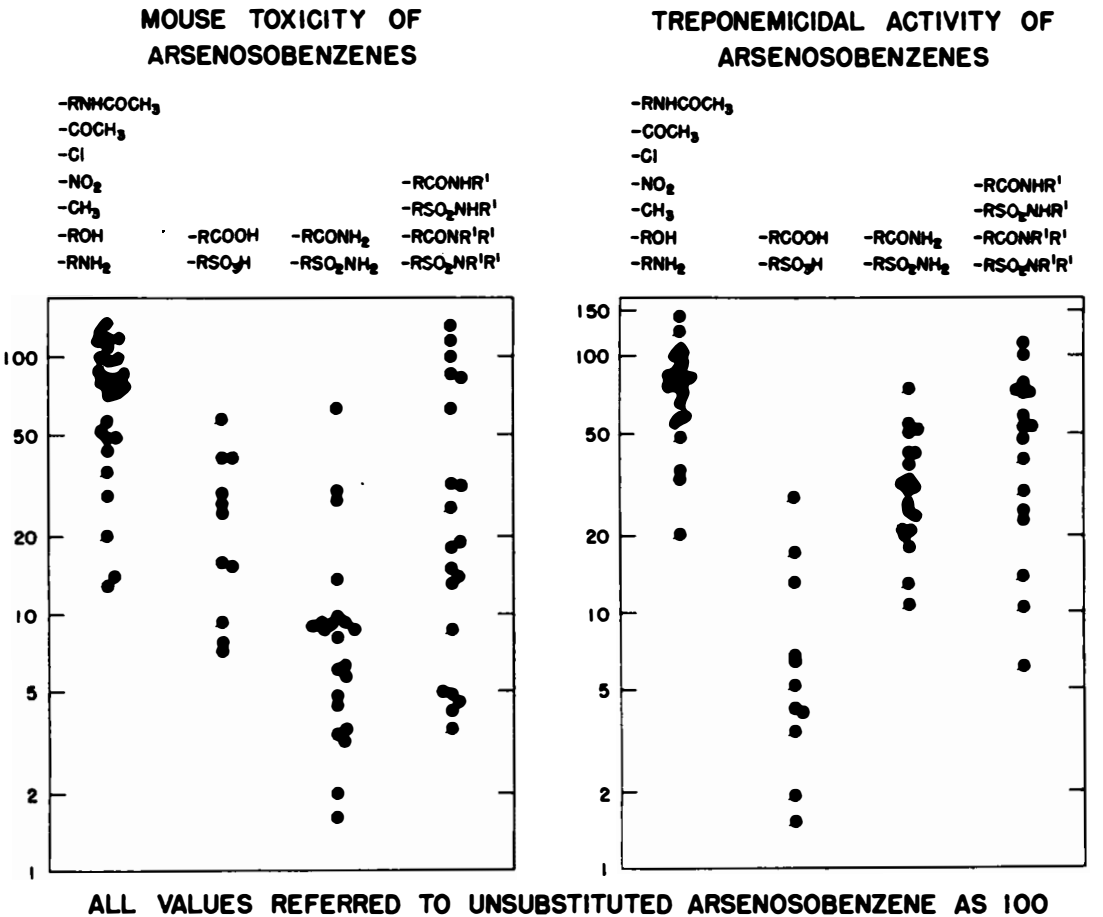


Fig. 6 The Effect of Various Types of Single Substituents on the Toxicity and Treponemicidal Activity of Representative Arsenosobenzenes

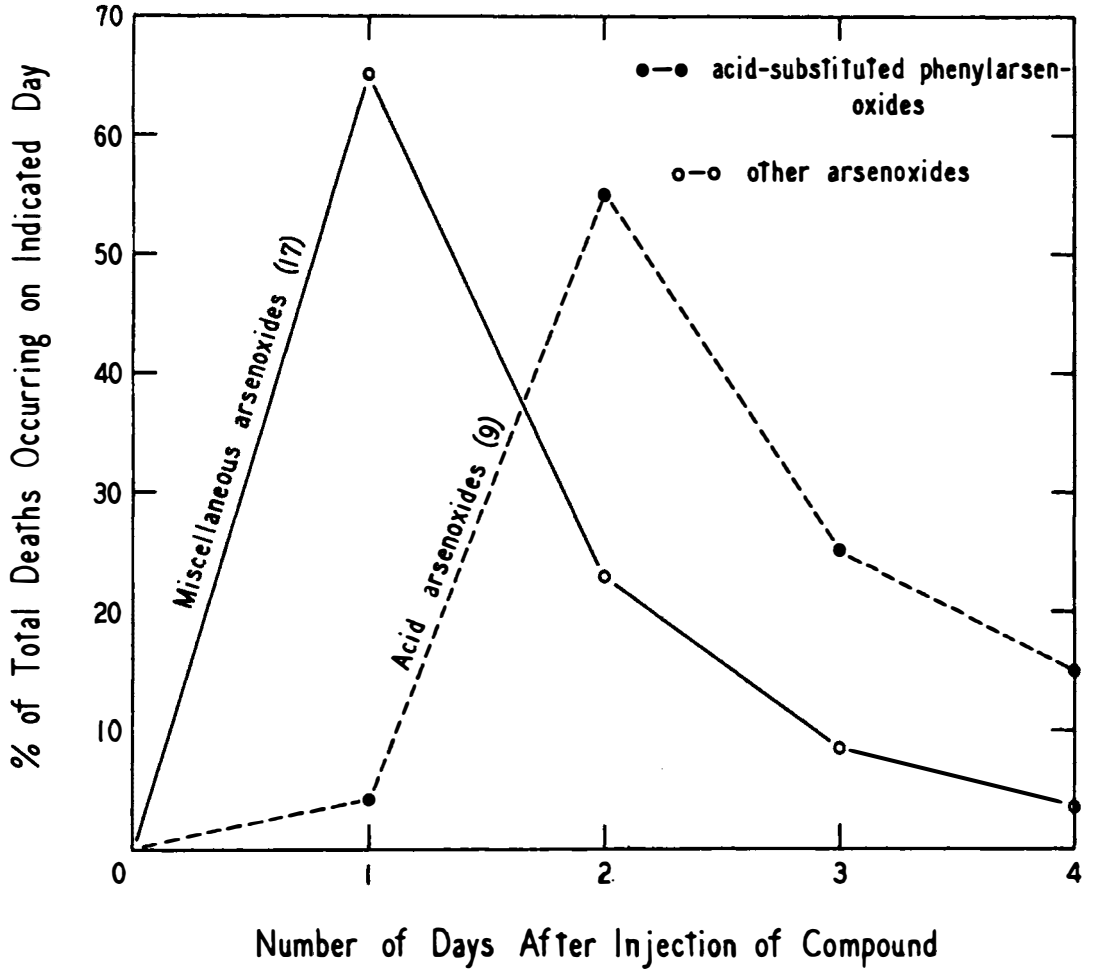


Fig. 7 The Characteristic Delay in the Death of White Mice Injected Intraperitoneally with LD_{40} to LD_{60} Doses of Acid-Substituted Arsenosobenzenes[37]

TABLE V

THE EFFECT OF VARIOUS SINGLE SUBSTITUENTS ON THE TOXICITY, TREPONEMICIDAL AND TRYPANOCIDAL ACTIVITY OF ARSENOSOBENZENE

(All values referred to that of unsubstituted arsenosobenzene as 100)

(Summarized from 18a, b, 20 and 21b)

Substituents	Toxicity in mice[1]	Treponemicidal activity in vitro (T. pallidum)	Trypanocidal activity in vitro (T. equiperdum)	Ratio of treponemicidal activity in vitro: toxicity	Ratio of trypanocidal activity in vitro: toxicity
"Inert" $-NO_2$, $-CH_3$, $-Cl$, $-NH_2$, $-OH$, $-F$	(17) 36-123 85	(17) 36-147 85	(7) 57-102 91	0.33-1.47 1.0	0.80-1.2 0.92
"Slightly active" $-RNH_2$, $-ROH$,[2] $-NHCOCH_3$, $R-NHCOCH_3$,[2] $-OCOCH_3$	(12) 6.7-76 23	(12) 21-78 38	(6) 31-71 34	0.38-4.8 1.76	1.2-6.0 2.2
Acidic (cf. Table VI) $-COOH$, $-SO_3H$, $-RCOOH$	(15) 5.8-41 16	(14) 0.7-28 4.7	(12) 0.06-54 3.0	0.04-2.7 0.24	0.002-6.1[3] 0.1
Acid amides (cf. Table VII) $-CONH_2$, $-SO_2NH_2$, $-RCONH_2$	(16) 3.2-14 7.2	(16) 11-52 30	(12) 1.4-73 25	2.3-6.0 4.5	0.4-7.4 3.8

1/ (17) = no. of compounds tested.
36-123 = range
85 = median

2/ Substituted amides not included.

3/ Anomalous result; next highest ratio was 0.9:(cf. Table VI).

TABLE VI

THE RELATIVE MOLAR TOXICITY AND PARASITICIDAL ACTIVITY OF ACID-SUBSTITUTED PHENYL ARSENOXIDES [17, 17a, 18a, 20]

Compound	pK	Toxicity * ≠	Relative activity in vitro against			
			T. pallidum	T. equiperdum	Schizatrypanum cruzi	L. donovani
Unsubstituted phenyl arsenoxide	-	100	100	100	100	100
o-SO_3H	<2	5.7	1.5	<0.02	<0.02	<0.02
p-COOH	4.0	41	6.7	0.45	0.04	0.21
p-CH_2COOH	4.35	41	4.2	4.7		
p-$(CH_2)_2COOH$	4.7	7.3	4.1	2.8		
p-$(CH_2)_3COOH$	4.9	8.7	1.9	54		32
p-$(CH_2)_4COOH$	5.23	7.4	-	27	47	30
p-$(CH_2)_5COOH$	5.35±	8.1	22	7.5	12	2.5
p-COOH	4.0	41	6.7	0.45	0.04	0.21
m-COOH	4.25	16	13	-	0.3	1.6
o-COOH	5.55	27	28	3.2		1.5±
3-NO_2-4-COOH	2.6	27	18	17	24	40±
p-OCH_2COOH	3.3	25	4.2	4.5	0.3	6.8
3-NH_2-4-COOH	4.7	15	20	4.0	0.05	0.12
p-$NHCO(CH_2)_2COOH$	4.75	7.7	6.4	0.4±		0.15±
3-NH_2-4-$(CH_2)_3COOH$	5.34	7.2	-	26	62	26

* Single intraperitoneal injection in white mice.
≠ All values referred to that of phenyl arsenoxide as 100.

These several lines of evidence indicate that the acidic group may be modified in the animal body, possibly by esterification or conjugation, to form a more toxic arsenical. The body mechanisms for defense against acid in this case apparently increase the reactivity of the compound with the tissues and increase its toxicity.

b. The Effect of pH on the Parasiticidal Effect of Arsenoso Compounds. There is reason to believe that such slight parasiticidal activity as acid-substituted arsenoso compounds possess is largely a function of the non-ionized molecule, and that the ion is usually relatively inactive. In preliminary studies there was a rough correlation between the pK of the acid and its treponemicidal activity in vitro.[27] The effect of pH on both treponemicidal and trypanocidal action was, therefore, studied in a larger series of compounds.[17]

The parasiticidal activity of arsenosobenzene and of derivatives containing non-acid substituents was independent of the pH of the testing medium over the total range of viability of the organism. With acid-substituted compounds, however, the activity against both Trypanosoma equiperdum and Treponema pallidum usually increased strikingly with the hydrogen ion concentration (Fig. 8). This effect of pH was referable to the fact that the ions were relatively inactive as compared with the undissociated acid. The activity changed with pH in relation to the degree of ionization of the compound, and was predictable from the pK of the acidic group. With strong acids, such as the p-SO_3H compound (pK = 2±), there was no demonstrable change in activity in the range of pH 8.5-5.5, throughout which the compound would be essentially completely ionized. With weak acids, however, the activity varied as much as a hundred-fold over the range pH 8.5-5.5 (cf. right hand portion of Fig. 5).

With several of the acids, the ionized form, while less active than the undissociated molecule, nevertheless had a definite parasiticidal action. With such compounds, the total parasiticidal activity at a given pH was therefore a sum of the (slight) activity of the ionized fraction and the activity of the non-dissociated free acid, the proportion of the latter at a given pH depending on the pK of the compound. Thus, the ions of the p-$(CH_2)_3COOH$, 3-NH_2-4-$(CH_2)_3COOH$ and 3-NO_2-4-COOH arsenosobenzenes had an unusually high trypanocidal activity which contributed significantly to the total activity noted at e.g. pH 7.0.

One may, therefore, conclude that in a series of acid-substituted arsenoso compounds, the most effective against a given organism would be that compound with the highest pK (i.e. with the largest proportion in undissociated form at body pH), the ion of which was also moderately active against the parasite. In a series of compounds with substituent groups R-COOH, or R-SO_3H, some interest therefore attaches to the effect of the radical R on the dissociation constant of the terminal acidic group (cf. Table VI). In the series -$(CH_2)_n$COOH, there was a progressive increase of approximately 0.25-0.35 pH units in the pK of the compound with each additional methylene group.

The high activity in vitro of some of these acid-substituted compounds against cultures of Schizotrypanum cruzi and Leishmania donovani is particularly to be noted. Infections with these two organisms have proved generally difficult to treat. In the case of T. equiperdum the direct trypanocidal activity in vitro had proved an accurate index to therapeutic activity in vivo (cf. page 16). This unfortunately was not the case with these two organisms, perhaps because their intracellular localization in the infected animal rendered them inaccessible to the drug. In addition, preliminary and unpublished data indicate that the susceptibility of the intracellular parasites to arsenoso compounds may be less than that of the cultured flagellate.

The mechanism of action of arsenoso compounds in general, and of these acid compounds in particular, and the explanation for the extraordinary difference between the parasiticidal activities of the free acid and its ion, will be discussed in a following section.

3. The Effect of Acid Amide and Ester Substituents.

A third group of substituents concerning which it was possible to make some generalizations with respect to biological activity were the acid amides. Compounds so substituted were remarkably uniform in their toxicity and parasiticidal activity. As shown in Table VII, their direct trypanocidal and treponemicidal activities were intermediate between those of the generally inactive acids and the highly active arsenosobenzene. However, the most important effect of the amide substituents was a regular and marked decrease in toxicity, so that the amide-substituted compounds had activity:toxicity ratios as much as six times higher than that of the parent

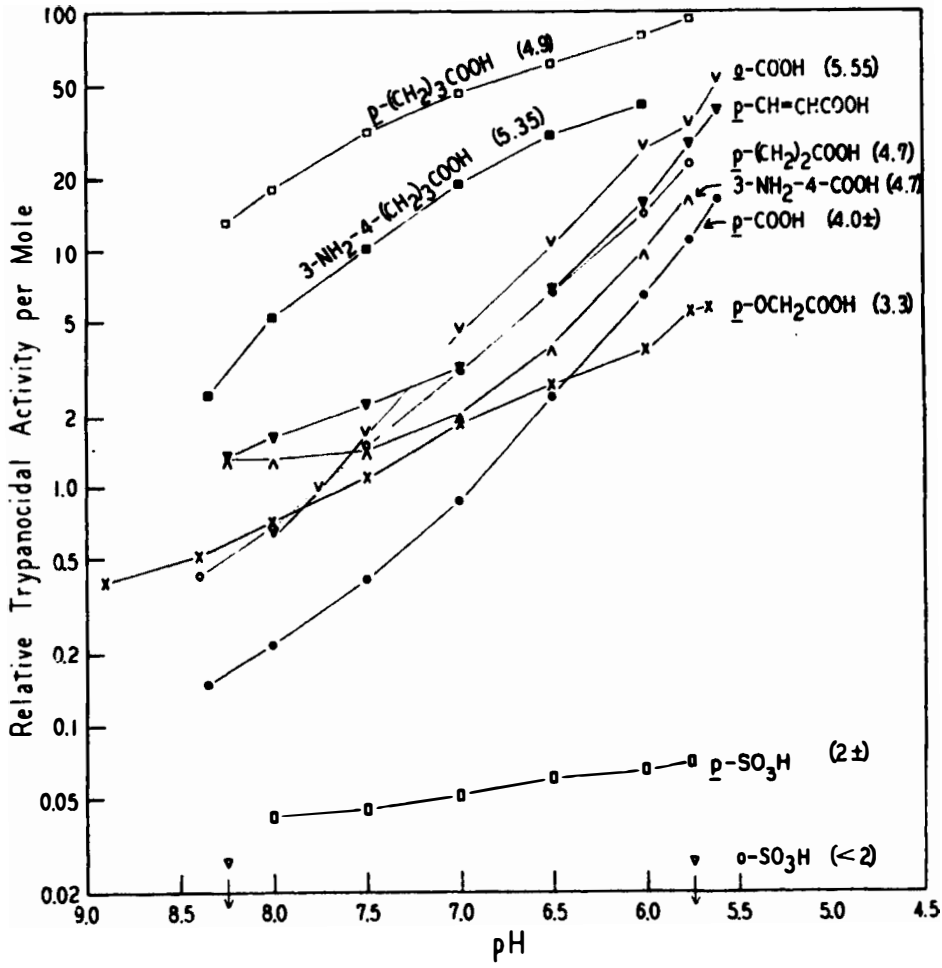


Fig. 8 The Effect of pH on the Trypanocidal Activity of Acid-substituted Arsenosobenzenes[17]

Figures given in parenthesis are the pK' values of the various acids

TABLE VII

THE RELATIVE MOLAR TOXICITY AND PARASITICIDAL ACTIVITY OF AMIDE-SUBSTITUTED ARSENOSOBENZENES

(All values referred to that of the unsubstituted arsenosobenzene as 100)[18a, 20, 21b]

Compound	Relative toxicity in white mice	Relative activity in vitro against:				
		T. pallidum	T. equiperdum	Arsenic-resistant T. equiperdum	Schizatrypanum cruzi	L. donovani
Unsubstituted phenyl arsenoxide	100	100	100	100	100	100
m-$CONH_2$	9.8	41	39	1.75	0.25	0.53
p-$CONH_2$	9.6	45	45	0.34	0.07	0.08
p-$CH{=}CHCONH_2$	9.7	42	73	-	-	0.98
p-CH_2CONH_2	8.6	20	31	-	-	-
p-$(CH_2)_3CONH_2$	13.5	33	60	8.15	8.3	4.5
p-$CONHCONH_2$	6.4	34	34	2.97	0.16	0.08
p-$CONHCH_2CONH_2$	3.86	24	15.0	0.68	<0.04	0.09
p-$CONHCH_2CH_2CONH_2$	3.2	13	-	-	-	-
p-$CH_2CONHCH_2CONH_2$	3.4	11	1.5	-	<0.08	5.2
p-OCH_2CONH_2	9.0	52	26	2.02	0.18	4.3
p-$NHCONH_2$	8.1	38	29	0.57	0.2	0.07
p-$NHCH_2CONH_2$	4.5	22	-	-	-	-
p-$NHCO(CH_2)_2CONH_2$	9.0	25	-	-	-	-
m-SO_2NH_2	6.1	21	7.8	-	-	-
p-SO_2NH_2	4.8	29	24	0.16	0.03	0.05
p-$SO_2NHCH_2CONH_2$	3.5	17	1.4	-	-	0.22
p-$CONHC_2H_4OH$	4.8	22	39	0.23	0.07	0.05
p-$SO_2NHC_2H_4OH$	4.2	23	9.0	0.18	-	0.13
p-$CONHCH_2CN$	4.5	27.0	19.5	0.42	0.08[†]	0.15
3-NH_2-4-$CONH_2$	5.6	28	52	2.76	0.06	0.15
3-OH-4-$CONH_2$	23	45	48	-	-	-

arsenosobenzene[21] (Table VII). A similar effect had been noted by Gough and King in studying amide-substituted arsonic acids in mice infected with Trypanosoma equiperdum.[33]

The favorable therapeutic effect of the amide groups was essentially independent of the particular acid used; sulfonamides and benzamides were similar in activity and toxicity. The position of the groups on the benzene ring was also unimportant. However, with both the p-$CONH_2$ and p-SO_2NH_2 compounds, the integrity of the terminal amide grouping was usually essential for the favorable effect on toxicity. Substitution of the amide hydrogens with methyl or ethyl groups successively increased the toxicity at a greater rate than the activity, so that the therapeutic index of these substituted amides was considerably less than that of the amides. The p-SO_2NH_2, p-SO_2NHCH_3 and p-$SO_2N(CH_3)_2$ arsenoso compounds had treponemicidal activities in vitro of 29, 72 and 112; relative toxicities of 4.8, 18 and 93; and activity:toxicity ratios of 6.1, 4.0 and 2.3, respectively. Similar effects of substitution of the p-$CONH_2$ compound are summarized in Table VIII.

In contrast to the $CONHCH_3$ or -$SO_2NHC_2H_5$ compounds, when the terminal grouping was one which was itself "eutherapeutic", i.e. depressed toxicity more than activity, the resulting compound was just as useful as the parent amide. Thus, the -$CONHC_2H_4OH$, -$CONHC_6H_4NHCOCH_3$ and -$SO_2NHC_2H_4OH$ compounds compared favorably with the parent amides with respect to toxicity and parasiticidal activity (cf. Table VIII).

Unlike amide-substitution, the esterification of acid compounds greatly increased both their parasiticidal activity and their toxicity. In consequence, such ester-containing compounds, when stable, were no better therapeutically than the parent arsenosobenzene. Many hydrolyzed in aqueous solution, so that their biological activity was the same as that of the acid-substituted compound.

4. Miscellaneous Substituents (-$NHCOCH_3$, -$OCOCH_3$, -$RNHCOCH_3$, -RNH_2)

Erratic results were obtained with arsenosobenzenes containing this group of substituents. Their parasiticidal activity varied between 3 and 78; their toxicity between 6.7 and 76; and their activity:toxicity ratio varied between 0.38 and 2.6 times that of the parent arsenosobenzene. In no instance was a compound obtained which was sufficiently active and non-toxic to suggest the possibility of its therapeutic use for the particular infections tested.

5. The Importance of the Terminal Grouping in a Substituent

Single substituents have thus been shown to have widely varying effects on the toxicity and parasiticidal activity of arsenosobenzene. In a number of the compounds, the substituent was a side chain of varying length. In general, and regardless of the length or nature of the side chain, the activity and toxicity of the compound were usually determined by the nature of the terminal functional groupings. Thus, the -CH_3 group had been found to be "inert", while the -$CONH_2$ and -SO_2NH_2 groups had a highly favorable effect on toxicity. The p-CH_2CONH_2, -$(CH_2)_2CONH_2$ and p-$(CH_2)_3CONH_2$ arsenosobenzenes all behaved as amides (Table VII). However, in the -$CONHCH_3$ and the -$CON(CH_3)_2$ compounds the favorable effect of the amide grouping was diminished or abolished by substituting methyl groups for the amide hydrogens (cf. above). Similarly, when an amide hydrogen was replaced by a group carrying a terminal acidic group (e.g. the -$CONHCH_2COOH$), the properties of the compound were determined by the terminal acidic group, and not by the amide.

6. The Effects of Multiple Substituents[18b]

The effects of multiple substitution on the toxicity and parasiticidal activity of arsenosobenzenes were difficult to interpret. Only a relatively small number of compounds were tested (cf. Table IV); and in no case could the effect of double substitution be anticipated from the effects of the two groups acting singly. Thus, six of the ten possible arsenosoaminophenols were prepared and tested for treponemicidal activity (Table IX). The -NH_2 and -OH substituents separately, whether o-, m-, or p- to the arsenoso group, had resulted in a uniform series of compounds, with treponemicidal activities varying only from 72 to 98, toxicities of 49 to 85, and activity:toxicity ratios of 1.0 to 1.46. The treponemicidal activity of the six aminophenol compounds was also remarkably uniform, varying only between 39 and 57; but their toxicities varied between 6.94 and 78.9, so that the activity:toxicity ratios varied ten-fold, from 0.54 to 5.5. The combination 3-NH_2-4-OH, which is the well-known compound oxophenarsine

TABLE VIII

THE EFFECT OF SUBSTITUTION IN THE $-CONH_2$ GROUP ON THE TOXICITY AND TREPONEMICIDAL ACTIVITY OF P-ARSENOSOBENZAMIDE[21a]

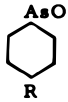
	Molar Toxicity	Molar Treponemicidal Activity	Ratio of Activity: Toxicity
$-CONH_2$	9.6	45	4.6
$-CONHC_2H_4OH$	4.8	25	5.2
$-CONHCH_3$	15	54	3.6
$-CONHCH_2COOH$	16	0.7	0.44
$-CON(CH_3)_2$	19	48	2.5
$-CONHC_2H_5$	26	59	2.3
$-CON(C_2H_5)_2$	64	53	0.83
$-CONHC_6H_5$	101	97	0.96

TABLE IX

THE TREPONEMICIDAL ACTIVITY AND TOXICITY OF AMINOPHENOL-SUBSTITUTED ARSENOSOBENZENES [18b]

Substituent Groups	Molar Toxicity	Molar Treponemicidal Activity	Ratio of Activity: Toxicity
$3-NH_2-4-OH$	6.9	42	6.1
$3-OH-4-NH_2$	10	41	3.9
$2-NH_2-3-OH$	10	34	3.3
$2-OH-5-NH_2$	22	39	1.8
$3-OH-5-NH_2$	74	57	0.78
$2-OH-3-NH_2$	79	43	0.54

("mapharsen") was the most favorable combination in the entire series. At the other extreme, the combination 3-NH_2-2-OH gave a less favorable therapeutic index than arsenosobenzene itself.

Any change in the 3-NH_2-4-OH combination, either by the introduction of a third substituent, extending either group on a side chain, or substitution of the hydrogen in either group, abolished its highly favorable properties.

Similarly variable and unpredictable results were obtained with the arsenosoaminobenzoic acids. Nine of the ten possible isomers were tested for direct trypanocidal activity. Unlike the example of the aminophenols just cited, the trypanocidal activities of these compounds varied 40-fold, from 0.6 to 23, with the 3-NH_2-2-COOH compound showing the highest activity.[20,17]

While no combination of two wholly inert substituents (-CH_3, -NO_2, -Cl, etc.) resulted in compounds with a highly favorable activity:toxicity ratio, one cannot say unequivocally that further search would not have revealed such a combination.

7. Thioarsenites

The reactivity of arsenicals with compounds containing thiol groups to form the corresponding thioarsenites was the subject of two patents by Kharasch in 1928 and 1934.[38] A number of thioarsenites have been used experimentally against trypanosomiasis[24], amebiasis and filariasis,[44] but none has as yet gained general clinical use.

A thorough study of the chemistry and pharmacology of the thioarsenites was undertaken by Barber[2] and by Cohen, King and Strangeways.[10] These authors prepared a number of such compounds and determined their efficacy in experimental trypanosomiasis in white mice.

A number of thioarsenites were also prepared in this laboratory by the condensation of arsenoso compounds with various thiols (cf. page 29). They are listed in Table X, together with their treponemicidal activity in vitro and their toxicity in white mice, both activities expressed relative to that of the unsubstituted arsenosobenzene as 100. As there shown, the thioarsenites were regularly less toxic and less activity parasiticidal than the parent compound; and in the treatment of rabbit syphilis their therapeutic index ($\frac{\text{therapeutic activity}}{\text{toxicity}}$) was no more favorable, and in several instances less favorable, than that of the corresponding arsenosobenzene. We have been unable to confirm the finding[45c] that the condensation of "mapharsen" with BAL (2,3-dimercaptopropanol) results in a compound more toxic than mapharsen itself. The LD_{50} of the addition compound prepared in this laboratory was 230 mg./kg. and 60 mM/kg., as compared with 43 mg./kg. and 12.4 mM/kg. for mapharsen.

Whether the activity and toxicity of the thioarsenites is determined solely by the amount of hydrolysis to the corresponding arsenosobenzene, or whether a thioarsenite may act directly has not been determined. It is true that an excess of thiol abolishes the parasiticidal activity of an arsenoso compound, both in vitro and in vivo (cf. Fig. 9), strongly suggesting that the hydrolysis of the thioarsenite may be essential for parasiticidal activity. It is, however, conceivable that the thioarsenite reacts directly with a tissue thiol, according to the equation:

$$RAs(SR')_2 + 2R''SH \rightleftharpoons RAs(SR'')_2 + 2R'SH$$

Thioarsenite	Tissue thiol	Tissue thioarsenite	Free thiol

In such case an excess of thiol would inhibit parasiticidal activity by competing with the tissue thiol for the thioarsenite.

C. The Selective Parasiticidal Action of the Arsenosobenzenes

The parent arsenosobenzene, and those arsenoso compounds with "inert" substituents, were highly toxic to the animal host, and were uniformly active in vitro against all the organisms tested. When substituents were introduced which modified the biological activity of the compound, they often developed a high degree of specificity. The amide-substituted compounds were relatively non-toxic for the mouse or rabbit host, uniformly treponemicidal, highly variable in their action on Trypanosoma equiperdum, and only negligibly active against Leishmania donovani or

TABLE X

THE RELATIVE TOXICITY AND TREPONEMICIDAL ACTIVITY OF THIOARSENITES[1] AND THE CORRESPONDING ARSENOSO COMPOUNDS[18a]

Substituent Group	Relative Molar Treponemicidal Activity in vitro			Relative Molar Toxicity in White Mice		
		 [1/]	Ratio		 [1/]	Ratio
o-CH_3	84	109	1.30	88	90	1.02
p-OCH_2CONH_2	52	32	0.61	9.0	7.3	0.80
p-$CONH_2$	45	39	0.87	9.6	4.6	0.48
		(a) 23	0.51		(a) 6.1	0.64
		(b) 21	0.47		(b) 5.1	0.53
p-$NHCONH_2$	38	21	0.55	8.1	2.9	0.36
p-$CONHCH_2CONH_2$	32	17	0.53	5.7	1.6	0.28
m-SO_2NH_2	21	22	1.05	6.1	3.3	0.54
p-SO_2NH_2	29	24	0.83	4.8	3.3	0.70
3-NH_2-4-OH	38	29	0.76	6.9	4.1	0.60
		(c) 0.86	0.023		(c) 1.4	0.2
m-COOH	13	11	0.85	16	15	0.94
p-SO_3H	3.4	3.6	1.06	29	24	0.83

[1/] All were dicysteinyl compounds, with the exception of the compounds indicated as (a), (b) and (c), which were the dithiosalicylate, dithioglycolate and dimercaptopropanol, respectively.

TABLE XI

THE VARYING PARASITICIDAL ACTIVITY OF ARSENOSOBENZENES AGAINST A NUMBER OF ORGANISMS

(Summarized from 18a, b, 20 and 21b)

Substituent Group (R ⬡ AsO)	T. pallidum	T. equiperdum		L. donovani	Schizotrypanum cruzi
		Normal	"Arsenic-fast"		
p-CH_3	102	102	70	96	47
p-$CONH_2$	45	45	0.34	0.08	0.07
p-OCH_2CONH_2	52	26	2	4.30	0.18
3-NO_2-4-COOH	18	17	-	40	24
p-COOH	6.7	0.45	-	0.21	0.04
p-$(CH_2)_3COOH$	1.9	54	38	65	32

TABLE XII

THE RELATIONSHIP BETWEEN THE TRYPANOCIDAL ACTIVITY OF ARSENOSOBENZENES AND THEIR COMBINING AFFINITY WITH THE ORGANISMS (AFTER EAGLE AND MUSSELMAN[22])

Original As concentration = 0.166 mgs.%; No. of trypanosomes = 250 x 10^6/cc;
Vol. of 10^9 sedimented organisms = 0.48 cc.

Substituted Arsenosobenzene R-AsO	Relative Trypanocidal Activity (referred to arsenosobenzene as 100)	Conc. of As in Trypanosomes, mg.%	Average ratio of $\frac{[As]_{trypanosomes}}{[As]_{supernatant}}$
p-SO_3H	0.06	0.41	2.4
p-$SO_2NHCH_2CONH_2$	1.4	0.68	5.2
p-OCH_2COOH	4.5	1.5	11
p-$CONHCH_2CONH_2$	15	5.7	53
3-NH_2-4-OH	27	6.5	87
p-$SO_2N(C_2H_5)_2$	35	7.4	93
p-$CONH_2$	45	7.7	113
m-OH	66	8.2	119
o-CH_3	91	9.5	186
Unsubstituted arsenosobenzene	100	9.7	224

Schizotrypanum cruzi. The acid-substituted compounds were generally only slightly parasiticidal. A few were, however, highly active against Trypanosoma equiperdum, Schizotrypanum cruzi and Leishmania donovani, and others were active against Treponema pallidum (cf. Table XI).

The basis for this selective action is discussed in the following section.

III. MODE OF ACTION OF ARSENOSO COMPOUNDS

A. The Reactivity of Arsenicals with Thiols

Ehrlich originally suggested that drugs were effective only to the degree to which they were bound by "chemoreceptor" groups in the parasite, and laid down the thesis that "corpora non agent nisi fixantur."[26] He further suggested that thiol groups might determine that fixation, although no definite chemical reaction was postulated.[25] (In 1908 Friedberger noted that arsanilic acid when mixed with thioglycollic acid increased markedly in both toxicity and trypanocidal activity.[30] In the light of subsequent developments, it seems clear that this effect was due to the reduction of the pentavalent compound to the more active trivalent form, and is not relevant to the mode of action of the latter.)

The reaction between arsenosobenzenes and thiol compounds to form thioarsenites has been briefly discussed in a preceding section. In 1923 Voegtlin, Dyer and Leonard[57] demonstrated that trypanosomes contained -SH groups, that organic compounds such as cysteine and glutathione containing -SH groups inhibited the trypanocidal action of trivalent arsenicals in vitro, and that the injection of such compounds immediately before an otherwise fatal dose of an arsenoso compound prolonged the life of the animal. They concluded that the effect of the arsenoso compounds in vivo was based on the reaction:

$$RAsO + 2R'SH \longrightarrow RAs(SR')_2 + H_2O$$

and that the specific sulfhydryl compound which acted as the "chemoreceptor" in determining toxicity was primarily glutathione. The possibility that other sulfhydryl-containing compounds might be involved was considered by Rosenthal.[57c] The inhibiting effect of sulfhydryl compounds on arsenicals was extended to T. pallidum by Eagle in 1939, who demonstrated that cysteine, glutathione and thioglycollic acid in excess abolished the treponemicidal action of arsenicals in vitro, as well as that of mercury and bismuth compounds.[14] In vivo also, thiol compounds in excess inactivated the arsenicals and abolished their trypanocidal and treponemicidal activity (cf. Fig. 9).

The inactivation of arsenicals by thiols as outlined in the foregoing paragraphs involves two quite different mechanisms. Thiol compounds in excess react with arsenicals to form thioarsenites, inhibit the hydrolysis of the thioarsenites to the free arsenoxides, and prevent the interaction of these arsenicals with the host tissues or with parasites, thereby decreasing their toxicity and parasiticidal activity. In addition, however, even after arsenicals have combined with the tissue cells or parasites, thiol compounds may reverse that combination, and remove the arsenical from its combination with the cell.[15] When cysteine was added to a suspension of T. pallidum which had previously been incubated with arsenicals and in which the larger proportion of the organisms had already been immobilized, not only was the treponemicidal action of the arsenical immediately halted, but a large proportion of the already immobilized organisms were revived.[15] The same phenomenon has been even more strikingly demonstrated with trypanosomes "killed" by arsenical, and then exposed to BAL (2, 3-dithiopropanol).[23] In vivo also, it is apparent from Fig. 9 that the parasiticidal action of arsenicals in vivo may be successfully reversed by thiol compounds given hours after the arsenical had been injected, and after the organisms had been given ample opportunity to combine with the arsenical injected.

The ability of thiol compounds to abstract arsenicals from the cell after they have already entered into combination with cell component is manifested not only in the revival of organisms already seriously damaged by the arsenicals, but may be demonstrated by the direct chemical analysis of the cells affected.[23] The arsenical presumably leaves the cell in the form of the thioarsenite.

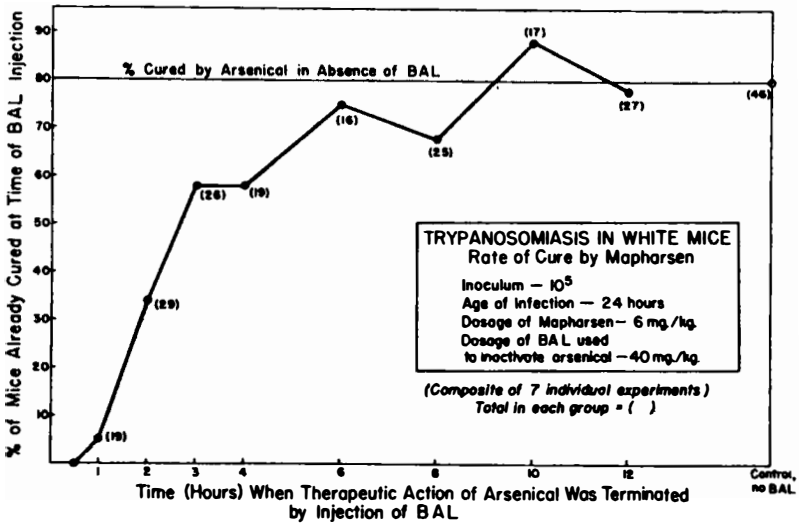


Fig. 9. The Inhibition of the Trypanocidal Action of Oxophenarsine in White Mice by 2,3-Dithiopropanol (BAL)[17b]

B. The Reactivity of Arsenicals with -SH Groups in Enzymes

The fact that arsenicals react strongly with thiol compounds to form thioarsenites, and the fact also that thiol compounds are able not only to prevent the combination of arsenicals with cells, but to remove the arsenic after it has already entered into combination with cellular constituents, do not however prove that a similar combination with cell thiols is primarily responsible for the toxic action of arsenicals. More recent work has indicated that this may actually be the case, and that the reactivity of arsenicals with -SH groups in protoplasm is the factor which determines their biological effects. It now seems unlikely that glutathione as such is necessarily or even primarily the cellular grouping affected.

In 1933 several workers reported on the role of thiol groups in the reversible deactivation of enzymes. Hellerman, Perkins, and Clark studied the inactivation of crystalline urease with mercurials such as phenylmercuric hydroxide;[36] and Bersin and Logermann noted that the activity of papain was destroyed by many oxidizing agents.[5] In both instances, the enzymatic activity was restored by the addition of either hydrogen sulfide or potassum cyanide. Maschmann[5] had suggested that such reactivation of papain or cathepsin by H_2S involved reductions of disulfide linkages in the enzyme to active thiol groupings essential for activity.

Bersin then correlated the work of Cohen, King and Strangeways[10] on the reaction between arsenicals and thiol compounds in vitro and the several previous indications as to the importance of thiol groups in the activity of many enzymes, and systemically studied the effects of a number of arsenic compounds on papain.[4] He showed that papain was inhibited by both arsenoso compounds and arsonic acids, and that this inhibition was reversed by glutathione. In explanation, he proposed the following alternative reactions:

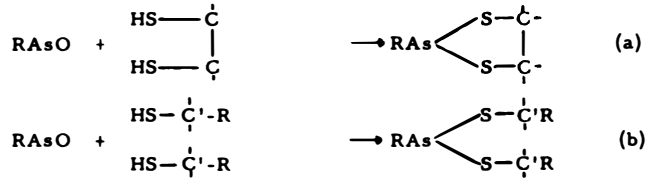
$$(1)\quad 4\ \text{Enz-SH} + RAsO_3H_2 \rightleftharpoons RAs(S\text{-Enz})_2 + \text{Enz-S-S-Enz}$$

$$(2)\quad 2\ \text{Enz-SH} + RAs(OH)_2 \rightleftharpoons RAs(S\text{-Enz})_2 + H_2O$$

A marked difference in the inhibitory actions of $p\text{-}CH_3CONHC_6H_4AsO_3H_2$ and $p\text{-}H_2NC_6H_4AsO_3H_2$ was explained on the basis of a difference in the equilibrium constant in reaction (1) above.

In the United States, Barron and his co-workers have thoroughly investigated the inhibiting effect of arsenicals on a number of enzyme systems,[3] including the enzymes involved in nitrogen, fat, and carbohydrate metabolism. Many were reversibly inhibited by arsenoso compounds and by the war-gas "lewisite" ($ClCH = CHAsCl_2$); and in such cases, -SH groups in the enzyme protein were believed to be essential to their enzymatic activity.

In England, working chiefly with lewisite, Peters and his co-workers have also investigated the reaction of enzymes and trivalent arsenicals.[45] They considered the primary site of attack of the arsenicals in the animal body to be on the pyruvate-oxidase system, which is particularly sensitive to trivalent arsenicals. Stocken and Peters[45b] reacted sodium arsenite and lewisite with kerateine in buffered solution and analyzed the precipitated protein for arsenic, sulfur and nitrogen. From 73 to 92 per cent of the bound arsenic had combined with sulfur in the ratio 1 : 2. Further, the protein sulfur was now resistant to oxidation, while the thiol groups in kerateine are normally highly susceptible. These workers therefore postulated that the arsenic had combined with two neighboring thiol groups on the protein molecule, i.e. that the protein contains a dithiol which is the primary point of attack by the arsenicals. Further, they stated that on physicochemical grounds the ring compound formed by the reaction of a dithiol with an arsenical (a) should be more stable than the straight chain thioarsenites formed with two molecules of a monothiol (b):



There may be several gaps in this argument. No dithiol such as that postulated by Stocken and Peters has yet been identified as a constituent of kerateine or any other protein in the amounts implied by their experimental data. It seems unlikely also that arsenicals would react with disulfide groups in proteins, or that the major portion of the free monothiols in a protein molecule would be arranged in pairs. The fact that the arsenic:sulfur ratio of the compound formed between thiols and kerateine in solution approaches 1 : 2 is not conclusive evidence for the presence of a dithiol, since the arsenic molecule could be bridging two molecules of kerateine. Further, in the absence of resonance there seems to be no basis for assuming that a bond between two atoms in a ring is necessarily stronger than the same bond in a straight chain compound. In a recent paper, Slater[50] has furnished strong evidence that the reaction between arsenicals and enzyme systems is probably more complex than that suggested by Peters and his co-workers. He has pointed out that while the reaction is supposed to be:

$$RAsO + 2\text{-HS-Enz} \rightleftharpoons RAs(S\text{-Enz}_2) + H_2O,$$

there may be no reactivation when the arsenical is removed or the reaction mixture is greatly diluted.

However, whether or not the reasoning of Stocken and Peters was valid in its entirety, it led to a significant advance in the therapeutic use of arsenicals. Following up the previously cited data, Stocken and Thompson[52] found that the dithiols were more effective than monothiols in protecting the pyruvate oxidase system in pigeon brain from arsenicals. In animals suffering lewisite burns or injected with toxic arsenicals, the dithiols greatly increased the rate of excretion of arsenic.[23, 53] Further, Whittaker found that dithiols which he believed to react with arsenic to produce strained rings were much less effective than dithiols which formed a stable ring structure.[59]

As an outcome of these studies, the English workers developed 2, 3-dimercaptopropanol ($HS\text{-}CH_2CHSH\text{-}CH_2OH$) for the treatment of lewisite burns. This compound (BAL = British Anti-Lewisite)[51] has found extensive use not only in the local treatment of arsenical skin burns, but also in the systemic treatment of arsenic poisoning[23, 41] and poisoning due to such heavy metals as mercury[42], antimony[19] and gold.[47a] It is ineffectual against cadmium[32] and of questionable value in lead poisoning.[31] The use of BAL has been thoroughly reviewed by Stocken and Thompson.[51]

Summarizing the above evidence, one may conclude that (a) arsenoso compounds combine with sulfhydryl compounds to form reasonably stable thioarsenites; (b) many enzyme groups contain sulfhydryl groups which are necessary in the intact state for the action of the enzyme, and such enzymes are inactivated by arsenoso compounds and other arsenicals; (c) this inactivation can be reversed by thiols, and in particular dithiols; and (d) thiol compounds, and particularly dithiols, protect animals and microorganisms against the toxic effects of arsenicals, can on occasion reverse the toxic action of arsenicals after they have already become manifest, and in such cases actually reverse the combination of the arsenicals with the cell or tissue.

On the basis of these facts it is now generally believed that the action of the arsenicals rests on their ability to combine with thiol groups in essential enzyme systems, both in the host (toxicity) and in the cell of the invading microorganism (therapeutic activity). This may be expressed schematically as:

$$RAsO + 2R'SH \longrightarrow RAs(SR')_2 + H_2O \qquad (A)$$

arsenoso compund	enzyme in host tissue or in parasitic cell	tissue or cell thio-arsenite

C. Possible Explanations for the Varying Reactivity of Different Arsenicals Against the Same Organism, and of the Same Arsenical Against Different Organisms

How does the theory of reactivity of arsenicals with enzyme -SH groups explain the experimental observations cited in detail in the preceding section (1) that chemically closely related arsenoso compounds may vary 100-fold in toxicity; (2) that different arsenicals may vary in their effect on a given microorganism, and (3) that the same arsenical may vary in its effect on different organisms? Thus, Tables VI, VII and XI compare the trypanocidal, treponemicidal, and leishmanicidal activity of a number of arsenosobenzenes. The striking effect of γ-(p-arsenosophenyl)-butyric acid against trypanosome infections[16] and its inactivity against Treponema pallidum is but one example of the parasite-specificity of many arsenic compounds (cf. page 26). Kuhs and Tatum[40] found that arsenicals capable of curing T. lewisi infections possessed aliphatic side chains with acidic groups, while the arsenicals which cured Trypanosoma equiperdum infections usually contained basic groups.

1. The Possibly Varying Reactivity of Different Arsenicals and Thiols as the Basis of Their Parasite Specificity

Reaction (A) above is freely reversible, being shifted to the left in alkaline solution and to the right in acid solution.[10] It is therefore conceivable that the variation in toxicity or activity of a series of arsenoso compounds might be a function of the hydrolysis constant of the formed thioarsenite. It is also possible that the rate of the above reaction is the determining factor. In either case, the usual relationships between chemical structure and reactivity should apply. In the particular case of equation (A), it should be possible to predict both the relative rate and the hydrolysis constant by means of Hammett's equation:

$$\log k - \log k^{\circ} = \rho\sigma$$

where k is the rate or hydrolysis constant for the substituted aryl groups, k° the rate constant for the unsubstituted group, ρ is a constant for the particular chemical reaction studied and which is independent of the groups, and σ is a constant for each substituent group and is independent of the reaction.[34] Under these circumstances, if either toxicity or parasiticidal activity were quantitatively dependent solely on the above reaction, that activity should be a function of the σ values, Actually, no correlation whatever was found between the Hammett σ values, and either the toxicity or the parasiticidal activity of the arsenoso compounds as previously described in Section II B.

Again on the basis of the Hammett equation, if the parasiticidal action of arsenicals and their toxicity to the host were quantitatively dependent upon their reactivity with thiol groups in the parasite and in the host, then the therapeutic index of a series of arsenoso compounds in a given infection should be reasonably uniform. The relative reactivity of an arsenoso compound with the two groups of thiols, one group in the parasites and one in the host cell, should be independent of the particular substituent on the benzene ring. Instead, that ratio varied as much as 60-fold in the arsenoso series alone. Even when the same compound was tested against the same organism (T. rhodesiense) but in different animal hosts, Tatum, Pfeiffer and Kuhs[55] obtained therapeutic indices of 11, 5 and 0 in rats, rabbits and dogs respectively.

It seems clear that possible variations in the rate or equilibrium constants of the reaction between arsenoso compounds and thiols to form thioarsenites are themselves inadequate to explain the known relations in the parasiticidal action or toxicity of these compounds.

Barron and Singer[3] studied the relative inhibitory action of various arsenicals against a number of enzymes in vitro, and their results are pertinent in this connection, although the quantitative aspects of their data are partially obscured by the varying amounts of impurities in the enzyme preparations capable of combining with and inactivating the arsenicals. The susceptibility of the different enzymes to the same arsenical was generally of the same order of magnitude.[3] Using eighteen different enzymes systems, they found that under the particular experimental conditions, most of the enzymes were almost completely inhibited (by 80 to 100 per cent), but that pyruvate dismutation was inhibited by only 35 per cent and pyruvate condensation by 56 per cent. The degree of inhibition obtained with five different arsenoso compounds acting on the same enzyme, succinoxidase, were also not significantly different.[3b] However, from the same laboratory it was later shown that d-amino acid oxidase and yeast carboxylase were inhibited by p-aminophenyldichlorarsine but not by lewisite.[3c] Also, trans-

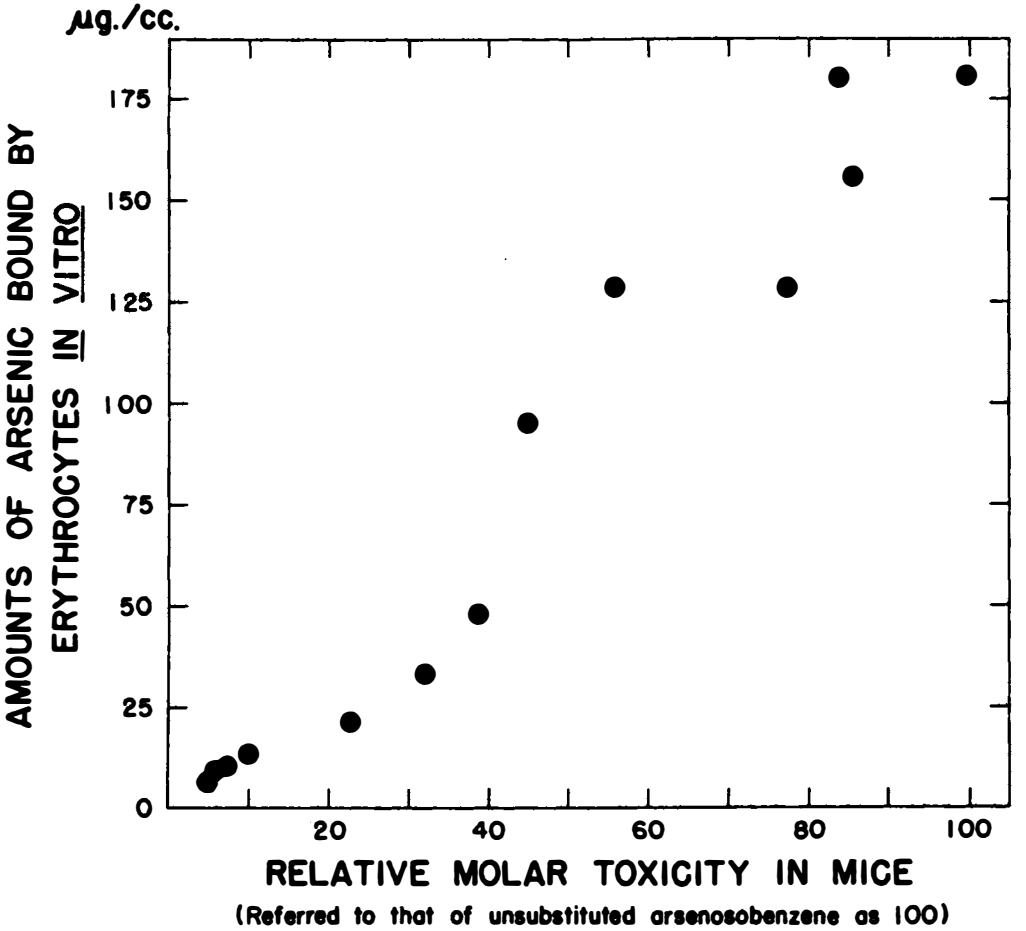


Fig. 10 The Correlation Between the Affinity of Arsenosobenzenes for Erythrocytes in Vitro and Their Systemic Toxicity in Mice (After Hogan and Eagle[37])

aminase was almost completely inhibited by p-arsenosobenzoic acid, but only 33 per cent inhibited by the same concentration of lewisite. The authors suggest that these differences may arise from a different spatial arrangement of the thiol group on the protein molecule.

2. The Possibly Varying Permeability of the Cell

In possible explanation of the varying biological effects of different arsenoso compounds, one may postulate as an alternative hypothesis that although their reactivity with a given -SH compound is reasonably uniform, the arsenicals vary widely in their ability to penetrate the cell wall, either in the microorganism or the host, and that it is these differences which largely determine the varying parasiticidal action and toxicity of these compounds. This thesis is strongly supported by the data on the binding of arsenicals by cell suspensions. It was first shown by Thuret that a suspension of red blood cells in vitro bound the highly active arsenoso compounds, but not the relatively inactive arsonic acids.[56] This finding was extended by Hogan and Eagle, who demonstrated that in a large series of arsenoso compounds the amount of arsenic bound by red blood cells in vitro under standard conditions was roughly proportional to the systemic toxicity of the arsenical,[37] strongly suggesting a causal relationship (Fig. 10). The corollary of this finding was also demonstrated: with compounds of widely varying toxicity, at dosages which produced equivalent toxic effects in vivo, essentially similar amounts of arsenic had been bound by the tissues.

(The binding of arsenicals by erythrocytes was also studied by Fink and Wright.[29] Using a single arsenical, mapharsen, these workers found an approximate linear relationship between the logarithm of the amount in the cells and the logarithm of the amount in the plasma, and suggested that the binding of arsenicals was largely physical in nature. They also found that from 60 to 90 per cent of the bound arsenic could be released from the red blood cells by simple resuspension in fresh plasma. These important findings are difficult to reconcile with the thesis that arsenoso compounds react with -SH groups in the cells as previously discussed, and further study seems indicated.)

Eagle and Magnuson[22] subsequently showed that, just as the degree to which arsenicals were bound by red blood cells was related to the systemic toxicity of the compounds, so the binding of arsenicals by trypanosomes was a measure of their parasiticidal activity. Their results with a large series of the arsenoso compounds and a normal strain of Trypanosoma equiperdum, are given in Table XII. The correlation between the amount of the arsenical bound under standard conditions, and its trypanocidal activity, was again so regular as to suggest a causal relationship. This quantitative study confirmed and extended the previous qualitative findings of Hawking[35], Yorke, Murgatroyd and Hawking[63] and Reiner, Leonard and Chao[47b], that actively trypanocidal compounds were bound by the organisms, while inactive compounds were not.

The wide differences in the amounts of arsenical bound by a trypanosomal or erythrocyte suspension could perhaps be due to the corresponding differences in the reactivity of the several arsenicals with a given cellular enzyme system. Differences of this order of magnitude have, however, not been demonstrated in the reaction of arsenoso compounds with thiols in vitro; and it seems improbable that the hydrolysis constant of a series of thioarsenites involving the same thiol and different arsenoso compounds would differ by a factor of e.g. 10^2-10^3 (cf. page 27 et seq.). The results are, however, consistent with the thesis that different arsenicals vary in the ease with which they can pass through the cell wall, and that it is this selective permeability which determines the varying activity of a series of closely related compounds against the same cell.

The results obtained with "arsenic-resistant" strains of trypanosomes offer additional strong evidence that the varying amounts of arsenical bound by the organisms reflect the varying permeability of the cell wall to the several compounds, rather than their varying reactivity with a cell thiol or thiols. Hawking[35] had shown that arsenosobenzene was as actively trypanocidal against a "resistant" strain of T. equiperdum as against a normal strain; but that arsenicals containing certain substituents, notably the -$NHCH_2CONH_2$ group were not effective against the resistant organism, and were not bound by it. Similar results were obtained by Yorke, Murgatroyd and Hawking,[63] using an arsenic-resistant strain of T. rhodesiense. King classified arsenoso compounds into three separate groups on the basis of their action on "resistant" strains of trypanosomes:[39] (1) arsenosobenzene, arsenosoxylene, and similar compounds which were active against both normal and "arsenic-resistant" strains, and which fall into the group of compounds with "inert" substituents as defined on page 16; (2) carboxyl substituted compounds and (3) amide substituted arsenicals. Compounds in either of the last two groups were relatively

inactive against one type of "arsenic-resistant" trypanosome, but normally active against the other. King concluded that while the actual intracellular process determining the death of the organism was the same in each instance, the several classes of compound entered the trypanosome by a different mechanism. Eagle and Magnuson,[22] working with a strain of Trypanosoma equiperdum which had spontaneously become arsenic-"resistant", found that arsenosobenzene and many of its simple derivatives (chloro, methyl, carboxy, etc.) was bound just as strongly by, and were just as active against, this resistant variant as the parent normal strain. On the other hand, arsenoso compounds containing amide and amino substituents were less active against the resistant strains by a factor of 1/5th to 1/200th with a corresponding reduction in the amount of arsenic bound by these organisms (Table XIII).

TABLE XIII

THE CORRELATION BETWEEN THE TRYPANOCIDAL ACTIVITY AND COMBINING AFFINITY OF ARSENOSOBENZENES FOR NORMAL AND "ARSENIC-RESISTANT" TRYPANOSOMES [20,22]

Compound Tested (R⬡AsO)	Relative Trypanocidal Activity vs.		Percentage of Arsenic Bound * by	
	Normal Trypanosomes	"Arsenic-Resistant" Trypanosomes	Normal Trypanosomes	"Arsenic-resistant" Trypanosomes
p-SO_2NH_2	15-21	0.11	45-50	0
p-$CONHCH_2CONH_2$	9	0.3	37-39	1.8
p-$(CH_2)_3COOH$	35-39	38	49-55	59
p-Cl	75	81	53-59	70
Unsubstituted phenyl arsenoxide	100	100	71-79	80

* 200-300 x 10^6 trypanosomes per cc.; 1.66 μg. As/cc.

The selective susceptibility of "arsenic-resistant" organisms to certain types of arsenicals, associated with their ability to bind those arsenicals, is thus well documented. If this selective action is not determined by a selective permeability, one must then assume (1) that each class of arsenical can combine only with certain thiols in the cell, and (2) that the normal and resistant trypanosomes differ qualitatively in the type of cellular thiol which is susceptible to arsenicals, and the inactivation of which determines the death of the cell.[35] The former assumption in particular seems improbable.

The importance of cell permeability is further indicated by the effect of pH on the parasiticidal activity of acid substituted arsenoso compounds (Eagle[17]). With aryl arsenoso compounds not containing acid substituents the trypanocidal activity proved to be independent of the pH of the solution. With acid substituted compounds, however, the activity, both against T. equiperdum and Treponema pallidum, decreased strikingly as the pH of the testing solution increased. As discussed in a preceding section, this was related to the fact that the ion was usually almost wholly inactive, while the undissociated molecule was highly trypanocidal. The effect of pH on the binding of acid-substituted arsenoso compounds paralleled its effect on their trypanocidal activity. One must conclude that the ion was usually inactive because it was bound by the organism to only a limited degree, while the highly active undissociated acid was highly active because it was strongly bound.

The striking differences shown in Tables XII and XIII and in Fig. 8 can hardly be related to the varying reactivity of the salt and the free acid with thiols: the salts of acid substituted arsenoso compounds reacted as readily with cysteine and other thiols as did arsenoso compounds with non-ionic substituents. The results are, however, consistent with the assumption that the ionized form passes through the cell wall of the trypanosome into the interior of the cell only to a limited degree as compared with the undissociated molecule. The many analogies in other cell types and compounds have been summarized by Eagle[17], by Albert[1] and by Simon and Blackman[49]. (The possibility that the arsenicals are bound on the outside wall of the organism, rather than within the cell, has been discussed by Reiner, Leonard and Chao[47b] and by Eagle[17]. The latter concluded the quantitative relationships between the amount of arsenical bound and the available surface area make it unlikely that the major portion of the arsenic bound by trypanosomes attaches to the cell membrane.)

On the permeability theory, the cell wall would be impermeable to certain arsenoso compounds, while others can penetrate with ease, and having penetrated are so firmly bound to some of the cellular constituents (thiols) that they no longer figure in the intracellular-extracellular equilibrium. In consequence, the concentration builds up inside the cell, and may attain 50, 100 and even 200 times that outside the cell. The factors determining which compounds can penetrate the cell wall and which cannot are not yet clear; and similarly unresolved are the nature of the changes in the cell which make it impermeable to, and thus, resistant to compounds which are normally highly active.

The phenomenon of drug antagonism, which has become so important of recent years in relation to the mode of action of bacteriostatic and bactericidal agents (the sulfonamides, antibiotics) and of growth factors, is of significance in this connection. Williamson and Lourie[60] showed that while the trypanocidal action of most arsenical drugs were not antagonized by p-aminobenzoic acid (P. A. B. A.), γ-(p-arsenosophenyl)-butyric acid was so antagonized. Similarly, melarsen oxide,[30b] a drug which is highly active against arsenic-resistant strains of trypanosomes, is antagonized by "surfen C",[60] a non-arsenic-containing compound somewhat similar to melarsen oxide in structure. Schleyer and Schnitzer have shown that many esters and amides of isocyclic and heterocyclic acids antagonize the action of oxophenarsine against Trypanosoma equiperdum, while the free acids do not.[48] (These drug antagonists did not, however, affect the toxicity of the arsenicals.) Unfortunately, there are no data as to whether these antagonists prevent the concentration of the arsenical within the cell. In such case, the antagonists might either prevent the passage of the arsenical across the cell wall, or by blocking the union of the arsenical with an intracellular compound, prevent its rapid accumulation and parasiticidal action. Work and Work, commenting on the striking antagonism of P. A. B. A. for γ-(p-arsenosophenyl)-butyric acid concluded that "the only explanation for this action is to assume that the drug acts ultimately in the same manner as other trypanocidal arsenicals, but that P. A. B. A. may be preventing or limiting its admission into the trypanosome cell."[61]

SUMMARY

1. Of the three types of arsenicals used in the treatment of spirochetal and protozoal diseases, only the arsenoso compounds have a direct parasiticidal activity, the arsonic acids and arseno compounds being active by virtue of their conversion to arsenoso compounds in the animal body.

2. Arsenosobenzene was one of the most toxic and most actively parasiticidal compounds in the entire series tested, with no demonstrable selective action among the organisms studied.

   (a) Substitution with a single $-CH_3$, $-NO_2$, $-Cl$, $-NH_2$, $-OH$ or $-F$ groups either did not significantly affect the toxicity or parasiticidal activity of the parent arsenosobenzene, or reduced them both to the same slight degree.

(b) Acid-substituents strikingly decreased both the direct parasiticidal and toxic action of arsenosobenzene. The ionized form was generally inactive, undissociated acid highly active, and the effect of pH on parasiticidal activity could be related to its effect on dissociation. There were, however, important exceptions to the general inactivity of the ionized compounds (e.g., the 3-NO_2-4-COOH and p-$(CH_2)_3COOH$ arsenosobenzene).

(c) Amide substituents caused a slight decrease in treponemicidal and trypanocidal activity, but a striking decrease in toxicity. Substitution in the amide hydrogens usually diminished its favorable effect on toxicity, exceptions being noted in groups which in themselves also reduced the toxicity of arsenosobenzene (e.g. $-C_2H_4OH$, $-C_6H_4NHCOCH_3$).

(d) Unlike amidification, esterification of acid substituents resulted in compounds approaching arsenosobenzene in their high toxicity and general parasiticidal action. Many of these compounds readily hydrolyzed in aqueous solution, their biological activity then corresponding to that of the free acid.

(e) The effect of complex substituents was usually determined by the nature of the terminal group in the substituent (e.g. $(CH_2)_3CONH_2$; $CON(CH_3)_2$).

(f) Two substituent groups had an effect which could not be predicted from those of the substituents taken singly. Unlike the case of the single substituents, in these di-substituted compounds the position on the benzene ring profoundly modified the activity of the compound.

(g) Both the toxicity and direct tryponemicidal activity of thioarsenites were somewhat less than those of the corresponding arsenosobenzene; and in the treatment of rabbit syphilis, the therapeutic index was also no better than that of the parent compound.

3. There is considerable evidence to support the view that the toxicity and therapeutic activity of arsenoso compounds are determined by the amounts which enter into combination with cellular components. The widely varying activity of a single arsenoso compound against different organisms, and of a series of such compounds against the same organism, would be related to the amount of arsenical bound.

4. Data have been summarized which indicate that arsenicals combine with -SH groups in protoplasm. Many essential enzymes contain sulfhydryl groups; and most such enzymes can be inactivated by arsenoso compounds and reactivated by sulfhydryl or other agents which can compete with the enzyme protein for the arsenical. It is a reasonable working hypothesis that arsenoso compounds combine reversibly with sulfhydryl groups in essential enzymes, and that their toxic effect on cells is due to that combination.

5. The selective action of particular compounds against certain cells could be explained on the basis of either (a) the varying affinity of different enzymes for the same arsenical, or of different arsenicals for the same enzyme; or (b) differences in the permeability of a given cell to different arsenicals. While the evidence is far from conclusive, the latter assumption appears best to explain the experimental data.

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DISCUSSION

DR. CARROLL KING (Northwestern University, Evanston, Illinois): I would like to ask Dr. Doak just how they plotted this information, in making an attempt to correlate this data with the sigma function.

DR. DOAK: Actually, no plot was possible between the Hammett-sigma values. One has, in the Hammett table, the p-amino compounds at the top, and a hydrogen in the middle, and a nitro at the bottom. We found that the activity or the toxicity of the p-amino compounds and p-nitro compounds were essentially the same. In attempting to plot either the log of the activity or the log of the toxicity, which must be a function of this sulfhydryl reaction, we made several attempts to isolate the actual reaction and study either the rate or the hydrolysis constants, and those studies are still going on. We have not been able, so far, actually to isolate the reaction and study either the rate or the hydrolysis.

DR. CARROLL KING: I would like to call attention to one factor in connection with the application of this sort of data. If you consider the Hammett equation, it states that the log of the ratio of two constants, is equal to sigma rho. Both sigma and rho are constants.

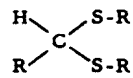
The validity of the equation has been established by application to fifty-two different reactions. I would just like to point out one thing. The sigma values have been established by many reactions. The rho value, is simply the slope of a line, and about all you can tell from the Hammett equation is whether the points fall on a line. So, if you plot log K against the sigma values, you may get a line whose slope is one in which case you have direct correlation of the type which, perhaps, you have searched for here. But you may get a line whose slope is minus, in which case, you correlate the other way, and, slopes may be much steeper, either negative or positive.

Specific examples of reactions in which the slope is large are known, and in which the slope is small are known, with the same sigma values. It may be that you simply have a case where the slope is very small.

DR. MAXWELL SCHUBERT (New York University College of Medicine, New York, New York): This is an enormously interesting paper which we have just listened to, and there are a great many things which would be worth discussing. There is just one point I would like to discuss at the present time, though, and that is the remark which Dr. Doak made that the BAL compounds with arsenic are not necessarily more stable than the open-chain ones.

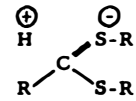
I am going to base this discussion on a paper which recently came out by Fennell and Carmack, in which they studied ultraviolet absorption spectra of organic sulfides and mercaptols.

The type of compound they had to deal with, if we take the mercaptal, was this type of



compound. It has generally been assumed, and this is a point which Dr. Doak was very careful to make, that in the absence of resonance, a compound of this structure would not necessarily be any less stable than a compound in which these R's were independently linked together.

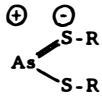
In the particular case of these mercaptals, as a result of the study of the ultraviolet absorption, a conclusion was arrived at that there is a resonance which may be described as follows:



One electron pair, which binds the carbon to the hydrogen, is pulled in by the carbon and shared with the sulfur. This particular sulfur atom becomes negative, and has, in effect, ten electrons around it. But the hydrogen becomes positive and is ionized under those conditions.

If we follow this thought and substitute our metal, whether it is arsenic or antimony does not make any difference at the moment, we would have a compound of this character. If the

same kind of resonance is possible here, there are two ways in which such electrons sharing could be imagined; one is by pulling in an electron from the sulfur; another is by pulling an electron pair out of the metal and sharing it with the sulfur. The set of possibilities could constitute a resonance system. In the second case, you would get an ionization effect, in which



the sulfur would become negatively charged. In that particular case, if you have an open-chain compound, this would be ionizable. In the presence of competing systems, which might want to combine with the metal, this would be more readily displaced.

However, in the case where the R's were combined independently, if one did become somewhat ionizable as a result of such resonance, it could not move away and, it would block the introduction of other elements there and give an appearance of greater stability.

DR. JOSEPH H. BURCHENAL (Sloan-Kettering Institute, New York, New York): This problem of resistant strains interests me tremendously, and I gather that the resistant properties of the trypanosomes are correlated with their ability to bind arsenic. I would like to ask two questions of Dr. Doak. In the first place, were these resistant strains naturally occurring or induced by suboptimal therapy or treatment in vitro?

Second, have you any suggestions as to the reason behind the ability of the resistant trypanosomes to bind one arsenical and not another type.

DR. EAGLE: If I may answer Dr. Burchenal's question this was a spontaneously occurring resistant variant, which developed in the absence of exposure to an arsenical. I wish we had some explanation of the fact that certain arsenicals are bound by these resistant strains just as actively as they are by normal trypanosomes, and are correspondingly active, while others are not bound at all, and are correspondingly inactive. Therein lies the crux to this problem, it seems to me: why certain substituents affect the permeability to the cells to arsenosobenzenes.

DR. D. W. WOOLLEY (Rockefeller Institute for Medical Research, New York, New York): In speaking of the effects of PAB on toxicity of arsenicals, there is one point which I think is worth bearing in mind, and that is an experiment which Sandground did some eight or ten years ago, in which it was shown that you could prevent, to a considerable degree, the toxicity of atoxyl and arsphenamine and neoarsphenamine, and various arsenical compounds, in animals by giving p-aminobenzoic acid.

The point I want to bring up is that, once the arsenic compound had been given, no amount of PAB would protect the animal. If PAB was given before the arsenical, then a good protection could be had. This might bear on the point brought up about penetration of the cell.

STRUCTURE-ACTIVITY RELATIONSHIPS IN PLANT GROWTH-REGULATORS

by

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Chemical Corps Biological Laboratories
Camp Detrick, Frederick, Maryland

CONTENTS

I. INTRODUCTION

A large number of organic compounds are known which have the property, in relatively small amounts, of influencing the mode or rate of the growth and developmental processes in plants. Such compounds, the majority of which have not as yet been demonstrated to occur naturally in higher plants, may be termed growth-regulators. The growth responses may be identical with, or similar to, those normally taking place or may be of a type not found in the intact healthy plant. Responses in the first category include acceleration of rate of cell elongation, acceleration or retardation of abscission, induction of parthenocarpy, stimulation or inhibition of development of dormant buds, and development of adventitious roots. Among the second type of responses may be mentioned production of galls and tumors, and modifications of leaves, flowers, and other organs. More or less profound changes in composition and metabolic processes doubtless cause, accompany, and result from these morphological changes, but these have been studied much less extensively.

Although the relationships between molecular structures and physiological potency of plant growth-regulators have been under consideration for some time, the present status is by no means satisfactory. Reference should be made at this point to a number of earlier publications dealing with certain of the topics to be treated[16, 21, 28, 29, 39, 44].

No attempt will be made in this review to catalog completely all the substances which have been stated to possess growth-regulatory activity or even to mention all the known types of responses. One notable omission is that of herbicidal activity, the study of which, although of great practical interest, has not yet reached the stage of profitable discussion in the present connection.

II. LIMITATIONS OF EXISTING DATA

Attempts to deduce general principles from the data now available are so greatly complicated by a variety of circumstances that present postulates must be regarded as provisional. Certain compounds possess activity in many of the types of response enumerated above, thus lending weight to the view that these responses, although not otherwise demonstrated to be related, may be diverse end-effects of a common fundamental reaction. On the other hand, some compounds exhibit high activity in one type of response but none at all in others. Regrettably, there has too frequently been a tendency to overlook this heterogeneity of behavior and to speak of structural requirements of growth-regulators in general. For this reason it appears advisable to discuss the structural requirements separately for each type of activity.

A further complication is introduced by evidence of more or less marked differences in sensitivity or responsiveness of similar organs of various species and also, for that matter, by the same organ at different stages of development. At present it is not clear to what extent such specificity may be due to fundamental differences in tissue or cellular reactivity and to what extent to secondary properties which may influence absorption, translocation, or destruction of chemicals applied.

Apart from the variability inherent in biological material, much of the uncertainty of current data is attributable to inadequate techniques. In relatively few cases have extensive series of compounds been compared by carefully standardized tests. A number of serious

inconsistencies have been reported by different investigators using a presumably standard technique, and even by the same worker in experiments performed at different times. Many of the published results are to be regarded as semi-quantitative at best; in numerous instances it has been determined only whether a compound is active or inactive at arbitrarily selected dosages. In development of test methods, conditions are commonly adjusted so as to result in a maximal response to a particular standard compound, frequently indole-3-acetic acid or 2,4-dichlorophenoxyacetic acid. The time of exposure or other experimental conditions may not be optimal for other substances which may enter, move, react, or be inactivated at appreciably different rates. In consequence, the relative activity of any substance, compared to another substance as standard, varies according to the method of assay. This situation, which was clearly recognized in the early investigations of structure and activity, has not been sufficiently emphasized in some of the later discussions of the subject. Only recently have attempts been made to devise tests so controlled as to permit expression of results in more or less absolute terms such as the dose of half-maximal response (ED 50)[5, 13, 19].

It is to be remarked that the responses under consideration include both stimulatory and repressive growth effects. In the normal economy of the plant both types play equally indispensable roles. From the experimental standpoint, however, much less ambiguity attaches to the study of stimulatory or inductive responses. Repression of growth may be brought about by almost any material in sufficiently high concentration, including even the essential nutrients and metabolites. Current experimental techniques are almost exclusively restricted to measurement of gross changes in complex organs so that the question arises to what degree the repressive or inhibitory activity of any particular compound may be regarded as specific for the response under investigation.

III. STIMULATION OF CELL ELONGATION

The opinion is widely held that stimulation of cell elongation represents the simplest morphological effect of growth-regulators, and in consequence the structural requirements for this response have been more extensively studied than for any others. Cell elongation is a process which is an important component of plant growth, and which is involved also in tropistic and etiolation responses. Compounds, whether of natural origin or not, which stimulate the elongation of cells have been termed "auxins" and although broader definitions have at times been given, the term will be used in this paper in this restricted sense.

Compounds having auxin-activity, therefore, are those which can be shown to induce cell elongation in standard tests. The available test methods depend upon measurements of overall growth of organs, or segments of organs, comprising a variety of tissues of different cell types. Such test objects, although less complex than the entire plant, nevertheless are far from simple, and part of the difficulty in interpretation and correlation of experimental results arises from the multiplicity of physiological processes involved. Some advantage might be derived from a test utilizing single tissues or cells but none such has yet been devised.

Four principal types of assay methods have been utilized in investigating activity of this type:

(a) Curvature or elongation of decapitated, but rooted, cereal coleoptiles growing in air. This comprises the classical *Avena* coleoptile curvature test, and its various modifications.

(b) Elongation of excised segments of etiolated coleoptiles or internodes immersed in solution.

(c) Curvature of slit halves of etiolated pea internodes immersed in solution.

(d) Curvature or elongation of stems, petioles or coleoptiles of green or etiolated intact plants to which small volumes of test material are applied at the responsive sites.

The physiological activity of any substances is a function not only of its effectiveness once it has gained access to the interior of the cell (primary activity) but also of various secondary properties such as rates of penetration into the test organ, transport through it, and ease of conversion within the tissue either to more active or less active form than the starting material. In methods (b), (c), and (d) the test material is applied in closer proximity to the reactive cells than in method (a) so that the necessity of internal movement may be presumed to be less. A number of compounds which exhibit little or no potency by method (a) are active when tested by one or more of the other techniques, presumably owing to this factor, whence the latter tests have been regarded as superior for the demonstration of primary activity. For the present purpose a substance active in any of these types of test will be regarded as possessing primary activity. There is reason to suspect, however, that all the available methods fall far short of the ideal test in which the intrinsic activity would be unobscured by factors such as entry into the cell.

Some hundreds of compounds have been investigated by these methods and a substantial number found to exhibit activity. A question of paramount interest is whether all active compounds possess identical primary activity modified to the observed potency by secondary differences in properties. Such a view has been advocated on the basis of rather limited experimental evidence[3, 37] but in the light of more recent results does not appear tenable.

The first systematic studies[10, 17] of the relationship of structure to auxin-type activity were published in 1935 and soon thereafter Koepfli, Thimann, and Went[16] were able, on the basis of results obtained with the pea test, to codify the multiplicity of active substances by postulating the following minimum structural requirements for primary activity:

(1) a ring system as nucleus

(2) a double bond in this ring

(3) a side chain

(4) a carboxyl group (or a structure readily converted to a carboxyl) on this side chain at least one carbon atom removed from the ring

(5) a particular space relationship between the ring and the carboxyl group

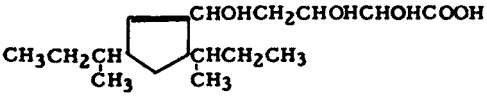
More recently these requirements have been restated as "an unsaturated ring system, with a side chain, adjacent to the ring double bond, of at least two carbon atoms ending in a carboxyl group"[38].

Although it is true that the majority of active compounds which have been discovered in the past fifteen years conform to these principles, there are known also a number of apparent exceptions. Conversely, the existence of some inactive compounds which also possess the stipulated structural features provides evidence that these have been incompletely defined. In the following paragraphs each of the putative requisite features will be taken up separately.

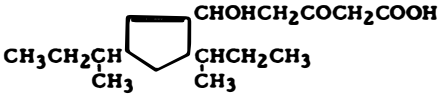
The ring system. Among compounds of demonstrated activity are found all the major types of rings: carbocyclic and heterocyclic, aromatic and alicyclic, simple and fused (Tables I, II). Active compounds are known with the following rings: Cyclopentene, cyclohexene, benzene, naphthalene, anthracene, acenaphthene, fluorene, indene, indane, benzofuran, indole, coumaran, thianaphthene. The activity of a compound containing one of these rings may be strongly influenced by the nature and position of attachment, both of the acidic side chain and of other ring substituents. In no ring series has a sufficient number of compounds been tested fully to determine these influences so that it appears premature to attempt to compare activities of various rings in general. In many of the ring series with active compounds there are also inactive members. Hence, in reaching conclusions as to the activity of any ring in general, it is necessary that a large number of derivatives be examined. The finding of one or a few inactive compounds, as e. g. certain of the acetic acid derivatives of pyridine, pyrrole, furan, thiazole, uracil, iminazole, carbazole, and dibenzofuran, does not furnish an adequate basis for

TABLE I

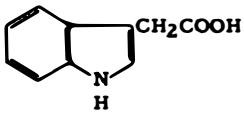
COMPOUNDS EXHIBITING HIGH AUXIN ACTIVITY



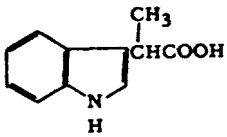
Auxin-a (Auxentriolic acid)



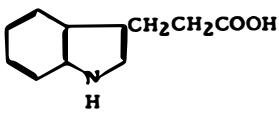
Auxin-b (Auxenolonic acid)



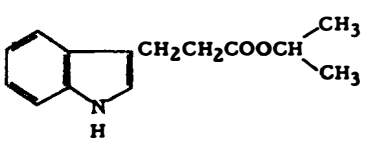
Indole-3-acetic acid



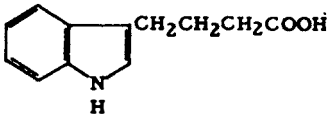
α-(Indole-3)-propionic acid



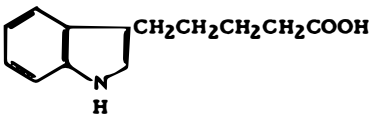
β-(Indole-3)-propionic acid



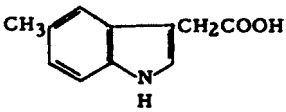
Isopropyl β-(indole-3)-propionate



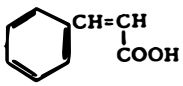
γ-(Indole-3)-butyric acid



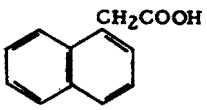
δ-Indole-3-valeric acid



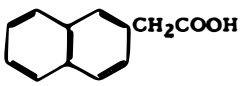
5-Methylindole-3-acetic acid



Allo (cis)-cinnamic acid

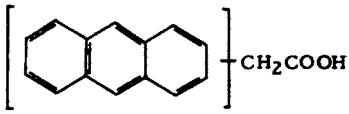


Naphthalene-1-acetic acid

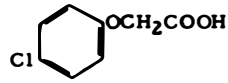


Naphthalene-2-acetic acid

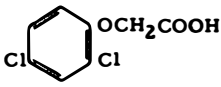
TABLE I (Cont.)



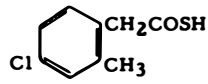
Anthraceneacetic acid



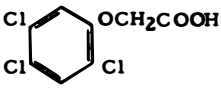
4-Chlorophenoxyacetic acid



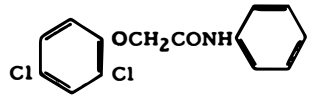
2,4-Dichlorophenoxyacetic acid



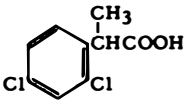
2-Methyl-4-chlorophenyl-thioacetic acid



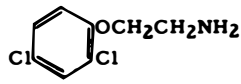
2,4,5-Trichlorophenoxyacetic acid



2,4-Dichlorophenoxyacetanilide



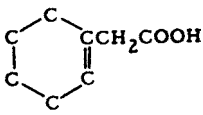
2,4-Dichlorophenyl-α-propionic acid



β-(2,4-Dichlorophenoxy)-ethylamine

TABLE II

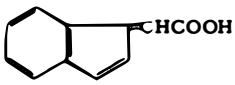
REPRESENTATIVES OF TYPES OF RING COMPOUNDS
ACTIVE IN STIMULATING CELL ELONGATION



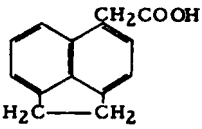
1-Cyclohexene-1-acetic acid

Indene-3-acetic acid

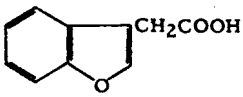
TABLE II (Cont.)



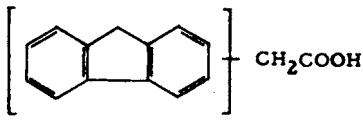
Benzofulvene carboxylic acid



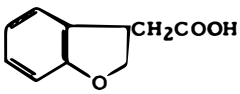
Acenaphthene-5-acetic acid



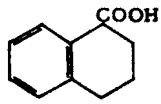
Benzofuran-3-acetic acid



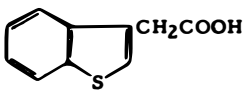
Fluoreneacetic acid



Coumaran-3-acetic acid



α-1, 2, 3, 4-Tetrahydronaphthoic acid



Thianaphthene-3-acetic acid

assuming that differently substituted derivatives of these nuclei also must be without activity. On the basis of present evidence, however, it appears that compounds with simple rings are in general less active than those with fused rings, the outstanding exceptions being auxins a and b. It has been postulated that the activity of a ring depends upon the presence of reactive double bonds therein but present information is insufficient to support this view. On the one hand, high activity is exhibited by some benzene derivatives which might be expected to possess relatively unreactive unsaturated linkages, while, on the other hand, low activity is shown by a number of derivatives with highly reactive double bonds in rings such as those shown in Table II. Indeed, even the requirement of the presence of a double bond in the ring adjacent to the side chain can scarcely be maintained in view of the activity of coumaran-3-acetic acid[39], α-1,2,3-tetrahydronaphthoic acid[31], and the so-called benzofulvenecarboxylic acid[28].

The carboxyl group. A considerable number of active compounds without a free carboxyl group in the side chain is known. Some of these, such as esters, amides, lactones, and nitriles may be hydrolyzed in vitro to yield a carboxyl but there is no direct evidence that these compounds become active in vivo only after hydrolysis, and indeed there is some evidence that this is not the case. If only the compound with free carboxyl were active it would be expected that the acid and its hydrolyzable relative would show parallel activity-concentration

curves. In the instance of naphthalene-1-acetic acid and its amide these curves are quite different; furthermore, no trace of ammonia could be detected at optimal concentrations of the amide[28].

Although in a number of examples esters and amides are less active than their corresponding acids this is not true without exception. The amides of 2,4-dichlorophenoxyacetic, γ-(2,4-dichlorophenoxy)-butyric, and ϵ-(2,4-dichlorophenoxy)-caproic acids are somewhat more active than the acids themselves[26]. The isopropyl ester of indole-3-acetic acid has activity equal to that of the free acid and the methyl ester of 2-methylindole-3-acetic acid is considerably more active than its acid. Also the methyl and isobutyl esters of phenylacetic acid exhibit equal activity although they may be presumed to be hydrolyzed at quite different rates[39].

Naphthalene-1-acetonitrile, while active in some tests, induces a much delayed response compared to the free acid[50], a result in harmony with the view that conversion of the former compound to the latter is involved. However, the possibility that some other factor, such as slow penetration, may be responsible for the delayed effect has not been excluded.

The activities of indole-3-acetaldehyde and of 2,3,5-trichlorobenzaldehyde quite conceivably may be due to in vivo oxidation to the corresponding acids. On the other hand, in the case of β-(2,4-dichlorophenoxy)-ethylamine, which has appreciable auxin activity, an oxidation and deamination would be required for conversion to an acid. The same would be true of β-(indole)-ethylamine which exhibits activity only after prolonged contact with the test plant[23].

Benzoyl oxide and benzoyl peroxide have been reported as active in a modification of the Avena test[24]. Activity of the latter compound could not be confirmed in either the Avena or the pea tests[16] but this may have been due to its rapid inactivation.

In any event, the stipulation of a carboxyl group appears too stringent, because compounds with other types of acidic functions also may possess activity. The acid grouping may be isosteric with carboxyl, as in the case of the aci-form of naphthyl-1-nitromethane, or not, as in potassium indoxylsulfate[33].

The side chain. The postulated requirement of a carbon side chain separating the acid group from the ring also cannot be maintained. Compounds in which a carbon in the side chain is replaced by a heteroatom, such as oxygen, nitrogen or sulfur have been shown to possess activity. The most obvious example is 2,4-dichlorophenoxyacetic acid. Others are N-(2,4-dichlorophenyl)-glycine, which has been tested in our laboratory, and S-(2-methyl-4-chloro)-thioglycollic acid[28].

Furthermore, there are now known several active substances in which the carboxyl group is attached directly to the ring. These include substituted benzoic acids, such as 2-bromo-3-nitrobenzoic acid, 2-bromo-3,5-dichlorobenzoic acid, and 2,3,5-trichlorobenzoic acid from among the many benzoic acids studied at Camp Detrick, 2,3,6-trichlorobenzoic acid[2], α-naphthoic acid and α-1,2,3,4-tetrahydronaphthoic acid[31].

Nevertheless, the character of the side chain appears to be of considerable importance. Thus, in the pea test the activity of phenylacetic acid is not appreciably diminished by substitution of a single methyl group in the α-position whereas activity is lost on introduction of a single phenyl or hydroxymethyl group, or of two methyl groups, or one methyl and one hydroxyl at this locus. Similarly, the primary activity of indole-3-propionic acid is not affected by introduction at the α-position of a methyl group but is completely nullified by carbomethyl (indole-3-succinic acid). This cannot be attributed merely to the presence of two carboxyls since other dicarboxylic acids (m-phenylenediacetic diethyl ester and indylene-1,3-diacetic acid) are active.

The carboxyl-bearing side chain may bear other functional groups without complete loss of activity as in o-aminophenylglyoxylic acid and indole-3-pyruvic acid. Tryptophane gives a delayed response suggesting need of transformation in vivo. Activity is not necessarily nullified by the presence of a double bond in the side chain, either adjacent to the ring as in 1,2,3,4-tetrahydronaphthylidene-1-acetic and benzofulvenecarboxylic acids, or elsewhere, as in cis-cinnamic acid.

The position of attachment of the side-chain in an unsymmetrical ring system may be of significance. Thus, appreciable differences of activity appear to exist between the members of the following pairs: coumaran-3-acetic > coumaran-2-acetic; 2-naphthoxyacetic > 1-naphthoxyacetic; naphthalene-1-acetic > naphthalene-2-acetic; β-naphthalene-2-propionic > β-naphthalene-1-propionic.

Length of the side chain also may be of importance. In the homologous series of omega-substituted phenyl aliphatic acids from phenylacetic to phenylvaleric only the first member is active. On the other hand, in the similar series from indole-3-acetic to indole-3-valeric all the members exhibit approximately equal activity. In the series from 2,4-dichlorophenoxyacetic to 2,4-dichlorophenoxycaprylic a clear-cut periodic effect of side chain length is apparent. The compounds with side chains containing an even number of carbon atoms are active, whereas the alternating odd-numbered members are inactive; the same effect is found among the amides of this series. Similarly, β-(2-naphthoxy)-propionic acid is inactive although the acetic and butyric homologs are active. A possible explanation of this periodicity in the 2,4-dichlorophenoxy series is to be found in the suggestion that β-oxidation of the acidic side chain precedes functioning of the higher homologs. In this way the active acetic homolog could be produced only from the even-numbered members, while the odd-numbered compounds would lead to unstable aryl carbonates[26].

Ring substituents. Relatively few derivatives of active compounds in the indole series have been available for assessing the role of substitution into the ring. Introduction of methoxyl at positions 5, 6, or 7 in indole-3-propionic acid leads to loss of activity, whereas the corresponding derivatives of indole-3-acetic acid remain active[8,16]. 1-Methyl-, 2-methyl-, 5-methyl-, and 2,5-dimethylindole-3-acetic acids are active, whereas the 2-ethyl derivative is not.

Introduction of one or two nitro groups into phenylacetic acid abolishes activity. Conversely, the activity of phenoxyacetic acid is markedly augmented by appropriate substitution of chlorine, bromine, or nitro into the ring. 3-Nitrophenoxyacetic acid is active but the 2- and 4-isomers are not. The activity of phenoxyacetic acid is enhanced also by a single halogen atom in the 3 or 4 position but not in the 2 position. However, introduction of two chlorine atoms in positions 2 and 4 leads to even greater increase in activity. 2,4,5-Trichlorophenoxyacetic has high activity, whereas the corresponding 2,4,6-compound has virtually none. On the other hand, 2,3,6-trichlorobenzoic acid has high activity, and 2,4,6-trimethylphenoxyacetic acid also is active[2,20].

From the information presently available, it does not appear possible to conclude generally that any particular position in any ring series must be substituted or unsubstituted in order to permit activity.

Spatial configuration. The spatial configuration of the molecule may be of great importance in determining activity. Two examples of the influence of optical isomerism are given by the α-(2,4-dichlorophenoxy)-propionic acids of which the D form has twice the activity of the racemic mixture, indicating virtually no activity for the L form[28] and by the 1,2,3,4-tetrahydronaphthoic acids of which the (-) form is much more active than the (+) form (Veldstra, cited by Thimann[28]).

The classical example of the importance of geometrical isomerism is that of the cinnamic acids, of which cis form is active, whereas the trans isomer is inactive. More recently, two additional pairs of isomers have been discovered - the 1,2,3,4-tetrahydronaphthylideneacetic acids and the naphthaleneacrylic acids - in each of which one form is active and the other not.

The importance of spatial configuration, together with other considerations, have lead Veldstra[29] to the following generalization of the structural requirements for auxin activity:

(1) A basal ring system with high surface activity,

(2) a carboxyl group (or its dipole) in a very definite spatial position with respect to this ring system.

The necessary spatial position is one in which the polar group is situated outside the plane of the ring system.

The evidence which has been assembled in support of the requirement of possession of high surface activity by the ring system cannot be reviewed here[4, 29, 31, 32]. It has been suggested that growth-regulatory activity is related to the relative lipophilicity (due to the surface-active properties of the ring) and hydrophilicity (contributed by the carboxyl group) of the molecule.

A number of otherwise puzzling cases may be explicable in terms of the spatial relationships of ring and side chain. One example is that of the three isomeric mononitrophenoxyacetic acids of which only the meta isomer has activity. This compound is incapable of mesomerism whereas the ortho and para isomers are able to resonate in equilibrium with respective quinoid forms in which the carboxyl group is claimed to be more restricted to the plane of the ring. Another example is that of α-1, 2, 3, 4-tetrahydronaphthoic acid which, in contrast to naphthoic acid, is active. Models indicate the carboxyl group of the former to be held outside the plane of the ring, whereas this is not true of the latter.

On the other hand there are many compounds, the activity of which have not yet been satisfactorily explained on the basis of Veldstra's postulates. Whatever the ultimate fate of this concept, it has the merit of providing a fresh working hypothesis for further experimentation.

Reference should be made at this point to a group of compounds which possess none of the above-postulated requisite structural characteristics but nevertheless include some of the most potent growth-regulatory substances known. These are carbon monoxide, the unsaturated hydrocarbons ethylene, propylene, butylene, and acetylene, and the halogenated hydrocarbons ethyl bromide, ethyl iodide, and propyl chloride. These gases, in low concentration, are capable of inducing differential rates of growth of upper and lower sides of petioles which result in growth curvatures of the organs. Ethylene is inactive in the pea test although it has been shown to accelerate the growth rate of certain other seedlings. What relation these substances may bear to the ring type of auxins is uncertain, but two points of interest may be mentioned. Firstly, unlike the great majority of the compounds previously discussed, ethylene is known to be a natural product of many kinds of plants, and there is considerable evidence that it may play an important role in plant metabolism and behavior. Thus, it appears to merit the designation phytohormone as well as, if not better than, any other known compound. Secondly, it has been shown that volatile emanations, having the physiological properties of ethylene, are produced by certain plants after treatment with growth-regulators such as indole-3-acetic acid[50].

Other physiological responses to ethylene and related compounds will be referred to in later sections.

IV. INITIATION OF ROOTS

A striking response, induced by a large variety of compounds, is the production of roots on plant organs, such as stems, leaves, and flowers, which do not normally bear them. Such adventitious roots may result from stimulation of pre-existent root primordia or may be initiated from cells of diverse tissues. Various fragments of many species of plants show a more or less strong tendency toward production of adventitious roots without the intervention of exogenous growth-regulators, a circumstance which has long been utilized in vegetative propagation. Consequently, in testing the root-initiating activity of chemical agents conditions should be selected so as to minimize the rooting of untreated test objects. Stem cuttings have been employed almost exclusively as test objects. Because of the great interest in practical application of rooting compounds much of the available information has been obtained from trials made with species of horticultural value, and relatively little attention has been given to development of standard tests. Accordingly, there is an abundant literature describing results of rooting tests with a wide variety of species. Unfortunately it is not always possible to compare the results of one worker with those of another as techniques have differed greatly and there has

been relatively little attempt to control influential environmental factors.

With these considerations in mind, some generalizations can be made. The structural requirements for root-initiating activity appear rather less stringent than those for auxin activity. Virtually every compound which possesses any activity in cell-elongation stimulation is active also in root initiation. In addition, several substances lacking the former type of activity have been reported as possessing the latter. A list of these compounds is given in Table III.

TABLE III

COMPOUNDS INACTIVE IN STIMULATING CELL-ELONGATION BUT REPORTED ACTIVE IN ROOT-INITIATION

Cinnamic acid	Naphthalene-1-methanesulfonic acid
Diphenylacetic acid	Uracil-4-acetic acid
Nitrocinnamic acid	N-Carbethoxymethylquinolinium chloride
Coumarin	4-Methylthiazole-5-acetic acid
Phenylacetamide	Desoxycinchotenine
	Desoxycinchotenidine

In Table IV are included compounds alleged to be active for root initiation but not heretofore tested for auxin activity. It should be pointed out that there has been no published confirmation of some of these reports.

TABLE IV

COMPOUNDS REPORTED ACTIVE IN ROOT-INITIATION BUT NOT TESTED FOR CELL-STIMULATION ACTIVITY

Tetrahydrofurfuryl alcohol	3,4-Dihydronaphthalene-1-acetic acid
Calcium furoate	1,2,3,4-Tetrahydronaphthalene-6-acetamide
Nicotine	Sodium tetralolacetate
Sodium naphthol-4-sulfonate	Aminophenylacetic acid
Naphthalene tetrachloride	p-Phenylenediamine
L-Proline	8-Hydroxyphenylthiocarbamate
Anthracene	8-Hydroxyphenyl-2-iminothiocarbonate
Anthraquinone-β-sulfonic acid	6,8-Dihydroxyphenyl-2-iminothiocarbonate
Anthranilic acid	Naphthalene-1-acetamide
Salicylideneacetamide	Naphthalene-1-thioacetamide

TABLE IV (Cont.)

α-(Naphthalene-1)-propionic acid
β-(Naphthalene-1)-propionic acid
γ-(Naphthalene-1)-butyric acid
γ-(Naphthalene-2)-butyric acid
δ-(Naphthalene-1)-valeric acid
ε-Naphthalene-1)-hexoic acid
Naphthalenediacetic acid
Naphthalene-1-acetylglycine
2-Methylnaphthalene-1-acetic acid
2-Methylnaphthalene-1-acetamide
4-Methylnaphthalene-1-acetic acid
4-Methylnaphthalene-1-acetamide
Vanillic acid
Vanillin
Sulfanilamide
Piperonal
Methoxysalicylaldehyde
α-(2-Chlorophenoxy)-propionic acid
α-(3-Chlorophenoxy)-propionic acid
α-(2-Chlorophenoxy)-n-butyric acid
α-(3-Chlorophenoxy)-n-butyric acid
α-(4-Chlorophenoxy)-propionic acid
α-(4-Chlorophenoxy)-n-butyric acid
α-(2,4-Dichlorophenoxy)-propionic acid
α-(2,4-Dichlorophenoxy)-n-butyric acid
α-(2,4-Dichlorophenoxy)-iso-valeric acid
2,4-Dibromophenoxyacetic acid
α-(2,4-Dibromophenoxy)-propionic acid
α-(2,4-Dibromophenoxy)-n-butyric acid
2,4-Diiodophenoxyacetic acid
2-Iodophenoxyacetic acid
3-Aminophenoxyacetic acid
4-Aminophenoxyacetic acid
2-Nitrophenoxyacetic acid
3-Nitrophenoxyacetic acid
α-(Phenoxy)-propionic acid
α-(Phenoxy)-n-butyric acid
α-(2-Methylphenoxy)-propionic acid
2-Methylphenoxyacetic acid
4-Methylphenoxyacetic acid
α-(2-Methylphenoxy)-n-butyric acid
α-(4-Methylphenoxy)-propionic acid
α-(4-Methylphenoxy)-n-butyric acid
α-(2,5-Dimethylphenoxy)-propionic acid
α-(2,5-Dimethylphenoxy)-n-butyric acid
Pyrrole-2-acetic acid
Furylpropionic acid
Hydantoinpropionic acid
α-(2,4,5-Trichlorophenoxy)-propionic acid
α-(2,4,5-Trichlorophenoxy)-n-butyric acid
2,6-Diiodo-4-carboxyphenoxyacetic acid
2,4,6-Triiodophenoxyacetic acid
2,6-Dibromo-4-aminophenoxyacetic acid
2,6-Diiodo-4-carboxyphenoxyacetic acid
Dithiobiuret
Salicylacetone
cis-Thiophane-2,5-dicarboxylic acid
Indole-3-acrylic acid

V. INDUCTION OF PARTHENOCARPY

In many species of plants development of fruit from floral tissues takes place only after pollination is accomplished. It has been known for nearly half a century that the stimulating role of the pollen is attributable to a substance, or substances, contained therein and is separate from its function in fertilization. During the past decade the availability of pure compounds of demonstrated activity in other types of plant growth response has resulted in the discovery that many of these are capable also of inducing parthenocarpy - the development of fruits without pollination. Such fruits are of course seedless.

Because of the practical applicability of artificial parthenocarpy, the bulk of presently available information has accumulated in much the same manner as that relating to root initiation. Attempts have been made to ascertain which compounds and formulations might be of greatest service under conditions of large-scale production of species of commercial interest, with due regard for ancillary effects of size, shape and quality of fruits and on the vegetative organs. Unfortunately, experimentation undertaken with such objectives, though of the greatest practical value, is frequently difficult of interpretation from the viewpoint of the relation between structure and activity. Only recently have quantitative tests which may be suitable for this purpose been devised[19] and virtually no results are yet available. There is considerable evidence indicating that in such studies a distinction should be made between the processes of setting and of subsequent growth of the fruit; frequently this has not been done.

For these reasons it seems desirable at present only to list the compounds which have been reported as parthenocarpically active without attempting quantitative comparison of activities (Table V). It is to be noted that particular compounds may show activity on some species but not on others, or under different circumstances.

TABLE V

COMPOUNDS ACTIVE IN INDUCING PARTHENOCARPY

2, 5-Dichlorobenzoic acid	2-Methylphenoxyacetic acid
Phenylacetic acid	α-(2-Methylphenoxy)-propionic acid
α-(Phenoxy)-propionic acid	2, 4-Dichlorophenoxyacetic acid
α-(Phenoxy)-n-butyric acid	α-(2, 4-Dichlorophenoxy)-propionic acid
2-Chlorophenoxyacetic acid	α-(2, 4-Dichlorophenoxy)-n-butyric acid
α-(2-Chlorophenoxy)-propionic acid	2, 5-Dichlorophenoxyacetic acid
α-(2-Chlorophenoxy)-n-butyric acid	2, 4-Dimethylphenoxyacetic acid
α-(3-Chlorophenoxy)-propionic acid	α-(2, 4-Dimethylphenoxy)-propionic acid
α-(3-Chlorophenoxy)-n-butyric acid	3, 4-Dimethylphenoxyacetic acid
4-Chlorophenoxyacetic acid	α-(3, 4-Dimethylphenoxy)-propionic acid
α-(4-Chlorophenoxy)-propionic acid	α-(2, 5-Dimethylphenoxy)-propionic acid
α-(4-Chlorophenoxy)-n-butyric acid	α-(2, 5-Dimethylphenoxy)-n-butyric acid

TABLE V (Cont.)

2, 4, 5-Trichlorophenoxyacetic acid	Fluorene-4-acetic acid
α-(2, 4, 5-Trichlorophenoxy)-propionic acid	Phenanthrene-9-acetic acid
α-(2, 4, 5-Trichlorophenoxy)-n-butyric acid	Pyrrole-2-carboxylic acid
2, 4, 6-Trichlorophenoxyacetic acid	Pyrrole-2-acetic acid
2, 4, 5-Trimethylphenoxyacetic acid	Indole-3-acetic acid
β-(2, 4, 6-Trichlorophenoxy)-β'-chlorodiethyl ether	β-(Indole-3)-propionic acid
Naphthalene-1-acetic acid	γ-(Indole-3)-n-butyric acid
Naphthalene-1-acetamide	Acenaphthene
Naphthalene-1-propionic acid	Skatole
Naphthalene-1-butyric acid	Sulfanilamide
β-Naphthoxyacetic acid	Oestrone
β-Naphthoxypropionic acid	Colchicine

Parthenocarpic activity, though exhibited by many substances active also in other responses, is by no means restricted to these. The structural requirements for parthenocarpic induction, like those for root initiation, appear considerably less circumscribed than in the cell-elongation or formative responses.

VI. MODIFICATION OF ORGANS

Certain types of compounds have been shown capable of profoundly modifying the size, shape, and texture of developing leaves. This so-called "formative" effect is due to production of closely packed, thick-walled parenchyma-like cells in place of the normal chlorophyllous mesophyll tissue and failure of lateral expansion of the leaf[35]. It is of interest that many, though not all, of the useful herbicidal growth-regulators show high formative activity and also that certain of the leaf modifications may resemble those of virus-infected plants.

Activity of this type was reported first for naphthoxyacetic acids[12]. Subsequently, a large number of substances have been tested for formative activity by workers at the Boyce Thompson Institute[25, 26, 40, 41, 42, 43, 45, 46, 47, 48, 49]. The use of a variety of test objects and techniques, some essentially qualitative or at best semi-quantitative, renders difficult a comprehensive comparison of the activities of all the compounds investigated. More recently, a quantitative assay method for formative activity has been devised at Camp Detrick[5]; ratings are made on the basis of the amount of growth-regulator which, after application to the terminal bud of a bean seedling, is required to repress the leaf expansion by 50 per cent while producing also the characteristic morphological modifications. The following discussion is based primarily

upon the heretofore unpublished Camp Detrick results, which are in general agreement with those of the Boyce Thompson investigations. It should be pointed out that injury of a non-formative nature is caused by many substances if applied in relatively large amounts. This sets a methodological limit to the doses that can be tested. Compounds which are rated as inactive at dosages up to this limit conceivably might exhibit activity if it were possible to test them at higher levels.

With few exceptions, all the compounds so far demonstrated to possess formative activity of this type are derivatives of phenoxyacetic, naphthoxyacetic, or benzoic acid. Many closely related compounds, some of which are highly active in other types of response, are ineffective.

Even among those series containing active members, details of molecular architecture play an important role. These will be discussed separately for the various series.

Phenoxyacetic acid itself is inactive, and activity is not conferred by introduction into any position of the ring of a single amino, nitro, or carboxyl group. Active compounds are obtained, however, by introduction of a methyl, oximinomethyl, methoxyl, or halogen into the ring in suitable position. Of these groups the halogens exert a much greater activating influence than the others, in the order F > Cl > Br > I. Of the monochloro- and monobromo-phenoxyacetic acids, of which all the possible isomers have been examined, only those substituted in position 4 are active. On the other hand, 3- and 4-methylphenoxyacetic acids show approximately equal activity, which is, however, of a low order.

Introduction of additional ring substituents into a highly active monosubstituted phenoxy-acetic acid usually tends to diminish the activity. Thus, as compared with 4-chlorophenoxy-acetic, 2,4-dichloro is about three-fourths as active and 3,4-dichloro about one-seventh. Slight activity is shown by 2,6-dichloro while the 2,5- and 3,5-isomers are inactive. So far as other dihalogenated compounds have been tested it appears that an inactivating tendency of similar magnitude is exerted by a second atom of iodine and even more strongly by fluorine or bromine. In this respect also the methyl-substituted compounds exhibit a somewhat different behavior, the 2,4-, 2,5-, and 3,4-dimethylphenoxyacetic acids showing activity approximately equal to that of the 3- and 4-methyl compounds. 2,4-Dinitrophenoxyacetic acid also has some activity, whereas the mononitro derivatives have none. Trisubstituted compounds are in general even less active than comparable disubstituted derivatives. Introduction of additional ring substituents may lead to complete loss of activity in some, though not in all, cases.

Propionic and butyric homologs are usually much less active than the related phenoxy-acetic acids although in some instances the reverse may be true. Replacement of the ether O atom by N greatly diminishes activity, while replacement by S nullifies it entirely.

Presence of a free carboxyl group is not essential for formative activity. Certain of the amides and esters exhibit even greater activity than the parent acids. Relatively high activity may be shown by some acid chlorides. Halogen-substituted phenoxy ethanols, ethyl ethers, and ethylamines also may possess activity though of a relatively low magnitude.

The phenoxy series provides the most comprehensive group enabling comparison of the structural requirements for stimulation of cell elongation and for formative effects. The curious situation exists that certain of these compounds have high activity in both types of response, whereas others possess auxin activity but not formative activity.

Too few naphthoxy acids have been tested to provide detailed information on the influence on formative activity of small changes in the molecule. As already noted, ring substitution is not essential for activity in this series.

No activity is shown by benzoic acid or any of its derivatives containing the following single substituents in the ring: amino, bromine, chlorine, fluorine, hydroxyl, iodine, nitro, carboxyl, and methyl. Some of the di- and tri- substituted derivatives are active however. In Table VI are listed active and inactive compounds of this type. A further group of diverse compounds without activity in this test is given in Table VII.

TABLE VI

FORMATIVE ACTIVITY OF SOME DI- AND TRI- SUBSTITUTED BENZOIC ACIDS

Active

2,5-Dichlorobenzoic acid
5-Bromo-2-iodobenzoic acid
2-Bromo-3-nitrobenzoic acid
3-Bromo-2-nitrobenzoic acid
3-Bromo-4-nitrobenzoic acid
2-Chloro-3-nitrobenzoic acid
2-Iodo-3-nitrobenzoic acid
2-Methyl-3-nitrobenzoic acid
5-Bromo-3-chloro-2-iodobenzoic acid
5-Bromo-2,3-dichlorobenzoic acid
5-Chloro-2,3-dibromobenzoic acid
2,3-Dibromo-5-iodobenzoic acid
3,5-Dibromo-2-iodobenzoic acid
3,5-Dichloro-2-iodobenzoic acid
2,3,5-Tribromobenzoic acid
2,3,5-Trichlorobenzoic acid

Inactive

3,5-Dicarboxybenzoic acid
2,4-Dichlorobenzoic acid
3,4-Dichlorobenzoic acid
2,4-Dihydroxybenzoic acid
2,5-Dihydroxybenzoic acid
2,5-Diiodobenzoic acid
3,4-Diiodobenzoic acid
2,3-Dimethoxybenzoic acid
2,5-Dinitrobenzoic acid
3,5-Dinitrobenzoic acid
2-Amino-5-iodobenzoic acid
5-Amino-2-hydroxybenzoic acid
2-Bromo-4-nitrobenzoic acid
2-Bromo-5-nitrobenzoic acid
3-Bromo-5-nitrobenzoic acid
3-Bromo-6-nitrobenzoic acid
4-Bromo-2-nitrobenzoic acid
4-Bromo-3-nitrobenzoic acid
2-Fluoro-3-nitrobenzoic acid
2-Hydroxy-3-methylbenzoic acid
2-Hydroxy-3-nitrobenzoic acid
5-Bromo-2-hydroxybenzoic acid
2-Hydroxy-5-iodobenzoic acid
5-Methyl-2-nitrobenzoic acid
2-Amino-3,5-diiodobenzoic acid
2-Bromo-3,5-dinitrobenzoic acid
2-Fluoro-3,5-dinitrobenzoic acid
3-Bromo-2-hydroxy-5-sulfobenzoic acid
3,5-Dinitro-2-hydroxybenzoic acid
2-Hydroxy-5-iodo-3-methylbenzoic acid
2,4,6-Trihydroxybenzoic acid
2,4,6-Trinitrobenzoic acid
3,4,5-Tribromobenzoic acid
3,4,5-Trichlorobenzoic acid
3,4,5-Trihydroxybenzoic acid
3,4,5-Triiodobenzoic acid

TABLE VI (Cont.)

Inactive

3,5-Diiodo-2-hydroxybenzoic acid

3,5-Diiodo-4-hydroxybenzoic acid

3,4,5-Trimethoxybenzoic acid

TABLE VII

MISCELLANEOUS COMPOUNDS INACTIVE IN FORMATIVE RESPONSE

Phenylacetic acid

4-Aminophenylacetic acid

4-Bromophenylacetic acid

4-Chlorophenylacetic acid

4-Hydroxypheny'acetic acid

4-Iodophenylacetic acid

4-Nitrophenylacetic acid

α-(2-Hydroxyphenyl)-phenylacetic acid

2,4-Dinitrophenylacetic acid

4-(Trimethylsilyl)-phenylacetic acid

Diphenylacetic acid

β-Methallyldiphenyl acetic acid

Allyldiphenylacetic acid

Hydrocinnamic acid

cis-Cinnamic acid

trans-Cinnamic acid

2-Chlorocinnamic acid

4-Chlorocinnamic acid

2,4-Dichlorocinnamic acid

2-Methoxycinnamic acid

cis-5-Chloro-2-hydroxycinnamic acid

α-Phenylbutyric acid

4-Hydroxyphenylglycine

3,5-Dibromo-L-tyrosine

Coumarilic acid

2-Thiophenecarboxylic acid

3-Pyridinecarboxylic acid (Nicotinic acid)

Nicotinamide

4-Pyridinecarboxylic acid (Isonicotinic acid)

2-Pyriolidinecarboxylic acid (Proline)

1-Naphthoic acid

2-Naphthoic acid

1-Hydroxy-2-naphthoic acid

3-Hydroxy-2-naphthoic acid

1-Naphthylacetic acid

2-Naphthylacetic acid

α-(1-Naphthyl)-propionic acid

Naphthalene-1,8-dicarboxylic acid

Indole-3-acetic acid

TABLE VII (Cont.)

Tryptophane	5, 7-Dibromo-8-quinolyloxyacetic acid
8-Quinolyloxyacetic acid	9-Allylfluorene-9-carboxylic acid

The impression may be given that the structural requirements for compounds active in producing formative responses are rather clearer and more restricted than for auxin activity or root initiation, and while this may be the case, it cannot be said to be proved at this time. The characterization of formative responses is a matter of great difficulty. Gross morphological abnormalities are not readily expressible. Certain compounds not containing phenoxy, naphthoxy, or benzoic groupings do induce changes in growth habit, but have not been included in comparative tests, so that their relative position is not known. In this category are such herbicides as maleic hydrazide, dichloral urea, and trichloroacetic acid.

VII. CONTROL OF ABSCISSION

There is a substantial body of evidence indicating that the process of abscission - the natural separation of leaves, foliage branches, floral parts, or fruits from the plant brought about by structural alterations in particular groups of cells - is regulated by a hormonal mechanism. Application to the plant of certain growth-regulatory compounds (Table VIII) has the effect of greatly delaying the onset of abscission. Such treatments have found extensive use for prevention of preharvest drop of orchard fruits and related purposes.

TABLE VIII

COMPOUNDS EFFECTIVE IN RETARDING ABSCISSION

Levulinic acid	1, 2, 3, 4-Tetrahydronaphthalene-6-acetamide
Indole-3-acetic acid	Naphthoxy-2-acetic acid
β-(Indole-3)-propionic acid	β-(naphthoxy-1)-propionic acid
γ-(Indole-3)-n-butyric acid (also esters)	4-Chlorophenoxyacetic acid
Naphthalene	2, 4-Dichlorophenoxyacetic acid (also esters)
Naphthalene-1-acetic acid (also esters)	α-(2, 4-Dichlorophenoxy)-n-butyric acid
Naphthalene-1-acetamide	2-Methyl-4-chlorophenoxyacetic acid
Naphthalene-1-thioacetamide	

On the other hand, other compounds are known to produce the opposite effect, namely, to accelerate or induce abscission. One of these, ethylene, is of particular interest in that it is a plant product and conceivably may play a role in the natural control of abscission. Additional compounds reported active in inducing abscission include calcium cyanamide, ammonium thiocyanate, carbon tetrachloride, ethylene chlorohydrin, 3,6-endoxotetrahydrophthalic acid, 3,6-endoxohexahydrophthalic acid, and various halogenated benzoic acids.

The evidence available from several types of investigations appears to support the view that abscission of an organ is induced when its content of auxin falls to a low level. The process may be retarded by supplying externally a substance with auxin activity and may be accelerated by any of a variety of means, physical as well as chemical, which result in diminution of the normal auxin content. The diversity of the chemical agents which can act as abscissants suggests that reduction of the auxin level can be accomplished by various mechanisms.

Only in the benzoic acids has the relation of activity to structure of a closely related series of compounds been investigated[36]. No activity is shown by benzoic acid itself or by any of its monohalogenated derivatives. Moderate activity is possessed by certain of the dihalogenated compounds while a number of the trihalogenated benzoic acids are highly effective (Fig. 1). It is worthy of comment that here the halogens arrange themselves in the order I > Br > Cl in activating influence, which is in the reverse direction to that found in the phenoxyacetic series in formative activity.

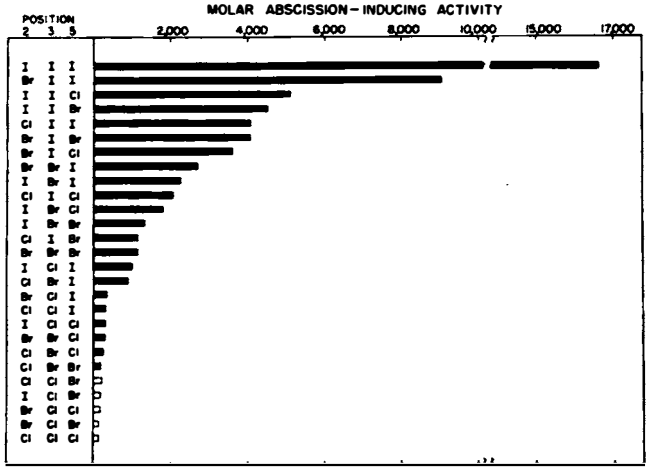


Fig. 1. Activity of 2, 3, 5-trihalogen benzoic acids in inducing abscission

VIII. CONTROL OF BUD DEVELOPMENT

A situation somewhat analogous to abscission is to be found in the control of bud development, which is also of great importance in the ontogeny of the plant. Here also dormancy appears to be under hormonal regulation. Endogenous auxins and related exogenous substances have the ability of maintaining or inducing dormancy, i.e. of suppressing bud development, whereas the reverse process of bud stimulation, or release of dormancy, can be accomplished by a congeries of rather diverse chemicals.

Among the compounds active in suppressing bud development are indole-3-acetic acid, β-(indole-3)-α-oximino-propionic acid, β-(indole-3)-acrylic acid, β-(indole-3)-pyruvic acid, γ-(indole-3)-n-butyric acid, naphthalene-1-acetic acid (and its esters), naphthalene-1-acetamide, naphthalene-1-thioacetamide, and coumaran-1-acetic acid. Although the number of compounds tested for activity of this kind is quite small, there appear to be rather narrow structural requirements; for example, cis-cinnamic acid which exhibits auxin activity in cell-elongation tests is inactive in suppressing bud development.

A number of compounds active in overcoming bud dormancy are listed in Table IX.

TABLE IX

COMPOUNDS EFFECTIVE IN OVERCOMING DORMANCY OF BUDS

Diethyl ether	Thioglycol
Chloroform	Sodium azido-dithiocarbonate
Ethyl bromide	Methyl disulfide
Ethyl iodide	Potassium sulfocarbonate
Ethylene chloride	Thioacetamide
Ethylene chlorhydrin	Ethyl carbylamine
Ammonia	Thiourea
Ethylene	Propylene
Trichloroethylene	Acetaldehyde
o-Tolylthiourea	Ethanol
Ammonium dithiocarbamate	Acetone
Thiosemicarbazide	Hydrogen cyanide
Hydrogen sulfide	Zinc sulfate
Ethyl mercaptan	Thiocyanates

IX. REPRESSION OF ROOT ELONGATION AND OF SEED GERMINATION

The repression of root growth of seedlings has been employed for many years as a technique for investigating inhibitory chemicals. More recently such methods have been employed extensively in preliminary screening for compounds with herbicidal activity. The root shows high sensitivity to a great diversity of substances, however, so that little purpose would be served by an enumeration of all the compounds found to exert repressive effects.

Interest in this response stems in part from the fact that certain of the compounds possessing auxin activity, e.g. indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid, are among the most active root inhibitors, producing measurable effects in concentrations as low as 10^{-6} to 10^{-8}M. Up to the present, however, the number of compounds that have been carefully assayed in both types of response is too few to warrant discussion of the comparative structure-activity relations.

A quite different class of substances, the arylcarbamic esters, also possesses activity of the same order in repression of root elongation but appears to be without effect in the other types of growth-regulatory phenomena so far discussed. The following generalizations concerning the carbamates are based upon preliminary tests at Camp Detrick. The very low solubility of many of these compounds presents a major methodological difficulty which may have introduced some bias into the data available. Hence, these conclusions are to be regarded as provisional.

Esters of carbamic acid itself are relatively inactive. Activity is conferred by substitution of one of the amino hydrogens by a phenyl radical or, in even greater degree, by certain substituted phenyl groups, particularly those containing halogen atoms. Compounds in which the second amino hydrogen is substituted, as by a methyl group, also may exhibit activity.

The nature of the esterifying radical plays a role which appears to depend upon the type of N substitution. Thus, among the N-phenyl carbamates the isopropyl ester is most active, followed closely by ethyl and various β-substituted ethyl esters, whereas in other series the n-propyl ester may equal or exceed the isopropyl in activity.

Several thionocarbamates are active while the corresponding thiolcarbamates are not. N,N-di-(carboethoxy)-aniline, which contains a second esterified carboxyl group, has been reported of equal activity with the related ethyl N-phenylcarbamate[27].

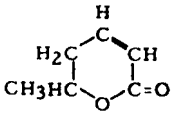
Closely related to suppression of root elongation is the inhibition of seed germination. The most obvious manifestation of germination is the elongation of the axes - root and shoot - of the embryo and this is commonly utilized as the criterion of the occurrence of germination. Ordinarily, root emergence precedes that of the shoot. At first, elongation of the root is due entirely to enlargement of cells pre-existing in the embryo but this initial phase merges so smoothly into that characteristic of subsequent growth, in which formation of new cells proceeds concomitantly with their expansion, that macroscopic observation permits no distinction between them. Hence separation of the germination stage from that of seedling development is entirely arbitrary. Whether there exist compounds capable of controlling specifically the initial phase of germination but inactive on later root growth is not clear at present.

Substances highly inhibitory to seed germination have been shown to occur in fruits and other organs of a wide variety of plants[7]. There is every reason to believe that some of these play an important role in the control of development. The term "blastokolin", which was introduced originally[15] to designate the germination-inhibiting substance present in the flesh of certain fruits, has been employed by some later workers in a generic sense. While the nature of many of these substances is still unknown, a number have been identified. Several classes of organic compounds are represented, including mustard oils, cyanophoric glycosides, aldehydes, carboxylic acids, essential oils, alkaloids, phenols, and unsaturated lactones. Relatively high concentrations, of the order of 10^{-2} to 10^{-3}M, of most of these substances are usually required for inhibition of germination. The wide diversity of structural types which exhibit activity of this kind bespeaks a multiplicity of inhibitory mechanisms having a common result.

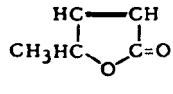
Only one of these groups of compounds - the unsaturated lactones - will be further considered. The most active of these are effective at concentrations of 10^{-4} to 10^{-5} M in root repression and at somewhat higher concentrations in prevention of germination. In Table X are shown a number of lactones which possess blastokolin activity. As these have been assembled from various investigations[1, 6, 11, 18, 22, 34] no comprehensive comparison of activities is possible. However, so far as can be judged, the most active of these compounds is protoanemonin, followed by coumarin. Substitution in either ring of the latter compound is stated to diminish activity. The diversity of structural types suggests that activity is a general property of α,β-unsaturated lactones; the relative activity is of course influenced by the specific structure of the whole molecule.

TABLE X

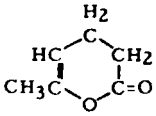
UNSATURATED LACTONES EXHIBITING BLASTOKOLIN ACTIVITY



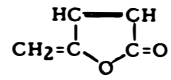
Hexene-2-olid-5, 1 (parasorbic acid)



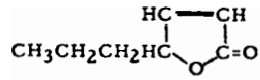
Pentene-2-olid-4, 1



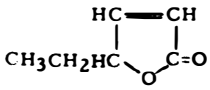
Hexene-4-olid-5, 1



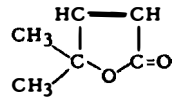
Protoanemonin



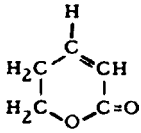
Heptene-2-olid-4, 1



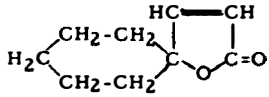
Hexene-2-olid-4, 1



4-Methylpentene-2-olid-4, 1

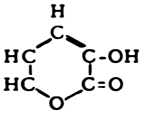


Pentene-2-olid-5,1 (δ-Pentenolactone)

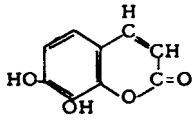


Lactone of 2-(1'-Hydroxycyclohexyl)acrylic acid

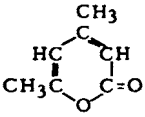
TABLE X (Cont.)



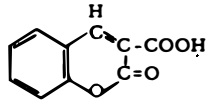
3-Hydroxy-α-pyrone



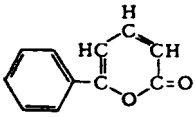
Daphnetin



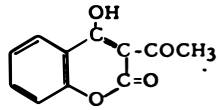
4,6-Dimethyl-α-pyrone



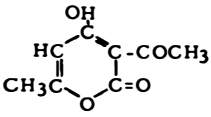
Coumarin-3-carboxylic acid



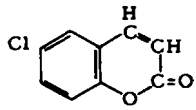
6-Phenyl-α-pyrone



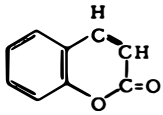
3-Acetyl-4-hydroxycoumarin



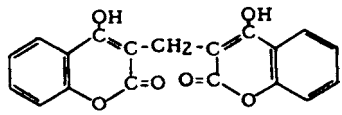
Dehydroacetic acid



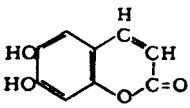
6-Chlorocoumarin



Coumarin



Dicoumarol



Esculetin

The root-inhibiting activity of an extensive series of coumarin derivatives has recently been studied[9]. Coumarin-3-carboxylic acid, 8-methylcoumarin, 7,8-dihydroxycoumarin (daphnetin), and 7,8-dihydroxy-4-methylcoumarin were found fully as active as coumarin itself. All the other compounds tested, nineteen in number, were appreciably less active. These included all the monomethyl isomers and various hydroxy, methylhydroxy, and methoxy substituted coumarins.

X. GENERAL DISCUSSION

Interest in the relationship between structure and activity stems from two principal sources. The increasingly widespread utilization of plant growth-regulators for practical ends in recent years has intensified the interest in this subject with the hope that promising directions of search for additional useful compounds may be indicated. In addition, it is expected that insight into the structural requisites will contribute to understanding of the mechanisms concerned in the regulation of plant development. To date neither of these hopes has been fulfilled. The search for new types of useful growth-regulators, which is being vigorously prosecuted in many laboratories, appears to be largely by trial and error.

The complexity of the physiological systems involved in the various processes influenced by plant growth-regulators has thus far defied interpretation on a biochemical level; in consequence there have been proposed a number of more or less incompatible hypotheses some of which appear to be based upon insecure experimental foundation. While an extended consideration of hypothetical mechanisms would be out of place in this paper one or two main points should be mentioned.

Arising primarily from work on plant tropisms and correlation, there has developed in the past quarter-century the concept of a system of endogenous growth-regulators in plants which control in a hormone-like manner many of the features of growth and development. It is believed that substances of high potency may be formed in certain organs, such as the bud or the leaf, and regulate or control normal phenomena of differentiation and development as well as many of the growth adjustments which follow injury or organ removal.

The nature and mode of action of such growth-regulators is very imperfectly understood. Omitting the case of ethylene, to which reference has already been made, only one substance, indole-3-acetic acid, or heteroauxin, has been certainly identified as an endogenous phytohormone. The versatility of this compound, an indication of which has been given in the foregoing sections, has led some investigators to the view that it may play a central and direct role in all, or most, manifestations of growth and development. Others believe that there exist in the plant diverse growth regulators, more or less specific for particular developmental phenomena, the functioning of which may be influenced by heteroauxin; thus a more indirect role is ascribed to the latter.

Opinions as to the fundamental mode of action of this phytohormone may be divided into two general categories. On the one hand it is suggested that heteroauxin participates as a coenzyme in some enzymatic metabolic reaction, a so-called master reaction, common to various kinds of plant cells and leading to diverse end-results according to environmental conditions and the physiological and morphological potentialities of the particular cells involved. It cannot be said that this view, which may be termed for convenience the biochemical hypothesis, is supported by any convincing evidence of direct nature. On the contrary, analogy with known cases of coenzyme action seems to speak against it.

The other suggestion is that the primary action of heteroauxin is to affect some more physico-chemical property of the cell, such as the degree of association or dissociation of protoplasmic proteins. In this way exposure or masking of active sites on enzymes might be brought about with resulting increase or decrease of enzyme activity. Further, the concomitant changes in structural viscosity of the protoplasm might be expected to influence physiological

processes such as cell expansion, permeability, and protoplasmic streaming. The postulated physico-chemical mechanism may be thought of as in some measure nonspecific with regard both to the active agents and to the types of proteins or enzymes affected. This view provides an explanation of the manner in which teratological and lethal effects can be produced by high dosages of compounds which at lower rates may act similarly to endogenous hormones in normal growth processes. A physico-chemical mode of action would appear to harmonize with the essentially physical requirements for auxin activity as postulated by Veldstra.

Whether the mode of action of growth-regulators be primarily biochemical or primarily physico-chemical, it must involve reactions peculiar to the green plant. Although comparative biochemistry does reveal similarities in the biocatalysts and the enzymically catalysed reactions involved in oxidations and energy transfer in unrelated groups of organisms, it must be pointed out that the degree of specificity of growth-regulators is apparently high. Few are known to influence organisms other than higher plants at comparable dosages though it must be admitted that the data on this point are extremely scanty.

The mechanism of action of exogenous growth-regulators will probably not be fully explicable until that of the endogenous growth hormones is understood. Nevertheless, knowledge of the structural requisites of the former may be very helpful in elucidating the role of the latter. There can be little doubt that in many cases the exogenous compounds act by participation in the same mechanism by which the native hormone operates. Thus, in some responses, such as stimulation of cell elongation, initiation of roots, induction of parthenocarpy, inhibition of bud development, and retardation of abscission, the exogenous growth-regulators appear capable of functioning as substitutes for the endogenous hormone. In certain other responses the relationship is less clear. In responses such as modification of leaf form, induction of abscission, and stimulation of bud development, the effects of the exogenous growth-regulators are the converse of those brought about by indole-3-acetic acid; in these phenomena, if the two classes of substances do act through a common basic mechanism, the relationship may be one of antagonism. There is some evidence that such is the case.

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No attempt has been made to document completely all the results included in this paper. Citations have in general been restricted to the more recent publications and to other summarizing articles in which references to most of the original literature may be found.

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DISCUSSION

DR. D. W. WOOLLEY (Rockefeller Institute for Medical Research, New York, New York): Mr. Chairman, in thinking of the relationship of structure to activity and, more particularly, in attempting to make generalizations about the observed data, I wonder if we can be too narrow in our view of the matter. I thought of this particularly when the activity of the naphthoic acid was mentioned.

In looking at a naphthalene ring, I wonder if we cannot regard it in two fashions: one, as a benzene ring, doubly substituted with an unsaturated side chain. In some respects it is analogous to dimethyl benzene. On the other hand, in looking at a naphthalene ring, I wonder if we cannot regard it as a benzene ring with a long alkyl side chain branch, part of it bent around and fused onto the benzene ring.

These may be mental gymnastics but, occasionally, it seems to me, they help in trying to formulate in our minds at least some order in the vast array of compounds which show a given activity.

DR. McKEEN CATTELL (Cornell University Medical College, New York, New York): I would like to comment on one point which seems to me of some importance to our general problem, and that is the question of the emphasis that should be given to the exceptions in the structural requirements necessary for a given activity.

These exceptions are always challenging, but it seems to me that, when we consider the complexity of the biological systems concerned, it need not be disturbing to find outstanding exceptions in structural relationships.

If we list the numerous factors which may influence the particular biological properties under observation, such as in the study of effects of growth, it becomes apparent that similar effects might be produced by many different types of action. It is evident that such exceptions are of interest not only in relation to further chemical development but particularly in relation to the analysis of the mechanism and site of action of an agent. Of course, to a certain extent, these complexities are eliminated when we are dealing with the simpler enzyme systems, and in these cases the correlation between structure and activity may be more significant.

REVIEW OF THE STRUCTURAL REQUIREMENTS FOR SYMPATHOMIMETIC DRUG ACTION

by

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A review of sympathomimetic amines, summarizing briefly a half-century of investigation, is appropriate at this time inasmuch as research in this field may be considered to have begun with the present century. Epinephrine ('Suprarenin', adrenalin) was isolated from the adrenal gland by Abel and Crawford[1], Aldrich[5] and Takamine[106] in the years 1899 - 1902. Chemical constitution was soon determined and synthesis followed in 1905[27,89]. Resolution of the racemic salt into its optical isomers was first described by Flacher[32] in 1908. This early research has been reviewed in some detail by Trendelenburg[107] and by Hartung.[44]

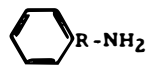
The investigations of Barger and Dale[14], published in 1909-10, extended greatly the field of investigation and emphasized the possibility of obtaining important new sympathomimetic drugs through synthesis. The intervening years have witnessed the appearance of a legion of synthetic compounds which influence activity of organs innervated by the sympathetic nervous system. In this communication, more than two hundred sympathomimetic amines have been reviewed and the significant pharmacologic "screening" data are listed in tables. Although the experimental results obtained by different investigators are often at variance, probably due to dissimilar test procedures and different criteria of effectiveness, the tabulated data will give a general indication of the relative potencies and toxicities. The text tables have been designed to emphasize the importance of variations in chemical structure on sympathomimetic action. Effects on blood pressure and toxicity have been most extensively investigated and will be considered first in this communication.

1. The Effect of Structural Modification of Sympathomimetic Amines on Vasopressor Action and Toxicity

Barger and Dale[14] established the importance of the basic group, β-phenylethylamine, for vasopressor action. The close analogs, α-phenylethylamine (No. 2), β-phenylisopropylamine (No 5) and γ-phenylpropylamine (No. 4) have been reported by most investigators to be distinctly less potent (Table I). Benzylamine (No. 1) and α-phenylpropylamine (No. 7) are likewise very weak vasopressor agents. Methyl substitution at the alpha carbon (No. 5) diminishes

TABLE I

THE EFFECT OF PHENYLALKYLAMINES ON VASOPRESSOR ACTION AND TOXICITY



Compound No.	Structure R	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
1	CH_2	cat	1000				14
2	$CH(CH_3)$	cat	1000				14
3	CH_2CH_2	cat	350-500				14
		cat	183				101
		dog	95				77
		dog	100	mouse	i.v.[b]	LD_{50}, 95±5[c]	60
				rabbit	i.v.	MLD, 60	45
				rat	i.p.	LD, 23	48
				rat	oral	LD, 50	48
4	$CH_2 \cdot CH_2 \cdot CH_2$	cat	1471				101
		dog	700	rat	i.v.	LD_{50}, 50	38

TABLE I (cont.)

Compound No.	Structure R	Pressor Action		Toxicity			Ref.
		Animal	Relative Potency	Animal	Admin.	mgm./kgm.	
5	$CH_2 \cdot CH(CH_3)$	cat	425				101
		dog	321	mouse	i.p.	ALD_{50}, 120	60
		dog	100-200	guinea pig	s.c.	$LD_{3/5}$, 52	7
		dog	250	rat	i.v.	LD_{50}, 20	38
		dog	237				74, 77
				rat	s.c.	MLD, 25	45
				mouse	s.c.	LD_{50}, 79.8	23
6	$CH(CH_3) \cdot CH_2$	cat	1015				101
		dog	500				37
		dog	600	rabbit	i.v.	LD_{50}, 55	109
		dog	350	mouse	i.v.	LD_{50}, 46 $\pm$ 1	60
		dog	254				74
				mouse	i.v.	ALD_{50}, 60-65	110
				mouse	s.c.	LD_{50}, 540	110
7	$CH(C_2H_5)$	cat	800				101
		dog	weak	rat	s.c.	MLD, 1000	45
				rabbit	s.c.	MLD, 50	45
8	$CH(CH_3) \cdot CH(CH_3)$	dog	800	rabbit	i.v.	LD_{50}, 38	109
9	$CH_2CH(C_2H_5)$	cat	500	rat	i.p.	LD, 70	48
				rat	oral	LD, 400	48
		dog	275				77
10	$C(CH_3)_2 \cdot CH_2$	dog	700	mouse	i.p.	ALD_{50}, 50	60
11	$CH_2 \cdot C(CH_3)_2$	dog	1000				60
		dog	600				109
12	$C(CH_3)_2 \cdot CH(CH_3)$	dog	1000	mouse	i.p.	ALD_{50}, 70	60
13	$CH_2 \cdot CH(C_3H_7)$	cat	almost inactive	rat	i.p.	ALD, 70	48
				rat	oral	ALD, 400	48
14	$CH(C_2H_5) \cdot CH(CH_3)$	dog	1000	rabbit	i.v.	LD_{50}, 38	109
15	$CH(i\text{-}C_3H_7) \cdot CH(CH_3)$	dog	1000	rabbit	i.v.	LD_{50}, 30	109

a/ Response obtained after intravenous injection
b/ i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous injection
c/ Values reported as the LD - lethal dose, MLD - minimum lethal dose, LD_{50} - lethal dose $\pm$ the standard error which kills 50 per cent within 24 hours, ALD - approximate LD_{50}

vasopressor potency less than a similar substitution at the beta carbon (No. 6). Increasing the number of carbon atoms in the side chain to more than three (Nos. 8-15) causes a great reduction in potency except in the case of β-phenylbutylamine (No. 9). As pointed out above, the optimum structure is N- $\overset{(1)}{C}$ - $\overset{(2)}{C}$ -phenyl and substitution reduces vasopressor potency in the following order: 1-methyl, 2-methyl 1-ethyl, 2,2-dimethyl, 1-1-dimethyl, 2,2-dimethyl-1,1-dimethyl. (The designation of the position of the carbon atoms as C-1 or C-2 will be used throughout this presentation). The greater effectiveness of compounds substituted by methyl at the first carbon may result from the central nervous system stimulation which these compounds are reported to produce[7,77,109] added to the peripheral cardiovascular effects. No such stimulation has been observed with derivatives substituted at the second carbon[85,109,110].

Substitution of the amine of these simple derivatives by a methyl group generally reduces pressor potency (Table II). Marsh[75,77] found β-phenylethylamine (No. 3) 1.4 times more active

TABLE II

THE EFFECT OF PHENYLALKYLMETHYLAMINES ON VASOPRESSOR ACTION AND TOXICITY

Compound No.	Structure R	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
16	$CH_2 \cdot CH_2$	cat	300-500				14
		dog	200	mouse	i.p.	ALD_{50}, 190	60
		dog	135-148				75
17	$CH_2 \cdot CH_2 \cdot CH_2$	dog	250	rat	s.c.	LD_{50}, 60	38
18	$CH_2 \cdot CH(CH_3)$	cat	695				93
		dog	(d) 116	mouse	i.p.	ALD_{50}, 70	65
			(d, l) 200	mouse	i.p.	ALD_{50}, 70	65
		dog	(l) 272	mouse	i.p.	ALD_{50}, 70	65
		dog	365				74
				rat	i.p.	MLD, 17	48
19	$CH(CH_3) \cdot CH_2$	dog	600	rabbit	i.v.	LD_{50}, 65	109
		dog	260				74
				rabbit	i.v.	LD_{50}, 72 ± 1.7	110
				mouse	i.v.	LD_{50}, 60 ± 3.4	110
				rabbit	s.c.	LD_{50}, 205 ± 15.9	110
				rat	s.c.	LD_{50}, 850 ± 37	110
				rat	i.p.	LD_{50}, 165 ± 9.2	110
20	$CH(CH_3) \cdot CH(CH_3)$	dog	1000	mouse	i.p.	ALD_{50}, 70	60
21	$C(CH_3)_2 \cdot CH_2$	dog	1000	mouse	i.p.	ALD_{50}, 110	60
22	$CH_2 \cdot C(CH_3)_2$	dog	1000				60
23	$C(CH_3)_2 \cdot C(CH_3)_2$	dog	1000	mouse	i.p.	ALD_{50}, 90	60

[a] See footnote, Table I, p. 75

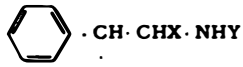
than the corresponding N-methyl analog (No. 16). Derivatives with four or five carbons in the side chain also show this reduction in potency with N-methyl substitution. Results obtained with β-phenylisopropylamine (amphetamine, No. 5) and its N-methyl analog (methamphetamine, No. 18) are somewhat variable. Data obtained with the cat[93, 101] and dog[7, 48, 74, 77] indicate less pressor action with the N-methyl derivative (No. 18). A careful evaluation of β-phenyl-n-propylamine (No. 6) and the N-methyl analog ('Vonedrine', No. 19) by Warren et al[109] did not disclose any important difference in potency. This has been confirmed by Marsh[74].

The toxicities of these phenylalkylamines, shown in Tables I and II, are relatively low. Increasing the size of the side chain apparently causes some increase in toxicity. Thus, β-phenylethylamine is only about one-half as toxic as β-phenylisopropylamine by intravenous injection to rats. It should be noted also that intravenous injection into rabbits of the larger molecules (Nos. 7, 8, 14 and 15) indicated some increase in toxic action over that of β-phenylethylamine (No. 3). The N-methyl derivatives may be slightly less toxic than their primary amine analogs.

The substitution of an alcoholic hydroxyl group at C-2 of phenylalkylamines is generally unfavorable for vasopressor action (Table III). When compared in the same experiments,

TABLE III

THE EFFECT OF PHENYLAMINOALKANOLS ON BLOOD PRESSURE AND TOXICITY



OH

Compound No.	Structure X	Structure Y	Pressor Action[a] Animal	Pressor Action[a] Relative Potency	Toxicity Animal	Toxicity Admin.	Toxicity mgm./kgm.	Ref.
24	H	H	cat	less active than No. 3	rabbit	i. v.[a]	MLD,[a] 80	24
			cat	300-500				14
			cat	124.5				25
					rabbit	i. v.	ALD, 30-90	6
					rat	i. v.	MLD, 140	96
25	CH_3	H	cat	60				101
			cat	80	rabbit	i. v.	MLD, 75	24
			dog	220	mouse	i. p.	ALD_{50}, 440	60
26	C_2H_5	H	cat	2262				101
			cat	1000 (approx.)	rabbit	i. v.	MLD, 50	24
27	C_3H_7	H	dog	none				47
			cat	depressor				101
28	C_4H_9	H	cat	depressor				101
29	C_6H_{13}	H	cat	depressor				101
30	H	CH_3	cat	less active than No. 24	rabbit	i. v.	MLD, 100	24
			dog	270	mouse	i. p.	ALD_{50}, 490	60

TABLE III (Cont.)

Compound No.	Structure X	Structure Y	Pressor Action[a] Animal	Pressor Action[a] Relative Potency	Toxicity Animal	Toxicity Admin.	Toxicity mgm./kgm.	Ref.
31	CH_3	CH_3	cat	(d,l) 190	rabbit	i. v.	MLD, 60	24
			cat	(l) 142	rabbit	i. v.	MLD, 60	24
			cat	(d) 420	rabbit	i. v.	MLD, 80	24
			dog	(d,l) 466	mouse	i. p.	ALD_{50}, 275	60
					(l)rabbit	i. v.	LD_{50}, 60 ± 3.2	110
					(l)rabbit	s. c.	LD_{50}, 175 ± 9.2	110
					(l)mouse	s. c.	LD_{50}, 600 ± 55	110
					(l)rat	s. c.	LD_{50}, 650 ± 109	110
					(l)mouse	s. c.	LD_{50}, 276.9	23
32	C_2H_5	CH_3	cat	1/6 activity of No. 3	rabbit	i. v.	MLD, 45	24
33	C_3H_7	CH_3	cat	depressor	rabbit	i. v.	MLD, 35	24
34	H	$CH(CH_3)_2$	dog	depressor	mouse	i. v.	ALD_{50}, 180	66
35	H	C_4H_9	cat	depressor	rabbit	i. v.	MLD, 50	24
36	CH_3	C_2H_5	cat	No. 31	rabbit	i. v.	MLD, 50	24
37	CH_3	C_3H_7	cat	depressor	rabbit	i. v.	MLD, 50	24
38	CH_3	$CH(CH_3)_2$	cat	sl. pressor	rabbit	i. v.	MLD, 40-50	24
39	CH_3	C_4H_9	cat	depressor	rabbit	i. v.	MLD, 15	24
40	CH_3	C_5H_{11}	cat	depressor	rabbit	i. v.	MLD, 20	24
41	$(CH_3)_2$	H	dog	1·000				60

[a] See footnote, Table I, p. 75

β-phenylethylamine (No. 3) is more pressor than 1-phenyl-2-aminoethanol (No. 24) and N-methyl-β-phenylethylamine (No. 16) more active than 1-phenyl-2-methylaminoethanol (No. 30)[24, 60]. This relationship is not clearly evident with the corresponding propyl derivatives. Tainter[101] found 1-phenyl-2-amino-1-propanol (No. 25) distinctly more pressor than β-phenylisopropylamine in cats. Similar results have been obtained with the dog[60]. However, results obtained with the corresponding methylamines are variable and not in agreement with the above findings for the primary amines. Results obtained with cats suggest higher activity for 1-phenyl-2-methylamino-1-propanol (No. 31), this relationship being reversed in experiments on dogs[24, 60, 65, 93]. Little or no pressor action was obtained with compounds in which the side chain contained more than three carbon atoms. As shown in Table III, most of these higher homologs lowered blood pressure.

A comparison of the most pressor compounds in Tables I-III suggest that the alcoholic hydroxyl at C-2 diminishes toxicity. The toxicity of β-phenylethylamine (No. 3) is probably slightly greater than 1-phenyl-2-aminoethanol (No. 24); β-phenylisopropylamine (No. 5) more than 1-phenyl-2-amino-1-propanol (No. 25). Increasing the size of the alkanol side chain increases toxicity, as previously pointed out for the phenylalkylamines. It is interesting to compare the activities and toxicities of the optical isomers of Nos. 18 and 31. In the case of

No. 18, greatest vasopressor activity is obtained with the d-isomer; with No. 31, with the l-isomer. This difference may result from the marked stimulating action on the central nervous system (possibly the vasomotor centers) observed with the d-isomer of No. 18 (see Table XXV, p. 108). However, this difference in physiological activity between the d- and l-isomers did not significantly influence acute toxicity inasmuch as there was no important difference between the optical isomers and the racemic mixture.

Substitution of the phenyl ring at the 4-position with an hydroxyl increases pressor action of the most pressor derivatives (Table IV). In order of increasing pressor potency, we find

TABLE IV

THE EFFECT OF 4-HYDROXYPHENYLALKYLAMINES ON BLOOD PRESSURE AND TOXICITY

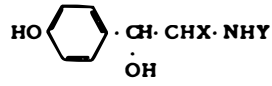
Compound No.	Structure X	Structure Y	Pressor Action[a] Animal	Pressor Action[a] Relative Potency	Toxicity Animal	Toxicity Admin.	Toxicity mgm./kgm.	Ref.
42	CH_2	H	cat	105				14
			cat	70				13
			dog	68	mouse	i.v.	LD_{50}, 280 ± 20	60
			dog	50-100	guinea pig	s.c.	$LD_{3/5}$, 2088	7
					mouse	s.c.	MLD, 2750	12
43	CH_2	CH_3	cat	140				13
			dog	68	mouse	i.p.	ALD_{50}, 760	60
44	$CH(CH_3)$	H	dog	50-100	guinea pig	s.c.	$LD_{4/5}$, 184	7
			dog	59				60
			dog	100	rat	i.v.	LD_{50}, 100	38
			cat	124				25
45	$CH(CH_3)$	CH_3	dog	250	rat	i.v.	LD_{50}, 100	38
46	$CH_2 \cdot CH_2$	H	dog	1750	rat	i.v.	LD_{50}, 170	38
47	$CH_2 \cdot CH_2$	CH_3	dog	750	rat	i.v.	LD_{50}, 170	38

[a] See footnote, Table I, p. 75

l-phenyl-2-aminoethanol (No. 24) < β-phenylethylamine (No. 3) < β-(4-hydroxyphenyl)ethylamine (No. 42). The N-methyl analog of the last compound (No. 43) is equal to or slightly less pressor than the primary amine. The introduction at C-2 of an alcoholic hydroxyl reduces pressor potency (Table V). Thus, l-(4-hydroxyphenyl)-2-aminoethanol (No. 48) is only one-half to one-fifth as pressor as the corresponding ethane derivative.

TABLE V

THE EFFECT OF 4-HYDROXYPHENYLAMINOALKANOLS ON BLOOD PRESSURE AND TOXICITY



Compound No.	Structure X	Structure Y	Effect on Blood Pressure[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
48	H	H	dog	E,[b] 200	mouse	i. p.	ALD_{50}, 600	66
			dog	E, 100				14
			cat	E, 500				78
49	H	CH_3	dog	E, 440	mouse	i. p.	ALD_{50}, 1000	66
			dog	E, 250	mouse	i. v.	LD_{50}, 270 ± 12	60
			cat	E, 116				104
			cat	E, 60-100	(l) mouse	s. c.	MLD, 700-800	58
					(d) mouse	s. c.	MLD, 1500	58
50	H	C_2H_5	dog	I, 600	mouse	i. p.	ALD_{50}, 600	66
			dog	I, 1000[c]				78
51	H	$CH(CH_3)_2$	dog	I, 350	mouse	i. p.	ALD_{50}, 370	66
			dog	I, 200[c]				78
					mouse	i. v.	LD_{50}, 144 ± 10	60
52	H	C_3H_7	dog	I, 1000[c]	mouse	i. p.	ALD_{50}, 300	66
53	H	C_4H_9	dog	I, 1000[c]	mouse	i. p.	ALD_{50}, 150	66
			dog	I, 1000[c]				78
54	H	$CH(CH_3)\cdot C_2H_5$	dog	I, 500				67
55	H	$C(CH_3)_3$	dog	I, 350	mouse	i. p.	ALD_{50}, 250	66
			dog	I, 350				78
56	H	$CH_2\cdot CH(CH_3)\cdot CH_3$	dog	I, very weak	mouse	i. p.	ALD_{50}, 220	66
57	CH_3	H	cat	E, 108	rabbit	i. v.	MLD, 130	24
			cat	E, 67				100
58	CH_3	CH_3	dog	E, 100-200				84
59	C_2H_5	H	dog	E, 1000				67

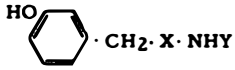
[a] See footnote, Table I, p. 75
[b] E, Multiples of the effective pressor dose of epinephrine
I, Multiples of the effective depressor dose of 'Isuprel'
[c] Approximately

In experiments on the dog, N-alkyl substitution of 1-(4-hydroxyphenyl)-2-aminoethanol (No. 48) results in depressor action when the N-alkyl substituent is larger than methyl. This depressor action has been expressed as multiples of the effective dose of 'Isuprel' (No. 103). The N-isopropyl (No. 51) and N-t.-butyl (No. 55) derivatives were the most potent depressor agents. Derivatives in which the N-alkyl group is propyl, butyl or isobutyl have low activity. This suggests that the structural requirements favorable for depressor action are $-NH\cdot\dot{C}\cdot$ in which the hydrogen atoms of this methyl group are replaced by one (No. 50), two (No. 51) or three (No. 55) methyl groups[64, 66, 67]. Derivative No. 54 in which the N-alkyl group is $-NH\cdot\dot{\underset{\dot{C}H_3}{\overset{H}{C}}}\cdot C_2H_5$ was found to be less potent than Nos. 51 and 55. When there is no methyl substitution, as indicated above, or when larger groups are substituted (Nos. 52, 53 and 56), depressor potency is greatly reduced or abolished. The alcoholic hydroxyl at C-2 is important for this action[66]. This will be discussed in a subsequent portion. Increase in the size of the side chain to propanol (Nos. 57 and 58) does not influence pressor potency; an increase to butanol (No. 59) causes a great reduction.

Hydroxyl substitution of the phenyl ring at the 3-position appears to influence pressor action of these ethane derivatives to no greater extent than does substitution at the 4-position (Tables IV and VI). A comparison of the pressor potencies of β-phenylethylamine (No. 3) with the

TABLE VI

THE EFFECT OF 3-HYDROXYPHENYLALKYLAMINES ON BLOOD PRESSURE AND TOXICITY



Compound No.	Structure X	Structure Y	Pressor Action[a] Animal	Pressor Action[a] Relative Potency	Toxicity Animal	Toxicity Admin.	Toxicity mgm./kgm.	Ref.
60	CH_2	H	cat	100				14
61	$CH(CH_3)$	H	dog	300	rat	i. v.	LD_{50}, 70	38
62	$CH(CH_3)$	CH_3	dog	125	rat	i. v.	LD_{50}, 60	38
63	$CH(CH_3)$	$CH_2\cdot C_6H_5$	dog	2000	rat	i. v.	LD_{50}, 35	38
64	$CH_2\cdot CH_2$	H	dog	300	rat	i. v.	LD_{50}, 90	38
65	$CH_2\cdot CH_2$	CH_3	dog	250	rat	i. v.	LD_{50}, 90	38
66	$CH_2\cdot CH_2$	$CH_2\cdot C_6H_5$	dog	2000	rat	i. v.	LD_{50}, 45	38
67	CH_2	$CH(CH_3)_2$	dog	depressor				

[a] See footnote, Table I, p. 75

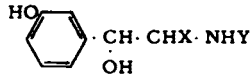
corresponding 3- (No. 60) and 4-hydroxyphenyl (No. 42) analogs suggest that the three compounds have activities of a similar order of magnitude. An increase in the size of the side chain to propyl and alkyl substitution of the amino group reduces pressor potency. Both β-(3-hydroxyphenyl)isopropylamine (No. 61) and γ-(3-hydroxyphenyl)propylamine (No. 65) have been reported to be less effective than β-(3-hydroxyphenyl)ethylamine (No. 60).

A comparison of toxicity data in Tables IV and VI suggest that the 3-hydroxyphenyl derivatives are more toxic than the corresponding 4-hydroxy analogs. A direct comparison of acute intravenous toxicity in the rat of β-(4-hydroxyphenyl)isopropylamine (No. 44) with β-(3-hydroxyphenyl)isopropylamine (No. 61) suggests that the latter is approximately one-third more toxic[38]. Similarly with the γ-phenylpropylamines (Nos. 46 and 64), the 3-hydroxyphenyl compound is about two times more toxic than the 4-hydroxy analog. The addition of an alcoholic hydroxyl at C-2 diminishes toxicity. This is illustrated by compound Nos. 43 (Table IV) and 49 (Table V). The ethanol derivative is approximately one-third less toxic. Unfortunately other toxicity data in Table V cannot be used for direct comparison with that in Table IV, but indicates toxicities of a relatively low order of magnitude.

There is a marked difference in the effect of the alcoholic hydroxyl at C-2, depending upon whether the phenolic hydroxyl is at the 3- or 4-position on the ring. A comparison of the pressor potency of 1-(3-hydroxyphenyl)-2-aminoethanol (No. 68) with that of the 4-hydroxyphenyl analog (No. 48) reveals a five to ten-fold greater potency for the former (Table VII). The N-methyl

TABLE VII

THE EFFECT OF 3-HYDROXYPHENYLAMINOALKANOLS ON BLOOD PRESSURE AND TOXICITY



Compound No.	Structure X	Structure Y	Effect on Blood Pressure: Animal	Relative Potency			Toxicity: Animal	Admin.	mgm./kgm.	Ref.
68	H	H	dog		E,	15-20	mouse	i.p.	ALD_{50}, 370-420	60
							rabbit	i.v.	ALD_{50}, 5-10	60
69	H	CH_3	cat	(d,l)	E,	15	mouse		1000	59
			cat	(d,l)	E,	5.6				100
			cat	(l)	E,	2.3				25
			dog	(l)	E,	7	mouse	i.p.	ALD_{50}, 140	65
			dog	(d,l)	E,	12	mouse	i.p.	ALD_{50}, 420	65
			dog	(d)	E,	77				65
							(l) rabbit	i.v.	LD_{50}, 0.5 ± 0.15	110
							(l) rabbit	s.c.	LD_{50}, 22 ± 2.2	110
							(l) rat	s.c.	LD_{50}, 27 ± 2.9	110
							(l) mouse	s.c.	LD_{50}, 22 ± 4.3	110
70	H	$CH(CH_3)_2$	dog		I,	500	mouse	i.p.	ALD_{50}, 320	66
71	CH_3	H	cat		E,	9.8				25
72	CH_3	CH_3	cat	(l)	E,	107.7				25
73	CH_3	$CH(CH_3)_2$	dog		I,	1000				67

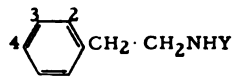
derivative (No. 69) is probably not significantly more pressor than the primary amine (No. 68). The difference in potency between the primary and secondary amine, when the phenolic hydroxyl is in the 4-position (Nos. 48 and 49), is somewhat more definite. Results obtained in this laboratory[66] indicate a definite reduction in activity with N-methyl substitution. The N-isopropyl analog of No. 68 (No. 70) is distinctly vasodepressor, although less potent than 'Isuprel'. Lengthening of the side chain reduces this activity.

These data indicate that the effect of the hydroxyl at C-2 and of N-methyl substitution is influenced by the presence of a phenolic hydroxyl in the 3-position. In its absence these substitutions either are without effect or diminish pressor potency. The increase in pressor activity of the 3-hydroxyphenylalkanolamines is associated with some increase in toxicity.

Hydroxy substitution of the ring at the 3- and 4- or 3-, 4- and 5-positions (Nos. 76 and 77) may increase activity somewhat but not enough to change the order of magnitude (Table VIII). The

TABLE VIII

THE EFFECT OF RING SUBSTITUTION OF β-PHENYLETHYLAMINE ON VASOPRESSOR ACTION



Compound No.	Structure 4	3	2	Y	Pressor Action[a] Animal	Relative Potency	Reference
3	H	H	H	H	cat	183	
					dog	111-116	75
16	H	H	H	CH_3	dog	135-148	75
42	OH	H	H	H	dog	68	60
					dog	53-66	75
					cat	100	75
74	H	OH	H	H	cat	100	14
75	H	H	OH	H	cat	500	14
76	OH	OH	H	H	cat	50	14
					dog	57	60
77	OH	OH	(5-OH)	H	dog	50	40
78	OH	OH	OH	H	cat	100	14
79	F	H	H	H	dog	74-80	75
					cat	120	75
80	F	H	H	CH_3	dog	97-100	75
					cat	220	75
81	Cl	H	H	H	cat	368	98
82	NO_2	H	H	H	cat	823	98
83	CH_3	H	H	H	cat	equals No. 3	43
84	C_2H_5	H	H	H	cat	No. 3	43

TABLE VIII (Cont.)

Compound No.	Structure 4	3	2	Y	Pressor Action [a] Animal	Relative Potency	Reference
85	CH_3	H	H	CH_3	cat	equals No. 3	43
86	H	CH_3	H	CH_3	cat	equals No. 3	43
87	H	H	CH_3	CH_3	cat	one-half as active as No. 3	43
88	NH_2	H	H	CH_3	dog	one-fifth as active as No. 3	18

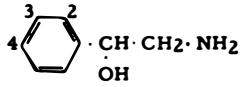
[a] See footnote, Table I, p. 75

2, 3, 4-trihydroxy substituted compound (No. 78) has approximately the same activity as the monosubstituted compounds, Nos. 42 and 60. The β-(4-fluorophenyl)ethylamine derivatives (Nos. 79 and 80) are approximately equal in activity to the unsubstituted compounds (Nos. 3 and 16). The activity of β-(4-chlorophenyl)ethylamine (No. 81) appears to be less than that of the corresponding fluoro-derivative. The 4-nitrophenyl analog is weak. Substitution of the ring with a methyl or ethyl group in the 4-position (Nos. 83-85) does not greatly influence pressor potency. Similarly, the 2- and 3-methyl substituted phenylethylamines (Nos. 86 and 87) have pressor activities comparable to that of the unsubstituted compounds. The 4-amino derivative (No. 88) appears to be distinctly less active than the corresponding unsubstituted compound (No. 16).

The alcoholic hydroxyl at C-2 greatly increases pressor potency when the phenyl ring is substituted by an hydroxyl at the 3-position (see above) or by 3, 4-dihydroxy substitution (No. 90, arterenol, norepinephrine) but is ineffective when the hydroxyls are substituted at the 2, 4- (No. 91) or 2, 5-positions (No. 94). The 2, 3, 4-trihydroxyphenyl derivative (No. 92) is depressor and the 3, 4, 5-trihydroxy derivative is inactive (Table IX). Maximum pressor activity is obtained

TABLE IX

THE EFFECT OF RING SUBSTITUTION OF 1-PHENYL-2-AMINOETHANOL ON VASOPRESSOR ACTION AND TOXICITY



Compound No.	Structure 4	3	2	Pressor Action [a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
48	OH	H	H	dog	200	mouse	i. p.	ALD_{50}, 600	45
89	H	OH	H	dog	20-40	mouse	i. p.	ALD_{50}, 370-420	60

TABLE IX (Cont.)

Compound No.	4	3	2	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
90	OH	OH	H	cat	(d,l) 1.5				14
				cat	(d,l) 1.2				99
				cat	(d,l) 0.5				25
				dog	(l) 0.63				71
				dog	(d) 27.5				71
						(l) rat	i.v.	LD_{50}, 0.1 ± 0.01	50
						(d,l) rat	i.v.	LD_{50}, 0.13 ± 0.02	50
						(d) rat	i.v.	LD_{50}, 1.4 ± 0.14	50
						(l) mouse	i.v.	LD_{50}, 5 ± 1	50
						(d,l) mouse	i.v.	LD_{50}, 7.5 ± 2	50
						(d) mouse	i.v.	LD_{50}, 60 ± 20	50
91	OH	H	OH		very weak				17
92	OH	OH	OH	rabbit	depressor				70
93	OH	OH	(5-OH)	rabbit	inactive				70
94	(5-OH)	H	OH	rabbit	weak				70

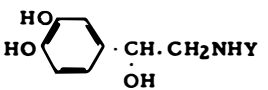
[a] See footnote, Table I, p. 75

with the primary amine (No. 90, nor-epinephrine) or the N-methyl homolog (No. 95, epinephrine). The N-ethyl derivative (No. 96) is both pressor and depressor. Intravenous injection causes an initial transient rise in blood pressure followed by a sharp fall. This diphasic response has also been observed with the N-propyl derivative (No. 97). The N-butyl and -amyl derivatives (Nos. 98 and 99) are depressor. The N- isopropyl compound (No. 100, isopropylarterenol, 'Isuprel', 'Aleudrine') is a potent vasodepressor substance and, for the purpose of this review, activities of other depressor amines are expressed as multiples of the effective dose of this drug. The N-sec. -butyl (No. 101) analog is somewhat less potent and the N-t. -butyl (No. 102) analog more potent than 'Isuprel'. The N-isobutyl (No. 103) and -cyclopentyl (No. 104) analogs are one-fifth to one-ninth as depressor as 'Isuprel'. The substitution of larger N-alkyl groups (Nos. 105-107) causes a marked reduction in depressor potency.

Epinephrine has a very high toxicity and is the most toxic amine tabulated in this review. The primary amine (arterenol) and N-ethylarterenol are slightly less toxic than epinephrine. The substitution of larger N-alkyl groups results in a distinct reduction in toxicity. 'Isuprel' is approximately thirty times less toxic than epinephrine. An examination of these data also shows that the toxicity of the l-isomers of both arterenol and epinephrine are distinctly more toxic than the d-isomers or the racemic mixtures. Similarly, pressor action results largely from the l-isomers inasmuch as the potency of the d-isomer is very small. This high toxicity appears to be closely related to the high excitatory sympathomimetic (vasopressor) actions of these drugs. The effective dose of 'Isuprel' is somewhat less than that for epinephrine[64] but, by comparison, has a very low toxicity. The inhibitory sympathomimetic action, represented by this response, does not appear to be closely related to toxicity. An examination of the toxicity of compound Nos. 48, 89 and 90 (Table IX) shows an increase with hydroxy substitution of the ring in the order 4-hydroxy < 3-hydroxy < 3,4-dihydroxy. The alcoholic hydroxyl at C-2 increases both pressor action and toxicity. However, with a single hydroxyl at the 4-position on the ring both vaso-pressor action and toxicity are diminished by the hydroxyl at C-2. These findings suggest that the increase in toxicity is directly related to the increase in pressor action.

TABLE X

THE EFFECT OF N-ALKYL SUBSTITUTION OF 1-(3,4-DIHYDROXYPHENYL)-2-AMINOETHANOL ON BLOOD PRESSURE ACTION AND TOXICITY



Compound No.	Structure Y	Effect on Blood Pressure[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Reference
90	H	cat	(d,l) E,[b] 0.5-1.5	(see Table IX)			14, 25, 99
		dog	(l) E, 0.63				71
		dog	(d,l) E, 1.35	mouse	i.p.	LD_{50}, 15.6 ± 3.8	64
				(d,l) mouse	i.v.	LD_{50}, 7.5 ± 2	50
95	CH_3		(l) E, 1.0				67, 97
		cat	(d) E, 20.0				
		cat	(d) E, 18.5 ± 0.7				
				(l) mouse	i.v.	LD_{50}, 2.7 ± 0.2	50
				(l) mouse	i.p.	LD_{50}, 4.6 ± 0.5	64
				(l) mouse	s.c.	LD_{50}, 3.86	23
				(l) rat	i.v.	LD_{50}, 0.4 ± 0.004	50
				(l) rat	s.c.	MLD, 5-10	19
				(l) rat	oral	MLD, 30	19
				(l) rabbit	i.v.	MLD, 0.2-0.3	19
				(l) rabbit	s.c.	MLD, 10-20	19
				(d,l) mouse	i.p.	LD_{50}, 7.8 ± 1.3	64
				(d,l) mouse	i.v.	LD_{50}, 4.0 ± 0.5	50
96	C_2H_5	cat	E, I, 20, 10	mouse	s.c.	ALD_{50}, 6	57
		dog	E, I, 5, 3	mouse	i.p.	LD_{50}, 27.5 ± 4.2	64
		dog	E, I, weak, 6-8	mouse	i.v.	LD_{40-60}, 8-12	79
		cat	E, I, weak, 2-5				79
97	C_3H_7	cat	I, weak	mouse	s.c.	ALD_{50}, >200	57
		cat	E, I, weak	mouse	i.p.	LD_{40-60}, 150-300	79
				mouse	i.v.	ALD_{50}, 95	67
98	C_4H_9	dog	I, 17	mouse	i.p.	LD_{50}, 347 ± 12	64

TABLE X (Cont.)

Compound No.	Structure Y	Effect on Blood Pressure[a]		Toxicity			Reference
		Animal	Relative Potency	Animal	Admin.	mgm./kgm.	
99	$n\text{-}C_5H_{11}$	dog	I, 4	mouse	i.v.	ALD_{50}, 36	67
100	$CH(CH_3)_2$	cat	I, 1	mouse	s.c.	ALD_{50}, 60-75	57
		cat	I, 1	mouse	i.p.	$LD_{40\text{-}60}$, 120-300	79
		dog	I, 1	mouse	i.p.	LD_{50}, 494 ± 13	64
				mouse	i.v.	LD_{50}, 77 ± 7	29
				rat	oral	LD_{50}, 2000	29
101	$CH(CH_3)\cdot CH_2\cdot CH_3$	dog	I, weak	mouse	i.p.	$LD_{40\text{-}60}$, 180-300	79
		dog	I, 1.3	mouse	i.p.	LD_{50}, 464 ± 8	64
102	$C(CH_3)_3$	dog	I, 0.4-0.6				79
		rabbit	I, weaker than No. 100	mouse	i.p.	LD_{50}, 370 ± 11	64
				mouse	i.v.	ALD_{50}, 47	67
103	$CH_2\cdot CH(CH_3)\cdot CH_3$	dog	I, 9				67, 97
104	Cyclopentyl	dog	I, 5	mouse	i.v.	ALD_{50}, 53	67
105	$CH(C_2H_5)_2$	dog	I, 25				67
106	$CH(CH_3)\cdot CH(CH_3)_2$	dog	I, 20				67
107	Cyclohexyl	dog	I, 31	mouse	i.v.	ALD_{50}, 67	67

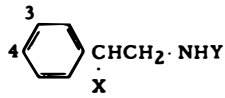
[a] See footnote, Table I, p. 75
[b] See footnote, Table V, p. 80

Significant vasodepressor action is observed with 1-phenyl-2-isopropylaminoethanol (No. 34) but is absent with N-isopropyl-β-phenethylamine[66]. Substitution of the phenyl ring of No. 34 by an hydroxyl at either the 3- or 4-position increases this depressor action, the increases observed being of a similar order of magnitude. Maximum action is obtained when both hydroxyls are present ('Isuprel') and absent when the alcoholic hydroxyl is removed from C-2.[64] The hydroxyl group at C-2 appears to be most important for vasodepressor action.

It would appear that the 3-hydroxy group on the phenyl ring is the key structure for vasopressor action and that the alcoholic hydroxyl at C-2 is similarly the key structure for vasodepressor action. Other substitutions are important insofar as they modify these actions. This is further illustrated by the data shown in Table XI. When the hydroxyl at C-2 of 'Isuprel' is

TABLE XI

THE EFFECT OF THE ALCOHOLIC HYDROXYL ON VASOPRESSOR ACTION AND TOXICITY



Compound No.	Structure 4	3	X	Y	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
3	H	H	H	H	cat	183				101
					dog	100	mouse	i.v.	LD_{50}, 95 ± 5	60
108	OH	H	O	H	cat					14
109	(2,5-dihydroxy)		O	H	cat	inactive				17
110	OH	OH	O	H	cat	23				14
111	OH	OH	O	CH_3	cat	23				14
					dog	52	mouse	i.p.	LD_{50}, 902 ± 25	64
112	OH	OH	O	C_2H_5	cat	15				14
113	OH	OH	O	C_3H_7	cat	140				14
114	OH	OH	O	$CH(CH_3)_2$	dog	>1000	mouse	i.p.	LD_{50}, 470 ± 15.6	64
42	OH	H	H	H	cat	70-105				13, 14
					dog	68	mouse	i.v.	LD_{50}, 280 ± 20	60
76	OH	OH	H	H	cat	50				14
200	(2,5-dimethoxy)		H	H	dog	+ 50 mm. Hg at 0.0025 mM/kgm.	mouse	i.p.	LD_{50}, 161	49
200a	(2,5-dimethoxy)		H	CH_3	dog	+ 50 mm. Hg at 0.002 mM/kgm.	mouse	i.p.	LD_{50}, 124	49
115	OH	OH	H	CH_3	cat	7				14
					dog	6.5	mouse	i.v.	LD_{50}, 770 ± 72	64
116	OH	OH	H	C_2H_5	cat	23				14

TABLE XI (Cont.)

Compound No.	Structure 4	3	X	Y	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
117	OH	OH	H	$CH(CH_3)_2$	dog	pressor or depressor	mouse	i.p.	LD_{50}, 500 ± 22	64
90	OH	OH	OH	H	cat	(d,l) 0.5-1.5				14, 25, 99
					dog	(l) 0.63				71
95	OH	OH	OH	CH_3		1	(See Table X)			
95a	OH	NH_2	OH	CH_3	cat	weak				32a
118	OH	OH	OH	C_2H_5		depressor	(See Table X)			
206	(2,5-dimethoxy)		OH	H	dog	+ 50 mm. Hg at 0.001 mM/kgm.		i.p.	LD_{50}, 131	49
206a	(2,5-dimethoxy)		OH	CH_3	dog	+ 50 mm. Hg at 0.0005 mM/kgm.		i.p.	LD_{50}, 94	49

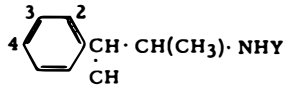
[a] See footnote, Table I, p. 75

replaced by =O (No. 114), the resultant compound is not depressor but is a weak pressor agent. The corresponding primary amine (No. 110) and the N-methyl analog (No. 111) are potent pressor drugs. All of the ketone derivatives shown in this table, except the 2,5-dihydroxyphenyl derivative (No. 109), have some pressor action. Similarly, removal of the hydroxyl at C-2 to give N-isopropyl-β-(3,4-dihydroxyphenyl)ethylamine (No. 117) may cause a rise in blood pressure. The corresponding primary amine (No. 76) and the N-methyl analog (No. 115, 'Epinine') are potent pressor agents.

The replacement of the phenolic hydroxyl at the 3-position on the phenyl ring by a methyl group (No. 119, Table XII) causes a great reduction in pressor potency (compare with No. 68).

TABLE XII

THE EFFECT OF RING SUBSTITUTION OF 1-PHENYL-2-AMINOPROPANOL AND 1-PHENYL-2-METHYLAMINOPROPANOL ON VASOPRESSOR ACTION AND TOXICITY



Compound No.	Structure 4	3	2	Y	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
25	H	H	H	H	cat	60-80	rabbit	i.v.	MLD, 75	2,13
							mouse	i.p.	ALD_{50}, 440	60
119	H	CH_3	H	H	cat	168				101

TABLE XII (Cont.)

Compound No.	Structure 4	3	2	Y	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
120	OH	CH_3	H	H	cat	288				101
121	CH_3	OH	H	H	cat	151				101
122	CH_3O	H	H	H		1/2 as active as No. 25	rabbit	i. v.	MLD, 35	47, 101
123	H	H	CH_3O	H		60-80				47, 101
					cat	226				101
124	CH_3O	H	CH_3O	H	cat	depressor				101
125	CH_3O	CH_3O	(5-CH_3O)	H	cat	very weak				101
126	(5-CH_3O)	H	CH_3O	H	dog	+ 50 mm. Hg at 0.0006 mM/kgm.	mouse	i. p.	LD_{50}, 92	49
126a	(5-CH_3O)	H	CH_3O	CH_3	dog	+ 50 mm. Hg at 0.001 mM/kgm.	mouse	i. p.	LD_{50}, 96	49
127	NH_2	H	H	CH_3	cat	153				101
127a	OH	NH_2	H	CH_3	cat	very weak				32a
128	OH	OH	NH_2	CH_3		very weak				72
129	Cl	H	H	H	cat	248				101

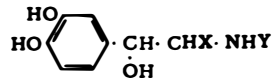
[a] See Footnote, Table I, p. 75

With a 3-methyl group present, the subsequent addition of a 4-hydroxy group (No. 120) causes a further reduction in potency. When these positions are reversed (No. 121), the resultant compound is almost twice as active with a corresponding increase in toxicity. The effect of methoxy substitution on the phenyl ring is variable. The 2-methoxy, 4-methoxy and 2, 5-dimethoxy derivatives equal the pressor activity of the unsubstituted compound (No. 25) or are one-half as active[47, 101]. The 2, 4-dimethoxy and 3, 4, 5-trimethoxy analogs are either very weak or inactive[49, 101]. Amino substitution at the 4-position (No. 127) decreases action and the 2-amino-3, 4-dihydroxy analog (No. 128) is almost inactive. The substitution of chlorine at the 4-position on the ring reduces pressor potency to about one-fourth that of the unsubstituted compound.

Increase in the length of the side chain by substitution at C-1 reduces pressor potency (Table XIII), particularly when the substituent is as large as ethyl (No. 131). Substitution of the amine by a methyl (No. 132) or larger alkyl group results in compounds with depressor action.

TABLE XIII

THE EFFECT OF VARYING THE LENGTH OF THE SIDE CHAIN OF 1-(3,4-DIHYDROXY-PHENYL)-2-AMINOALKANOLS ON BLOOD PRESSURE ACTION AND TOXICITY



Compound No.	Structure X	Structure Y	Effect on Blood Pressure[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
90	H	H	cat	(d,l) E,[b] 0.5-1.5		(See Table IX)		14, 25, 99
			dog	(d,l) E, 1.35				64
130	CH_3	H	dog	E, 2				84
				E, 12	rat	i.v.	LD_{50}, 8	99
			cat	E, 3.3				105
131	C_2H_5	H	cat	I, 300				101
			dog	E,I, 200,200	mouse	i.v.	LD_{50}, 117±1	63, 102
95	H	CH_3		E, 1		(See Table X)		
132	CH_3	CH_3	dog	I				84
100	H	$CH(CH_3)_2$		I, 1		(See Table X)		
133	CH_3	$CH(CH_3)_2$	dog	I, 7	mouse	i.v.	ALD_{50}, 42	60
134	C_2H_5	$CH(CH_3)_2$	dog	I, 16	mouse	i.v.	LD_{50}, 57±2	63
104	H	cyclopentyl	dog	I, 5	mouse	i.v.	ALD_{50}, 53	67
						(See Table X)		
135	C_2H_5	cyclopentyl	dog	I, 14	mouse	i.v.	LD_{50}, 83±7	63
107	H	cyclohexyl	dog	I, 31		(See Table X)		
					mouse	i.v.	ALD_{50}, 67	67
136	CH_3	cyclohexyl	dog	I, 440	mouse	i.v.	ALD_{50}, 47	60
137	C_2H_5	cyclohexyl	dog	I, 500	mouse	i.v.	LD_{50}, 83±4	63

[a] See footnote, Table I, p. 75
[b] See footnote, Table V, p. 80

Depressor action is also dimished by lengthening of the side chain. Thus with the N-isopropyl analogs, the effect on pressor potency of substitution at C-1 is H > CH_3 > C_2H_5 (Nos. 100, 133, 134). Similar results were obtained with the N-cyclopentyl and N-cyclohexyl derivatives. These data suggest that a two carbon side chain is optimum for both pressor and depressor action.

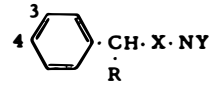
Toxicity of pressor compounds diminishes along with the reduction in pressor potency. This is illustrated by the primary amines in Table XIII (Nos. 90, 130 and 131). Increase in the length of the side chain by substitution at C-1 reduces both pressor action and toxicity. The butanol derivative (No. 131) has about 1/200 - 1/300 the pressor action and about 1/15 the

toxicity of the primary amine (No. 90). All of the depressor compounds have relatively low toxicities. There does not appear to be any clear correlation between the size of the side chain and toxicity with these compounds.

Dialkyl substitution of the amino group (Table XIV) greatly diminishes or abolishes pressor

TABLE XIV

THE EFFECT OF DIALKYL SUBSTITUTION OF THE AMINE GROUP OF VARIOUS PHENYLALKYLAMINES ON VASOPRESSOR ACTION



Compound No.	Structure 4	3	R	X	Y	Pressor Action[a] Animal	Relative Potency	Ref.
138	H	H	H	CH_2	$(CH_3)_2$	dog	weak	83
139	H	H	H	CH_2	$(C_2H_5)_2$	dog	variable	83
140	H	H	H	CH_2	$(C_3H_7)_2$		weak	20
141	H	H	OH	$CH(CH_3)$	$(CH_3)_2$	cat	weak	24
142	H	H	OH	$CH(CH_3)$	CH_3, C_2H_5	dog	(1) very weak	16
143	H	H	OH	$CH(CH_3)$	$(C_2H_5)_2$	cat	very weak	57
144	OH	H	H	CH_2	$(CH_3)_2$	cat	variable	101
145	NH_2	H	H	CH_2	$(CH_3)_2$	dog	>No. 134	18
146	OH	H	OH	CH_2	$(CH_3)_2$	dog	1000	67
147	OH	H	OH	CH_2	$(C_2H_5)_2$	dog	>2000	67
148	OH	H	OH	CH_2	$[CH(CH_3)_2]_2$	dog	inactive	67
149	OH	OH	OH	CH_2	CH_3	cat	25	39
						cat	10-20	88
						dog	25-35	90
						cat	25	108
						dog	40	108

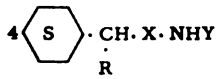
[a] See footnote, Table I, p. 75

action. Only the N-dimethyl analog of arterenol (No. 149) possesses significant vasopressor action. The N-diisopropyl analog (No. 148) of the active vasodepressor compound, 1-(4-hydroxyphenyl)-2-isopropylaminoethanol, is inactive. Toxicity data have been available to the writer for only a few compounds. One of these, No. 149, has been reported to have an intravenous lethal dose in rabbits of 40 mgm./kgm. and a subcutaneous lethal dose in mice of 250 mgm./kgm. The corresponding secondary amine, epinephrine, is approximately 100-200 times more toxic.

The effect of hydrogenation of the phenyl ring on vasopressor action is shown in Table XV.

TABLE XV

THE EFFECT OF HYDROGENATION OF THE PHENYL RING OF VARIOUS SYMPATHOMIMETIC AMINES ON BLOOD PRESSURE AND TOXICITY



Compound No.	Structure 4	R	X	Y	Pressor Action[a] Animal	Relative Potency	Toxicity Animal	Admin.	mgm./kgm.	Ref.
150	H	H	CH_2	H	cat	weak	mouse	i.p.	ALD_{50}, 360	41
					dog	565	mouse	i.p.	ALD_{50}, 150	60
						274				74
151	H	H	CH_2	CH_3	dog	324	mouse	i.p.	ALD_{50}, 125	61
					dog	230				74
152	H	H	CH_2	C_2H_5	dog	350	mouse	i.p.	ALD_{50}, 100	61
153	H	CH_3	CH_2	H	dog	539	mouse	i.p.	ALD_{50}, 90	60
					dog	516				74
154	H	CH_3	CH_2	CH_3	dog	312	mouse	i.p.	ALD_{50}, 100	60
					dog	435				74
155	H	OH	CH_2	CH_3	dog	1000	mouse	i.p.	ALD_{50}, 240	60
156	OH	OH	CH_2	CH_3	dog	>1000	mouse	i.p.	ALD_{50}, 660	60
157	OH	OH	CH_2	$CH(CH_3)_2$	dog	>2000				60
158	H	H	$CH(CH_3)$	H	dog	475				92
					dog	225	mouse	i.p.	ALD_{50}, 60	60
					dog	333				74
					cat	200-400	mouse	i.p.	ALD_{50}, 60	41
159	H	H	$CH(CH_3)$	CH_3	dog	(d,l) 290	mouse	i.p.	ALD_{50}, 70	65
						(d) 400	mouse	i.p.	ALD_{50}, 70	65
						(l) 160	mouse	i.p.	ALD_{50}, 70	65
					dog	(d,l) 479				74
					dog	(d,l) 710				92
160	H	H	$CH_2 \cdot CH_2$	H	cat	200-400	mouse	i.p.	ALD_{50}, 120	41
161	H	H	$CH_2 \cdot CH_2$	CH_3	dog	250	mouse	i.p.	ALD_{50}, 125	61

[a] See footnote, Table I, p. 75

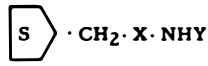
Examination of these data discloses a marked reduction in pressor potency in most instances. Thus, β-cyclohexylethylamine (No. 150) is one-third to one-fifth as potent as the corresponding phenyl analog. A comparison of the activity of the isomers of N-methylcyclohexylisopropylamine (No. 159) with the corresponding phenyl analogs is of interest. The l-isomer of the cyclohexyl derivative is 2.5 times more active than the d-isomer, whereas with the phenyl analog, this relationship is reversed. Although the racemic cyclohexyl derivative is weaker than the corresponding phenyl compound, the activity ratio between the optical isomers of the two compounds is approximately the same, indicating that hydrogenation reduces activity of both isomers to about the same extent but causes a change in the sign of optical rotation. As with the phenyl compounds, both the 4-hydroxyl group on the ring and an alcoholic hydroxyl at C-2 diminish pressor potency. The cyclohexyl analogs of active vasodepressor compounds are weak pressor agents. Saturation of the ring is unfavorable for vasodepressor action.

The effect on toxicity of hydrogenating the phenyl ring is inconstant. The toxicity of cyclohexylisopropylamine is identical with that of the phenyl analog; N-methyl-β-cyclohexylethylamine is about four-fifths as toxic as the phenyl analog. The addition of a 4-hydroxyl and/or an alcoholic hydroxyl at C-2 reduces toxicity (see Nos. 151 and 156). In this respect, they resemble the corresponding phenyl analogs (see Nos. 16 and 49).

Reduction in the size of the ring from cyclohexyl to cyclopentyl may cause some further reduction in pressor potency (Table XVI). A comparison of the pressor potency of β-cyclopentyl-

TABLE XVI

THE EFFECT OF CYCLOPENTYLALKYLAMINES ON VASOPRESSOR ACTION AND TOXICITY



Compound No.	X	Y	Pressor Action[a]		Toxicity			Ref.
			Animal	Relative Potency	Animal	Admin.	mgm./kgm.	
162	CH_2	H	dog	710				92
163	CH_2	CH_3	dog	300	mouse	i.p.	ALD_{50}, 125	61
164	CH_2	C_2H_5	dog	inactive	mouse	i.p.	ALD_{50}, 115	61
165	$CH(CH_3)$	H	dog	350				92
166	$CH(CH_3)$	CH_3	dog	190	mouse	i.v.	LD_{50}, 41.6 ± 1.5	92
					mouse	oral	LD_{50}, 168.7 ± 19.7	92
167	$CH(CH_3)$	C_2H_5	dog	475				92

[a] See footnote, Table I, p. 75

ethylamine (No. 162) with that of the cyclohexyl analog (No. 150) reveals a reduction in potency of thirty to sixty per cent. By contrast with the phenyl series, N-methylation of both the cyclopentyl and cyclohexyl derivatives causes some increase in pressor action over that of the primary amine. This is particularly marked with the cycloalkylisopropylamines (Nos. 158 and 195; Nos. 165 and 166). Swanson and Chen[92] found cyclopentylisopropylamine (Nos. 165) and N-methylcyclopentyliso-

propylamine (No. 166) distinctly more pressor than the corresponding cyclohexyl derivatives (Nos. 158 and 159). Both cyclohexyl- and cyclopentylalkylamines are of comparable toxicity.

The vasopressor action of several aliphatic amines is shown in Table XVII. Barger and

TABLE XVII

THE EFFECT OF ALIPHATIC AMINES ON VASOPRESSOR ACTION

Compound No.	Structure	Pressor Action[a]		Ref.
		Animal	Relative Potency	
168	1-aminobutane	cat	weak	14
		cat	depressor	101
169	1-aminopentane	dog	4800	91
		cat	581	101
170	1-amino-3-methylbutane	dog	3500	91
		cat	variable	101
171	2-aminopentane	dog	2500	91
172	1-aminohexane	dog	1300	91
		dog	525	76
		dog	500	69
173	1-methylaminohexane	dog	400	69
174	2-aminohexane	dog	1100	91
		dog	455	76
175	1-aminoheptane	dog	590	76
		dog	3600	91
176	2-aminoheptane	dog	(d, l) 324	52, 91, 94
			(l) 456	94
			(d) 237	94
			(d, l) 222	76
177	1-amino-3-methylhexane	dog	385	76
178	2-amino-2-methylhexane	dog	inactive	76
179	2-amino-3-methylhexane	dog	900	76
180	2-amino-4-methylhexane	dog	227	76
		dog	280	91
181	2-amino-5-methylhexane	dog	286	76
182	2-methylaminoheptene	dog	< No. 176	3
183	3-aminoheptane	dog	2000	76
184	4-aminoheptane	dog	2500	76

TABLE XVII (Cont.)

Compound No.	Structure	Pressor Action[a]		Reference
		Animal	Relative Potency	
185	1-aminooctane	dog	inactive	91
186	2-aminooctane	dog	1970	91
201	2-amino-2-methylheptane	dog	263	78a
202	2-amino-3-methylheptane	dog	710	78a
203	2-amino-4-methylheptane	dog	590	78a
204	2-amino-5-methylheptane	dog	400	78a
205	2-amino-6-methylheptane	dog	256	78a

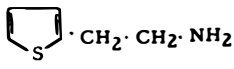
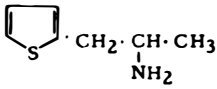
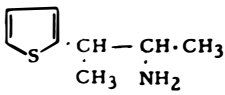
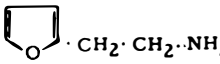
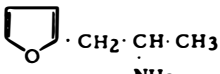
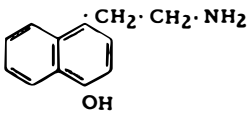
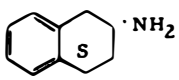
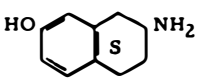
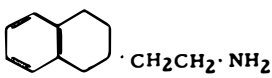
[a] See footnote, Table I, p. 75

Dale[14] and Tainter[101] have shown that there is little or no action on blood pressure with derivatives smaller than 1-aminobutane (No. 168). There is a progressive increase in activity as the length of the chain is increased to six or seven carbon atoms. Greatest vasopressor potency was obtained with 2-amino-4-methylhexane (No. 180) and 2-aminoheptane (No. 176). Further increase in size is not favorable. Thus, 2-aminooctane (No. 186) is much weaker than 2-aminoheptane and 1-aminooctane (No. 185) is inactive. Swanson and Chen[91] have also reported that introduction of a double bond into the aliphatic chain decreases potency, 2-aminohexene, 2-amino-5-methylhexene and 2-amino-6-methylheptene being distinctly less pressor than their saturated analogs. Swanson, Steldt and Chen[94] have examined the optical isomers of 2-aminoheptane. They found the d-isomer to be two times more active than the l-isomer. In this respect, 2-aminoheptane resembles phenylisopropylamine and the N-methyl analog. Toxicity data are not presently available for most of the compounds described in Table XVII. Warren and Werner[110] have described the toxicity of 2-aminoheptane. They have reported the following data (mgm./kgm.): rabbit, i.v., 22 ± 14; i.m., 85 ± 5.7; s.c., 130 ± 14.4; rat, i.p., 34 ± 2.2; s.c., 135 ± 6.1 to 160 ± 13.0 (light and heavy animals respectively); mice, s.c., 115 ± 11.6.

Miscellaneous compounds, evaluated for vasopressor action, are shown in Table XVIII. The thiophene derivatives (Nos. 187-189) have approximately the same potencies as the corresponding phenyl analogs. Similarly, the toxicities are approximately equal to those of the phenyl analogs (Nos. 5 and 6). Other compounds shown are variable or depressor in action and probably do not have true sympathomimetic action. Many amines, when injected intravenously in doses of 0.5-2.0 mgm./kgm., will cause similar variable responses.

TABLE XVIII

THE EFFECT OF THIENYL- AND FURYLALKYLAMINES ON VASOPRESSOR ACTION AND TOXICITY

Compound No.	Structure	Pressor Action[a]		Toxicity			Ref.
		Animal	Relative Potency	Animal	Admin.	mgm./kgm.	
187		cat	183 equals No. 3				98 15
188		dog dog	100 620	mouse rabbit	i.p. i.v.	LD_{50}, 114 LD_{50}, 35	9 109
189		dog	less active than No. 188	rabbit	i.v.	LD_{50}, 63	109
190		rabbit	variable - often depressor				33, 101
191		dog	300	mouse	i.p.	LD_{50}, 348	9
192		cat	depressor				101
193		cat	variable				101
194		cat	depressor				101
195		cat	depressor				101

[a] See footnote, Table I, p. 75

2. The Effect of Structural Modification of Sympathomimetic Amines on Bronchodilator Action

In the preceding section, the vasopressor and depressor actions of sympathomimetic amines have been described. These effects are the net resultant of several factors such as vasoconstriction, alterations in the rate and amplitude of cardiac contraction and, in some instances, alterations in the respiratory movements of the thorax and diaphragm. Thus, a weak vasodilator drug which stimulates the heart or increases the amplitude of respiration may actually cause a rise in blood pressure due to increased filling of the arterial side of the vascular system. Inhibitory sympathomimetic potency can be determined more accurately when only a single organ is involved and where the predominant response is one of inhibition. The smooth muscles of the bronchioles are convenient for such an evaluation. Isolated lung preparations have been most often used for this purpose and the method of Sollmann and von Oettingen as modified by Tainter[103] has become a standard screening test for the evaluation of sympathomimetic bronchodilator action.

The bronchodilator action of various phenyl and hydroxyphenyl derivatives is shown in Table XIX. Most of the phenyl compounds either cause bronchoconstriction or are ineffective.

TABLE XIX

BRONCHODILATOR ACTION OF SYMPATHOMIMETIC AMINES - PHENYL AND HYDROXYPHENYL DERIVATIVES

Compound No.	Structure 4	3	R	X	Y	Bronchodilator Action: Preparation	Bronchodilator Action: Relative Potency[a]	Ref.
24	H	H	OH	H	H	cat, dog	inactive	96
6	H	H	CH_3	H	H	rabbit	constricts	38
5	H	H	H	CH_3	H	rabbit guinea pig	constricts constricts	37, 38 103
25	H	H	OH	CH_3	H	guinea pig	constricts	103
31	H	H	OH	CH_3	CH_3	guinea pig	385	103
127	NH_2	H	OH	CH_3	CH_3	guinea pig	178	103
120	OH	CH_3	OH	CH_3	H	guinea pig	constrictor	103
42	OH	H	H	H	H	cat, dog	constricts	95
48	OH	H	OH	H	H	guinea pig	inactive	66
49	OH	H	OH	H	CH_3	guinea pig dog, cat	weak 375	66 42
50	OH	H	OH	H	C_2H_5	guinea pig	weak	66
51	OH	H	OH	H	$CH(CH_3)_2$	guinea pig	weak	66
68	H	OH	OH	H	H	guinea pig	inactive	67

TABLE XIX (Cont.)

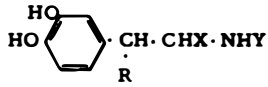
Compound No.	Structure 4	3	R	X	Y	Bronchodilator Action Preparation	Relative Potency[a]	Ref.
69	H	OH	OH	H	CH_3	guinea pig	(d,l) 63.7	103
						guinea pig	(d,l) >3000	67
						dog	weak	81a
70	H	OH	OH	H	$CH(CH_3)_2$	guinea pig	50-100	67
72	H	OH	OH	CH_3	CH_3	guinea pig	21.7	103
1-N-Ethylephedrine (No. 142) has a bronchodilator action in rabbit, cat and guinea pig lungs equal to that of ephedrine[16].								

[a] Expressed as multiples of the effective dose of epinephrine

Only ephedrine (No. 31) and the 4-aminophenyl analog (No. 127) induce measurable bronchodilation and they are relatively weak drugs. The hydroxyphenyl derivatives in which the hydroxyl is in the 4-position are also quite weak or inactive. Compounds in which the hydroxyl is in the 3-position on the ring are distinctly more effective. Compound Nos. 69, 70 and 72 are moderately effective in dilating the bronchioles of the isolated guinea pig lung. A comparison of β-(3,4-dihydroxyphenyl)ethylamine (No. 76) and the N-methyl analog (No. 115) indicates that they are not significantly more effective than 1-(3-hydroxyphenyl)-2-methylaminoethanol (No. 69). Greatest bronchodilator action is obtained when there are hydroxyl groups at both the 3- and 4-positions on the phenyl ring and when there is an alcoholic hydroxyl at C-2. The importance of the alcoholic group is illustrated by results obtained with compound Nos. 115 and 95. The former

TABLE XX

BRONCHODILATOR ACTION OF SYMPATHOMIMETIC AMINES - CATECHOL DERIVATIVES



Compound No.	Structure R	X	Y	Bronchodilator Action Preparation	Relative Potency[a]	Ref.
76	H	H	H	dog, cat	50	10
				guinea pig	weak	10
				guinea pig	350	60
196	H	CH_3	H	dog, cat	50	10
115	H	H	CH_3	guinea pig	50.5	103
				dog	10-20	22
117	H	H	$CH(CH_3)_2$	guinea pig	150	62
111	O:	H	CH_3	guinea pig	167	62

TABLE XX (Cont.)

Compound No.	Structure R	X	Y	Bronchodilator Action Preparation	Relative Potency[a]	Ref.
90	OH	H	H	guinea pig	(d,l) 7.4	103
					(d,l) 280	64
					(d,l) 10-14	79
					(l) 17	71
					(d) 1000	71
95	OH	H	CH_3	guinea pig	(l) 1.0	
96	OH	H	C_2H_5	guinea pig	1.0	87
100	OH	H	$CH(CH_3)_2$	guinea pig	0.12	62
97	OH	H	C_3H_7	guinea pig	6-10	79
				guinea pig	0.5	86
98	OH	H	C_4H_9	guinea pig	0.72	86
99	OH	H	C_5H_{11}	guinea pig	0.30	86
101	OH	H	$CH(CH_3)CH_2 \cdot CH_3$	guinea pig	1.0	87
102	OH	H	$C(CH_3)_3$	guinea pig	0.2	79
104	OH	H	cyclopentyl	guinea pig	0.05-0.1	86
107	OH	H	cyclohexyl	guinea pig	0.6-0.8	86
130	OH	CH_3	H	guinea pig	14.7	103
				dog	100 approx.	22
133	OH	CH_3	$CH(CH_3)_2$	guinea pig	very weak	67
131	OH	C_2H_5	H	guinea pig	71.1	103
					6.1	63
134	OH	C_2H_5	$CH(CH_3)_2$	guinea pig	0.45	63
135	OH	C_2H_5	cyclopentyl	guinea pig	0.12	63
137	OH	C_2H_5	cyclohexyl	guinea pig	6	63

[a] See footnote, Table XIX, p. 99

('Epinine') has only one-tenth to one-fiftieth as much bronchodilator action as the latter (epinephrine). The primary amine, 1-(3,4-dihydroxyphenyl)-2-aminoethanol (arterenol, No. 90) is a weak bronchodilator agent. N-Alkylation greatly increases potency. Epinephrine appears to be at least seventeen times more active than the primary amine (arterenol). The N-isopropyl derivative (No. 100, 'Isuprel') is eight to ten times more effective than epinephrine. A further increase in potency is obtained when the N-alkyl group is cyclopentyl (No. 104). Lengthening of the side chain does not increase bronchodilator action. Compounds with four carbons in the side chain (Nos. 134-137) are somewhat less effective than the corresponding analogs in which there are only two carbons. Almost no bronchodilation was observed with 1-(3,4-dihydroxyphenyl)-2-

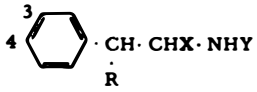
isopropylaminopropanol (No. 133). These data suggest that the order of importance of the various substituents is: (1) alcoholic hydroxyl at C-2, (2) an N-alkyl substituent, (3) an hydroxyl at the 3-position on the ring and (4) hydroxyls at the 3- and 4-positions.

3. The Effect of Structural Modification of Sympathomimetic Amines on Uterine Action

The uterus may be either stimulated (contracted) or inhibited (relaxed) by sympathomimetic amines. Results obtained with isolated non-gravid uteri are shown in Tables XXI and XXII.

TABLE XXI

THE EFFECT OF SYMPATHOMIMETIC AMINES ON UTERINE MOTILITY - PHENYL AND HYDROXYPHENYL DERIVATIVES



Compound No.	Structure 4	3	R	X	Y	Uterine Action[a] Animal	Action	Relative Potency[b]	Ref.
3	H	H	H	H	H	rat	I[c]	weak	66
						guinea pig	E	weak	24, 66
						rabbit	E, I or N		51, 66
19	H	H	CH_3	H	CH_3	rabbit	E	very weak	109
						guinea pig	E	very weak	109
24	H	H	OH	H	H	rabbit	E	280	72
						rabbit	E		51
						guinea pig	E		24
30	H	H	OH	H	CH_3	guinea pig	E		24
35	H	H	OH	H	C_4H_9	guinea pig	E		24
25	H	H	OH	CH_3	H	rabbit	E		51
31	H	H	OH	CH_3	CH_3	guinea pig	E		24, 51
						rat	I	5000	35
42	OH	H	H	H	H	rabbit	E, E or I		28
						rabbit	E		66
						rat	I	300,000-1,000,000	2, 35, 66
						cat	E or I		28
						guinea pig	E		66
48	OH	H	OH	H	H	rat	I		66
						rabbit	E		66
						guinea pig	E		66
49	OH	H	OH	H	CH_3	rat	I		66
						rabbit	N		66
						guinea pig	N		66
						cat	I	70-100	58

TABLE XXI (Cont.)

Compound No.	Structure 4	3	R	X	Y	Uterine Action[a] Animal	Action	Relative Potency[b]	Ref.
50	OH	H	OH	H	C_2H_5	rat	I		66
						rabbit	I		66
						guinea pig	N		66
51	OH	H	OH	H	$CH(CH_3)_2$	rat	I		66
						rabbit	I		66
						guinea pig	N		66
57	OH	H	OH	CH_3	H	rabbit	E or I		51
68	H	OH	OH	H	CH_3	rat	I	10	59
						rabbit	E		19a
71	H	OH	OH	CH_3	H	rabbit	E		51
						guinea pig	E		51
142	1-N-ethylephedrine					guinea pig	E	weak	16
						rabbit	E	weak	16

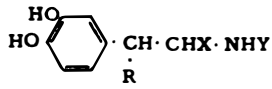
[a] Isolated non-gravid uteri were used
[b] Multiples of the effective dose of epinephrine
[c] E, contraction; I, relaxation; N, no effect

The phenylalkylamines are excitatory substances of relatively low potency (except in the case of the rat in which uterine relaxation is obtained with all sympathomimetic amines). The presence of an alcoholic hydroxyl at C-2 is not alone sufficient for inhibitory action. This action is not apparent, to a significant degree, until three of the four important substitutions have been made. Compound Nos. 49, 50 and 51 containing an hydroxyl group at the 4-position on the ring, an alcoholic hydroxyl at C-2 on the side chain and an N-alkyl group are predominantly inhibitory in action. Relatively slight changes in structure cause a return of excitatory action. This is illustrated by the predominantly excitatory compounds, 1-(4-hydroxyphenyl)-2-aminoethanol (No. 48), in which the N-alkyl group is missing and by tyramine (No. 42) in which both the N-alkyl group and the hydroxyl at C-2 are missing.

The catechol derivatives (Table XXII) may induce either contraction or relaxation, depending upon the kind of substitutions made at C-2 and at N. Hydroxytyramine (No. 76) has been reported to have a weak inhibitory action on the rat and cat uterus[35, 42]. The N-methyl analog ('Epinine', No. 115) is excitatory to the rabbit uterus. The presence of an alcoholic group at C-2 increases excitatory action. Epinephrine (No. 95) is four times more potent than 'Epinine'. The primary amine (arterenol, No. 90) is only slightly less potent than epinephrine. Inhibitory action is dominant with N-alkylarterenols in which the alkyl substituent is larger than ethyl. Branching of this group is important inasmuch as both the N-sec.-butyl (No. 101) and N-t.-butyl (No. 102) compounds are more active than the N-n.-butyl analog (No. 98). When the side chain is increased to four carbons, inhibitory action is weak in the absence of an N-alkyl group (No. 131). However, both the N-isopropyl and N-cyclopentyl analogs (Nos. 134 and 135) are potent inhibitory agents. The N-cyclohexyl (No. 137) analog is weak. In this respect, the cyclopentyl group most nearly resembles the isopropyl and t.-butyl groups (see Sec. 1).

TABLE XXII

THE EFFECT OF SYMPATHOMIMETIC AMINES ON UTERINE MOTILITY - CATECHOL DERIVATIVES



Compound No.	Structure R	X	Y	Uterine Action[a] Animal	Action	Relative Potency	Ref.
76	H	H	H	cat	I	90	42
				rat	I	2000-6000	35
115	H	H	CH_3	rabbit	E	4	64
				rat	I	20	62
				rat	I	120	35
117	H	H	$CH(CH_3)_2$	rabbit	I	400	64
				rat	I	10	62
111	O:	H	CH_3	rabbit	E	400	64
				rat	I	2	62
				rat	I	25	35
114	O:	H	$CH(CH_3)2$	rabbit	N		64
90	OH	H	H	rabbit	(d,l) E	4-5	64, 111
				rabbit	(l) E	1	71
				guinea pig	(l) E, I	10-20	71, 79
				rat	(d,l)	100	111
				rat	(l) I	30	71
				rat	(l) I	10	62
				cat	(d,l) I	10	111
95	OH	H	CH_3	rabbit	(l) E	1.0	
				guinea pig	(l) E, I	1.0	
				rat	(l) I	1.0	
96	OH	H	C_2H_5	rabbit	E	4	64
				guinea pig	E	40	64
				guinea pig	I	10	79
				rat	I	0.5-1.0	35
100	OH	H	$CH(CH_3)_2$	rabbit	I	1.0	64
				guinea pig	I	1.0	64
				rat	I	0.5-1.0	35
98	OH	H	C_4H_9	rabbit	I	40	64
				guinea pig	I	40	64
101	OH	H	$CH(CH_3)\cdot CH_2\cdot CH_3$	rabbit	I	1.0	64
				guinea pig	I	2.0	79
102	OH	H	$C(CH_3)_3$	rabbit	I	4.0	64
				guinea pig	I	40.0	64
				guinea pig	I	2.0	79

TABLE XXII (Cont.)

Compound No.	Structure R	X	Y	Uterine Action[a] Animal	Action	Relative Potency	Ref.
131	OH	C_2H_5	H	guinea pig	I	weak	63
134	OH	C_2H_5	$CH(CH_3)_2$	guinea pig	I	2.0	63
135	OH	C_2H_5	cyclopentyl	guinea pig	I	2.0	63
137	OH	C_2H_5	cyclohexyl	guinea pig	I	weak	63

[a] See footnote, Table XXI, p. 102

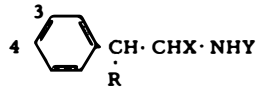
The non-gravid uteri of most species of animals are stimulated by phenylalkylamine derivatives. Both excitatory and inhibitory actions may be increased when there is an alcoholic hydroxyl present at C-2. Inhibitory action is enhanced by N-alkyl substitution, particularly by branched groups such as isopropyl or t. -butyl. The significance of this grouping has been recently reviewed[66a] and will be discussed in this communication in the summary section. The above data suggest that inhibitory action on the uterus is favored by substitution, in the order (1) an alcoholic hydroxyl at C-2, (2) N-alkyl substitution, (3) an hydroxyl at the 4-position on the phenyl ring and (4) hydroxyls at the 3- and 4-positions. Excitatory action is favored by (1) hydroxy substitution of the ring at the 3- and 4-positions, (2) an alcoholic hydroxyl at C-2 and (3) by a N-methyl group.

4. The Effect of Structural Modification of Sympathomimetic Amines on Intestinal Action

Results obtained with isolated intestinal segments are shown in Tables XXIII and XXIV. In the absence of an hydroxyl at C-2, the predominant action is excitatory (No. 3). Inhibitory and excitatory actions have been reported for 1-phenyl-2-aminoethanol (No. 24). The N-alkyl derivatives of No. 24 are inhibitory. The phenylpropylamines (Nos. 5, 25 and 31) are variable

TABLE XXIII

THE EFFECT OF SYMPATHOMIMETIC AMINES ON INTESTINAL MOTILITY - PHENYL AND HYDROXYPHENYL DERIVATIVES



Compound No.	Structure 4	3	R	X	Y	Intestinal Action[a] Animal	Part	Action	Relative Potency[b]	Ref.
3	H	H	H	H	H	guinea pig	ileum	E[c]	weak	66
						rabbit	sm. int.	E, I	weak	24
						rabbit	ileum	E		51
						rabbit	colon	E		51

TABLE XXIII (Cont.)

Compound No.	Structure 4	3	R	X	Y	Intestinal Action[a] Animal	Part	Action	Relative Potency[b]	Ref.
24	H	H	OH	H	H	rabbit	duodenum	I	±1200	96
						cat	sm. int.	I,E	weak	96
						rabbit	sm. int.	I		24
						rabbit	ileum	E,I		51
						rabbit	colon	I		51
30	H	H	OH	H	CH_3	rabbit		I		24
34	H	H	OH	H	$CH(CH_3)_2$	guinea pig	ileum	I	weak	66
35	H	H	OH	H	C_4H_9	rabbit	sm. int.	I		24
5	H	H	H	CH_3	H	rabbit	jejunum	I	weak	109
25	H	H	OH	CH_3	H	rabbit	ileum	I		51
						rabbit	colon	I		51
31	H	H	OH	CH_3	CH_3	rabbit	sm. int.	E		24
						rabbit	ileum	E,I		51
						rabbit	colon	E,I		51
19	H	H	CH_3	H	CH_3	rabbit	jejunum	I	weak	109
42	OH	H	H	H	H	guinea pig	ileum	N		66
						rabbit	sm. int.	E		95
						cat	sm. int.	E,I		95
						rabbit	sm. int.	I,E		80
48	OH	H	OH	H	H	rabbit	jejunum	I	500	78
49	OH	H	OH	H	CH_3	rabbit	jejunum	I	500	78
						rabbit	sm. int.	I	100	58
						rabbit	ileum	I		51
						rabbit	colon	I		51
						(1) rabbit	sm. int.	I	1000-10,000	31
50	OH	H	OH	H	C_2H_5	rabbit	jejunum	I	500	78
						guinea pig	ileum	I	weak	66
51	OH	H	OH	H	$CH(CH_3)_2$	rabbit	jejunum	I	500	78
						guinea pig	ileum	I	weak	66
55	OH	H	OH	H	$C(CH_3)_3$	rabbit	jejunum	I	<500	78
						guinea pig	ileum	I	weak	66
53	OH	H	OH	H	C_4H_9	rabbit	jejunum	I	500	78
						guinea pig	ileum	N		66
57	OH	H	OH	CH_3	H	rabbit	ileum	I		51
						rabbit	colon	I		51
69	H	OH	OH	H	CH_3	rabbit	sm. int.	I	12	59
						rabbit	sm. int.	I	10	31

TABLE XXIII (Cont.)

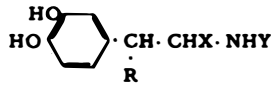
Compound No.	Structure 4	3	R	X	Y	Intestinal Action[a] Animal	Part	Action	Relative Potency[b]	Ref.
71	H	OH	OH	CH_3	H	rabbit	ileum	I		51
						rabbit	colon	I		51

[a] Isolated intestinal segment used
[b] Multiples of the effective dose of epinephrine
[c] E, contraction; I, relaxation; N, no effect

in action and may cause either excitation or inhibition. Tyramine, like β-phenylethylamine, is excitatory. The addition of an hydroxyl at C-2 gives rise to compounds that are inhibitory. Thus 1-(4-hydroxyphenyl)-2-aminoethanol is predominantly inhibitory whereas tyramine may cause excitation. Comparison of 1-(3-hydroxyphenyl)-2-methylaminoethanol (No. 69) with 1-(4-hydroxyphenyl)-2-methylaminoethanol (No. 49) suggests that the hydroxyl at the 3-position on the ring is more important than that at the 4-position. The former compound is ten to fifty times more potent than the latter. Substitution of hydroxyls at the 3- and 4-position on the ring in the absence of an hydroxyl at C-2 (No. 76) gives an inhibitory compound but the inhibitory action does not exceed that of 1-(3-hydroxyphenyl)-2-methylaminoethanol. The subsequent addition of an hydroxyl at C-2 (No. 90, arterenol) causes a marked increase in inhibitory potency.

TABLE XXIV

THE EFFECT OF SYMPATHOMIMETIC AMINES ON INTESTINAL MOTILITY - CATECHOL DERIVATIVES



Compound No.	Structure R	X	Y	Intestinal Action[a] Animal	Part	Action	Relative Potency	Ref.
76	H	H	H	rabbit	sm. int.	I	20-40	42
				cat	sm. int.	I	17	42
115	H	H	CH_3	guinea pig	ileum	I	17	62
				guinea pig	ileum	I, E[c]	5, 1-2.5	64
				rat	colon	I	100-200	35
117	H	H	$CH(CH_3)_2$	guinea pig	ileum	I	200	35
111	O:	H	CH_3	rabbit	sm. int.	I	25-50	51
				guinea pig	ileum	I	20	64
				rat	colon	I	20	35
114	O:	H	$CH(CH_3)_2$	guinea pig	ileum	N		64

TABLE XXIV (Cont.)

Compound No.	Structure R	X	Y	Intestinal Action[a] Animal	Part	Action	Relative Potency	Ref.
90	OH	H	H	(d,l) rabbit	sm. int.	I	2	51
				(d,l) rabbit	jejunum	I	2-4	79
				(d,l) rabbit	ileum	I	2	111
				(l) rabbit	ileum	I	1	71
				(d) rabbit	ileum	I	60	71
				(d,l) cat	ileum	I	1	111
				(d,l) rat	ileum	I	3	111
				(d,l) guinea pig	ileum	I	5	64
				(d,l) guinea pig	ileum	I	2	79
				(l) guinea pig	ileum	I	1.37	71
				(d) guinea pig	ileum	I	35-40	71
				(l) guinea pig	ileum	I	0.5	62
				(d,l) rat	colon	I	0.2-1.0	35
96	OH	H	C_2H_5	rabbit	jejunum	I	4	79
				guinea pig	ileum	I	2	64
100	OH	H	$CH(CH_3)_2$	rabbit	ileum	I	2	79
				guinea pig	ileum	I	1-2	62, 79
				rat	colon	I	1	35
101	OH	H	$CH(CH_3)CH_2CH_3$	rabbit	jejunum	I	>20	79
				guinea pig	ileum	I	1-2	64
102	OH	H	$C(CH_3)_3$	rabbit	jejunum	I	>20	79
				guinea pig	ileum	I	10-20	64, 79
98	OH	H	C_4H_9	guinea pig	ileum	I	20	64
134	OH	C_2H_5	$CH(CH_3)_2$	guinea pig	ileum	I	110	63
135	OH	C_2H_5	cyclopentyl	guinea pig	ileum	I	100	63
137	OH	C_2H_5	cyclohexyl	guinea pig	ileum	I	320	63

[a] See footnote, Table XXIII, p. 106

The major portion of this activity is attributable to the l-isomer. The d-isomer has only one-thirty-fifth to one-fortieth the activity of the l-isomer[71]. Comparison of results obtained with epinephrine, which is the l-isomer of the N-methyl analog, indicates that these l-isomers are of comparable potency. The substitution of larger groups for the N-methyl group does not increase intestinal inhibitory action. Thus the N-isopropyl derivative (No. 100, 'Isuprel') is approximately equal to epinephrine and arterenol; the N-sec.-butyl and N-t.-butyl derivatives are less potent. An increase in the size of the side chain to four carbons is not favorable for inhibitory action. Thus the butanol derivative, No. 134, is distinctly less potent than the corresponding ethanol derivative, No. 100.

Youmans, Aumann and Haney[112] have made a qualitative comparison of intestinal inhibitory action of several sympathomimetic amines in dogs with Thiry-Vella fistulas. The drugs were administered intravenously and the response to epinephrine was used as a basis for comparison. The drugs studied with results, expressed in terms of the effective dose of epinephrine, are shown below:

epinephrine	1.0
(d,l) arterenol (No. 90)	1.5-4.0
'Cobefrine' (No. 130)	2.5-10.0
'Epinine' (No. 76)	10-25
'Kephrine' (No. 111)	25-100
'Neo-Synephrine' (1, No. 69)	25-100
'Sympatol' (No. 49)	660-2500

These results are in general agreement with those obtained with the isolated intestinal segment. Addition of an hydroxyl at C-2 increases inhibitory action ten to twenty-five times ('Epinine' vs. epinephrine). A comparison of the activity of (l) 'Neo-Synephrine' with that of (d,l) 'Sympatol' reveals the importance of the hydroxyl at the 3-position on the ring. Although the comparison is made with the l-isomer of the former and the d,l form of the latter, the results obtained suggest different orders of activity, the 3-hydroxyphenyl analog being about ten times more potent than the latter.

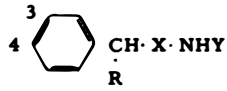
The data in Tables XXIII and XXIV suggest that intestinal excitatory action of phenylalkylamine derivatives is weak and is suppressed or obscured by any one of the four basic substitutions. In the presence of an hydroxyl at C-2, inhibitory potency is increased by substitution of an hydroxyl (1) at the 3-position on the phenyl ring and (2) at the 3- and 4-positions on the ring. An N-alkyl group does not appear important for this action.

5. The Effect of Structural Modification of Sympathomimetic Amines on Central Nervous System Stimulation

A quantitative comparison of the stimulating action of sympathomimetic amines on the central nervous system has been made in a few instances. These data are shown in Table XXV.

TABLE XXV

STIMULATING ACTION OF SYMPATHOMIMETIC AMINES ON THE CENTRAL NERVOUS SYSTEM



Compound No.	Structure 4	3	R	X	Y	Central Nervous System Stimulation: Animal	Dose Used mgm./kgm.	Admin.	Effect	Ref.
3	H	H	H	CH_2	H	rat	80	s.c.	+	85
						mouse	30	i.p.	+	48
30	H	H	H	CH_2	CH_3	rat	160	s.c.	±	85
						mouse	>50	i.p.	±	48
4	H	H	H	$CH_2 \cdot CH_2$	H	rat	>10	s.c.	0	85

TABLE XXV (Cont.)

Compound No.	Structure 4	3	R	X	Y	Central Nervous System Stimulation: Animal	Dose Used mgm./kgm.	Admin.	Effect	Ref.
5	H	H	H	$CH(CH_3)$	H	rat	(d, l) 2.5	s.c.	++	85
						rat	(d, l) 50	oral	+++	48
						mouse	(d, l) 0.55	i.p.	+	109
						rat	(d) 2.5	s.c.	++	85
							(l) 10.0	s.c.	+	85
18	H	H	H	$CH(CH_3)$	CH_3	rat	(d, l) 0.25	s.c.	+	81
						rat	(d) 0.125	s.c.	+	81
						rat	(l) 2.0	s.c.	+	81
							(d, l) 0.9	i.p.	+	48
19	H	H	CH_3	CH_2	CH_3	rat	50	oral	0	109
9	H	H	H	$CH(C_2H_5)$	H	mouse	8	i.p.	+	48
13	H	H	H	$CH(C_3H_7)$	H	mouse	14	i.p.	+	48
197	H	H	H	$CH(CH_3)$	C_2H_5	rat	4	s.c.	+	81
198	H	H	H	$CH(CH_3)$	C_4H_9	rat	>128	s.c.	0	81
199	H	H	H	$CH(CH_3)$	C_5H_{11}	rat	>128	s.c.	toxic	81
25	H	H	OH	$CH(CH_3)$	H	rat	40	s.c.	+	85
26	H	H	OH	$CH(C_2H_5)$	H	rat	>320	s.c.	0	85
27	H	H	OH	$CH(C_3H_7)$	H	rat	320	s.c.	+	85
28	H	H	OH	$CH(C_4H_9)$	H	rat	>80	s.c.	0	85
31	H	H	OH	$CH(CH_3)$	CH_3	rat	(d, l) 16	s.c.	+	85
						rat	(l) 5	s.c.	+	85
							(d) 40	s.c.	+	85
							(d, l) 19	i.p.	+	48
42	OH	H	H	CH_2	H	rat	>320	s.c.	0	85
43	OH	H	H	CH_2	CH_3	rat	160	s.c.	+	85
44	OH	H	H	$CH(CH_3)$	H	rat	80	s.c.	+	85
49	OH	H	OH	CH_2	CH_3	rat	>320	s.c.	0	85
58	OH	H	OH	$CH(CH_3)$	CH_3	rat	>32	s.c.	0	85
76	OH	OH	H	CH_2	H	rat	80	s.c.	+	85
90	OH	OH	OH	CH_2	H	rat	>2	s.c.	0	85
						rat	(l) 0.25-2.0	s.c.	+	71
95	OH	OH	OH	CH_2	CH_3	rat	0.25	s.c.	+	85
							(d) >40	s.c.	0	85

TABLE XXV (Cont.)

Compound No.	Structure 4	3	R	X	Y	Central Nervous System Stimulation: Animal	Dose Used mgm./kgm.	Admin.	Effect	Ref.
130	OH	OH	OH	$CH(CH_3)$	H	rat	>5	s.c.	0	85
132	OH	OH	OH	$CH(CH_3)$	CH_3	rat	>40	s.c.	0	85
131	OH	OH	OH	$CH(C_2H_5)$	H	rat	>160	s.c.	0	85

The most effective stimulating drugs are β-phenylisopropylamine (No. 5) and its N-methyl analog (No. 18). The d-isomers appear to be ten to twenty times more stimulating than the l-isomers[81, 85]. β-Phenylethylamine (No. 3) is almost inactive. An increase in the number of carbons in the side chain to four (1-phenyl-2-aminobutane, No. 9) or five (1-phenyl-2-aminopentane, No. 13) decreases central excitatory action. Similarly, the substitution of groups larger than methyl at N causes a decrease in activity in the order methyl, ethyl, butyl, amyl[81]. Substitution of an hydroxyl at C-2 greatly reduces excitatory action. 'Propadrine' (No. 25) and ephedrine (No. 31) are distinctly less potent than amphetamine (No. 5) and N-methylamphetamine (No. 18). Hydroxyl substitution on the ring is likewise unfavorable for stimulation of the central nervous system. The threshold stimulating dose of β-(4-hydroxyphenyl)isopropylamine (No. 44, 'Paredrine') is one-thirty-seconds that of amphetamine[85]. The hydroxyphenyl analog of methylamphetamine (No. 45, 'Paredrinol') is inactive. The corresponding catechol analogs (Nos. 133 and 135) also have been reported to be inactive. Jacobsen et al.[55] have reported results in man similar to the above data.

Gunn and Gurd[41] observed excitation in mice after intraperitoneal injection of various cyclohexylalkylamines. These results are not in agreement with those of Lands, et al.[65], who compared N-methylcyclohexylisopropylamine (No. 159) with the corresponding phenyl analog and found that hydrogenation of the ring greatly reduced excitatory action. Swanson and Chen[92] found amphetamine forty times more potent than N-methylcyclopentylisopropylamine (No. 166). Aliphatic amines have little or no stimulating action on the central nervous system[85].

These data establish the importance of phenylisopropylamine (No. 5) for stimulation of the central nervous system. Modification of this basic structure diminishes or abolishes excitatory action.

SUMMARY

The most important modifications of the sympathomimetic actions of β-phenylethylamine are obtained by (1) substitution of the phenyl ring by an hydroxyl at the 3-position, (2) disubstitution of the ring at the 3- and 4-positions, (3) substitution of C-2 of the side chain by an hydroxyl and (4) alkyl substitution of the amine.[14] The relative importance of each of these substitutions in modifying the activity of β-phenylethylamine is difficult to determine by an examination of the published experimental data. Variations in test procedures and insufficient attention to quantitative methods of testing have given rise to important differences between the results obtained by different investigators. However, some generalizations are suggested by the data tabulated in this communication.

The substitutions listed above differ in importance when different test organs are used. Excitation of the cardiovascular system is favored by an hydroxyl at the 3-position on the ring. The subsequent addition of the alcoholic hydroxyl causes a distinct increase in potency. However, the alcoholic hydroxyl does not appear to be the key structure for this action inasmuch as its presence causes a distinct reduction in pressor potency when the phenolic hydroxyl is in the 4-position on the ring. Methyl substitution of the nitrogen atom does not greatly influence pressor potency, except in the case of hydroxytyramine ('Epinine'). The pressor potencies of 1-(3-hydroxyphenyl)-2-aminoethanol and 1-(3,4-dihydroxyphenyl)-2-aminoethanol are essentially the same as that of their N-alkyl analogs.

When depressor action is considered, quite another picture is obtained. The substitution of the second carbon of the side chain of β-phenylethylamine by an hydroxyl causes a distinct reduction in pressor potency and this is further reduced by the addition of an hydroxyl to the ring at the 4-position and by an N-methyl group. If an N-ethyl or N-isopropyl be substituted in place of the N-methyl group, the dominant action of the compound is depressor. N-Isopropylarterenol ('Isuprel', 'Aleudrine') is a very potent vasodepressor agent. The removal of the alcoholic hydroxyl abolishes depressor action, a weak pressor response resulting in most instances[64]. Ring hydroxyls are not necessary for depressor action inasmuch as 1-phenyl-2-isopropylamino-ethanol is an effective depressor agent. The corresponding ethane analog is almost inactive[66], indicating further the importance of the alcoholic hydroxyl for depressor action.

Bronchodilator action is not usually obtained unless there is both an alcoholic hydroxyl at C-2 and a phenolic hydroxyl at the 4-position. Both 1-(4-hydroxyphenyl)-2-methylaminoethanol and the 3-hydroxy analog are weak bronchodilator agents. Arterenol, with both hydroxyls on the ring. is somewhat more potent. The N-methyl analog (epinephrine) is fifty times, the N-isopropyl analog one hundred times more potent than the primary amine. The alcoholic hydroxyl is most important for this action. Thus, little or no bronchodilation is obtained with N-isopropyl-β-(3,4-dihydroxyphenyl)ethylamine. The structural requirements for bronchodilation and vaso-depression are apparently quite similar. Failure to obtain bronchodilation with weak vasode-pressor agents may result from the strong bronchoconstriction employed in the usual test methods.

By contrast to the above results, inhibition of the intestine appears to be favored by structural modifications favorable for vasopressor action. The inhibitory action of 1-(4-hydroxy-phenyl)-2-aminoethanol is about one-five hundredths that of epinephrine; the 3-hydroxy analog is about one-tenth to one-twelfth that of epinephrine. Addition of the second hydroxyl to the ring increases intestinal inhibitory potency as it does pressor action. The substitution of an N-methyl, N-ethyl or N-isopropyl group does not increase this action, these derivatives being no more effective than the primary amine. Removal of the alcoholic hydroxyl reduces potency but the resultant compound retains strong inhibitory action. The ethane analogs of arterenol and epineph-rine have one-fifth to one-fortieth the inhibitory potency of epinephrine.

Uteri of various species differ greatly in their response to drugs. Pharmacologic evaluation is further complicated by differences in response resulting from variations in the physiological state of this organ. With the rat uterus, which is relaxed by all sympathomimetic amines, potency is greatly increased by the addition of the alcoholic hydroxyl. Gaddum et al.[35] reported l-arterenol to be about twenty times more inhibitory than hydroxytyramine. The presence of an N-alkyl substituent causes some further increase in potency[35,63,71]. This is in general agreement with results obtained for vasodepression and bronchodilation. The responses of rabbit and guinea pig uteri are variable and these organs may be either contracted or relaxed by sympathomimetic amines. Although our data are incomplete, the results tabulated here suggest that the response obtained represents the net resultant of these two opposing actions. Weak sympathomimetic amines such as β-phenylethylamine or tyramine usually cause contraction. Relaxation is obtained when there is an alcoholic hydroxyl and/or an N-alkyl group larger than methyl. The N-isopropyl analog of hydroxytyramine is a weak uterine inhibitor. Addition of the alcoholic hydroxyl increases greatly this inhibitory action. The primary amine, arterenol, may either contract or relax the guinea pig uterus[71,79] and the rabbit uterus is contracted. The presence of an N-alkyl group larger than methyl suppresses excitation, the balance now being favorable for relaxation. Inasmuch as both the N-alkyl group and the alcoholic hydroxyl are important for inhibition of the uterus, the inhibitory pattern of this organ most nearly resembles that of the vascular system and bronchioles.

The effect of structural modifications of β-phenylethylamine divides these sympathetically innervated organs into two functional groups (1) vasodepressor action (relaxation of the arterioles and capillaries), bronchodilation and uterine relaxation; (2) vasopressor action (contraction of the

arterioles and capillaries), uterine contraction and intestinal relaxation. The alcoholic hydroxyl at C-2 on the side chain appears to be the key structure for the first and an hydroxyl at the 3-position on the phenyl ring for the second. The addition of each of the other substituents to the key compound will then increase potency, as indicated above.

The relative effectiveness of sympathomimetic drugs on various other organs innervated by the sympathetic nervous system has been determined in some instances. These data are reviewed here in terms of the above generalization. Sympathomimetic action on the heart must be considered for effects on both rate and amplitude inasmuch as each of these actions may be influenced by drugs to a different degree. Effects on heart rate suggest that there is no distinct differentiation of effects such as is found with the peripheral vascular system. Structural modifications which favor either vasoconstriction or vasodilatation are favorable for cardiac acceleration. Thus, the highly potent vasodepressor drug, 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol ('Isuprel') and the N-t. -butyl analog are about four to ten times more effective than epinephrine in increasing the rate[64, 79]. Similarly, 1-(4-hydroxyphenyl)-2-isopropylaminoethanol is four times more effective than the corresponding N-methyl analog, 'Sympatol'[78]. An alcoholic hydroxyl at C-2 of the side chain is important. This is easily demonstrated with 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol and the corresponding ethane analog. The former compound is three hundred to five hundred times more potent than the latter. Excitatory action is retained in 1-phenyl-2-isopropylaminoethanol, indicating that the ring hydroxyls only increase potency.[64] An hydroxy at the 3-position on the ring is more favorable for cardiac acceleration than at the 4-position. Comparison has shown 1-(3-hydroxyphenyl)-2-methylaminoethanol ('Neo-Synephrine') to be twenty to fifty times more stimulating than the 4-hydroxyphenyl analog ('Sympatol') in experiments on the denervated hearts of dogs.[112] Hydroxyls at both the 3- and 4-positions on the ring are more favorable than either alone. Racemic epinephrine is approximately ten to twenty-fives times more effective than 'Neo-Synephrine' and two hundred to twelve hundred times more effective than 'Sympatol'. From the above data it readily can be seen that each of the four substitutions to the β-phenylethylamine nucleus contribute importantly to cardiac accelerating action.

There are little quantiative data available with regard to the effects of these drugs on the force of cardiac contraction. Garb[30] has reported arterenol somewhat more effective than either epinephrine or 'Isuprel'. Similarly, Tainter has reported an increased force of contraction with arterenol which was greater than that obtained with epinephrine and more prolonged[26]. Marsh et al.[79] and Ahlquist[4] reported equal effects for d,l-epinephrine and arterenol with the perfused cat and rabbit hearts but greater effect was obtained with 'Isuprel'. Removal of the hydroxyl at the 3-position on the ring reduces activity to about one-hundredth that of the corresponding catechol analog[78]. Crismon and Tainter[26] found that the simpler amines, 'Propadrine', ephedrine and 'Benzedrine', exerted an influence on rate but were without important effect on amplitude. These data are in general agreement with those for effect on rate except for the effect of the N-methyl group, the primary amines being equal to or slightly more effective than the N-methyl analog in increasing the force of contraction.

The cat nictitating membrane has been used as an indicator of excitatory action. Bacq,[11] found epinephrine more effective than arterenol whereas Gaddum et al.[35] reported the two drugs equally potent. Removal of the hydroxyl from the 4-position only on the ring reduces activity to about one-quarter; from the 3-position only to about one-thirtieth to one-one hundred-twentieths that of epinephrine. The alcoholic hydroxyl is important for this action. Both 'Epinine' and 'Kephrine' are distinctly weaker than epinephrine. Hydroxytyramine has one-hundredth the stimulating action of epinephrine[35]. Ahlquist[4] found 'Isuprel' ineffective and Gaddum et al.[35] have reported relaxation of the nictitating membrane after injection of this drug. These data suggest that stimulation of the cat nictitating membrane is favored by the same structural changes which favor vasopressor action, uterine contraction and intestinal relaxation.

Ahlquist[4] has postulated two receptors for sympathomimetic drug action; an alpha adrenotropic receptor for vasoconstriction in the viscera and skin, for contraction of the uterus and nictitating membrane and for relaxation of the intestine, ureter and dilator pupillae, a beta receptor for vasodilation in skeletal muscle, coronary vessels and viscera, for inhibition of the uterus and bronchioles and for cardiac stimulation. The data presented here are in general agreement with such a functional classification of organs innervated by the sympathetic nervous system. The key configuration for stimulation of the α-receptor probably is β-(3-hydroxyphenyl)-ethylamine with subsequent substitution of the alcoholic hydroxyl on the side chain and the second hydroxyl to the ring each increasing potency. N-Alkyl substitution is less important and may actually diminish potency with groups larger than methyl. The key configuration for stimulation

of the β-receptor is probably 1-phenyl-2-aminoethanol, the alcoholic hydroxyl at C-2 of the side chain playing an important role. Substitution of an hydroxyl at the 4-position on the ring and N-alkyl substitution each increase inhibitory potency. With both key structural requirements present in the molecule, mixed effects are frequently observed, as for example the responses which follow an intravenous injection of epinephrine. The cardiac receptor for sympathomimetic drug action either is different from those described above or is undifferentiated and may be considered as an α β structure, responding to both excitatory and inhibitory drugs by increased cardiac action.

Lengthening of the side chain decreases both excitatory and inhibitory actions. The propanol derivative, 'Cobefrine', is somewhat less pressor than the ethanol derivative, arterenol. An increase in the number of carbon atoms in the side chain to four practically abolishes pressor action. Inhibitory action on the bronchioles, uterus and intestine are also diminished. These results suggest that stimulation of both the α and β systems is diminished. Further evidence of this is furnished by the low stimulating action of this compound on the heart[102], an organ in which both functional systems may cause increased action.

Modification of the key compounds, as indicated above, causes similar changes in action. Thus, 1-(3-hydroxyphenyl)-2-aminopropanol is approximately ten times more pressor than 1-(4-hydroxyphenyl)-2-aminopropanol[24, 25]. The alcoholic hydroxyl at C-2 does not increase vasopressor action, in the absence of an hydroxyl at the 3-position on the ring. This is readily seen by comparing the pressor action of β-(4-hydroxyphenyl)isopropylamine with that of 1-(4-hydroxyphenyl)-2-aminopropanol. The former is 1.5-2.0 times more effective than the latter[13, 24, 100]. The presence of an N-alkyl group increases stimulation of the β-receptor. The action of 1-(3,4-dihydroxyphenyl)-2-methylaminopropanol is predominantly depressor[4, 84]; that of 1-(3,4-dihydroxyphenyl)-2-isopropylamino-1-butanol strongly depressor but less so than 'Isuprel'[63]. Again, using the heart as an indicator of the sum of sympathomimetic action, we find the stimulating action of 1-(3,4-dihydroxyphenyl)-2-isopropylamino-1-butanol much weaker than 'Isuprel'.

The data shown in Table XV suggest that hydrogenation of the phenyl ring abolishes stimulating action on the β-receptor but only diminishes stimulation of the α-receptor. The pressor action of β-cyclohexylethylamine and 1-cyclohexyl-2-methylaminoethanol is about one-half that of the corresponding phenyl analogs[41, 60, 74]. The cyclohexyl analog of the effective vasodepressor compound, 1-(4-hydroxyphenyl)-2-isopropylaminoethanol, is inactive[60]. The effect of cyclopentyl or α-thienyl substitution in place of the phenyl ring likewise reduces pressor potency. Available data do not permit other comparisons.

Aliphatic amines appear to stimulate the α-mechanism but have little or no effect on the β-mechanism. Thus, they generally raise blood pressure but cause bronchoconstriction and contraction of the intestine and uterus[8, 30, 76, 101]. Two of these, 'Octin' and 'Tuamine' have clinical importance as vasoconstrictors.

Excitatory action on the central nervous system observed with some sympathomimetic amines does not fit into the pattern of action previously described. The most effective stimulating drugs are phenylisopropylamine and its N-methyl analog. An increase in the length of the side chain or of the N-alkyl group reduces central excitatory effect. Substitution of an hydroxyl on the side chain or on the ring greatly diminishes or abolishes excitation[81, 85]. Ephedrine (alcoholic hydroxyl at C-2) is about one-sixteenth as active as amphetamine; 'Paredrine' (phenolic hydroxyl at the 4-position) has almost no stimulating action[85]. Epinephrine has been reported to cause some stimulation[85]. Gaddum and Kwiatkowski[34] and Lawrence, Morton and Tainter[68] have reported potentiation of the pressor action of epinephrine by ephedrine and suggested that this might be due to the inhibition of amine-oxidase thus preventing the destruction of epinephrine. Similar action has been reported by Jang[56] for 'Benzedrine" and 'Propadrine'. One might assume that the stimulation observed with these drugs is secondary and results from the protection of epinephrine, produced naturally at synaptic junctions, from enzymatic destruction. This seems improbable in the light of the results obtained by Lewis[69] who observed marked epinephrine potentiation with various aliphatic amines, although aliphatic amines have been reported to be devoid of central stimulating action[76, 85]. Another barrier to the acceptance of this concept is suggested by the work of Marrazzi[73] and Bulbring and Burn[21] who have shown that physiological quantities of epinephrine inhibit synaptic transmission in sympathetic ganglia. The observed elevation of mood and increased alertness that follow amphetamine or N-methylamphetamine could hardly result from increased synaptic resistance, if these central synapses are similar to

those in sympathetic ganglia. The stimulating action of these amines on the central nervous system appears to involve receptor systems other than the α and β types found in sympathetically innervated organs.

This review of sympathomimetic amines has listed representative data of the type usually supplied by the pharmacologist to the medicinal chemist as a guide to further synthesis. It is the hope of the writer that such a collection will be useful to those engaged in the synthesis and evaluation of sympathomimetic drugs. The limitations of the data are obvious. It is readily apparent that these correlations are based upon a two-dimensional picture of a three-dimensional structure. There is also relatively little known about differences in physical and chemical properties of these molecules which may contribute importantly to the physiologic actions observed. Future research must provide us with information with regard to the nature of the reactions that take place within the effector organs and elucidate the importance of structural variation on these reactions. The progress of sympathomimetic drug research during the first half of this century is of such magnitude that we may look forward to the second half century with great expectations. It may not be too much to hope that, as a result of an increasing life expectancy, many of us who have participated in the work of the first half may witness also the full achievements of the second half century.

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'Suprarenin', 'Isuprel', Cobefrine', 'Neo-Synephrine', 'Sympatol', and 'Kephrine' are trademarks of Winthrop-Stearns Inc.

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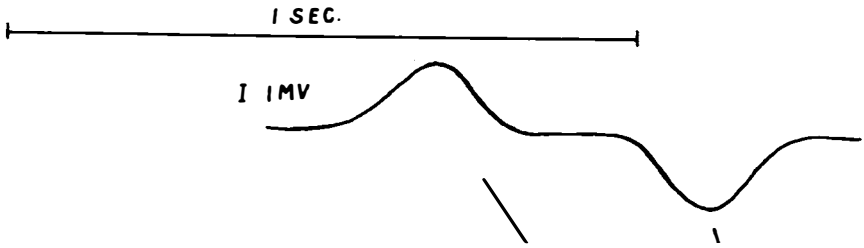
DISCUSSION

DR. T. C. BARNES (Hahnemann Medical College and Hospital of Philadelphia): I should like to congratulate Dr. Lands on this fine paper and I should like to know if he would consider the possibility that the structural modifications of drug molecules have electrical effects which may explain their action. We have found that a series of sympathomimetic drugs have distinctive phase-boundary potentials. A triglyceride oil is capable of picking out sympathomimetic drugs from a solution and leaving all the parasympathetic drugs in the aqueous medium. We feel that structural modifications such as those described by Dr. Lands are simply factors which influence the oil solubility of the compounds and the extent of their ionization within the oil phase. Thus the pharmacodynamic action on the tissue may be produced by the electrical potential generated by the drug.



Oil-cell apparatus for measuring phase-boundary potentials of drugs.

(Slide) Our methods are rather unusual so I am showing this slide of the glass tube containing the oil layer forming a phase-boundary with saline. With triacetin in the apparatus electrical potential is generated with sympathomimetic drugs but not by acetylcholine.

(Slide) This shows the apparatus for producing phase-boundary potential directly on nerve. We extract some of the lipoid from the frog sciatic with ether and treat the nerve with the adrenergic oil-triacetin. The nerve gives a greater potential with amphetamine than it did before the triacetin was added. Nerve from an animal not having an adrenal like the lobster is not sensitized in this way. We believe that the sympathetic and parasympathetic nerves contain different lipoid.

(Slide) This apparatus shows a glass model of a cellular receptor upon which a drug is supposed to act. The center capillary tube is filled with an adrenergic oil on which amphetamine for example produces the same negative potential as it does on a wide surface of oil 5 cms. in diameter. In other words, membrane potential is independent of area. There is no need to postulate the presence of receptor spots on living cells.

(Slide) This shows the Speedomax chart of the negative phase-boundary potential produced by tyramine on triacetin. Tyramine can be used to detect adrenergic oils. Catechol is electrically inactive.

(Slide) This shows a series of parasympathetic and sympathetic drugs which were tested on adrenergic oils. Acetylcholine, curare, methacholine and atropine are inactive on triglyceride oil on which the sympathomimetic drugs generate potential (including the aliphatic members, octin, oenethyl and aranthol). Nicotine is also active on adrenergic oils suggesting its pressor action is related to that of epinephrine.

ACTIVITY OF CHEMICALS ON ANDRENERGIC OILS
(all concentrations 0.05%)

Acetylcholine	0
Choline	0
Mecholyl	0
Pilocarpine	0
Eserine	0
Prostigmine	0
Atropine	0
Curare	0
Etamon	0
Aranthol	19 millivolts
Octin	32 millivolts
Oenethyl	15 millivolts
Priscol	45 millivolts
Yohimbine	77 millivolts
Dibenamine	6 millivolts

In view of the above evidence I should like to ask Dr. Lands if he would agree that the phase-boundary potential of an adrenergic drug is a factor in its pharmacodynamic action on the living cell. We suggest that epinephrine, for example, stimulates the heart exactly like an electric current.

DR: MARRAZZI (Department of the Army, Army Chemical Center, Maryland): I think it will be apparent that the data have carried us or, rather Dr. Lands, far in the correlation of structure to function. I believe another point of view might carry us further, even more significantly further, and deserves some emphasis. Lest your first impression be that the addition of an unnecessary complication is involved, I would like to call your attention, very briefly, to some preliminary considerations.

Recapitulating some of Dr. Lands' remarks, the characteristic activity of what we might call the sympathomimetic nucleus, namely the β-phenylethylamine group, is that it will, in fact, exhibit all of the actions of the sympathomimetic amines.

All of the actions in the case of sympathomimetic amines can be readily divided into two types, and summarized as excitatory and inhibitory actions; that is, the stimulating action on the heart, for example, is an excitatory action; the bronchodilator action is an inhibitory action.

The sympathomimetic nucleus mentioned is the basic member of the series (β-phenylisopropylamine is the basic member of an analogous series) in which the commonest substituents, i.e. a meta phenolic hydroxyl, a para phenolic hydroxyl, an alcoholic hydroxyl on the beta carbon and a methyl group on the nitrogen, all four individually or collectively (adrenaline) always produce enhancement of both types of characteristic activity.

Whether you agree with me in detail or not, the point I would like to make is that these modifying groups here and, possibly, with other compounds, are producing a multiplicity of actions which ordinarily can be summarized into a few groups, usually into excitation and inhibition; and that, for fundamental understanding, it is sufficient to focus on only one action or on one type of action because, ordinarily (in fact, I know of no exceptions), all of the actions are simultaneously being affected. So I would plead for further emphasis on what you occasionally saw in Dr. Lands' slides; namely, a correlation to both types of action simultaneously or to the ratio of excitatory to inhibitory actions or the other way around, as may be desired. Since a change in chemical constitution produces a simultaneous change in all of the activities elicited by a compound, one can only hope to correlate the chemical change to the complete picture of activity.

Obviously, there will be difficulties. One will have to choose an excitatory action which is characteristic of the whole group, and an inhibitory action characteristic of the whole group. One of the final remarks Dr. Lands made may have been left in your memory as a possible conspicuous exception to the ready classification of actions into related excitatory and inhibitory groups. He pointed out that the inhibitory action of adrenaline on the brain which we[1(73a)] have described, would seem to be quite different from and unrelated to any other central action but this is because one has been familiar hitherto with only the central so called excitatory actions of sympathomimetic amines. We have, however, described a cerebral inhibitory action for adrenaline and several other sympathomimetic amines which is characteristically similar to the one we have previously described in sympathetic ganglia[2(73b)] and shown to be analogous[3(73)] to inhibition at adrenergic neuroeffector junctions. Thus in keeping with the concept we are proposing, even these central actions of the sympathomimetic amines are apparently divisible into excitatory and inhibitory and for adequate, more meaningful and accurate analysis the correlation of chemical structure should be made to something expressing both actions, i.e. to the ratio of the two.

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DR. LANDS: With regard to Dr. Barnes' comments, I think it probable that sympathomimetic amines act at some easily accessible surface inasmuch as the effect is rapidly elicited and readily reversed. However, I would prefer to ask Dr. Barnes a question rather than attempt to answer the one he has proposed. In a system such as the one shown in his slides, would the dextro and levo isomers of potent sympathomimetic amines such as epinephrine have different solubilities and ionize to different degrees in the "sympathomimetic oil" postulated?

I would like to comment briefly on Dr. Marrazzi's statements. The interrelationship between the actions of these sympathomimetic amines are undoubtedly complex. The substituents which are effective in increasing exicitatory action are also important for inhibitory action so that you have a mélange of effects. However, I would like to emphasize the observation that it is not possible to elicit important inhibitory action in the absence of the alcoholic hydroxyl. Excitatory action is not similarly dependent upon this group. 'Epinine', N-methyl-β-(3,4-dihydroxyphenyl)ethylamine, is a potent pressor amine. On the other hand, this drug has little inhibitory action. The inhibitory action obtained may be non-specific inasmuch as papaverine has comparable potency.

In conclusion, I believe that out of all this has emerged some indication as to the structural requirements important for excitatory and inhibitory sympathomimetic actions.

ORGANIZATION, OBJECTIVES AND PROGRESS OF THE CBCC

by

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On entering the auditorium this morning each of you received a small green booklet entitled "The Chemical-Biological Coordination Center of the National Research Council". This booklet, written by the staff, describes in some detail the History, Organization and Objectives of the Center together with a description of the procedures which have been developed for the assembly and organization of chemical-biological data. These procedures involve the use of data sheets, prepared by our abstracters, chemical card files, a chemical code and biological codes and punched cards. Some examples are given to illustrate the way the Center utilizes these facilities in answering questions concerning chemical-biological correlation. Dr. Marsh of West Virginia University will elaborate on this problem in his discussion of antihistamines and Dr. Anderson of the American Cyanamid Co. in his discussion of antithyroid compounds. We are extremely grateful to both of these gentlemen for their contribution to this portion of the Center's participation in the Symposium. Information is provided regarding the screening program which is being sponsored by the Center and the booklet contains a list of the publications which have been issued by the Center, to date. Incidentally, these publications are displayed in a case in the lobby on the left as you make your exit from the auditorium. The booklet also provides information on the organizations and individuals who are eligible to utilize the Center's facilities, and the present limitations of the service it can render.

I hope that each of you who received a copy of this booklet will read it carefully. There are a few points which I would like to emphasize. The Center will be four years old on July 1, 1950. The major portion of this time has been spent in developing the procedures which are now in use. We have about fifty-five thousand data sheets in our files which contain chemical-biological information on some nineteen thousand chemicals. These data have been taken from declassified war reports, which are not generally available, from our screening program, from unpublished reports solicited from government, university and industrial laboratories and from current, selected scientific periodicals. Our abstracters are routinely preparing data sheets from about one hundred and fifty selected journals starting with January 1946. An analysis of chemical-biological literature made by our staff indicates that if this list is extended to two hundred and fifty journals the Center will be in position to record over ninety per cent of the "useful" chemical-biological data. In order to cover an additional one hundred journals and also to include the literature prior to 1946 we need the services of additional abstracters. The Center reimburses its abstracters at a fixed rate per data sheet prepared. If you know of qualified biologists who would be interested in cooperating with this program we would be very pleased to receive their names.

Each of the chemicals listed on the data sheets have been coded by the Chemical Code which will be described by Dr. Geer. We also have three sets of chemical card files which are filed by serial number order, by empirical formula and by their Chemical Abstracts name so that it is relatively easy to locate data sheets for any chemical. A good start has been made in coding the biological data on the data sheets by the new detailed biological code which Dr. Beard will describe and a large fraction of the data sheets have been coded by the General Biological Code. Much of the coded data are contained on punched cards in our files and hence available for machine use.

In regard to the screening program the Center has made about twenty-five hundred compounds available to the thirty-odd laboratories which are cooperating in this phase of its activity and it has received about forty-five hundred reports on their biological actions. About fifty of these compounds have possessed sufficiently interesting activities to warrant further investigation. In addition to providing compounds to our regular screening agencies the Center is also furnishing selected chemicals to a group of virus investigators and also to a group of biochemists.

I want to take this opportunity to publicly express the thanks of the Center's staff to Dr. Winternitz, Chairman of the Center's Executive and Advisory Committees, who originally conceived the idea of organizing the Center, to the members of these committees, and to the seventy-five members of the Center's subcommittees and panels for the outstanding service and cooperation which they have rendered to the Center during the four years of its existence. The Center is a fine example of team work between chemists and biologists.

Finally, I wish to express the Center's sincere appreciation to the agencies which are providing the financial support for its activities. These include the Chemical Corps, the Corps of Engineers, the Surgeon General's Office and the Quartermaster Corps of the Department of the Army, the Medical Sciences Division of the Office of Naval Research, the American Cancer Society and the National Cancer Institute of the U.S. Public Health Service. Without their generous support this entire development as well as this Symposium would not have been possible.

THE NATIONAL RESEARCH COUNCIL CHEMICAL CODE

by

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The National Research Council Chemical Code was developed by the Chemical Codification Panel under Dr. C. Chester Stock as Chairman. The primary purpose in developing this code was to provide a means of describing the structure of a chemical compound linearly for transcription to a punched card and thus permit the selection of types of compounds by machine methods. Cataloguing of compounds by empirical formula and chemical name has been used extensively, and certain systematic classifications such as Beilstein and the Wiselogle classification have also been employed. More recently the Dyson system as well as several other methods of classifying chemicals have been developed and, in some instances, applied to punched cards. To locate a single compound, the empirical formula offers a simple and sure method; to locate types of compounds, the chemical name or any other of the various classification systems permits the location of many compounds. Oftentimes, however, a group is obscured because of the presence of another group or groups which take precedence in the classification scheme. By expressing the component parts of a compound by individual numbers or code designations and transferring them to a punched card any desired component or components may be searched for.

The NRC Chemical Code describes a chemical structure by assigning code designations to the component parts without showing specifically how the parts are attached to one another. According to the principles of this Code, the component parts or groups are divided into four main divisions (organic, organoheteroid, inorganic and indeterminate structures), which are further divided into so-called families. Within each family three numbers or letters describe the various groups in these categories. A fourth digit is used to record how many times the group occurs in the structure coded. In all families, space has been left for future expansion. The classification in Division I or the organic group which includes Families 0, 1, 2 . . . 9, A, B . . .N, Ø* is based upon a coding system developed by Dr. Frear and his associates at Pennsylvania State College. The families in Division I are listed in order of decreasing complexity with respect to the number of elements present. Groups containing N, O, S and halogen in addition to C and H are in Family 0-- and so on through Family Ø-- containing only noncyclic C. Cyclic groups are classified in separate families from the noncyclic, e.g., Family 6-- classified noncyclic groups containing N and O (in addition to C and H) whereas Family 7-- codes ring structures containing C, N and O. In Division I, the specificity with which the individual group is defined has been regulated to a large degree by the frequency with which the group in question occurs. For example, dithiocarboxylic acids and esters are coded by the same code designation. Carboxylic acids and esters on the other hand are not only separated from each other but are further divided according to the nature of the acid and the alcohol forming the ester. Carboxylic acids are listed as RCOOH and separate code designations assigned depending upon the nature of R, i.e. whether it is heterocyclic, aromatic carbocyclic or either alicyclic or aliphatic. Likewise esters are listed as RCOOR' and separate designations assigned depending upon the nature of both R and R'.

The organoheteroid family (P--) classifies elements attached to C other than C, H, N, O, S and halogen according to their combining power. Combining power is defined as the number of electrons the atom in question furnishes for sharing or transfers to other atoms. Inorganic compounds are coded as central atoms (Family R-- or T--) with other groups coordinated to them (Family S-- or U--). In the case of ionic structures, the cation and anion are coded as separate units; the fourth digit of the code designation records the number of times the group occurs in the ion rather than the number of times it occurs in the compound. The nonmetallic elements have been arranged in a series in order of increasing electronegativity proceeding from selenium through fluorine. The elements so listed are coded in Family T-- if they occur as free elements or central atoms of neutral molecules. Other elements are coded in Family R-- when free or central atoms of neutral molecules. Simple cations or central atoms of complex cations are coded in Family R--; simple anions or central atoms of complex anions in Family T--. The second and third digits of the code designations classify the elements of these families according to their oxidation state, which is calculated in the conventional way by assuming that enough shared electrons to fill the outer shell of the more electron-attracting atom belong to that atom.

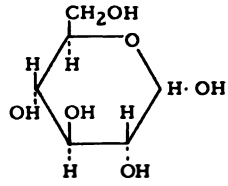
Families S--, U-- and V-- classify coordinate or solvate groups which act as units. Groups coordinated to an R-group are coded in Family S--; those coordinated to a T-group in Family U--; groups coordinated to a P-group in Family S-- or U-- depending upon the electronegativity of the P-element.

* Ø is used to distinguish the letter O from zero.

Hydrates and solvates are coded in Family V--; indeterminate structures in Family Z--.

In the code for a compound, code designations are listed in the order in which they appear in the List of Group Numbers, i.e. 0-9 and A-Z. The coding of a compound is accomplished by selecting from the List of Group Numbers the first group which describes a component part of the structure. If no code designation is found which describes an entire group, it may be split into two or more groups for coding purposes. One systematically proceeds through the List of Group Numbers until all parts of the structure have been coded. The examples shown below illustrate the procedure.

D-Glucose

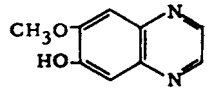


H4M. 1-H8A. 3-H8K. 1-IH2. 1-ø99. 1

($H4M = H_2C(OH)_2$; H8A = R-CHOH; H8K = RCH_2OH, R is heterocyclic; IH2 = tetrahydropyran; ø99 = C_1)

Since only C, H and O appear in this compound, the first family which codes a group in this structure is the H or (CH)O noncyclic family. H4M, shown in the code as $H_2C(OH)_2$, is the first group in this family which describes a part of the structure. The hydrogens in this group may be replaced by R.

6-Quinoxalinol, 7-methoxy-



F50. 2-GFR. 1-H67. 1-H74. 1-NYI. 1-ø99. 1

(F50 = R : N (: may be resonating double bond); GFR = pyrazine; H67 = ROR', R and R' are aromatic carbocyclic and alicyclic or aliphatic; H74 = ROH, R is aromatic monocarbocyclic; NYI = benzene fused to a heterocyclic structure; ø99 = C_1)

C, N and O are all present in this compound, but only CN and CO groups occur as component groups indicated in the code. F50. 2 codes the ring nitrogens and GFR. 1 the heterocyclic ring. Fused heterocyclic rings are always coded as component monoheterocyclic rings. With the exception of the C_5N ring, no distinction is made between fused and unfused heterocyclic rings. Heterocyclic rings of varying degrees of unsaturation are indicated by separate code designations. Because of the frequency of occurrence of C_4N_2 rings, these have been separated into pyrazine, pyrimidine and pyridazine, but in all other cases the location of the heterocyclic atoms is not indicated, only the size of the ring, the number of heterocyclic atoms and the degree of unsaturation. Polycarbocyclic rings are coded as a unit and are not separated into their component structures. With the exception of a 6-membered ring fused to a heterocyclic structure, the same code designation is used for carbocyclic structures fused to a heterocyclic ring as for those which are not. As in the heterocyclic structures, the degree of unsaturation is indicated.

Benzene, arso- $C_6H_5AsO_2$

NYR.1-P1J.1-U63.2

(NYR = benzene, P1J = As^5, U63 = (:O) or -O-)

Since the organic groups precede the organoheteroid and inorganic in the List of Group Numbers, the benzene ring (NYR) is coded first. P1J.1 shows arsenic with a combining power of 5 attached to carbon. The two oxygens coordinated to the arsenic are coded in Family U-- since As is listed in the electronegativity series.

Potassium sodium nitrocobaltate (III), monohydrate

$K_2NaCo(NO_2)_6 \quad H_2O$

RDØ.1-RGØ.1-T6Ø.1-U7V.6-V61.1

(RDØ = K^{+1}, RGØ = Na^{+1}, T6Ø = Co^{+3}, U7V = nitro group, V61 = H_2O)

Only one K^+ is indicated because in ionic structures the fourth digit shows the actual number of atoms present in the ion. Co is the central element of a complex anion and is therefore coded in Family T--.

Fig. I Chemical Card

The Chemical Code is placed on the punched card as shown in Fig. I. The serial number of the compound is in the first 8 columns. This allows for a six digit number with two additional numbers to express salts of the parent compound. The code for the chemical structure is placed in columns 17-52. Structures which contain more than nine groups are given a designating punch, and the additional groups are punched on second cards which are kept in a separate file. The average number of groups in the code for a compound is between five and six. To facilitate sorting, the families present (Col. 9-11) and the empirical formula (Col. 53-66) are also given on the card. The empirical formula is not a true empirical formula inasmuch as the actual number of atoms of every element present in a molecule is not recorded. In columns 52 and 53, the number of carbon atoms up to 99 is shown; in subsequent columns, the number of Br, Cl, F, I, N and S are indicated up to six and oxygen to nine. In columns 59 to 66, the presence of any other element is shown by a single unique punch. The remainder of the space on the card is unassigned and may be used for physical properties when a physical property code is developed.

A serial number as well as a so-called rotated file is prepared for all chemical punched cards. In the rotated file as many cards are prepared as there are groups in the chemical code of a compound. The groups are so rotated that each one in turn appears in the first field (Col. 17-20), which is used for filing purposes. In this way hand selection of all compounds containing any simple specified group may be made directly.

To locate types of structures by machine methods it is important to define the question carefully. The definition of the term analogs often proves troublesome. Since only definite component groups may be searched for, it is necessary to decide what component groups fulfill the definition of analogs. Although the chemical code was developed for the express purpose of selecting types of structures in conjunction with certain biological activities, in the illustrations given here only the chemical code is employed. As was stated earlier the chemical code is not specific for a chemical structure and the specific structure can seldom be reconstructed from the code. As a result when sorting for types of structures a certain number of structures which do not fulfill the desired requirements will be obtained. It is necessary to check the answers obtained to determine whether or not they fulfill the original conditions laid down.

The method of searching the files for all aminophenols is described in order to illustrate the methods used in answering a typical question. The term aminophenol was arbitrarily defined as including those compounds which contained both an amino and a hydroxy group attached to the same benzene ring. The first digit of the serial number has been assigned according to the elements present in a compound and hence only serial numbers beginning with 5 or 9 need to be used here. No others contain C, N and O in the same compound.

Since no group specifically defines an amino group attached to a benzene ring, it was necessary to include all amino groups attached to an aromatic carbocyclic structure. An amino group of this type may be indicated by any one of the following groups: F51 (R-NR'); F53, F55, F57, F58, (RR'R''N with one or more R groups aromatic carbocyclic); F5C, F5E, F5G, (RR'NH with one or both R groups aromatic carbocyclic) and F5L (RNH_2, R is aromatic carbocyclic). The code designation, H74, represents only hydroxy groups attached to a monocarbocyclic ring. From the rotated file the amino or F cards listed above are selected, merged in serial number order and collated with the cards filed under H74.

At the time that this sort was made, there were approximately 15,000 coded structures in the file. Three hundred twenty-seven contained at least one of the F groups as well as H74. It was then necessary to ascertain how many of these compounds were answers to the question. The 327 serial numbers were listed in numerical order by the tabulator, and visual examination of these structures in the serial number chemical card file was carried out. Two hundred ninety-nine proved to fulfill the conditions and 28 did not. Of the 28 structures, two were quinoline compounds with a hydroxy and amino group on the carbocyclic ring, the other 26 contained the hydroxy and amino groups on different rings.

The NRC Chemical Code has been published and may be purchased from the

National Research Council
Publications Office
2101 Constitution Avenue, N. W.
Washington 25, D. C.

THE BIOLOGICAL CODES

by

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The present development of biological codes is the result of cooperative effort on the part of the staff at the Chemical-Biological Coordination Center, the Biological Codification Panel, and the several subcommittees interested in the biological phases of the Center's activities.

The purpose of the biological codes is to classify biological information in a manner permitting the representation of data by punched cards. Such a classification needs to be of sufficient detail to permit correlations, particularly those relating to chemical structure and biological response, but not so detailed that each bit of information becomes unique. It needs to be an indexing system, but one permitting a multiplicity of cross referencing. A system of this kind, embracing physiology, pharmacology, toxicology, and enzymology, and considering all organisms, is not easy to construct with logical and consistent patterns. The adaptation of the system to punched cards imposes some limitations, but the program has been pursued on the premise that only by the use of mechanical aids will it be possible to handle efficiently the mass of information which ultimately will be in the files at the Center. Because no previous system applicable to the punched-card machines and all fields of biology was available to serve as a model, development proceeded in an experimental manner. As a result, several coding systems have been tested at the Center for their application to the problem.

At the outset it seemed desirable to devise a general code, chiefly for utilitarian purposes, to describe the broad fundamental actions of many compounds and to indicate the extent to which the compounds had been tested on biological systems. Impetus to this was given by the accumulation of data resulting from the war-stimulated screening programs particularly for anti-malarials, insecticides, rodenticides, fungicides, and plant growth regulators. The General Biological Code was thus developed to meet the practical needs of the moment. It was designed to occupy the space on a punched card not occupied by the code which describes the chemistry of the test compound and was to be used in conjunction with this chemical description. This space amounts to twenty-three columns each of which contains twelve punching positions, or a total of two hundred and seventy-six. The General Biological Code is based on the principle of direct coding, in which each punch designates a single unique concept which is independent of all other concepts. Thus there is opportunity for expressing two hundred and seventy-six different ideas. Of these, two hundred and eleven have been utilized, which represent subdivisions of the following major considerations:

Taxonomic groups of microorganisms and plants
Physiologic actions on plants
Taxonomic groups of invertebrates
Physiologic action on invertebrates
Population control of microorganisms
Population control of invertebrates
Chemotherapeutic action against parasitic infections
Enzymes
Carcinogenic and carcinoclastic action
General physiologic actions pertaining to microorganisms, plants, and animals
Taxonomic groups of vertebrates
Morphology and pharmacology of vertebrates

The unit represented by the punched card is the chemical compound. All the biological information, classified according to the code, is indicated on the one card together with the chemical information. This code provides for recording positive or negative activity, but the degree of activity is not expressed.

The usefulness of this code is largely limited to providing a general summary of the actions of a compound and to designating the fields of study in which the compound has been investigated. Many desired correlations cannot be made because of the lack of specificity in many of the categories and because the degree of activity is not indicated. Moreover, the application of direct coding introduces difficulties in machine operation which require special handling in entering new information, in maintaining useful files, and in seeking answers to questions put to the system.

In order to augment the General Biological Code and to answer questions on biological actions more specifically, detailed codes have been worked upon concurrently, and an integrated Detailed Biological Code is in use at the present time. This represents an expansion of the ideas cataloged by the General Biological Code as well as additional concepts and details. As applied to punched cards, the two codes differ fundamentally in what the cards represent. Used with the General Biological Code, the punched card describes the chemical compound and all its actions. Used with the Detailed Code, the punched card contains a coded description of one observation on the effect of a compound on a biological system, with details of the particular system affected, technique of application, and type and degree of response. This card is designated as the Biological Card in distinction to the Chemical Card which, in code form, describes the chemical structure of the compound tested. A third card, designated as the Chemical-Biological Card, combines the chemical information with certain of the more fundamental biological information. This card permits direct correlation of those elements expressed on it. In addition, because of the information common to it and to the Biological Card, correlation is possible between any of the details expressed on the Biological Card and those of the Chemical Card. Replicas of the three basic cards are filed in such a way as to facilitate the search for cards having one or more characteristics in common. In such a search, hand selection and machine sorting are used for the greatest economy of time and effort.

As used with the Detailed Code, the punched card is divided into fields each of which consists of the space required for the code number expressing a given idea. The various fields of immediate interest are as follows:

- Test chemical
- Organism
  - Test organism
  - Host organism (or non-living test environment)
- Sex and stage of development of the test organism
- Primary organ system (organ system responding to test chemical)
- Secondary organ system (organ system experimentally modified, or site of application when organ is not responding)
- Tissues, cells, fluid (concerned with action and/or primary organ system)
- State of organism, organ, or tissue tested (experimental condition)
- Action
  - Direction of action (increases, decreases, inhibits, etc.)
  - Specific action
  - General action (includes ". . . cidal" actions (i.e. fungicidal), terms embracing more than one specific action, conditions of ill-defined origin)
- Estimate of effectiveness (quantitative summary expressing effectiveness in one of five ranges)
- Secondary chemical (chemical which may be affected, antagonized, synergized, produced, or which serves as a standard for comparison)
- Technique
  - Size of inoculum in chemotherapeutic studies
  - Route and manner of administration of inoculum
  - Route and manner of administration of test chemical
  - Route and manner of administration of secondary chemical
  - Part treated
  - Method of expressing dosage
  - Concentration component of dosage
  - Quantity component of dosage
  - Time component of dosage
  - Frequency of dose
  - Duration of action
  - Time of evaluation
  - Criterion of response
  - Slope of the dosage-response curve
  - Physical state of the compound when applied
  - Physical state of the compound when response is read
  - Presence of conditioning agents
  - Properties of the compound studied; indication of additional information

All of the quantitative data are expressed in ranges rather than in absolute values.

Although the above outline seems to give undue emphasis to technique, this is actually subordinate to the more fundamental information and serves merely to give additional information and to qualify the estimate of the chemical's effectiveness in producing the recorded action. Of chief interest are the test organism, the organ system responding, and the biological response, and it is upon the basis of these three components that the card files are established.

Some of the concepts expressed, or the way in which they are expressed, are incorporated in the code on a trial basis. Experience in use will determine whether or not they become permanent features of the code.

A few examples will serve to illustrate the use of the code and the language employed in expressing data.

The observation that "Indole-butyric acid induces root formation in branch cuttings of Atrocarpus communis" would be represented, according to the General Biological Code, on the punched card for indole-butyric acid by two concepts: (1) Growth modification and (2) Information available relative to Angiosperms. It should be pointed out that these two items are added to any other concepts which may have been recorded for this compound, and the several items bear no relationship to each other. On the other hand, this observation would be represented according to the Detailed Code by a punched card recording the information as follows:

Indole-butyric acid	(test chemical)
Induces	(direction of action)
Tissue development	(specific action)
(in the)	
Shoot	(primary organ system)
(as an) Isolated structure	(state of organ)
(of)	
Atrocarpus communis	(test organism)

This illustrates that the language of the report has to be "translated" into the code language. In developing the code an attempt was made to use the common terminology of a given discipline. In some cases, however, different disciplines use different phraseology to express similar concepts, and compromises were required. The important thing is the coding of ideas and not words.

The observations that "Parathion causes 50 per cent inhibition of bee brain cholinesterase at 1×10^{-6} molar concentration; its LD_{50} to the honeybee is 3.5 μg/g" are indexed according to the General Biological Code by three categories on the punched card for diethyl p-nitrophenyl thiophosphate (= parathion). These are (1) Information available relative to Insecta (2) Esterases, phosphatases, sulfatases, and (3) Insecticide. These items are so general that almost all the details are lost. As described by the Detailed Code, these observations might read as follows:

(1)	Diethyl p-nitrophenyl thiophosphate (=parathion)	(test chemical)
	Inhibits	(direction of action)
	Cholinesterases	(action)
	(in the)	
	Brain	(primary organ system)
	(of)	
	Apis mellifera (=honeybee)	(test organism)
	(being)	
	Highly effective ($In_{50} = 1 \times 10^{-6}$ or less)	(estimate of effectiveness)
(2)	Apis mellifera (=honeybee)	(test organism)
	Acute toxicity	(specific action)
	Insecticide	(general action)
	Effective ($LD_{50} = .2 - 3.5$ μ g/g)	(estimate of effectiveness)

In these cases, the details are preserved except for the exact quantitative data, which are placed in ranges instead of being reported in absolute values.

A third example involves vertebrate pharmacology. "Dimercaprol (12.5 mg./kg. injected intramuscularly after intravenous injection of lead acetate) increases the excretion of lead in the bile of anesthetized rabbits." By the General Biological Code this would be indexed on the card for 1-propanol, 2,3-dimercapto- (= dimercaprol) as (1) Information available relative to rodents other than rats or mice, (2) Elimination, (3) Liver function. By the Detailed Code the information is coded quite literally:

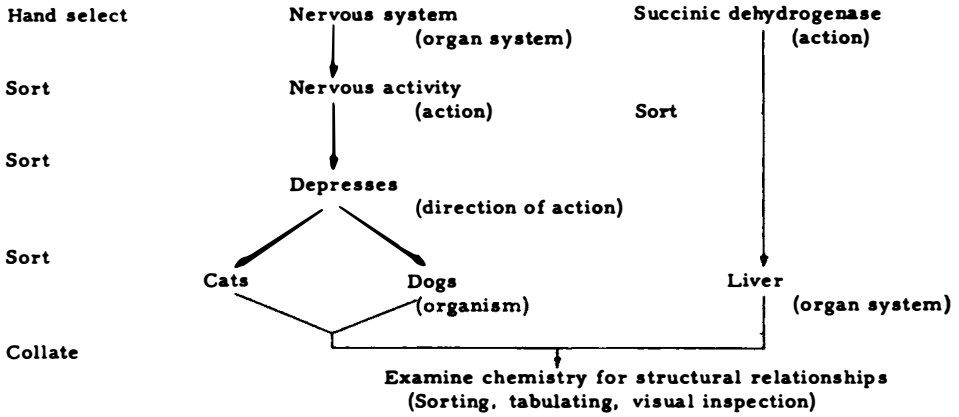
1-propanol, 2, 3-dimercapto (= dimercaprol)	(test chemical)
5.1-25 mg/kg	(range of dose administered)
Intramuscular injection	(route of administration)
Test drug given after secondary chemical	(signa)
Lead acetate	(secondary chemical)
Intravenous injection	(route of admin. of second chemical)
Rabbit	(test organism)
Anesthetized	(state of test organism)
Biliary elimination of secondary chemical	(action)
Increased	(direction of action)

To understand how the cards can be used as an aid in answering questions it should be explained that data sheets, on which may be found information abstracted from original papers, are filed according to the test chemical. As mentioned before, the punched cards describing the chemistry of test chemicals (Chemical Cards) are filed in one series according to the serial numbers assigned to the chemicals and in another series according to the code designations for the structural groups for each compound. The latter file is the "rotated" file in which for a given compound there are as many cards as there are structural groups present. The Biological Cards and the Chemical-Biological Cards are replicated and filed under "test organism", primary organ system", and "action".

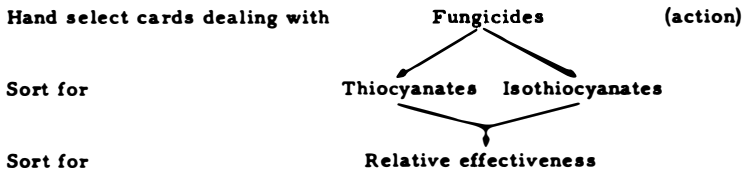
In many respects the simplest type of question to answer is that involving information on a single compound, as "Has p-chlorophenoxyacetonitrile been tested as a plant growth regulator?". This can be found by referring directly to the data sheet file under the serial number of this compound, which happens to be 900,249.

Other types of questions require the use of the punched cards and the aid of the machines. It is beyond the scope of this paper to explain the use of machines, but some idea of the procedures followed in answering questions can be obtained from a few examples.

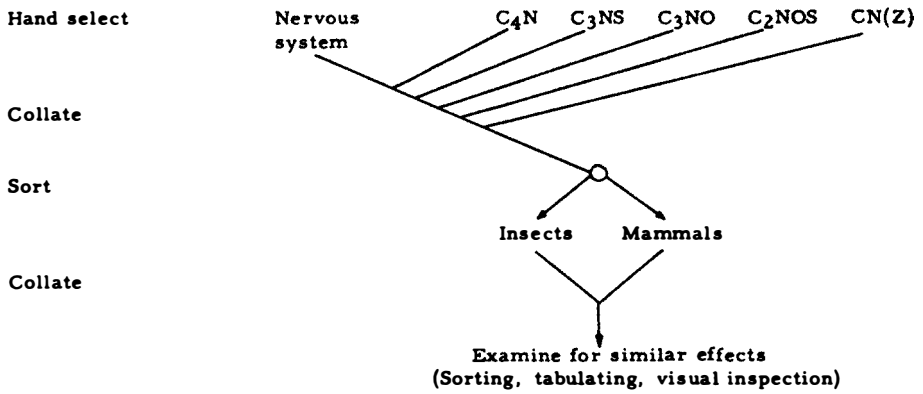
One type of question involves a primary search among the biological data. "What structural relationship is exhibited by compounds known to be C.N.S. depressants in cats and dogs and which also are known to interfere with succinic acid dehydrogenase metabolism in the liver?" In answering this, cards dealing with the Nervous System can be hand-selected from the file based on Primary organ system, and cards dealing with Succinic dehydrogenase can be hand-selected from the file based on Action. These cards then can be handled by machine according to the following scheme:



Another type of question calls for use of the Chemical-Biological Card file, which permits direct correlation between chemical and biological information. "Which compounds possessing the thiocyanate or the isothiocyanate groups have been tested as fungicides and what is their relative effectiveness?" In answering this question,



A still different procedure can be followed in answering these questions: "Which compounds having 5-membered rings containing N in the ring have been studied for their effects on the nervous system of insects? Which of these compounds have similar effects on mammalian nervous tissue?" From the Biological Card file based on the Primary organ system those cards dealing with the Nervous System can be hand selected, and from the Chemical rotated file those cards dealing with the desired structural groups can be hand selected. These groups of cards then can be handled as follows:



These suggested procedures are not the only way in which these questions can be answered. A skilled machine operator, familiar with the files, can outline procedures which require the minimum number of cards to be handled and the minimum number of machine operations.

The systems outlined above were not designed to replace the thinking process, nor can they be expected to yield a completed manuscript ready for publication. They were designed to serve as a research tool to facilitate experimental work and to provide leads toward new investigations.

Note: Since the above was presented, certain modifications of detail in card arrangement and filing procedures have been made at the Coordination Center. The principles, however, remain the same.

DEMONSTRATION OF MACHINE BIOCHEMORPHOLOGY

CORRELATION OF ANTIHISTAMINIC ACTIVITY AND CHEMICAL STRUCTURE

BY MEANS OF

THE NATIONAL RESEARCH COUNCIL CHEMICAL-BIOLOGICAL COORDINATION CENTER

IBM PUNCH CARDS

by

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West Virginia University
Morgantown, West Virginia

INTRODUCTORY NOTE

The following material has been prepared to illustrate the type of machine derivation of simple generalizations between biological activity and chemical constitution, and correlative data, that the Center can supply. There are certain limitations inherent in any coding and machine sorting procedure; these will become apparent. With certain types of questions, the machines can save months of effort by employing the punch cards already prepared from the extensive data compiled by the Center. The present machines are not intended to replace the various abstracting journals for answering simple search questions. For coordination work, any problem that involves much less than a thousand compounds or entries can usually be handled by visual inspection. Simple card systems, such as the Keysort, are usually adequate for five hundred to twenty-five hundred entries if a limited number of types of data are recorded. The machines become useful when a large number of compounds with a large number of different or qualified types of data are available. With such problems the machines can rapidly sort the compounds into groups so that only a few cards are left for final analysis.

The present problem, that of determining any possible generalizations concerning the relationships between chemical constitution and antihistaminic activity, has been chosen since the field is a large one, most biological and chemical workers are generally aware of the results, and a large number of modern entries are available. Since the antihistaminic structure-activity relationships have been surveyed in several excellent review articles and are widely known, the problem is an excellent one to demonstrate the usefulness of the procedure and to stimulate the formulation of future problems. Since the general solution to the problem is known to most workers in this field, the development may seem naive.

As far as humanly possible, no recourse to prior knowledge has been used to reach any preconceived or expected results, and the machines have used only such cards as would be routinely available at the time of any sorting. Obviously, errors committed by the original worker, publisher of journal article, abstracter, coder, punch operator, or machine operator will not be eliminated by the machines. In a general problem, a few mistakes are not important. However, any compound that is apparently missing in a series, or any compound or experimental result that appears out of place can be easily checked by referring to the chemical file, the data sheets, or the original publication.

It is necessary to have some definition of terms. Antihistaminic activity may have several meanings. Considerable confusion exists in some of our minds concerning "activity" and "use". For example, some of you know that clinical use of a few of the agents in this discussion is not predicated on their relative activity at all, nor on available published data, and some of the very active compounds are not clinically available for patent or other economic reasons. Obviously, the machines will not have this information. In general, a chemical is said to have antihistaminic activity if it has little or no direct action on a biological structure but will prevent any typical action of histamine on this structure, or if the histamine is already present, if the chemical will overcome this effect or return the condition of the structure toward the normal.

Since the first demonstration of this subject was presented in May, 1950, numerous additional publications concerning antihistaminics have appeared. Consequently, the entire procedure has been rerun, and is complete, as far as the Center is concerned, through May, 1951. Of course, some published articles are still in the possession of abstracters, some data sheets remain to be coded, and some cards to be punched. This, coupled with the delays involved in publication, means that the demonstration is several months out of date; for purposes of illustration of the general method, this is of little practical importance. The present demonstration has followed the general line of attack of the earlier one, although some items have been followed more thoroughly and some have been discarded as simple duplications of included material.

It should not be overlooked that the Center has several types of information with which to work. The abstracter prepares a work sheet to contain pertinent data about each compound listed in an original article. This data sheet is the primary reference to the original publication. The

chemical agent is next listed in the chemical file and given a reference number, or if it is already listed in the chemical file, the reference number is supplied. All the data sheets of a given chemical are kept together according to this CBC number, with secondary numbers to indicate individual data sheets in case there are more than one. The information on the sheet is coded, and the coded material is punched on a standard IBM card. This punch card has the compound number which is also the data sheet number, a secondary number to indicate which data sheet is involved if there is more than one, and the coded material printed on the top of the card, as well as having it punched into the body of the card. It is possible, then, to go from punch card to chemical file to find just the formula of the compound, or to the name file to find its names or synonyms, or to the sheet to check the coded data or to find the reference so that the original article may be found and examined.

For rapid determination of generalizations between structure and activity, the investigation should be limited to the punch cards with the use of the chemical or formula file as necessary. The data sheets should be avoided as far as possible as they are very bulky and relatively clumsy to handle if any number of items is involved. It is necessary that the questions asked be in a form that the machines can handle. Careful examination of the general biological code and the chemical code will help to formulate reasonable questions, but final consultation with the machine operator is advantageous; his experience in handling the cards and the system will often enable him to use shortened or simplified procedures or to point out impractical questions. This is necessary, since the CBCC is accumulating punch cards so rapidly that it will soon require a matter of days or even weeks to make a single sort of all the available cards. Replacing the sorted cards in numerical order also takes much machine time.

I am grateful to Dr. W. R. Kirner and Dr. C. P. Huttrer of the Center for suggesting the problem and much assistance, to Mr. Ballard for carefully explaining the machine operations and carrying out numerous sorting procedures, and to Dr. Filippi and Dr. Geer for assistance with the Biological and Chemical codes.

MACHINE QUESTIONS

One primary question has been asked and nine subsidiary groups of questions. Obviously, the answer to the first question is the summarized data of the answers of the other nine. The questions, which are the same sort one might ask for any problem relating biological activity and chemical constitution, regardless of primary subject, are listed below.

1. Is there any relationship between antihistaminic activity and chemical constitution or chemical structure?

2. Since I am relatively unacquainted with the biological code, are there appropriate headings that will allow for the isolation of punch cards that refer to compounds that have antihistaminic activity; i.e., agents that prevent, overcome, or inhibit the effects of histamine?

3. In a sort or search under these headings, how many compounds or cards are found?

4. Have all of these compounds been tested by the same pharmacological method?

5. If not, are there any outstanding or common methods that have been used in sufficient numbers that a correlation might be possible?

6. If two or more methods have been used to determine antihistaminic activity, is it possible to rank the compounds according to increasing activity by these methods, and are the more active compounds and less active compounds always found in the same place regardless of the method used? If they are not, then the test methods are not comparable and any correlation will be limited.

7. By visual examination of the most active compounds of any classification, are there any common chemical features that are found? Do the very active compounds occur in only one chemical family or are they not chemically related at all or do they occur in a few chemical types or groups? If they occur only in one family or a few families, are any of the compounds in the inactive or relatively inactive groups that also belong to this family?

8. Have the compounds that have been tested for antihistaminic activity been tested for any other type of biological activity? If so, is there any group or groups of data that might be correlated with antihistaminic activity? Are the most active compounds in any group also the most antihistaminic or is there no apparent correlation?

9. Have any of these compounds been tested in man? If so, is there any relationship between the relative activity of compounds tested in man and any tests carried out in animals?

10. Of the compounds that have been found with high antihistaminic activity, are there any common chemical features? Do they all have a common chemical feature, such as a methoxy group, a nitrogen atom, a benzene ring, an ethylene chain, or a halogen atom? If any chemical grouping or pair of chemical groupings seems common in the high or moderately active compounds, what is found when the entire groups of compounds in the files is searched for this? Are compounds found with this grouping that have been inactive on test? Are compounds found that have not been tested as antihistaminics?

RESULTS

2. With any general problem, it should be possible to approach from either the chemical or biological side to demonstrate any possible correlation. In this particular instance we are assuming that we know nothing of the chemistry of any antihistaminic and can only approach the problem from the biological side for the first approximation.

For an understanding of the application of the general biological code, reference to the division of the punch card is helpful. The general biological card consists of 80 vertical columns, each 10 number spaces high with space for alphabetical overpunches. The columns 1 - 6 are used for the CBCC serial number, columns 7 and 8 if a particular salt of the compound is mentioned, columns 12 - 14 to specify which of several data sheets with this particular CBC number is involved. If an additional, or secondary chemical is involved in the test, such as a mutual antagonism problem, it is listed by CBC number in columns 44 - 49 (field H of the biological code). Or a comparison standard chemical may be listed in 44 - 49. Column 19 is used for the route of administration of the secondary chemical (if any is used) and column 22 is used for the route of administration of the primary chemical listed in columns 1 - 6. The various routes are listed in field K of the biological code. Columns 33 - 35 (field T) are for the response criterion, 53 - 60 (field A-1) to describe the animal species used, 62 - 64 (field C-1) to describe the part of the animal involved, 68 (field F-1) indicates the direction of response, 69 - 72 (field F-2) lists

the specific action of the primary chemical on the organism, while 73 - 75 (field F-3) may give the general action of the primary chemical. If it is possible to express the potency in a quantitative summary according to field G of the biological code; this is listed in column 76.

Insufficient data may be given to use all of the columns and fields listed and it may not be necessary to use all of the information in these columns for a particular problem. It is also possible to have data that mean essentially the same thing listed in more than one way in the biological code, depending on whether the activity has been listed in a general way according to field F-3, as a specific action in field F-2, or as acting against some other chemical H in field F-1, or acting like some other chemical H in field F-1.

In the present problem, the general action "antihistaminic" is found in F-3 punch 206. Compounds are also listed as having an antagonistic action, punch 3 or L in field F-1, against the secondary chemical histamine (found in the name file to be CBC 800,199) listed in field H. There is the possibility that a compound may be listed as having activity similar to some other well-known antihistaminic which would be listed in field H but with a different verb in F-1. Compounds with related activity and possibly related structure may be found in field F-2 under 8A1 or 8A11 for anaphylaxis, or in F-3 under 208 for antispasmodic since histamine is often considered a spasmogenic agent. Several sorts of the biological cards will be necessary to isolate all the possible antihistaminic entries from the general file.

3. As a result of sorting for these headings, 3,447 cards are found. These involve the results of biological tests of 1182 parent compounds, which with the various salt modifications actually represent 1315 compounds. By use of the collator it is very simple to remove the duplicate numbers or eliminate the salts.

4. Unfortunately these compounds have been tested by many procedures and on several different animal species. As a preliminary breakdown, the cards have been separated into groups according to animal species (field A-1).

Code Designation of Test Organisms		Number of Cards		Common Name
(No test organism given)		197		
A420101	Rana pipiens	1		Frog
A610201	Gallus domesticus	1		Chicken
A710101	Bos primegius	3		Cattle
A720101	Canis	26	140	Dog
A7201011	Canis familiaris	114		Dog
A720102	Urocyon cenereo	4		Gray fox
A720201	Felis	37	107	Cat
A7202011	Felis domestica	70		Cat
A730201	Mus	9		Mouse
A7302011	Mus musculus	49		Mouse
A730202	Rattus	12		Rat
A7302022	Rattus norvegicus	36		Rat

(Cont.)

Code Designation of Test Organism		Number of Cards		Common Name
A730401	Cavia	1957	2484	Guinea pig
A7304011	Cavia porcellus	527		Guinea pig
A7306011	Cricetus frumentarius	2		Hamster
A740101	Homo	46	204	Man
A7401011	Homo sapiens	158		Man
A770101	Lepus	1		Rabbit
A7701011	Lepus americanis	1	198	Rabbit
A770102	Oryctolagus	115		Rabbit
A7701021	Oryctolagus cuniculus	81		Rabbit

Perhaps the most startling find of this particular count or sort is that 197 cards, apparently prepared from scientific journals, purport to describe the antihistaminic activity of various compounds but do not state what experimental animal was used! The count also indicates that a comparative survey of antihistaminic activity in various species is not yet complete.

5. The groups of cards under guinea pigs, rabbits, and man are sufficiently large to warrant further sorting. Since several different procedures have been used, the groups under dogs and cats do not yield enough cards of a single type to be interesting.

Dividing the guinea pig cards according to the test procedure used, yields four definite groups:

gI	Action against histamine on isolated intestine (F-2 813, C-1 66 67 or 68, F-1 3 4 L M or 5)	1206
gII	Protective action against histamine aerosol (H 800199, F-1 3 4 L M or 5, F-2, 11 111 or 16, Y i)	190
gIII	Protective action against parenterally administered histamine (Same as group II except Y 5)	82
gIV	Protection against anaphylactic shock (F-2 8A11, F-1 L or M)	11

The remaining 995 guinea pig cards were rejected since they did not fit one of these four classes, or had inadequate data, or could not be grouped together.

The cards in the four groups were classified according to the quantitative activity in field G (column 76), with 0 active but amount not stated, 1 relatively inactive and increasing up to 9 as most active.

Rank	gI	gII	gIII	gIV
0	83 - 69	20 - 20	1 - 1	1 - 1
1	527 - 396	34 - 29	10 - 9	-
3	292 - 256	37 - 24	23 - 18	-
5	202 - 155	64 - 39	26 - 17	2 - 2
7	67 - 39	20 - 11	11 - 6	2 - 2
9	48 - 27	18 - 15	15 - 11	6 - 4

The left hand columns are the total number of cards in this activity class; the right hand columns have had the duplicate compounds removed. These are not true duplicates, but are instances in which different workers, or the same workers in different references have found the same activity for a given compound by the same experimental procedure.

Two reasonably sized groups of cards were found among the rabbit entries. These included 9 items tested on uterine muscle and 63 items tested on intestinal muscle against histamine produced spasm. The 204 cards referring to antihistaminic activity in man were rather discouraging in that a common method had been used in only 21 instances and 11 of these were found to be listed as inactive.

6. As a check on the relative uniformity of the assay procedures, the number of duplicating cards in various activity levels was determined for the largest group of items (those listed under gI):

gI CARDS COMMON TO OTHER ACTIVITY LEVELS

Rank	1	3	5	7	9
0	20	6	10	2	1
1	396	45	25	1	3
3	45	256	28	5	3
5	25	28	155	11	7
7	1	5	11	39	8
9	3	3	7	8	27

It is readily apparent from this table that there is some overlapping between adjacent activity levels, but that the number of items reported as having both very high and very low level activity is relatively small. This is probably some tribute to the majority of pharmacologists that have used this method. The duplicate entries of the same activity were eliminated before this table was constructed.

There were thirteen CBC numbers that appeared in three activity classifications 1, 3, 5 or 1, 3, 7 as determined by the guinea pig intestinal strip method. These compounds were taken back to the original data sheets, and as the cause for the multiple listing did not appear obvious from these, the original references were consulted. The compounds all originated in

the same laboratory, were listed as slightly active as antihistaminics in a Federation Proceedings abstract, listed with various activities on an arbitrary scale in a paper describing their chemical synthesis, and finally listed in terms of activity against definite amounts of histamine in a pharmacological paper. The interpretation of their activity by various abstracters and coders led to their multiple listing. This is really a semantic error, if it is an error at all, and can be expected to occur in any system that tries to reduce biological data to symbols. Some of the duplicate entries may have occurred in a similar manner, since many of the compounds are described in both "chemical" and "pharmacological" papers.

The other aspect of the assay correlation problem, which compounds appear as high activity compounds by more than one investigative technique, is encouraging. If one combines the 7 ("high activity") and 9 ("most active") levels of each group, but subtracts the common compounds of each group, then: gI 39 + 27 - 8 = 54; gII 11 + 15 - 3 = 23; gIII 6 + 11 - 4 = 13, and gIV 2 + 4 - 2 = 4. Of the 23 gII items, 10 are common to gI; of the gIII items, 10 are common to gI and 1 additional is found in gII; and all of the items in gIV are listed in gI. On a percentage basis this is a remarkably good correlation among the active compounds. As a total check of all the active and inactive compounds, a table has been constructed of the gI, gII, and gIII entries.

CORRELATION OF QUANTITATIVE ACTIVITY VERSUS TEST METHOD

Number of Common Compounds

gI 1						gII 1						gIII 1					
II	1	1	III	1	5	I	1	1	III	1	0	I	1	5	II	1	0
	3	6		3	6		3	0		3	0		3	3		3	1
	5	7		5	7		5	0		5	0		5	3		5	1
	7	4		7	1		7	0		7	0		7	3		7	2
	9	4		9	2		9	0		9	0		9	2		9	1
gI 3						gII 3						gIII 3					
II	1	0	III	1	4	I	1	6	III	1	1	I	1	6	II	1	0
	3	11		3	10		3	11		3	1		3	10		3	1
	5	9		5	6		5	5		5	3		5	3		5	0
	7	4		7	1		7	4		7	3		7	2		7	1
	9	1		9	2		9	3		9	2		9	0		9	1
gI 5						gII 5						gIII 5					
II	1	0	III	1	3	I	1	7	III	1	1	I	1	7	II	1	0
	3	5		3	3		3	9		3	0		3	6		3	3
	5	8		5	11		5	8		5	2		5	11		5	2
	7	5		7	4		7	5		7	3		7	8		7	8
	9	6		9	4		9	10		9	3		9	3		9	4
gI 7						gII 7						gIII 7					
II	1	0	III	1	3	I	1	4	III	1	2	I	1	1	II	1	0
	3	4		3	2		3	4		3	1		3	1		3	3
	5	5		5	8		5	5		5	8		5	4		5	3
	7	6		7	4		7	5		7	2		7	4		7	2
	9	3		9	5		9	8		9	6		9	3		9	2
gI 9						gII 9						gIII 9					
II	1	0	III	1	2	I	1	4	III	1	1	I	1	2	II	1	0
	3	3		3	0		3	1		3	1		3	2		3	2
	5	10		5	6		5	6		5	4		5	4		5	3
	7	8		7	3		7	3		7	2		7	5		7	6
	9	3		9	5		9	3		9	3		9	5		9	3

Since all the duplicating entries are listed, some activity groups appear to have more compounds than the total number of compounds listed for that activity group. The overall correlation is fair, with more entries listed in low-low and high-high in general than in crossed divisions as low-high. The gII class has a lower over-all activity rating than gI and gIII or else the gI and gIII are biased toward the higher activity levels.

One compound (800,422) is listed as having activity at the following twelve levels: gI 3, 5, 7, and 9; gII 3, 5, and 7; gIII 5, 7, and 9; and gIV 5 and 9. One other compound is listed nine times and one eleven times. This is the result of using these compounds for comparative purposes or standard reference chemicals as disclosed by sorting field H for compounds listed as using -03 or -04 criteria in field T.

The rabbit data is not correlatable with the guinea pig data. Only one compound is listed in both groups. The name file discloses that it is atropine. The formula file shows that the compounds are almost exclusively esters. Reference to data sheets indicates that the compounds were primarily tested for atropine-like activity and for antihistaminic activity secondarily. Since there is no overlapping of active compounds, it is impossible to decide if the method is adequate for determining useful antihistaminic activity.

As a tentative conclusion, based on the punch cards alone, the guinea pig test methods most commonly used do adequately separate the various chemical compounds, with the most active compounds as determined by one technique being most active by other techniques, and the less active compounds almost always being less active. This is true within the normal limits of operator error.

7. For visual examination of the active compounds, it is necessary to refer to the chemical formula file. The gI 7 and gI 9 punch card numbers have been removed from the chemical file. Of this group of 54 cards, 11 have formulas that correspond to R-O-C-C-N= with large groups for R, such as benzhydryl, minor substituted benzhydryl, or benzylphenyl, no substituents on -C-C-, and two methyl groups on the N or a very small heterocyclic group. Twenty-three have formulas that correspond to $Ar\text{-}CH_2\text{-}\underset{\displaystyle Ar'}{\overset{\displaystyle |}{N}}\text{-}C\text{-}C\text{-}N=$ with aromatic ring groups such as benzene, thiophene, or pyridine for Ar and Ar', with minor ring substituents in some instances, no substituents on -C-C-, and two methyl groups on the N. The remaining compounds comprise a very miscellaneous group as far as structural chemistry is concerned.

The gII high activity group only contributes two more ethylenediamine derivatives, two more ether derivatives and three compounds that are related to $Ar\text{-}\underset{\displaystyle R}{\overset{\displaystyle |}{N}}\text{-}C\text{-}C\text{-}X$ with X either chlorine or bromine. The gIII compounds that are different only contribute one ethylenediamine compound. All high activity compounds in gIV are listed in gI.

Reference to the CBCC name file, for the names of active compounds of ether or ethylenediamine derivatives listed above, reveals that 16 compounds are listed in gI that are commercially available on the open market under a variety of trade and common names.

Of the miscellaneous compounds, the name file lists theophylline, epinephrine, nicotinamide, meperidine, ephedrine, papaverine, arterenol, Orthoxine, prophenpyridamine, and chlorcyclizine as highly active. Most of these miscellaneous compounds are not usually considered antihistaminics by definition, and the two that are increase the number of active commercially available compounds to 18.

For many purposes, this type of correlation between chemical structure and high activity would be sufficient. Another aspect of the problem is the determination of the correlation between chemical structure and low activity. For this, the chemical structure file was consulted and the cards corresponding to the 396 gI 1 numbers found. Of these formulas, 196 were RR=N-C-C-N=RR, 108 were R-O-C-C-/-N=RR, and the rest miscellaneous. The ethylenediamine derivatives included several acetamidine and imidazoline compounds, some compounds with very low molecular weight or very large molecular weight or size, with very large aromatic substituents or large alicyclic substituents, and with these exceptions were similar to the high activity compounds in gI 7 and 9. The same remarks may be made for the ether derivatives. Consulting the name file for the miscellaneous compounds revealed that the list

included such items as quinidine, atropine, nicotinamide, meperidine, Trasentin, procaine, papaverine, Dibucaine, cocaine, Benodane, methadon, alpha-fagarine and lysolecithin. Two of these miscellaneous compounds were among those listed as having high activity as antihistaminics. Consulting the name file for the low activity ethylenediamine and ether derivatives did not reveal that any of these relatively inactive agents have a common, public, or trade name, although many of them have serial or laboratory numbers of one kind or another.

Since most of the active compounds that are commercially available are of the type $Ar\text{-}CH_2\text{-}\underset{\underset{Ar'}{|}}{N}\text{-}CH_2CH_2\text{-}N(CH_3)_2$, $(Ar)_2{=}\underset{\underset{R}{|}}{C}\text{-}O\text{-}CH_2CH_2\text{-}N(CH_3)_2$, or with a phenothiazine ring replacing the $Ar\text{-}CH_2\text{-}\underset{\underset{Ar'}{|}}{N}\text{-}$, or with minor substitutions in one of the Ar rings, and since most of the apparently possible major modifications of these two types of compounds are listed in the lesser active groups, further endeavor along these two chemical lines might not be very profitable. More promising leads for further synthetic work might be found by critical examination of the miscellaneous group in the lesser active classes with the possibility that new synethetic relatives might be more active, or else to investigate types of compounds that have not been listed at all as having been investigated.

8. In order to find if compounds with antihistaminic activity may be in other classes of pharmacologically active compounds, it is necessary to know what other types of activity antihistaminics may have. Such a search or correlation might be useful to find other uses for both old and new antihistaminic agents. Consequently, all of the punch cards having CBC numbers that have been found as having any antihistaminic activity have been separated. Preliminary sorting of these cards reveals that antihistaminics are listed as having apparently been tested 3427 times for some 81 other types of activity. Of these, 255 are not definitely listed on the punch card under the common F-2 heading, 1544 are listed as having some effect on muscle tone, 858 refer to toxicity determination (667 "acute" toxicity), 260 on histamine shock, 160 on blood pressure, 67 on the toxicity of a secondary chemical, 30 on body secretions, 26 on relief of action of a secondary toxic chemical, 26 on anaphylaxis, 15 on permeability, 13 on growth, 13 on ability to produce ulceration, 12 on respiration, 11 on sedative activity, and the balance with only a few chemicals being tested for a particular activity.

It should be remembered that these groups include both negative and positive results. With a particular card selected at random, the top of the punch card reads 500,990 005 A7401011 2 329 6 W 842 and use of the biological code indicates that man (A7401011) was given CBC 500,990 orally (2) and that the permeability (842) of the capillaries (329) of the skin (6) was not affected (W) by the dose given. The reference is on data sheet 005 of compound 500,990 which, according to the name file, is diphenhydramine. The machines in this instance could serve for a very rapid cross search of activity. Compound 500,990 is listed as being tested 121 times of the total 3427 entries for secondary activity.

The correlation of the secondary actions and antihistaminic activity is not very good. In the first place, some of these cards are additional entries of material that has already been covered. Of the 1544 cards referring to action on muscle tone, 1496 refer to actions on muscle that has been made spastic with histamine or acetylcholine and have already been considered in gI. Similarly the 260 histamine shock items and 67 and 26 items involving a secondary chemical have been considered in gII and gIII and the 26 anaphylaxis items have been considered in gIV.

The 160 items involving blood pressure include 84 items in dogs and cats relating to the ability to overcome histamine vasodepression and the other items are insufficiently limited to one animal species to indicate any possible correlation. These duplicate entries of activity have to be rejected for this type of correlation, although they have a very useful purpose in that they insure that any misinterpretation of the biological code will not invalidate the entire system.

The only reasonably sized group of secondary actions remaining is the 667 "acute" toxicity items. Most of these involve rats as the species and can be broken into two approximately equal sized groups according to route of administration since they are almost all oral or intraperitoneal with only a few intravascular administrations listed. Unfortunately, correlation of these compounds with those listed in gI does not yield any positive results, since there are almost as many high rodenticidal activity as low rodenticidal activity cards in both the high and low antihistaminic activity entries. This simply means that rodenticidal activity and antihistaminic activity are not related.

Therefore, while it is obviously desirable to test antihistaminics for rodenticidal activity before testing in man, it does not seem that all efficient rodenticides should be tested for antihistaminic activity, either in guinea pigs or man.

Consequently, there does not seem to be any secondary activity that is directly related to antihistaminic activity (other than antianaphylactic), nor is there any other general type of activity that might be searched for compounds that would be expected to have antihistaminic activity.

9. The most desirable test of the data summarized in answers 6 and 7 is its applicability to results found in man. The available published data of the investigation of antihistaminics in man is inadequate, discontinuous, and non-uniform as indicated by the 21 cards in which the antihistaminic had been tested for the ability to prevent the local effects of histamine in the skin out of the total 204 listed tests in man. The 11 compounds listed as inactive in man were also listed in gI 1, 3, or 5. Two cards with 7 activity in man were listed in gI 9 as was one card with only 1 activity in man. One card with activity not stated (0) was listed in gI 7. The remainder of the cards were duplicate entries.

The gI 9 card that was listed as only a 1 activity in man was referred to the data sheet. Apparently a high enough dose to produce a high level of activity could not be tolerated in man. With this expected type of exception, the data in man, as little as it is, does correlate well with the data obtained by the most commonly used test procedure. The two high activity compounds by test in man are commercially available; it is unfortunate that comparable testing of the other commercially available compounds has not been undertaken.

By the time this symposium is published, such a study may have been undertaken with the common commercially available compounds, but I doubt if adequate data will ever be published for the other gI 9 compounds. Many of these are available to only limited numbers of individuals; in general, if a compound is tested in man by a commercial house, and not found superior to some comparison compound that is widely used, there is not much incentive to publish the results. For chemical-biological correlation to have any eventual application, the results of such tests of compounds that appear only mediocre or even relatively inactive must be made available.

10. It is scarcely reasonable to carry out any profound chemistry sorts when the cards have already been put in small groups by the biological sorting. If all the antihistaminic agents had been investigated by one common technique, or all the techniques could be related in any way, then the machines could be used for chemical type and frequency analysis. A few trials have been run to indicate the possibilities.

A frequency analysis of the number of carbon atoms versus the relative activity of the CBCC compounds in gI has been made.

NUMBER OF CARBON ATOMS IN ANTIHISTAMINIC

The analysis is biased since some of these compounds contain -S- or -N= or -O- as isosteric replacements for -CH= or -CH=CH- in some aromatic rings. On this basis, most of the compounds with 14 carbon atoms belong either in the 15 or 16 class. Most of the compounds investigated have 14 to 18 carbon atoms, including most of the relatively active and inactive agents. There is only a very minor shift of activity toward the lower number of carbon atoms as the activity increases.

A more interesting analysis is based on the frequency distribution of the chemical code groups in the gI compounds. By use of the rotating file it is possible to place any group first in the chemical code designation. The 155 compounds in gI 5 require 82 code groups for all of the chemical structures although the total number of separate groups is 924 or an average of about 6 groups to name a compound.

The incidence of common groups is even greater with gI 7 and 9. With gI 7 that has only 20 compounds after the gI 5 entries have been removed, only 40 characters are required and these have occurred only 153 times with 6 characters accounting for 71 of these. With gI 9 the number of groups has dropped to 38 with 107 entries, and 5 characters account for 51 of these.

Analysis of these code designations for the gI 9 entries in the chemical code indicates that some type of aliphatic substituted tertiary amine has occurred 29 times, that a pyridine ring has occurred 6 times, pyrimidine 2, ether oxygen 8, alcohol 2, furan 1, benzene or phenyl 12, four carbon atom chain 2, three carbon atom chain 5, two carbon atom chain 11, and one carbon atom methyl or methylene 12. These are the findings one would expect from the visual examination of the data in 7.

If the effect of a certain substituent on antihistaminic activity is desired, it can be readily found. For example, the effect of introducing a non-ionic halogen atom into an aromatic ring can be found from columns 55 and 56 of the chemical cards. Of the 108 such compounds found in the gI series only 4 are found in the highly active group. It would probably be easier with this small group of active materials to find the non-halogenated congeners in the formula file than to run the collator on the parent groups of 1182 compounds. Visual examination of the formulas of the compounds against the non-halogenated parents indicates that halogenation may increase the activity or decrease it, and that this is a function of the position of the halogen atom on the aromatic ring. This type of position isomerism is a feature that the CBCC chemical code is not equipped to handle; i. e., the code does not differentiate between ortho, meta, and para substitution of a benzene ring, since the number of such isomers is always small enough that they can be

separated visually.

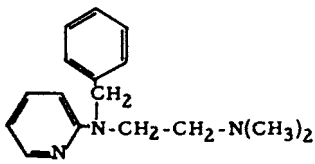
SUMMARY

1. The answer to the fundamental question, "Can the CBCC system show any correlation between chemical constitution and antihistaminic activity by use of the IBM machines" is generally in the affirmative as developed in section 7.

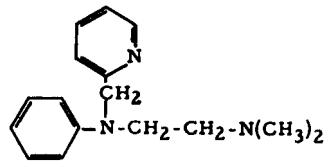
It has been pointed out that the biological data is limited by lack of use of a universal test procedure for all compounds and that the relationship of effects in man to effects in small animals is limited by lack of published data of experiments in man, although there is no lack of general clinical data. There is good correlation between man and animals in the data available, and the data obtained by one test procedure is generally equivalent to that obtained by other test procedures.

The results can only be as good as the original data. If the data is not uniform, or based on uncontrolled experiments, clinical impressions, or non-comparable methods, then the Center cannot function. It may be able to indicate groups of data, or trends, so that future work may be better carried out, or in rare instances to find an isolated compound with a desirable action so that families of compounds can be prepared to find better compounds with this action.

Certain aspects of the problem have not been considered. The third component of chemical biological correlation, that of time, whether expressed as duration, uptake, fate, or excretion, has been ignored, since there is so little available data on this subject as far as the antihistaminics are concerned. Another problem, that of position isomerism and biological activity, is beyond the scope of the machines, the code, and our present chemical knowledge. For example, by any classification system you wish to use CBC 801,638 and 800,422 have the same chemical components, the same size and shape, and very similar chemical properties.



800,422



801,638

The both have the same molecular weight, two aromatic rings, the same constituents, the same shape, and even the Hirschfelder models look identical. The 800,422 is a highly active antihistaminic that is commercially available as tripelennamine and is widely used in man; the 801,638 is an inactive, or relatively inactive, antihistaminic that is worthless. This is one instance when we do not know enough of the underlying chemistry of the two molecules to know how they differ, and until we have this information, we cannot expect to know why the biological activity differs.

As abstract journals do not list all actions, particularly minor or secondary actions of compounds in their indices, the CBCC serves as a reservoir of such information and secondary activity studies may lead to many investigations, either to develop compounds with better primary

activity, or with more desirable secondary activity in some instances.

This demonstration should not be considered complete as many items are not listed for lack of space. For example, the Center has a combination chemical-biological card which will be valuable for rapid searches of a limited nature, and the true value of the chemical code has probably not been emphasized. Much data about intermediate activity compounds has not been included in this brief presentation, and only limited examples of the numerous inactive compounds. Such information would be valuable to an investigator, and it is available in most of the modern review articles of the subject.

Examination of the finally selected formulas by visual inspection indicated that high activity antihistaminics belong to three classes: a group of ethylenediamine derivatives with specific limitations as to substituents, a group of 2-dialkylaminoethyl ethers with specific limitations as to substituents, and a miscellaneous group of compounds that had no common chemical features. It must be remembered that such correlations are non-limiting in a dynamic field such as biological science. Other compounds, which are entirely different in type of structure, may have very similar activity or be highly active. Similarly, all compounds with the same or similar structural components and similar size and shape as one of the two limited groups above will not necessarily be active. However, all highly active compounds of these basic types will have this size and shape, and when all the possible ones of this size and shape are made, it will not be necessary to make all of their derivatives or derivatives outside of these dimensional limits.

A DEMONSTRATION OF THE USE OF THE CBCC SYSTEM IN THE FIELD OF ANTITHYROID COMPOUNDS

by

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When this demonstration was planned, it was hoped that most of the antithyroid data in the literature would be recorded on CBCC punch cards and available for use before the Symposium. Thus, it would be possible to make correlations of chemical structure with antithyroid activity, utilizing the same basic information which was recently used in a review article[1]. Advantages and disadvantages of the CBCC system should become apparent. However, revision of the detailed CBCC biological code caused sufficient delay that not all of the antithyroid literature could be abstracted and the data coded, punched and sorted for a complete study. The results to be described here were nevertheless sufficient to indicate that the CBCC system is workable, and can be used conveniently to answer specific questions in the antithyroid field.

This demonstration is based on fifteen questions which were designed to disclose the most active antithyroid compounds, and to bring out correlations of chemical structure with antithyroid activity. A continuation of the process would, of course, yield more information, and other approaches could be used to get the same results.

Before proceeding with a discussion of the machine operations used to obtain data from the punched cards, a few general remarks about the nature of the information there are in order. Antithyroid compounds, which may be defined as those compounds which depress the secretion of the thyroid hormone, have largely been developed since 1942. Something like eight hundred compounds have been tested and reported in the literature. Most of the testing has been done in rats. Generally, the compound was fed in the diet or drinking water to young animals for ten days to two weeks and the effect on the thyroid gland was then determined. An active compound will cause cellular hyperplasia, an increase in weight of the gland, and a decrease in iodine content. All of this arises because of an interference with the synthesis of the thyroid hormone. Organically bound iodine is not stored, and the gland increases in size in a compensatory action caused by the thyrotropic hormone from the pituitary.

The antithyroid effect was measured in the early work by the hyperplasia, and in the later, more precise investigations, by the effect on thyroid weight and iodine content. The latest method depends upon the rate of uptake of radioactive iodine by the thyroid gland. This method has been of particular value in determining relative activities in man and monkey.

Antithyroid activities relative to a standard, which is usually 2-thiouracil, have been reported in the literature for tests in rats, and also for the limited tests in man and monkey by the radioactive iodine method. Also, where relative activities have not been given, they can be estimated from dose-response data. The biological code of the CBCC is suited to this, since doses and responses are recorded as falling in ranges.

Thus, most of the animal evaluation of antithyroid activities of chemical compounds has been done in rats. This information is either summarized in the form of the author's evaluation relative to 2-thiouracil as a standard, or can be put in this form by comparing dose-response data. Finally, these relative activities in rats can be compared to those reported for man as a test animal.

With this background, the following questions were devised:

(Group I. Designed to select the most active compounds in rat tests, and see which have been tested in man.)

1. How many compounds tested in rats have an activity of 0.05 or greater? (Thiouracil = 1)

[1] Medicinal Chemistry, Vol. I, pp. 1-150, John Wiley and Sons, Inc., N.Y., 1951.

2. Which compounds tested in rats have an activity of 0.5 or greater? (Thiouracil = 1)

3. How many compounds showed a +2 hyperplasia of the thyroid gland when administered orally to rats for a week or more in daily doses of 125 mg./kg./day or less? (Should give compounds 0.2 as active as thiouracil or greater, based on Astwood's (1943) results.)

4. Which of the compounds in Question 3 showed a +2 hyperplasia or greater when tested at a dose of 25 mg./kg./day or less? (Should give compounds as active as thiouracil or greater.)

5. Which of the compounds in Questions 1 and 3 have been tested in man?

6. How many of the compounds in Questions 1 and 3 have not been tested in man?

7. Which of the compounds in Question 5 have an activity of 0.05 or greater in man? (Thiouracil = 1)

8. Which of the compounds in Question 5 have an activity of <0.05 in man?

9. Which of the compounds in Questions 2 and 4 have not been tested in man?

10. Based on thyroid weights alone, how many compounds have an activity of Class 5 or higher (Gives a group of compounds which could be further analyzed as in the questions above.)

(Group II. To correlate chemical structure with activity.)

11. Do compounds in Questions 1, 3 and 10 have any common chemical grouping?

12. Are there chemical groupings which confer inactivity?

13. Are heterocycle compounds more active in general than nonheterocycles?

14. Knowing that compounds containing a -SH or =S group may be active, are any -SR compounds active? (R is anything but H)

15. Are any compounds of molecular weight >500 active?

We can now proceed to the demonstration.

The plan followed in the CBCC machine room by Mr. Ballard, supervisor, was to first select a basic group of cards containing the information desired and then classify these. The classification was actually done by two procedures, and a description of both will illustrate the possibilities of the CBCC system.

Since demonstrations in both the antihistamine and antithyroid field were planned for this symposium, a group of cards containing data in both fields was sorted out. For Procedure I, 1432 cards were available. Later, the number of cards increased considerably as the work of abstracters and coders flowed in, and all of these were used in Procedure II. Results are given in Table I.

TABLE I

ANALYSIS OF BASIC CARDS

Operation (machine)	No. of Cards
Procedure I (used on preliminary data)	
Start	1432
1. Separate cards in Field F-3 (general action), punched in 207 in Columns 73-75 (antithyroid)	862
Rejects	570
2. Divide the antithyroid cards according to the test organism (Field A-1); get	
A4 (frogs)	9
A6 (chickens)	4
A7 (mammals)	849
3. Breakdown of A7 in (2):	
A72 (carnivora) [2 dogs, 1 cat]	3
A73 (rodents)	824
A74 (primates) [all man]	22
4. Breakdown of A73 (rodents)	
A730401 (guinea pigs)	2
A730201 (mice)	21
A730202 (rats)	801
5. Cards in activity classes 3, 5, 7, 9 relative to thiouracil as a standard (Field H) Quantitative Summary (Fields T + G), for rats	39
Procedure II (simplified from I; used on final data)	
1. Cards in Field F-3, punched 207 (antithyroid)	2081
2. Number of cards in response classes 3, 5, 7 and 9 (all animals; some cards counted twice because of double punches)	1041
3. Activity breakdown for rats - total cards	935
Class 3	383
Class 5	328
Class 7	142
Class 9	154
(double punches are present)	1007

In Procedure I, the cards punched for antithyroid action were separated. These were then sorted by machine according to the test animal. Since the questions deal with tests in rats and man, the cards for these were selected for further study. As well as giving the desired cards for further study, operations 1-2 also disclose that most of the antithyroid testing has been done with mammals, and rats in particular, as was expected. Since the questions in general deal with quantitative responses, the cards for rats were further treated by operation 5. Field T of the CBCC system records responses, and Field G gives quantitative summaries in five classes (1, 3, 5, 7 and 9). When activities are given relative to a standard (specified in Field H), activity ratios are classified according to:

Class	Ratio of $\frac{\text{Test Chemical}}{\text{Standard}}$
1	<0.05
3	0.051 - 0.5
5	0.51 - 5
7	5.1 - 50
9	>50

Much of the antithyroid data in the literature is given as activities relative to thiouracil as a standard. A compound twice as active as thiouracil would fall in Class 5, for instance. Thus, operation 5 gave the separation of cards necessary to answer the Group I questions.

When more cards were available, time was saved by using Procedure II (Table 1) to obtain the same grouping of cards resulting from operation 5 of Procedure I.

The fact that the machine counted 1007 punches from 935 cards indicates "double punching". This is sometimes done in the CBCC coding because the information available shows a range of activity greater than any one digit in Field G indicates (see above Classes vs. Ratio of Test Chemical to Standard).

A given activity may be recorded in two or more ways, depending upon the criteria used in the evaluation. For instance, the antithyroid activity of a compound may be a response (a) relative to thiouracil as a standard; (b) based on hyperplasia; (c) based on thyroid weight; (d) based on iodine content of the gland; or (e) miscellaneous minor responses. If the recorded response involves two criteria, both may be punched but on separate cards.

Summarizing the basic procedures, (I) gave incidental information as well as the basic cards to answer the questions and is an exploratory type operation; (II) was a direct approach to the basic cards. This illustrates that information on punches cards can be extracted by various procedures. When a large number of cards is involved, the actual running time of the machine is important; a skilled operator can reduce it to a minimum using an efficient pre-filing system.

The answers to the Group I questions are given in Table 2. Questions 1 and 2 require no comment.

Question 3 was chosen to include the earlier investigations where the results were not expressed relative to thiouracil. From Astwood's early and later papers wherein thiouracil was tested by both methods, the estimate was made that the answer to Question 3 should give compounds which are two-tenths as active as thiouracil or greater (particularly for Astwood's results). The CBCC code given under Question 3, Table 2, involves a rating also of verbal statements of activity, and the answer here probably covers other papers, which is all the better. The eighteen compounds found can be considered a basic group, of interest comparable to those of Question 1. Question 4 selects the more active ones, and is to be compared with Question 2. It so happens that the three compounds turned up have all been retested relative to thiouracil, but only 6-methyl-2-thiouracil was as active as thiouracil. A check of the thirty-two compounds in Question 2 confirms this - it is the only one of the three listed there. Therefore, the basic assumption that the procedure should turn up compounds as active as thiouracil or better is a little too broad. Incidentally, the values found by Astwood were 0.4 for 1,3-diethylthiourea, 0.3 for tetramethylthiourea, and 1 for 6-methylthiouracil. Also, all three have had clinical trial in man. 6-Methylthiouracil is twice as active as thiouracil clinically, and is widely used for the treatment of hyperthyroidism. The other two were not as good as thiouracil but have not been definitely rated.

TABLE 2

ANSWERS TO GROUP I QUESTIONS

Question 1

Compounds tested relative to Thiouracil = 1, breakdown:

Class 3	42
5	24
7	7
9	1
Total (answer)	74

Question 2

The cards in activity classes 5, 7 and 9 (32 in number) of the group separated for Question 1 are examined for CBCC code number, and this is looked up in the files to give the name.

Question 3 Answer: 18 compounds

Composed of compounds of Classes 5, 7 and 9 in Field G, punch -01 (Author's evaluation) or punch -02 (Coder's or Abstracter's evaluation) of the response criteria (in Field T):

Class			Verbally stated in abstract
1	0	0	inactive
3	+		slight or mild
5	++	+	active or moderate
7	+++	++	very active
9	++++	+++	extremely active

Question 4 Answer:

CBCC Number	Name
801.456	urea, 1,3-Diethyl-2-thio-
801,602	urea, Tetramethylthio-
900,059	uracil, 6-Methyl-2-thio-

TABLE 2 (Cont.)

		CBCC Number	Compound
Question 5	Answer:	800,253	urea, Thio-
		800,694	2-Benzimidazolethiol
		900,059	uracil, 6-Methyl-2-thio-
		900,587	uracil, 2-Thio-
		800,569	2-Thiazoline-2-thiol
		900,899	barbituric acid, 5,5-Diethyl-2-thio-
		901,796	uracil, x-Methyl-2-thio-
Question 6	Answer:	70 compounds	
Question 7	Answer:	CBCC Number	Compound
		800,253	urea, Thio-
		800,569	2-Thiazoline-2-thiol
		900,899	barbituric acid, 5,5-Diethyl-2-thio-
		901,796	uracil, x-Methyl-2-thio-
Question 8	Answer:	None	Indicates that 800,694, 900,059 and 900,587 in Question 5 were not rated as to relative activities.
Question 9	Answer:		CBCC numbers given for 24 compounds of activity class 5, seven in class 7, and one in class 9. The numbers then looked up for the structure of the compounds.
Question 10	Answer:	26 compounds	

Question 5 necessitated the use of the collator. For this purpose, the cards from Question 1 were recombined with those from Question 3. These were then reassembled by the collator in order of the CBCC numbers. Thus, this stack was ready for comparison with the cards indicating tests in man, which were obtained by an operation on all the residue cards of Procedure II. The machine was then set to select cards on the basis of identical CBCC numbers in both stacks. The numbers and compounds obtained are listed in Table 2.

Question 6 is an easy one to answer, since it merely involves a count of the rejects from Question 5. The answer was seventy compounds. The purpose of this question was to indicate that there is a large group of compounds highly active in rats which might logically be investigated clinically in man.

Question 7 shows that four of the seven compounds tested in man are active. Question 8 gave the answer of none. This indicates that the residual compounds in Question 5 were not rated as to relative activities. This was easily checked by observation of the cards.

Question 9 involved use of the collator as done for Question 5. The machine operation also gave the activity classes, which from the nature of the activity were Class 5, 7 or 9. There were twenty-four 5's, seven 7's, and one 9. The purpose of the question was to make available a list of highly active compounds (in rats) which might logically be tried in man. Question 6 told us that there were seventy highly active compounds by the rat tests which had not been tested in man, and Question 9 extracted and listed the most active thirty-two. From the results of the above questions, particularly No. 4, we might safely assume that all of these thirty-two are at least one-half as active as thiouracil in rats.

The last question concerned with activity tests is No. 10. It gives a group of active compounds not included in the above. These were excluded from the detailed treatment because

some of the papers which report activities by the effect on thyroid weight alone are not of very high quality. The compounds in this group might well be analyzed as in the question above.

In summary, the purpose of these ten questions was to test whether or not the CBCC system of recording and handling data on antithyroid compounds is useful in indicating compounds which might logically be further tested in man. The answer is - yes, it is useful. But since the field of antithyroid compounds is comparatively small, a critical study of the published papers would be possible for a clinician desiring to enter the field, and it would probably give better results. It is to be remembered that the CBCC system involves little criticism. Nevertheless, the results such as obtained here would be a good starting point for a newcomer in the field. The value of the CBCC system grows as the quantity of published data in a field increases.

The second phase of this investigation on antithyroid compounds (Group II questions) is concerned with correlations of chemical structure with biological activity, and Questions 11 through 15 are associated.

The procedure was to compare the cards obtained from Questions 1, 3 and 10 with the rotated chemical cards which were made up from all the basic antithyroid group including active and inactive compounds. You will recall that the rotated cards are obtained by duplicating each chemical card in such a way that each chemical group in turn appears first in the sequence of chemical code designations. Actually the Center had time to process only those cards from the Question 1 group. However, the procedure would be the same for the Question 3 and Question 10 cards. The result was a machine-typed listing of chemical code designations and their frequency of occurrence. That is, the number of cards for each chemical field was given, and these were also broken down according to all activity classes 1, 3, 5, 7 and 9. Question 11 asks for activity classes 3, 5, 7 and 9. Question 12 requires study of the activity class 1 cards, and Questions 13 and 14 use all activity classes.

This sheet contained 180 compounds with chemical groups occuring 720 times. Duplicates of any one group in a given compound were excluded. Seventy compounds had antithyroid activity and 110 were inactive. These numbers are probably not great enough for detailed statistical analysis.

Question 11 was written to see if a chemical grouping such as -N-$\overset{|}{\underset{|}{C}}$-SH could be detected, since published reviews indicate that this group is found in most of the more active antithyroid compounds. This particular group does not appear in the CBCC code. It might appear from a detailed procedure of comparing the most active CBC groups which do occur, and analyzing them for common structural features. When a larger basic group of cards on antithyroid compounds is available, this process will be worthwhile. For the moment, no common chemical sub-grouping is apparent by inspection of the active compounds. Answer to 11: none apparent.

Question 12 also got a negative answer.

Question 13 - to answer this, a count of heterocycles in both the active and inactive classes was made. Sixty-four heterocycles, or 27 per cent of the 241 groups in the active classes, were found. There were eighty heterocyclies in 479 groups in the inactive class, or 17 per cent. One might conclude that heterocycle groups are favorable to antithyroid activity.

Question 14 was answered by observing that five compounds containing sulfide, or R-S-R' groups, were active. If these had not been observed, an investigation of such groups as Code 825, which includes thioureas and pseudothioureas, would be necessary. The pseudothioureas are $>$G-SR compounds, and the thioureas $>$C=S compounds.

When Question 15 was written, the author did not know that molecular weights were not coded. Coding of physical data is not now done, but there is considerable interest in such at the Center. The staff kindly calculated the molecular weights for the one hundred and six active compounds and the three hundred and nineteen inactive ones. Only one turned up in the active group which had a molecular weight greater than 500. This was thyroxine, which did not belong there anyhow, and slipped in by a mistake in punching (although it is an indirect antithyroid compound). Four inactive compounds of molecular weight greater than 500 were found. This is not a significant number. Perhaps someone should test more high molecular weight compounds.

These questions were, of course, only examples of what can be handled by the CBCC system. Many interesting things might well turn up on statistical analysis of the frequency of occurrence of chemical groups as related to biological activity. As the quantity of data grows, the value of the work done by the CBCC will increase. Perhaps some day we shall call it indispensable

PANEL DISCUSSION

ON

ANTIMALARIALS

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New York, New York

THE BIOLOGICAL BASIS FOR ANTIMALARIAL TESTS

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The present paper is an attempt to give the fundamental biological background for the chemotherapeutic papers which follow. These papers will deal with the question: "How active is a given compound as an antimalarial?" Before considering this quantitative question, however, we must ask a more fundamental one, namely: "What kind of antimalarial action does the drug exhibit?" It is obvious, of course, that the type of malaria must be specified. More important than this, however, we must indicate the stage of development of a particular malaria, for, unlike the bacteria, viruses, rickettsiae and most of the protozoa, the malarial parasites have an exceedingly complex life-cycle, a cycle involving several stages of development which differ from one another both microscopically and physiologically.

We shall be dealing with at least three kinds of antimalarial activity - prophylactic, suppressive and curative. Since each requires destruction of a different phase of development of the parasites, we must consider how all these phases fit together in the total life-cycle of the malarial organisms.

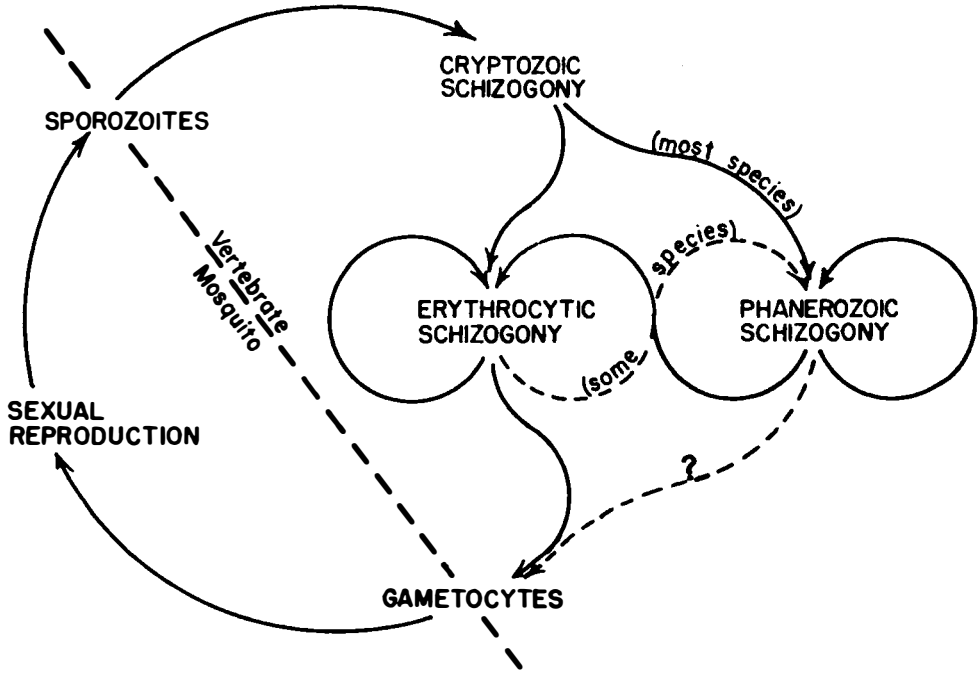

Figure 1

Figure 1 represents the general life-cycle of members of the genus Plasmodium as exhibited by those species which have been most thoroughly studied. Infection of the vertebrate host begins with inoculation of sporozoites from the salivary glands of an infected mosquito. These sporozoites invade certain cells of the tissues of the vertebrate and initiate a process of growth and multiple division known as cryptozoic schizogony. In bird malarias the parasites of this phase, known as cryptozoites, are found in macrophages, the large cells of the skin, liver, spleen, etc., which take in and destroy foreign particles[1]. In malarias of monkeys and man the cryptozoic schizogony is said to occur in the hepatic parenchyma, the liver cells proper[2], although it should be pointed out that there is disagreement on this point, investigators claiming either that the evidence is not yet conclusive or that at least some of the cryptozoites are in macrophages[3].

In those malarias which have been best studied, there are one or two generations of cryptozoic schizogony, occupying two to ten days, depending on the species. At the end of this time the young parasites produced give rise to two new kinds of parasites. One kind, designated phanerozoites, is similar to the cryptozoites. In the avian malarias they occur in macrophages or the endothelium lining most blood vessels. In the primate malarias, those of monkeys and man, they are said to develop in the liver cells. The phanerozoic schizogony is self-perpetuating. It persists throughout the total duration of the vertebrate infection, serving as a reservoir of parasites which may give rise, at times, to the erythrocytic parasites discussed below.

There is evidence that cryptozoites and phanerozoites are similar not only in appearance and cellular localization but also in the physiological attributes concerned with drug susceptibility. The two cycles together, constituting those stages in the vertebrate which occur outside of erythrocytes, are commonly called exoerythrocytic schizogony. It is proposed here that the parasites of this phase be designated by the etymologically dubious but convenient term exerythrozoites. The first one or two generations, occurring during the incubation period before the blood infections become established, are the cryptozoites (pre-erythrocytic stages of some authors). Later generations are the phanerozoites.

Simultaneously with the phanerozoites there arises also from the cryptozoites a type of parasite undergoing schizogony in the red blood cells. These are the classical parasites causing clinical malaria. They are commonly known as erythrocytic schizonts, but it is suggested that the term erythrozoites would be more convenient. Their reproduction is not self limited. They may be transferred to a new host by blood inoculation and will continue to reproduce there. However, in time, acquired immunity of the host brings an end to the acute attack of malaria by suppressing them to levels at which they may be detected with difficulty or not at all. At least in the primate malarias, relapse occurs by reinitiation of the erythrocytic cycle from the persisting phanerozoites. In at least some avian malarias erythrozoites may give rise to phanerozoites. In the primate malarias there is indirect evidence that this does not occur.

The final stage in the vertebrate host is the gametocyte, a sexual parasite arising in red blood cells from erythrozoites and perhaps also from phanerozoites. Once mature, the gametocytes do not develop further in the vertebrate host. Ingested by a mosquito they initiate the sexual phase of the cycle, which results eventually in the production of sporozoites infective for the vertebrate.

Before describing the test procedures for the various kinds of antimalarial activity it may be useful to review the essentials of the above life cycle as seen in the groups of parasites which interest us especially. The most widely used avian malarias are Plasmodium gallinaceum of chicks, P. lophurae of ducks, chicks and turkeys and P. cathemerium of canaries. They have two generations of cryptozoites, occupying two to three days. Phanerozoites and erythrozoites appear at the end of this period, become abundant, and are then suppressed to quite low levels, where they remain for months or years. Erythrozoites may arise from the phanerozoites and, at least in P. gallinaceum and P. cathemerium, may in turn produce phanerozoites.

In P. vivax of man and P. cynomolgi of monkeys incomplete evidence indicates that there are one or two generations of cryptozoites, lasting about nine days. Erythrozoites and phanerozoites arise together at the end of this time. The former become abundant, causing acute malaria, and are then eliminated from the blood. The persisting phanerozoites often repopulate the blood with erythrozoites at intervals of one to many months, initiating relapses. Erythrozoites apparently cannot produce phanerozoites.

In P. falciparum of man the cryptozoites occupy about the first seven days of the infection They then give rise to erythrozoites, but apparently not to phanerozoites. When the erythrocytic schizogony is suppressed, the infection terminates.

We may now consider the various kinds of antimalarial activity. Four are of major importance - prophylactic, suppressive, curative and gametocidal.

A drug showing prophylactic activity prevents establishment of infection in the vertebrate host. It is clear from Figure 1 and the above discussion that prophylactic action depends on destruction of sporozoites or cryptozoites or both. Tests for such activity vary in detail but show a similar pattern[4]. The test drug is administered to animals or human volunteers during the incubation period after inoculation of sporozoites. Determination of drug activity is based on whether infection develops in the test hosts.

There arises immediately the question of interpretation of what is usually called partial prophylactic action, namely a delay in the appearance of blood infection. Clearly a drug which merely delays infection has no practical value in the control of malaria. Such delay may result from at least three mechanisms. First, the drug may persist in the host long enough after administration to exhibit action against the erythrozoites. Obviously such action must be ruled out, and usually experiments can be so designed that it is not troublesome. Second, the drug may merely inhibit development of the cryptozoites. Finally, the drug may destroy some but not all of the pre-erythrocytic stages. Either of these last possibilities is of interest if it expresses practical potentialities. Drugs which show complete prophylactic activity at adequate dosage are partially effective in smaller doses. It is not known which of the two mechanisms is responsible. In any event, partial activity is of interest since it suggests follow-up studies which may reveal compounds of practical value.

It has been pointed out that prophylactic action may be directed against either sporozoites or cryptozoites. Either possibility would be useful, and in general experiments are not designed to distinguish between them. However, the best indications are that the known prophylactic drugs act on cryptozoites. No drug has been shown to be specifically active against the sporozoites injected by the mosquito.

There are two general expressions of suppressive activity. The first is clinical cure of acute malaria without eradication of infection. The second is prevention of acute malaria without prevention of infection. Both involve inhibition or destruction of erythrozoites. This is the action of quinine, quinacrine or chloroquine when given to a patient with acute clinical malaria or when administered continuously to exposed individuals. It is clear that drugs effective only against the erythrozoites cannot cure an infection, such as P. vivax, in which phanerozoites are present. Such drugs cure P. falciparum, however, for this infection does not exhibit persisting exerythrozoites.

Tests for suppressive activity in the avian malarias are simplified in accordance with the principle that we are interested merely in the effect of drugs on the erythrozoites. Birds inoculated with parasitized blood are treated with test drugs during the first few days of infection. Comparison of their course of infection with those of controls reveal action of the drugs on the erythrozoites. In the human infections suppressive activity is studied in tests imitating the clinical treatment of acute malaria. Effective drugs ameliorate or cure the malarial attack.

Curative activity is shown by complete termination of infection in the vertebrate host. In the case of P. falciparum this requires merely the destruction of all erythrozoites. In the other malarias, in which phanerozoites may initiate relapses, drugs can cure infection only if they destroy the phanerozoites or both the phanerozoites and erythrozoites. Experiments designed to detect curative drug action obviously must utilize mosquito-induced infections. Effectiveness of the test drugs may be demonstrated by various methods of determining the absence of infection in the treated hosts. However, the ultimate test for curative activity is failure of the treated infections to relapse.

Finally, gametocidal action of drugs is determined by the fate of gametocytes in treated infections induced by either blood parasites or sporozoites. While elimination of gametocytes has theoretical value in malaria control, since it reduces the reservoir of infection, this action is not at present considered of much practical significance.

Some, at least, of the above types of drug activity are qualitatively different. For instance, there is a host of suppressive drugs, such as quinine, quinacrine and chloroquine, which have no prophylactic, curative nor gametocidal action. On the other hand, the 8-amino-quinolines, such as pentaquine, are prophylactic and curative for P. vivax yet exhibit very slight suppressive action in this infection. An extreme case is seen in the esters of para-guanyl benzoic acid, which are partially prophylactic in avian malarias yet have no detectable suppressive activity. There are indications that common physiological actions are involved in prophylactic, curative and gametocidal effects, but the number of kinds of drugs known to have such actions is still too small to warrant more than a supposition. The above discussion should have made it clear that for the present, each type of drug action must be sought by appropriate specific methods.

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STRUCTURE OF 4-AMINOQUINOLINES IN RELATION TO ANTIMALARIAL ACTIVITY IN AVIAN INFECTIONS

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The present paper is an effort to make some generalizations concerning the relationship of chemical structure to one kind of antimalarial activity. Before examining the data we should indicate the source and some of the limitations of the information used. All the data discussed were selected from the Survey Tables[1]. The specific test methods are detailed in connection with those tables.

While there is a great deal of other information about the compounds considered, we shall deal only with their suppressive activity against Plasmodium gallinaceum of chicks and P. lophurae in ducklings. The quantitative measures of antimalarial activity given in Tables I - V are quinine equivalents. They are obtained by dividing the dosage of quinine base producing a given degree of suppressive action by the dosage of test drug showing a comparable degree of activity. We may well question the fundamental significance of these numbers. The fact that a drug exerts antimalarial action in small dosage does not necessarily mean that it is a superior drug. Further, it should be realized that the figure represents a summation of the various factors involved in absorption, localization, metabolic alteration, excretion and specific antiparasitic actions of the compound. However, it is certainly true that, for a given host-parasite combination, differences in quinine equivalents among related compounds have some quantitative meaning.

The reproducibility of the figures has been discussed by Wiselogle[1]. In general, two-fold differences are considered marginal. Four-fold or greater differences are definitely significant.

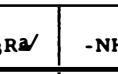
Table I shows the effect of various terminal groups on the side-chain in chloroquine and camoquin analogs. Certain facts are apparent from examination of the aminopropylamine series, column one. The primary amine is weak, as are the long-chain groups. The moderate-sized terminal groups are very similar in their effect on quantitative activity as long as they are simple hydrocarbons. The alcohol is definitely less favorable.

The remaining columns of Table I agree well with the above generalization. The last column suggests an additional principle. While the differences are rather close to the threshholds of significance, it appears that activity falls off faster in the chains with even numbers of carbon atoms than in those with odd numbers. This is reminiscent of much biochemical information on the enzymatic manipulation of fatty acids.

Table II compares those analogs of chloroquine in which the intermediate portion of the side-chain is varied, keeping the terminal diethylamine. Considering tests with Plasmodium lophurae we find that the peak of activity falls in the compounds with four- or five-carbon chains. Those with two-, three- or six-carbon chains have significantly lower activity. In the compounds with branched chains activity seems to be determined rather by the length of the chain than by the total number of carbon atoms. There is a suggestion that the position of a methyl group on a butyl chain makes some difference. Hydroxyl groups do not appear to affect activity. The significance of spatial effects is emphasized by the two cyclohexyl isomers studied. Finally, the β-phenyl butyl compound and the compounds with chains interrupted by oxygen or sulfur show definitely reduced activity.

TABLE I

EFFECT OF TERMINAL GROUP ON SIDE CHAIN

R	Side chain in position 4 on 7-chlor-quinoline			
	$-NH(CH_2)_3R$ [a]	$-NH(CH_2)_4R$ [a]	$-NHCHMe(CH_2)_3R$ [b]	 [a]
NH_2	0.8		4	6
NHMe			3	
NMe_2		15		
NHEt	20		6	
NHPr	15			
NHisoPr	30			
NEt_2	30	15	15 [c]	20 [d]
NHBu	10		8	
NHisoBu			8	
NHEtMe	15		8	
$NHCMe_3$	30			
$N(CH_2CH_2)_2CH_2$				30
NHcyclohexyl	10			
NPr_2				20
NBu_2				20
NAm_2				3
$NHex_2$	0.3			0.4
$NHept_2$				1
$NOct_2$	0.3			0.2
$N(C_2H_4OH)_2$	0.3			

a. Gallinaceum quinine equivalents
b. Lophurae (duck) quinine equivalents
c. Chloroquine
d. Camoquin

TABLE II

EFFECT OF SIDE CHAIN -NH-R-N Et_2 IN 4 POSITION ON 7-CHLOR-QUINOLINE

R	Lophurae	Gallinaceum	C length	Total C
$(CH_2)_2$	3	10	2	2
$(CH_2)_3$	6	30	3	3
$(CH_2)_4$	15	15	4	4
$(CH_2)_6$	3	6	6	6
$CHMe(CH_2)_3$	15	15	4	5
$CHMe(CH_2)_4$	15	15	5	6
$(CH_2)_3CHMe$	8	8	4	5
$CH_2CHOH\ CH_2$	3	20	3	
$(CH_2)_2CHOH(CH_2)_2$	15	30	5	
cyclohexyl	15*, 4#	20*, 40#	4	6
$CH_2CH\ Ph(CH_2)_2$	2	8	4	
$(CH_2)_3O(CH_2)_2$	4	4		
$(CH_2)_2S(CH_2)_2$	3	10		

* 12,108 m. p. 151°, tested as diphosphate
\# 14,477 m. p. 223-226°.

The data on Plasmodium gallinaceum in Table II are less clear, although they do not disagree with the above generalizations.

Table III shows a few of the many compounds in the survey tables illustrating the effects of various ring substitutions. Those selected here are the ones available in both chloroquine and camoquin series. The general agreement in order of activity between the two series is excellent. It indicates clearly that the mechanisms of antimalarial action in the two groups are related. Further, it enhances the significance of the most outstanding discrepancy, the fact that a 7-methyl group was beneficial in the camoquin series and not in the chloroquine series.

It should be noted that the last seven compounds in both series are the same. However, five of the chloroquine analogs were inactive at maximum dosage, whereas all of the camoquin analogs were active. The camoquin side-chain, given various substituents para to the hydroxyl group, has antimalarial activity in itself. It is possible that the side-chain alone is responsible for the activity of the last compounds in the series.

TABLE III

EFFECT OF RING SUBSTITUENTS ON P. GALLINACEUM QUININE EQUIVALENTS

CHLOROQUINE ANALOGS		CAMOQUIN ANALOGS	
7 Cl	15	7 Cl	20
7 Br	15	7 Br	15
7 I	10	7 CH_3	10
5,7 Cl	4	7 I	8
3CH_3 7Cl	4	6 OCH_3	8
6 Cl	2	7 OCH_3	6
6 OCH_3	2	3CH_3 7 Cl	6
7 OCH_3	2	6,7CH_3	6
7 CH_3	1	(None)	3
(None)	1	6 Cl	3
6,7 CH_3	0.8	5,7 Cl	3
2 ph.	0.4	8 OCH_3	0.8
8 OCH_3	0.4	8 Cl	0.6
8 Cl	<0.8	2 ph.	0.4
3 CH_3 5 Cl	<0.8	2 ph. 7 Cl	0.3
2 ph 7 Cl	<0.8	3 CH_3 5 Cl	0.3
5,8 Cl	<0.8	5,8 Cl	0.2
2 ph 6 OCH_3	<0.8	2 ph 6 OCH_3	0.2

Table IV illustrates a fact that is often neglected in investigations such as these. Students of chemotherapy may tend to think in terms of structural formulae rather than of chemical compounds. Thus, the 6-methyl analog of camoquin shows a quinine equivalent of 10, the 7-methyl compound, 4. It might be supposed that a compound with both would be superior.. That the chemistry of the compound is important is demonstrated by the fact that the composite drug exhibits activity not significantly higher than that of the weaker mono-substituted compound. Throughout the table this fact is evident. The combinations show activity in the range of that of the weaker member of the pair.

TABLE IV

QUININE EQUIVALENTS WITH MULTIPLE RING SUBSTITUTIONS IN CAMOQUIN ANALOGS

(P. gallinaceum)

6 CH_3	4	7 CH_3	10	Both	6
6 CH_3	4	8 CH_3	0.8	Both	0.6
6 Cl	3	7 Cl	20	Both	4
6, 7 Cl	4	8 Cl	0.6	All three	<0.3
2 phenyl	0.4	7 Cl	20	Both	0.3
3 phenyl	0.3	7 Cl	20	Both	0.8
6 OCH_3	8	7 OCH_3	6	Both	2

If, despite the warning expressed above, one were to concentrate on structural formulae, one might construct a suggestive hypothesis. It may be that in a series such as this a substitution or a hydrogen atom is favorable not if it adds to the activity of a weak parent drug but if it detracts little from the activity of a hypothetical strong compound. This would explain the fact that the combination of a relatively favorable with a relatively unfavorable substitution generally gives an unfavorable result.

Table V gives additional information bearing on this point, taken from the series of chloroquine analogs. The table is to be interpreted according to the following example. The second figure in column one (0.8) is the quinine equivalent for the 6,7-dimethyl compound. The first figure in column two (1) is the equivalent of the 3-methyl compound. The second figure in column two (0.4) gives the equivalent for the combination, namely the 3,6,7-trimethyl compound.

Table V shows that the tentative hypothesis suggested above can be held only with qualifications. The exceptions are some of the 7-chloro and 7-bromo compounds. In several cases, and especially in the 6-methoxy, 7-chloro compound, the combination is affected more by the more favorable than by the less favorable substitution. This may be explained by the possibility that the 7-chloro substitution creates a new chemical series which must be considered separately from the parent series. The examination of composites may thus reveal pharmacologically significant chemical differences. In any event, it is obvious that the chemistry of the compounds is fundamental.

One specific fact observable in Tables III and V is noteworthy. Substitutions next to the ring nitrogen, in positions 2 or 8, appear to be uniformly unfavorable.

The possibilities for general conclusions from these data are limited. Their principal general significance lies in their internal self-criticism. They show what should be obvious on *a priori* grounds, that the superficial comparison of structural formulae has very little possibility for fundamental conclusions. It is clear that chemical as well as spatial effects determine antimalarial action. One may wish for the chemical and biochemical information necessary to a basic interpretation of the data.

TABLE V

QUININE EQUIVALENTS WITH MULTIPLE RING SUBSTITUTIONS IN CHLOROQUINE ANALOGS

(P. gallinaceum)

Substituent B	Substituent A					
	(None)	3 CH_3	2 phenyl	5 Cl	6 Cl	7 Cl
(None)	1	1	0.4	0.6	2	15
6,7 CH_3	0.8	0.4				
5 Cl	0.6	<0.6				
6 Cl	2	<0.4				
7 Cl	15	4	<0.8	4		
7 Br	15	3				
7 I	10	1				
8 Cl	<0.2	<0.2		<0.4	<0.2	
6 OCH_3	2	<0.4	<0.2			15
7 OCH_3	2		<0.2			
8 OCH_3	0.4	0.2				

1. Wiselogle, F. Y., ed. 1946 - A Survey of Antimalarial Drugs, 1941-1945.

STRUCTURAL TYPES TESTED FOR CURATIVE ACTIVITY AGAINST LOPHURAE MALARIA IN THE CHICK

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At the war's end, emphasis in the antimalarial program at Parke, Davis and Company shifted from the development of a good suppressive drug to the search for chemical types having curative activity against vivax malaria. The new policy was implemented by a curative test, using lophurae malaria in the chick, which was developed by Dr. Paul E. Thompson and which was set up by him on a routine basis during the summer of 1948. Usefulness of the test was indicated by positive results obtained with the 8-aminoquinolines in conjunction with quinine, by positive results obtained with the naphthoquinone, SN 12,320*, and also by negative results obtained with a number of compounds known to be devoid of curative action against vivax in man, for example, quinine, quinacrine, chlorguanide, sulfadiazine, SN 6771*, and SN 8617*.

By means of the new procedure, representative members of the following suppressive series were tested for curative activity: quinine analogs, 4-aminoquinolines, acridines, α-aminocresols, chlorguanide analogs, sulfonamides, dithiocarbamates, miscellaneous dyes, and other structural types. The significance of the results will be briefly discussed in terms of the structure-activity relationships within each suppressive series. Although no new series showing curative activity has yet been discovered in this program, the wide structural variations among suppressive drugs strongly suggests that other curative types eventually will be found.

* Key to Survey Numbers:

SN 6771 6,6'-Diallyl-α,α'-bis(diethylamino)-4,4'-bi-o-cresol.

SN 8617 4-(6-chloro-2-methoxy-9-acridylamino)-α-diethylamino-o-cresol.

SN 12,320 2-[3-(decahydro-2-naphthyl)propyl]-3-hydroxy-1,4-naphthoquinone.

DISCUSSION

DR. W.C. COOPER (National Institutes of Health) discussed trials of 4-aminoquinoline compounds in man. The group provides some of the most useful antimalarials now available. Of the more than two hundred members of the group known to be active in the avian malarias, eleven have been tried in man, and four (chloroquine, SN 10,751, sontochin, and oxychloroquine) have had field trials. Chloroquine and SN 10,751 have proved so far to be the best. Chloroquine has had the widest use: it combines rapid action against erythrocytic parasites, delayed elimination from the body (permitting brief therapeutic regimens or weekly suppressive dosage), absence of serious toxicity, and no acquired resistance by parasites. SN 10, 751 (amodiaquin or camoquin) appears to have analogous properties. The 4-aminoquinolines are not effective against fixed-tissue parasites and are not causal prophylactic agents. They do not produce radical cure of relapsing vivax malaria. Their mode of action is unknown.

DR. FREDERICK WISELOGLE (Squibb Institute for Medical Research, New Brunswick, New Jersey): I would like permission to tell a story. During the hectic days of the malaria program, we were in the habit of sending drugs out just as fast as we could, and expecting very prompt replies from the persons who were carrying out the tests. Because Dr. Porter was so far away, we asked him, in special cases, to reply by telegram. We were particularly interested in one 4-aminoquinoline for which we had high hopes; that is, we had hopes for a quinine equivalent of 15 or 30, or perhaps 50.

One afternoon, just after lunch, we received a telegram that this 4-aminoquinoline had a quinine equivalent of 425. We were all set to close up the office and quit work and declare the malaria problem solved.

Then we began looking at that number, and were more and more intrigued as to why Dr. Porter picked such a number, which was so extraordinarily high. We were so disturbed that we finally called him up and asked him what the quinine equivalent of this compound was. He said, "Let me look it up." He looked it up and said, "It is 4 to 5," and that is what he had told the telegraph girl, who interpreted it very literally as 425.

I would like to ask a few questions, one of Dr. Porter, or perhaps some other person who can answer it, and that is whether anything has been done on mammalian malaria which, I believe, was found, right at the end of the war, in a bat, and was successfully transferred to a mouse. I would like to know whether there has been any quantitative study of those compounds found active in the avian malarias.

The second question I would like to ask of Dr. Moore is purely a question of information, and that is, whether, in mixing quinine with these other substances listed in his first Table at the bottom of page 1, it is true that the tolerated dose of quinine was significantly lowered by the addition of these other substances. I think that is quite interesting, because I thought you gave quinine alone, 400 mgm. per kilo, then dropped down as low as 100; or is that just that you carried it down to a lower dose?

DR. A.M. MOORE: The answer to the question is a technical one. The drugs were administered in the diets, and a definite percentage was mixed with each diet. The amount which a bird ate depended on how toxic the mixture was. In the case of quinine, as I recall, there was 0.4 per cent in the diet; that is on page 2, second slide, second line down. But in the case of pamaquine plus quinine, the dose of quinine was cut to one-half the maximum tolerated dose, which would have been 0.2 per cent in the diet. But, when pamaquine or pentaquin was added, the mixture was very, very toxic, because of the pamaquin or pentaquin, and the birds just did not eat so much as when the diet contained quinine alone.

CHAIRMAN ELDERFIELD: I wonder if Dr. Fieser would care to elaborate very briefly on the naphthoquinones.

I might say, in arranging this program, due to the large number - I think it was somewhere around sixty-eight - of chemical classes of compounds which have shown some antimalarial activity, in order to avoid spending a week or more in discussing the relation between structure and activity, it was necessary to trim the thing down rather drastically, and a somewhat arbitrary selection of groups for discussion was made, based solely on the amount of information, as far as antimalarial activity is concerned, in a given particular group.

The naphthoquinones of Dr. Fieser are, I think, one of the most interesting series of substances which have been turned up.

However, it seemed to us that possibly there was not quite as much information available dealing with the naphthoquinones as there was in the other groups, and that is the reason for the somewhat arbitrary choice.

I see that Dr. Fieser is here and, if he would like to elaborate for a few minutes on his naphthoquinones, I am sure everybody would be glad to hear him.

DR. LOUIS F. FIESER (Harvard University, Cambridge, Massachusetts): I feel like an outsider in this group, and feel rather badly about it. Some seven years ago I became very much excited about the problem of malaria chemotherapy and, as a chemist, welcomed the opportunity for active cooperation with medical scientists in advancing the whole methodology of chemotherapeutic research. I thought that, with cooperation of chemists and pharmacologists, the standard method of analyzing and expressing bioassay data could be improved upon. I hoped that techniques could be found for promoting better absorption of orally administered drug. I thought that development of a precision method for studying the rate of metabolic drug deactivation in the naphthoquinone series might influence work on other series of antimalarials. I thought our demonstration that in the naphthoquinone series biological potency can be correlated with chemical structure only when allowance is made for varying distribution characteristics of the compounds concerned might suggest useful developments in the study of other series of antimalarials and might have some impact on chemotherapeutic research in general.

I did indeed have some very able cooperation at the time from such individuals as A. P. Richardson, W. B. Wendel, and others. However, my impression tonight is that, in respect to American antimalarial research, these hopes were largely in vain. The discussions of the evening sound exactly like those that I heard in 1944. The chemist is still dutifully synthesizing compounds and nothing more; the pharmacologist is still analyzing his often very good assay data by the same sloppy method of matched doses; and the clinician is still the supreme authority who passes judgement of "yes" or "no" on a compound or series without reference to points of possible importance in the organic, physical, or biochemical properties of the compounds.

Our attempts to develop a satisfactory antimalarial in the naphthoquinone series, continued in the face of initial failure and against the judgement and ruling of the medical directors of the CMR, and involving such unorthodox procedures as introduction into a chemical laboratory of a Warburg apparatus and a colony of infected ducks, eventually led to apparent success in the form of the drug that we have called lapinone. The curative and prophylactic effect of the naphthoquinones had been well demonstrated in avian infections and here, in lapinone, was a member of the series that, according to numerous quantitative tests in normal humans, resisted metabolic degradation and afforded high blood levels of material that retained its activity for very long periods. After a long and hard battle, we were ready for another clinical trial.

But we did not belong to the club. We were not working on 8-aminoquinolines or on other compounds derived from or related to quinine, atabrine, or plasmochin. We were outsiders. We lacked the right contacts. And we had to go all the way to the American University at Beirut, Lebanon, to find the active cooperation needed. There were, and are, plenty of difficulties. In this progressive Arab state malaria cases have become hard to find. When my Lebanese friends succeeded in getting a few tests that proved most encouraging, American doctors said, "But what kind of malaria do they really have in Lebanon, is it really good vivax? You should get a trial against the Chesson strain; we know all about that." Two attempts to try the Chesson strain have been made without success. In the second attempt, Dr. Martin Young in South Carolina prepared a suspension of Chesson sporozoites in serum on a certain Thursday and shipped it to us by air. My associate met the plane Thursday night, replenished the ice, took the night train to New York

to deliver the thermos the next day to the steward of an Arabian-American Oil Co. plane just about to leave for Saudi-Arabia. The flight went off as planned, fresh ice was obtained at Gander and at Rome, and the material was promptly transshipped to Lebanon and injected into ten patients in the mental ward. But the infection did not take.

Thus, we will have to try some other desperate expedient for getting evaluation of lapinone. We can, however, report that of nine patients with primary vivax infection that were given lapinone intraveneously for four days, all nine showed prompt relief of fever and parasites, and no drug symptoms, and six were without relapse after periods of from thirteen to fifteen months after treatment. It seems to me that this is a better performance, in the direction of curative action, than that of any of the nitrogen-compounds of orthodox types that I have heard discussed by the regular members of the symposium.

THE RELATIONS BETWEEN CHEMICAL STRUCTURE AND TOXICITY AMONG THE 8-AMINOQUINOLINES

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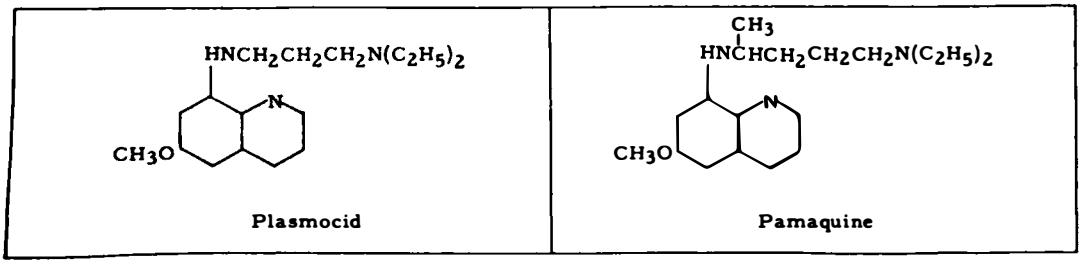
Some of the members of this audience may wonder why a dissertation on pharmacology, and more specifically on toxicology, should be included in tonight's panel discussion on the relations between chemical structure and biological activity. The most likely reason is that toxicological studies have played a uniquely important role in the development of curative drugs belonging to the 8-aminoquinoline series. The clinician has leaned heavily on the results of such studies in selecting new 8-aminoquinolines for clinical trial, while the chemist has used such results as a primary guide to synthesis of new compounds. There are two explanations for this unusual importance of pharmacological studies. In the first place, until quite recently, there was at hand no experimental malaria which yielded assessments of curative activity having application to the human disease. Secondly, 8-aminoquinolines as a group exhibited a variety of striking and undesirable toxic reactions, some of which precluded administration to the human subject, and most of which could be closely related to specific chemical structures.

In the time allotted to this discussion, an attempt will be made to accomplish three things: first, to describe the sequence of events which led to the unorthodox practice of using the rhesus monkey as a test object for routine pharmacological studies; secondly, to describe the different reactions evoked by various 8-aminoquinolines when administered to the rhesus monkey; and finally, and principally, to set forth what appear to be firmly supported generalizations on the relationships between the chemical structures of the 8-aminoquinolines and various aspects of their toxicity.

First, let us consider the sequence of events which led to the selection of the rhesus monkey as the test object for evaluating the pharmacological properties of the 8-aminoquinolines. When interest was evoked in this group of drugs, during the latter stages of World War II, data on the effects of the compounds on larger animals were meager if non-existent. Since a base-line of information was deemed essential to rational exploration of the above chemical series, it was decided to make a careful study of the reactions of various animal species to the old German drug pamaquine (Plasmochin). Supplies of a close relative, Plasmocid, were also at hand; for this reason the projected study was expanded to include work with this latter compound. As will be apparent from the discussion which follows, the fortuitous availability of Plasmocid was a remarkably fortunate circumstance.

Before proceeding to a discussion of the experimental observations, attention should be called to the chemical structures of pamaquine and Plasmocid (Chart I). These compounds have

CHART I



the same quinoline nucleus, with a methoxyl group substituted at position 6 and an amino group at position 8. The side chains of the compounds have the same terminal grouping, diethyl amine. The compounds differ, however, in the alkyl groups which separate the side chain nitrogens. In the case of pamaquine, a 1-methyl-butyl group is present, while Plasmocid has an n-propyl grouping.

The studies with pamaquine and Plasmocid included work with the mouse, rat, dog, and rhesus monkey. Whereas in all of these animal species there were quantitative differences in the toxicity of the above quinolines, there were no qualitative distinctions in the toxemias which developed in the rodents and in the dog. The situation in the rhesus monkey was quite different (Chart II).

CHART II

TOXIC REACTIONS OF THE MONKEY (Macaca mulatta) TO PAMAQUINE AND PLASMOCID

Pamaquine	Plasmocid
Leucopenia	Hyperesthesia
Neutropenia	Nystagmus
Anemia	Loss of pupillary reflexes
Bilirubinemia	Loss of vision
Methemoglobinemia	Loss of equilibrium
Cyanosis	Dysmetria
Loss of appetite	Dysbasia
Loss of weight	Dysergia
Lassitude	Paralysis of lower limbs
Generalized muscular weakness	Pathological changes in brain and cord; highly localized lesions involving the nuclei of cranial nerves III, IV, VI and VIII and associated cell groups
Pathological changes involving bone marrow, liver, spleen, kidneys, heart and brain (nuclei of nerves III, IV and VI)	

Monkeys, intoxicated with pamaquine, exhibited marked changes in the formed elements of peripheral blood, including leucopenia, with neutropenia approaching agranulocytosis, anemia, methemoglobinemia, cyanosis, severe abdominal cramping, anorexia, lassitude, and a generalized muscular weakness. These more obvious symptoms were associated with a depletion of the myeloid elements of bone marrow and lesions of moderate severity in heart muscle, liver, spleen, and kidneys. Moderately severe lesions were present in the central nervous system in the supraoptic and paraventricular nuclei and minimal lesions in the nuclei of cranial nerves III, IV, and VI. Chart III contains an example of the peripheral blood changes which occur during pamaquine intoxication.

CHART III

LEUCOPENIA AND NEUTROPENIA INDUCED BY PAMAQUINE

Day of Treatment	WBC per cmm. x 1000	Distribution of Leucocytes - Per Cent				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
0	18.5	54	38	4	2	2
5	2.6	9	81	4	1	5
9	1.35	3	91	5	1	0
13	1.1	1	96	2	0	1

Monkeys intoxicated with Plasmocid exhibited an entirely different picture from that just described (Chart III). In Plasmocid intoxication, there appeared a remarkable set of symptoms referable to injury to the central nervous system. These symptoms, which appeared within one to two days after first exposure to the drug, included hyperesthesia, muscular rigidity, nystagmus (either vertical, horizontal or mixed) loss of pupillary reflexes, and loss of equilibrium and ability to coordinate muscular movements. These symptoms were associated with severe and widespread yet comparatively localized lesions in the brain involving many of the principal nuclei in the proprioceptive, vestibulo-cerebellar, auditory, visual reflex, and extrapyramidal motor pathways, the olfactory areas and to a lesser degree the anterior horn cells. A list of the principal areas of the brain affected by Plasmocid is given in Chart IV. A typical area of injury is given in Chart V, the corresponding normal region being presented for comparison.

CHART IV

LOCATION OF LESIONS IN THE CENTRAL NERVOUS SYSTEM OF THE RHESUS MONKEY ASSOCIATED WITH FATAL PLASMOCID INTOXICATION

Proprioceptive System	Visual Reflex System
Column of Clarke	Abducens nucleus
Large cells in the central gray	Trochlear nucleus
Lateral reticular nucleus	Lateral oculomotor nucleus
Lateral cuneate nucleus	Central nucleus of Perlia
Mesencephalic V nucleus	Edinger-Westphal nucleus
Centrum medianum nucleus	Interstitial nucleus of Cajal
Posterolateral ventral nucleus	Magnocellular area of the lateral geniculate body

CHART IV (Cont.)

Vestibulo-cerebellar System	Extrapyramidal Motor System
Descending vestibular nucleus	Substantia nigra
Medial vestibular nucleus	Subthalamic nucleus
Lateral vestibular nucleus	Some of the pontile nuclei
Superior vestibular nucleus	Globus pallidus (in part)
Nucleus intercalatus	Large cells of the caudate and putamen
Nucleus prepositus	Anterior ventral nucleus
Nucleus of Roller	
Interfascicular nucleus	Olfactory Areas
All cerebellar nuclei	Lateral mammillary nucleus
Nucleus ruber (both parts)	Ansapeduncular nucleus
Lateral ventral nucleus	Magnocellular preoptic nucleus
	Lateral habenular nucleus
Auditory System	
Cochlear nuclei	Other Areas
Superior olivary nucleus	Some anterior horn cells
Trapezoid nucleus	Large cells of reticular formation
Nucleus of the lateral lemniscus	Supraoptic and paraventricular nuclei

CHART V

Lateral oculomotor nucleus, left, and medial longitudinal fasciculus, right, monkey #1126 control. Paraffin, Morgan's stain; X 160

CHART V (Cont.)

Lateral oculomotor nucleus, left, and medial longitudinal fasciculus, right, monkey #1194, after 12 days' treatment with Plasmocid. Complete absence of all neuron cell bodies, glial replacement; degeneration and glial replacement in the medial longitudinal fasciculus. Paraffin, Morgan's stain; X 160.

Taken from "Neutrotoxicity of the 8-aminoquinolines. I. Lesions in the central nervous system of the rhesus monkey induced by administration of Plasmocid." by Ida G. Schmidt and L. H. Schmidt.

Reprinted from Journal of Neuropathology and Experimental Neurology, VII, No. 4, October 1948.

By the time these observations on pamaquine and Plasmocid had been completed, nine additional 8-aminoquinolines were made available through the work of the cooperating chemists. These compounds were administered to rats, dogs, and monkeys, the same techniques being employed as in the work with pamaquine and Plasmocid. Again there were quantitative differences in the toxicity of these compounds for the rat and dog, but no qualitative distinctions. In the monkey the situation was again different (Chart VI). Certain of the compounds produced effects on peripheral blood and bone marrow, analogous to the effects of pamaquine. Others produced symptoms of central nervous system injury, comparable to reactions to Plasmocid. Still others produced cardiac arrhythmias and symptoms indicative of a postural hypotension.

CHART VI

REACTIONS OF RHESUS MONKEYS TO 8-SUBSTITUTED-6-METHOXY-QUINOLINES

Drug Code No.	8-Substituent	Nuclear Substituent	Primary Effect on
1452	$HN(CH_2)_3NH_2$		Heart - circulation
3114	$HN(CH_2)_2N(C_2H_5)_2$		CNS
3115	$HN(CH_2)_3N(C_2H_5)_2$		"

CHART VI (Cont.)

Drug Code No.	8-Substituent	Nuclear Substituent	Primary Effect on
11889	$HN(CH_2)_3N(C_2H_5)_2$	(5) $-OCH_3$	CNS
5832	$HN(CH_2)_3N$ 		"
971	$HNC(CH_3)(CH_2)_3N(C_2H_5)_2$		Blood - bone marrow
12322	$HN(CH_2)_6N(CH_3)_2$		"
11191	$HN(CH_2)_6N(C_2H_5)_2$		"
7672	$HN(CH_2)_3S(CH_2)_2N(C_2H_5)_2$		"
10309	$HN(CH_2)_2$ 		Heart - circulation
11888	$HN(CH_2)_3$ 		"

On the basis of these observations, it was decided to make routine use of the rhesus monkey as the test animal in evaluating the toxicity of 8-aminoquinolines. This decision led to studies on some one hundred forty-six 8-aminoquinoline derivatives. These compounds have been administered to more than nine hundred rhesus monkeys, in doses ranging from one-sixteenth of the lethal to twice the lethal dose. In all cases, a subacute method of studying toxicity has been employed, divided daily doses being administered for periods up to fourteen days.

The results which have emerged from these studies have demonstrated remarkable, wholly unexpected and completely unexplained relations between chemical structure and pharmacological activity. Three distinctly different reaction patterns were produced by the various quinolines: (1) depression of myeloid activity of peripheral blood and bone marrow with methemoglobinemia and anemia; (2) a complex group of neurological symptoms associated with severe and widespread lesions in the spinal cord and brain stem; and (3) disturbances in the heart and circulation, associated with highly localized lesions in the dorsal motor nucleus of the vagus. Remarkable as it may seem, every compound thus far studied has produced reactions which fall primarily into one of these patterns. There are instances in which compounds which effect peripheral blood changes also exert slight effects on the heart and circulation. Generally speaking, however, there is little overlapping in the pattern of toxic reactions evoked by a given compound.

The general chemical distribution of one hundred forty of the derivatives with respect to the 8-amino substituent is shown in Chart VII. Ten of the compounds had alkyl side chains terminating in a primary amino group. Sixty-four had terminal secondary amino groups. Fifty-one had terminal tertiary amino groups, while ten had the terminal nitrogen in piperidyl linkage. Within each of these major groups there were broad variations in the alkyl group which separated the side chain nitrogens. In the case of the secondary and tertiary amino derivatives there were also broad variations in the terminal alkyl grouping on the side chain and in the nuclear substituent.

CHART VII

GENERAL DISTRIBUTION OF 8-AMINOQUINOLINES EXAMINED FOR THEIR EFFECTS ON THE RHESUS MONKEY

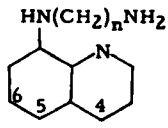
No. Compounds Studied	Type of 8-amino-substituent	
10	$-(CH_2)_nNH_2$	n = 2 to 6
64	$-(CH_2)_nNH$ Alkyl	n = 2 to 6
51	$-(CH_2)_nN(Alkyl)_2$	n = 2 to 11
10	$-(CH_2)_n$ Piperidyl	n = 2 to 7 (piperidyl 1, 2, or 4)
5	-Miscellaneous	
Total = 140		

The work with the ten primary amines (Chart VIII) demonstrated that, irrespective of the nuclear substituent, the type of toxic reaction was determined by the number of methylene groups which separate the side chain nitrogens. When the side chain contained 2, 3, 4 or 5 methylene groups in straight linkage, the compounds exerted their primary effects on the heart and circulation. However, when the side chain contained 5 methylene groups in a branched chain or 6 in a straight chain primary effects were on the formed elements of peripheral blood and bone marrow. No primary amine in the group studied evoked symptoms of central nervous system injury such as those produced by Plasmocid.

CHART VIII

RELATIONS BETWEEN STRUCTURE AND TOXICITY AMONG 8-AMINOQUINOLINES WITH SIDE CHAINS TERMINATING IN PRIMARY AMINO GROUPS

(10 compounds)



(6) $-OCH_3$ (5) $-OCH_3$ (4) $-CH_3$

1. Irrespective of nuclear substituent, type of toxic reaction is determined by number of CH_2 groups separating amino groups in the side chain.

   (a) When the side chain contains 2, 3, 4 or 5 methylene groups in a straight chain, toxic reactions involve the heart and circulation, primarily.

CHART VIII (Cont.)

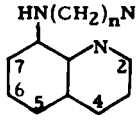
(b) When side chain contains 5 methylene groups in a branched chain, or 6 in a straight chain, toxic reactions involve the formed elements of peripheral blood and bone marrow, primarily.

The studies with derivatives having secondary or tertiary terminal amino groups (Chart IX) show that the types of reactions evoked by the compound were independent of the character of the terminal alkyl substituent. Reactions were determined, however, both by the alkyl chain which separated the side chain nitrogens and by the position of the nuclear substituent. When there was a nuclear substituent at positions 7, 6, and/or 5 the type of reaction was determined solely by the methylene linkage separating side chain nitrogens. When the linkage comprised 2 or 3 methylene groups (branched or straight) the drug produced symptoms of CNS intoxication identical with those of Plasmocid. When there were 4 (branched or straight) or 5 (straight) methylene groups present, the symptoms produced were referable to effects on the heart and circulation. When the side chain nitrogens were separated by 5 methylene groups (branched) or 6 or more branched or straight, toxic reactions involved primarily the formed elements of peripheral blood and bone marrow.

CHART IX

RELATIONS BETWEEN STRUCTURE AND TOXICITY AMONG 8-AMINOQUINOLINES WITH SIDE CHAINS TERMINATING IN SECONDARY OR TERTIARY AMINO GROUPS

(115 compounds)



(7)	(6)	(5)	(4)	(2)
$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$
$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$
	-OH	-OPh		
	-OPh	-Cl		
	-OEtOH	$-NH_2$		
	-Cl			
	$-NH_2$			

1. Irrespective of the nuclear substituent at positions 7, 6 or 5 the type of toxic reaction is determined by the number of CH_2 groups separating amino groups in the side chain.

   (a) When the side chain contains 2 or 3 (branched or straight) methylene groups, toxic reactions involve the CNS, primarily.

   (b) When the side chain contains 4 (branched or straight) or 5 (straight) methylene groups, toxic reactions involve the heart and circulation, primarily.

   (c) When the side chain contains 5 methylene groups in a branched chain or 6 or more in either branched or straight chain, toxic reactions involve the formed elements of peripheral blood and bone marrow, primarily.

CHART IX (Cont.)

2. The type of toxic reaction is influenced both by the nuclear substituent at positions 4 and 2 and by the number of CH_2 groups separating the amino groups of the side chain.

   (a) When a methoxyl group is substituted at position 4, the type of toxic reaction follows the pattern set by side chain variations with derivatives having substituents at 7, 6 and 5.

   (b) When a methyl substituent is present at position 4, toxic reactions are independent of side chain variations and involve the formed elements of peripheral blood and bone marrow, primarily.

   (c) When either methyl or methoxyl substituents are present at position 2, toxic reactions are independent of side chain variations and involve the formed elements of peripheral blood and bone marrow, primarily.

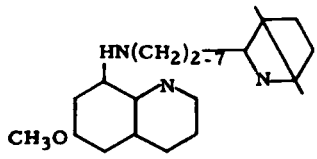
The situation differed considerably when the 2 or 4-position of the nucleus was substituted. In that case the reactions evoked depended both upon nuclear substituent and the alkyl group separating the nitrogens of the side chain. With a methoxyl at position 4, the reactions followed the pattern set by side chain variations among compounds with nuclear substituents at positions 7, 6 and/or 5. With a methyl group at position 4, or a methyl or methoxyl group at position 2, toxic reactions were independent of side chain variations and involved the formed elements of peripheral blood and bone marrow, primarily.

The final group of compounds comprises those in which the terminal nitrogen was in piperidyl linkage (Chart X). Six unsubstituted 2-piperidyl derivatives, with from 2 to 7 methylene groups in the side chain and one 4-piperidyl derivative all produced effects on the heart and circulation. One nitrogen substituted derivative and one derivative in which the side chain attachment was through the piperidyl nitrogen produced effects on the central nervous system indistinguishable from those of Plasmocid.

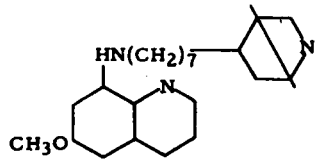
CHART X

RELATIONS BETWEEN STRUCTURE AND TOXICITY AMONG 6-METHOXY-8-AMINOQUINOLINES WITH SIDE CHAINS TERMINATING IN PIPERIDYL GROUPINGS

(10 compounds)

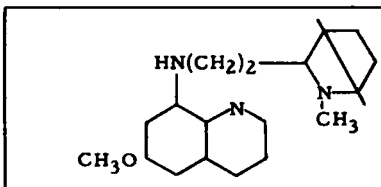


Toxic reactions involve heart and circulation

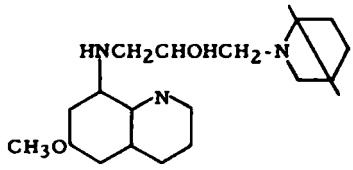


Toxic reactions involve heart and circulation

CHART X (Cont.)



Toxic reactions involve CNS



Toxic reactions involve CNS

It would be satisfying if in concluding this report information could be presented would explain the radically different types of reactions evoked by 8-aminoquinolines of such slightly differing chemical structures. Unfortunately, despite substantial efforts, no tenable explanation is at hand. As has been said often, the facts are here, underlying reasons not yet forthcoming.

PRIMAQUINE, SN 13,272

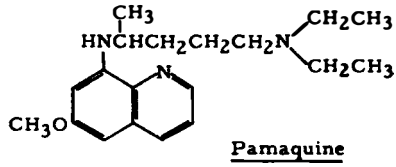
A NEW CURATIVE AGENT IN VIVAX MALARIA: A PRELIMINARY REPORT[1,2]

John H. Edgcomb[3], John Arnold[3], Ernest H. Yount, Jr.[3], Alf S. Alving and Lillian Eichelberger (from the Malaria Research Unit, Department of Medicine, University of Chicago) and Geoffrey M. Jeffery, Don Eyles and Martin D. Young (Laboratory of Tropical Diseases, Microbiological Institute, National Institutes of Health)

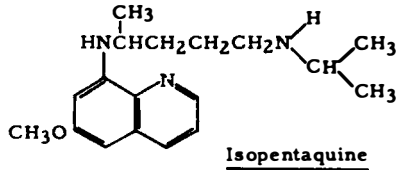
Introduction

This paper reports the preliminary clinical experience with primaquine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline, or SN 13,272, in reducing the relapse rate of the Chesson strain of vivax malaria. This compound was first tested in man early in 1948 as part of a comprehensive study of compounds related to pamaquine, using methods previously described by Alving, et al. (1948).

Primaquine differs chemically from pamaquine by having a primary amine substituted for the tertiary terminal amine on the aliphatic side chain in the 8-position of the quinoline nucleus. Pamaquine, isopentaquine and the new compound, primaquine, may be considered as a family of compounds, the members of which differ only in the characteristics of the terminal amino group. Thus:



Pamaquine (Tertiary terminal amine)

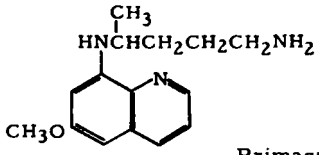


Isopentaquine (Secondary terminal amine)

[1] Reprinted from The Journal of the National Malaria Society, Vol. 9, 285-292, 1950. Additional patients have been added to those originally reported in the first table on page 192.

[2] These investigations were, in part, supported by a grant-in-aid from the United States Public Health Service and, in part, carried out under contract between the Office of the Surgeon General of the United States Army and the University of Chicago. The clinical studies were also aided by the participation of Army Medical Officers assigned to the project. The studies would not have been possible except for the valuable cooperation and help given by Warden Joseph E. Ragen of Stateville Penitentiary and other administrative officials of the State of Illinois.

[3] Captain, MC-AUS



Primaquine

(Primary terminal amine)

Procedures and Methods

In the therapeutic trials primaquine was given in the form of the diphosphate salt (56. 9% base) and doses have been calculated in terms of base weight. [4/] The details of the testing program have been reported elsewhere. Briefly the general procedure was as follows: White, healthy inmate volunteers from the general population of the Illinois State Penitentiary (Stateville Branch) who had had no previous experience with malaria were selected. A standard method of inoculation by the bites of ten infected mosquitoes was used because this gives a consistently severe infection. Drug administration was begun early in the course of the clinical attack in order to reduce acquired immunity to a minimum. During the therapeutic trials the patients were hospitalized. Parasite counts were made daily during the immediate follow-up period and at frequent intervals for periods up to one year. No case with a follow-up of less than six months has been included. It is known that 98% of all relapses, in patients infected by this technique and treated with 8-aminoquinolines, occurs before one hundred fity days (unpublished observation). Inasmuch as the variety of toxic manifestations is similar to that of pamaquine, toxicity has been expressed in terms of pamaquine equivalents. All drugs were administered orally.

Choice of clinical material.

All primary cases treated were characterized by having an incubation period of less than fifteen days. A limited number of patients that had relapsed after treatment with a heterogeneous group of drugs have also been included in this report, but only subjects who relapsed within thirty days after end of therapy were chosen. Craige, et al. (1947) have shown that patients with short prepatent or latent periods offer a severe challenge to curative drugs. Under these experimental conditions the relapse rate after treatment of primary attacks with suppresive drugs approaches 100% (Table I).

TABLE I

RELAPSE RATE AFTER TREATMENT OF PRIMARY ATTACKS OF CHESSON STRAIN VIVAX MALARIA (STANDARDIZED SPOROZOITE INFECTIONS) WITH SUPPRESSIVE DRUGS

Drug	Relapse Rate
Quinine	18/18
Quinacrine	4/4
Chlorguanide	8/8
Chloroquine	8/8
Total	38/38

[4/] Primaquine was first synthesized by Dr. Robert C. Elderfield, Department of Chemistry, Columbia University, New York City. Later supplies of primaquine have been provided by Eli Lilly and Company, Indianapolis, Indiana, and by the Abbott Laboratories, North Chicago, Illinois.

Results

When primaquine was given alone in six divided doses daily, curative effect was demonstrated in doses as low as 22.5 mgm. (base) per day (Table II).

TABLE II

CURATIVE EFFECT OF PRIMAQUINE WHEN ADMINISTERED ALONE DURING PRIMARY ATTACKS OF VIVAX MALARIA (CHESSON STRAIN)

Cases	Days From End of Rx to First Relapse		Follow Up (Days)	Ratio: Subjects Relapsed/ Subjects Treated	Symptoms	MET* HGB %	Mean Plasma** Concentration of Primaquine (gamma/liter)
	F/102+	Para					
DAILY DOSE OF 22.5 mgm. ***							
1.	16	15	-		None	9.6	9
2.	17	14	-		Abd. +	9.4	10
3.	12	13	-	4/5	None	13.9	5
4.	16	12	-		None	15.8	4
5.	-	-	330		Abd. ++	8.0	4
DAILY DOSE OF 45 mgm. ***							
1.	-	-	292		None	20.0	43
2.	18	16	-		Abd. ++	22.0	75
3.	-	-	333	1/5	None	18.1	45
4.	-	-	333		Abd. +	16.9	33
5.	-	-	284		Abd. +	23.8	30

* Expressed as % of total hemoglobin (average for last five days of treatment)
** Determined by method of Brodie, Udenfriend and Taggart (1947)
*** Drug administered in six divided doses daily for two weeks
Abd. + = mild, transient abdominal cramps
Abd. ++ = moderate, repeated abdominal cramps

Subsequent experience with the action of primaquine against trophozoite-induced infections suggests that many, if not all "relapses" reported in Table II were really recrudescences, that is, were due to incomplete eradication of trophozoites because the parasitemia recurred very early. When primaquine was given in conjunction with 1.64 Gms. quinine (base) daily, considerably greater curative effect resulted (Tables III, IV and V).

TABLE III

CURATIVE EFFECT OF PRIMAQUINE WHEN ADMINISTERED TOGETHER WITH 1.64 GMS. QUININE DAILY IN PRIMARY ATTACKS OF VIVAX MALARIA (CHESSON STRAIN)

Cases	Days From End of Rx to First Relapse		Follow Up (Days)	Ratio: Subjects Relapsed/ Subjects Treated	Symptoms	MET* HGB %	Mean Plasma** Concentration of Primaquine (gamma/liter)
	F/102+	Para					
DAILY DOSE OF 15 mgm. ***							
1.	19	16	-	4/5	None	4.8	13
2.	66	65	-		Anorexia	15.1	32
3.	58	57	-		None	2.2	14
4.	50	47	-		None	4.9	12
5.	-	-	451		None	3.3	12
DAILY DOSE OF 30 mgm. ***							
1.	-	-	370	0/5	Abd. +	8.7	12
2.	-	-	369		None	12.1	15
3.	-	-	365		None	9.8	11
4.	-	-	365		None	11.0	14
5.	-	-	356		None	7.7	13
DAILY DOSE OF 60 mgm. ***							
1.	-	-	403	0/4	Abd. +++	8.2	27
2.	-	-	405		Abd. +	6.8	58
3.	-	-	377		Abd. ++	9.1	42
4.	-	-	367		Abd. ++	9.2	21

* Expressed as % of total hemoglobin (average for last five days of treatment)
** Determined by method of Brodie, Udenfriend and Taggart (1947)
*** Drug administered in six divided doses daily for two weeks
Abd. + = mild, transient abdominal cramps
Abd. ++ = moderate, repeated abdominal cramps
Abd. +++ = severe, persistent abdominal cramps

TABLE IV

CURATIVE EFFECT OF PRIMAQUINE WHEN ADMINISTERED IN DOSAGES OF 22.5 MGM. TOGETHER WITH 1.64 GMS. QUININE DAILY* IN PRIMARY ATTACKS OF VIVAX MALARIA (CHESSON STRAIN)

Cases	Days From End of Rx to First Relapse		Follow Up (Days)	Ratio: Subjects Relapsed/ Subjects Treated	Symptoms	MET** HGB %	Mean Plasma*** Concentration of Primaquine (gamma/liter)
	F/102+	Para					
1.	-	-	370	0/10	None	5.3	8
2.	-	-	520		None	7.9	-
3.	-	-	365		Diarrhea	3.4	10
4.	-	-	415		None	6.3	8
5.	-	-	364		None	5.8	5
6.	-	-	226		None	4.1	1
7.	-	-	341		None	8.7	4
8.	-	-	373		None	12.2	9
9.	-	-	373		None	8.6	6
10.	-	-	389		None	10.3	4

* Both drugs administered in six divided doses daily for two weeks
** Expressed as % of total hemoglobin (average for last five days of treatment)
*** Determined by method of Brodie, Udenfriend and Taggart (1947)

TABLE V

CURATIVE EFFECT OF PRIMAQUINE WHEN ADMINISTERED IN DOSAGES OF 22.5 MGM. WITH 1.64 GMS. QUININE DAILY* - IN CASES REPRESENTING THE FIRST OR SECOND RELAPSE AFTER OTHER THERAPY

Cases	Days From End of Rx to First Relapse		Follow Up (Days)	Ratio: Subjects Relapsed/ Subjects Treated	Symptoms	MET** HGB %	Mean Plasma*** Concentration of Primaquine (gamma/liter)
	F/102+	Para					
FIRST RELAPSES							
1.	-	-	175		None	4.6	3
2.	-	-	277		None	8.3	9
3.	-	-	312		None	5.7	3
4.	-	-	330		None	6.0	6
5.	-	-	337		Abd. ++	6.5	2

TABLE V (Cont.)

Cases	Days From End of Rx to First Relapse		Follow Up (Days)	Ratio: Subjects Relapsed/ Subjects Treated	Symptoms	MET** HGB %	Mean Plasma*** Concentration of Primaquine (gamma/liter)
	F/102+	Para					
FIRST RELAPSES							
6.	-	-	307		None	6.7	2
7.	-	-	364	0/13	None	3.4	4
8.	-	-	316		None	5.1	3
9.	-	-	346		None	8.4	6
10.	-	-	343		None	8.1	2
11.	-	-	245		None	4.5	-
12.	-	-	161		Abd. ++	-	-
13.	-	-	285		None	8.3	4
SECOND RELAPSES							
1.	-	-	362		None	4.7	17
2.	-	-	369		Abd. +	5.3	13
3.	76	74	-		Abd. +	5.6	25
4.	-	-	349	1/8	None	1.9	5
5.	-	-	365		None	9.3	9
6.	-	-	365		Anorexia	7.7	-
7.	-	-	349		None	9.4	5
8.	-	-	349		Abd. +	7.5	6

* Both drugs were administered in six divided doses daily for two weeks
** Expressed as % of total hemoglobin (average for last five days of treatment)
*** Determined by method of Brodie, Udenfriend and Taggart (1947)
Abd. + = mild, transient abdominal cramps
Abd. ++ = moderate, repeated abdominal cramps

A daily dose of 22.5 mgm. of primaquine given concurrently with 1.64 Gms. of quinine (base)[5/] prevented relapse in practically 100% of cases. (Tables IV and V). Increasing the dose

5/ Subsequent studies have shown that 1.64 Gms. of the base is in excess of the amount of quinine needed. A dose of 0.82 Gms. of base (1.0 Gms. quinine sulfate) is certainly sufficient and possibly even as little as 0.547 Gms. of base may suffice. The smallest effective dose of quinine has yet to be determined.

of primaquine to 60 mgm. increased the toxicity without concurrent therapeutic advantage (Table III).

Toxicity

Toxicity studies were carried out on volunteers unsuited for therapeutic trials.[6] The drug was given during attacks induced by intravenous malaria. The same drug dosage regimen was followed. The toxic manifestations observed during administration of primaquine at 120 mgm. daily (alone, and in conjunction with other drugs) is shown in Table VI. The toxicity of primaquine tends to be cumulative; in some instances symptoms began late in the course of drug administration and continued for several days after its discontinuance.

TABLE VI

TOXICITY STUDIES OF PRIMAQUINE (SN 13,272)

Days Rx	Case	Age	Weight	Symptoms	Laboratory Findings	MET* HGB %	Mean Plasma** Concentration of Primaquine (gamma/liter)
DAILY DOSE OF 120 mgm. ***							
14	1.	21	145	Abd. ++	Normal	20.5	201
14	2.	28	145	Abd. + and Nausea	Normal	21.7	228
14	3.	39	168	Abd. +	WC 4700****	18.3	308
14	4.	24	150	Abd. ++	Normal	19.6	144
14	5.	43	152	Abd. +	Normal	20.7	171
DAILY DOSE OF 120 mgm. GIVEN CONCURRENTLY WITH 1.64 gms. QUININE***							
14	1.	36	140	Abd. +++	Normal	9.0	77
14	2.	24	172	Abd. +++	Normal	9.8	85
14	3.	28	189	Abd. ++ and Nausea	Normal	8.8	132
14	4.	39	137	Abd. + and Anorexia	Normal	15.5	126
14	5.	30	139	Abd. + and Anorexia	Normal	11.1	74
14	6.	22	180	Abd. +++ and Vomiting	Normal	5.5	-

* Expressed as % of total hemoglobin (average for last five days of treatment)
** Determined by method of Brodie, Udenfriend and Taggart (1947)
*** All drugs given in six divided doses daily
**** 5% immature granulocytes, 18% mature granulocytes, 76% lymphocytes (returned to normal five days after last dose of drug)
Abd. + = mild, transient abdominal cramps
Abd. ++ = moderate, repeated abdominal cramps
Abd. +++ = severe, persistent abdominal cramps

[6] Extensive studies of toxic and therapeutic effect of primaquine in mammals and primates have been done by Dr. L. H. Schmidt (The Christ Hospital Institute for Medical Research, Cincinnati, Ohio).

Two hundred and forty mgm. (base) probably represents the maximum dose that can be administered with safety for periods longer than a week even under close observation in hospital (Table VII). Although toxic manifestations were severe, no irreversible damage was noted. In contrast, the maximum tolerated dose of pamaquine is probably 90 mgm. (base) per day; and, for pentaquine (SN 13,276) is 120 mgm., but severe damage to the nervous system may result from its administration at that dose. Of the curative antimalarial drugs extensively studied, only isopentaquine (SN 13,274) can be given safely at a dose of 240 mgm. (base) per day for an extended period.

It is of interest to note the effect of quinine on the production of methemoglobin. At high doses of primaquine with quinine the methemoglobin is roughly 50% as great as that formed by the same dose of primaquine given alone (Table VI).

TABLE VII

TOXICITY STUDIES OF PRIMAQUINE (SN 13,272)

Days Rx	Case	Age	Weight	Symptoms	Laboratory Findings	MET* HGB %	Mean Plasma** Concentration of Primaquine (gamma/liter)
DAILY DOSE OF 240 mgm. GIVEN CONCURRENTLY WITH 0.199 gms. METHYLENE BLUE***							
9	1.	24	134	Abd. ++	WC 2000#	7.5	395
DAILY DOSE OF 240 mgm. GIVEN CONCURRENTLY WITH 1.64 gms. QUININE***							
11	1.	39	160	Abd. ++++	WC 3200##	9.6	213
14	2.	36	172	Abd. +++	WC 11,900	10.0	131

* Expressed as % of total hemoglobin (average for last five days of treatment)
** Determined by method of Brodie, Udenfriend and Taggart (1947)
*** All drugs given in six divided doses daily
\# 13% immature granulocytes, 3% mature granulocytes, 79% lymphocytes (returned to normal seven days after last dose of drug)
9% immature granulocytes, 30% mature granulocytes, 56% lymphocytes (returned to normal fourteen days after last dose of drug)
Abd. ++ = moderate, repeated abdominal cramps
Abd. +++ = severe, persistent abdominal cramps
Abd. ++++ = intolerably severe abdominal cramps

Discussion

The therapeutic significance of the change in character of the terminal amino groups on the side chain of pamaquine-like compounds can be seen by the following comparison. (For greater homogeneity of data only primary cases with a standardized infection are summarized):

Drug*	Daily Dose** (base weight)	Relapse Ratio	Estimated Dose for 100% Cure
Pamaquine	60 mgm. +	10/20	90-120 mgm.
Isopentaquine	60 mgm.	6/20	90 mgm.
Primaquine	22.5 mgm.	0/20	22.5 mgm.

* administered concurrently with quinine
** given in six divided doses for two weeks
\+ this is equal to 133 mgm. of the salt, pamaquine naphthoate

It is apparent that on an equal weight basis, primaquine is about four times as active as the best of the other members of the family. Comparison of the subjective toxicity in the pamaquine family in terms of estimated pamaquine equivalents is as follows:

Drug.	Toxicity		Chemotherapeutic Index+
	In Terms of Symptomatology at 60 mgm./day	In Terms of Maximum Tolerated Dose	
Pamaquine	1.00	1.00	1
Isopentaquine	0.75	0.33	2 1/2
Primaquine	1.00	0.33	10

\+ chemotherapeutic index is the ratio of largest tolerated dose divided by the smallest dose capable of preventing nearly all relapses

Although not the subject of this paper, which stresses comparative curative effect and toxicity of the three drugs studied under standard conditions, it should be mentioned further that primaquine can establish a high prophylactic and curative ratio when administered in therapeutically safe single daily doses. This is not possible with either pamaquine, pentaquine or isopentaquine. These latter three drugs have been shown in field studies to be active in doses one-half to one-third as great as those necessary to produce equivalent results against our standard test strain of vivax malaria (Most, et al., 1946), (Alving, 1948), (Coggeshall and Rice, 1949). Observation of a limited number of patients suggests that naturally acquired infections likewise can be cured with much smaller doses of primaquine than reported here.

Primaquine is superior to both pamaquine and isopentaquine because it will cure severe infections of vivax malaria in dosages that are relatively non-toxic in white subjects and because it has a wide range between the clinically effective dose and the maximum tolerated dose.

ACKNOWLEDGEMENTS

We wish to acknowledge the invaluable nursing and technical assistance rendered in this study by Miss Shirley Mock, Mrs. Katherine Chellew and Mrs. Lorraine Gruben, and to the many hard-working and conscientious inmate nurses and technicians assigned to the malaria project.

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ADDENDUM

The toxicity of primaquine in darkly pigmented races and in children is unknown. Until these problems have been adequately investigated therapeutic use of the drug should be limited to adult white subjects.

5-ARYLOXY-2,4-DIAMINOPYRIMIDINES AS ANTIMALARIALS

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A major project in our laboratories for a number of years has been a study of the role of pyrimidine derivatives and related substances in the biosynthesis of nucleic acids[1]. A screening test was established using Lactobacillus casei as the test object, in a manner which allows one to test for antifolic acid, anti-thymine and antipurine effects as well as for stimulatory activity. By means of reversal experiments the nature of the blocks which the inhibitors produce can be examined in more detail. For example (Figure I) 2,4-diamino-5-p-chlorophenoxypyrimidine had been found in the screening test to have a strong antifolic acid activity. The figure shows a more detailed study of this effect. Over a considerable range of concentration the effect of the inhibitor can be overcome completely by the addition of more folic acid. Moreover, the level of growth obtained depends rather closely on the ratio of the pyrimidine to folic acid and is nearly independent of the absolute concentration. This constitutes a rather good example of competitive inhibition and probably indicates that the two substances are competing for some surface in or on the microorganism.

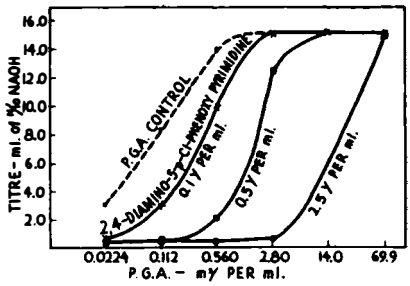

Figure I

The inhibitory effect shown by this aryloxydiaminopyrimidine is a general property of 2,4-diaminopyrimidines and condensed systems containing the diaminopyrimidine structure.[2] These all inhibit the growth of L. casei in the folic acid system. When reversal experiments are

carried out one finds that these substances form a whole spectrum of inhibitors. Some of them, like the aryloxydiaminopyrimidines, are reversed only by folic acid. Others, like diaminopurine, are reversed very little by folic acid but readily by a purine, and all gradations between these two extremes can be found.

In the course of our work a number of commercially available substances had been tested, and among these, both paludrine and quinine have effects in the Lactobacillus casei test which resemble those of the diaminopyrimidines. Paludrine (chlorguanide) lies quite far toward the antifolic end of the spectrum, quinine somewhat nearer the center, that is, the inhibition by quinine can be relieved to a considerable extent by the addition of purines.

This was one reason for testing the compounds for antimalarial activity.

A second reason was that there is a certain structural resemblance between chlorguanide and the aryloxypyrimidine derivatives. This is shown on the slide (Figure II). If chlorguanide is written in a cyclic form, you will see that the resemblance between it (IV) and the p-chloro-phenoxy derivative (V) is close, and perhaps closer to that of the p-chlorophenoxy-6-methyl-pyrimidine (VI). This type of structural resemblance - as between a cyclic and an acyclic compound - probably is not very good chemistry, but sometimes results in leads to the solution of practical problems.

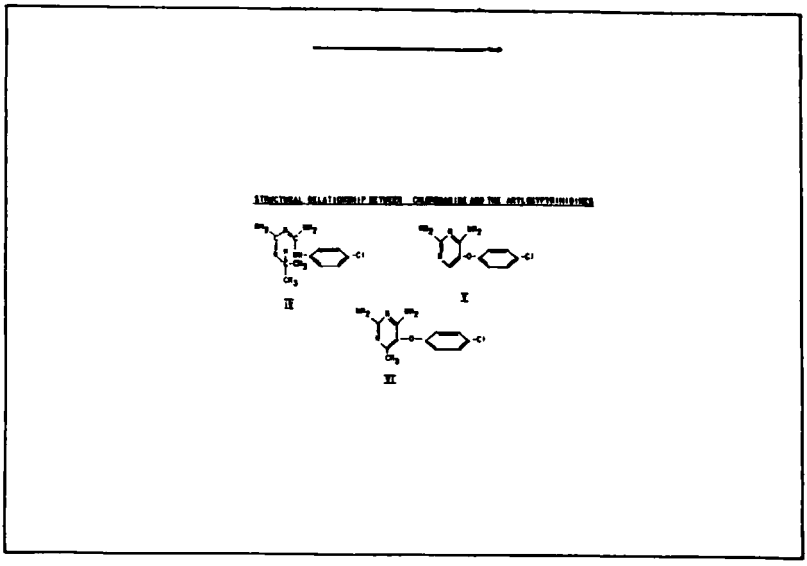

Figure II

At any rate, these pyrimidines were submitted for trial as antimalarials and both found to be active, V being about as active as quinine and VI about four times as active. So we were introduced to antimalarial research by the back door, as it were.

Looking through the literature one finds that a considerable amount of work on pyrimidine derivatives as antimalarials was carried out during the wartime search for such substances, but all these pyrimidines contained basic side chains attached to the pyrimidine nucleus. With the wisdom of hindsight, it is now possible to see that these basic side chains are not only not necessary, but definitely reduce the activity. This may be illustrated by the substances shown

on the next slide (Figure III). VII is one of the substances studied by the I. C. I. group[3]. On their scale the 2-p-chloroanilino-4-diethylaminoethylamino-5-phenoxypyrimidine shows ± activity at 160 mg./kg. and was negative at 80 mg. per kg. At about the same time Todd and co-workers[4] reported on compound VIII which had an amino group in place of the anilino group in the 2-position. The activity of this was rated a + at 120 mg. per kg. and a ± at 60 mg. per kg. In our own work, the 2,4-diamino-5-phenoxypyrimidine (the corresponding substance with removal of the basic side chain) would rate at least +++ on the I. C. I. scale and is thus more active than either of the more complex substances, although it is not now considered to be a very active substance.

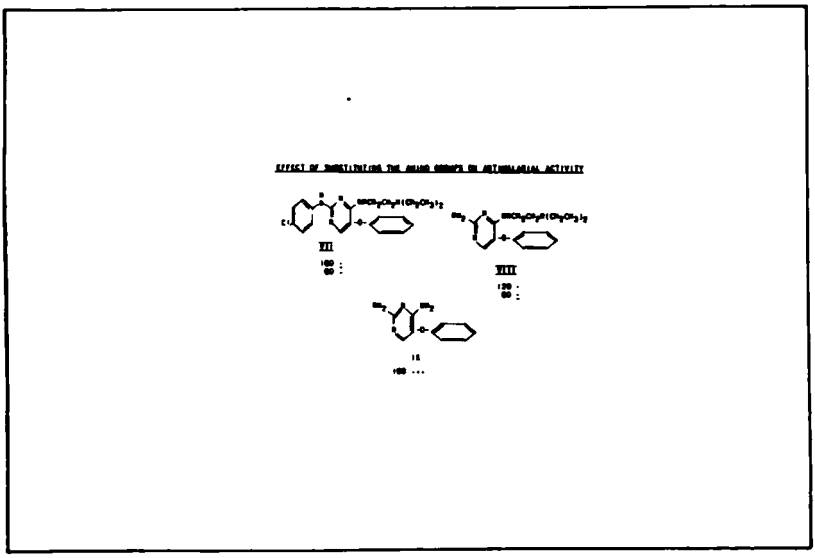

Figure III

For the benefit of the chemists the next two slides show general methods for the preparation of the aryloxypyrimidines. Figure IV shows the preparation of the 6-unsubstituted variety by formylation of aryloxyacetic esters, condensation with guanidine, followed by chlorination and amination. The 6-alkyl derivatives are prepared from acyl acetic esters (illustrated on Figure V by acetoacetic ester) by way of the α-aryloxyacylacetic ester and then by the above route to the diaminopyrimidine.

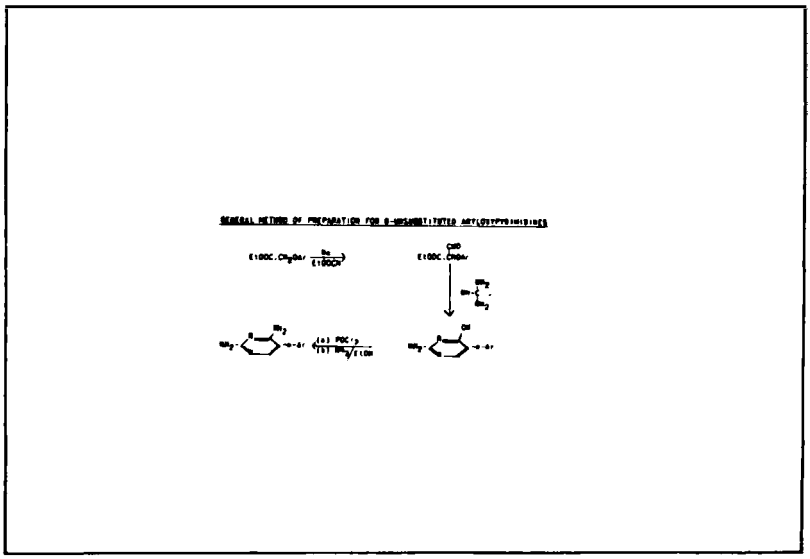

Figure IV

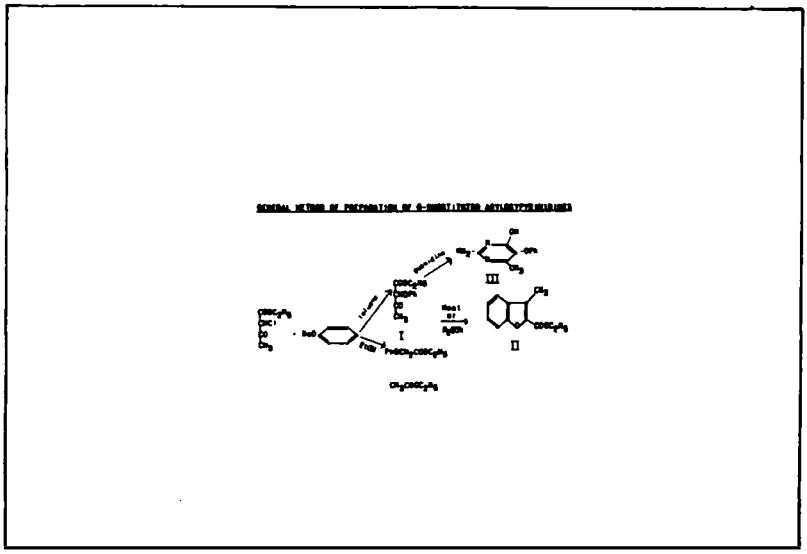

Figure V

The elaboration of this lead toward new antimalarials is being pursued as a cooperative project between the Wellcome Research Laboratories, Tuckahoe, and the Wellcome Laboratories for Tropical Medicine in London under Brigadier J. S. K. Boyd. At just about the time our work began, Plasmodium berghei infection in the mouse became available through the courtesy of Professor Shortt of the London School of Tropical Medicine and Goodwin[5] studied the action of several antimalarial drugs in parallel against P. gallinaceum in chicks and P. berghei in mice and found some interesting differences (Figure VI). The dose of quinine required to suppress the parasites is much the same on the two organisms, and chloroquine and our 2,4-diamino-5-p-chlorophenoxy-6-methylpyrimidine have about the same quinine coefficients against both experimental malarials. On the other hand, mepacrine is more active against P. berghei than against P. gallinaceum while chlorguanide and especially pamaquin are less active against P. berghei Our compounds all have been tested against both types of experimental malarias, and we hope that eventually data bearing on the suitability of P. berghei as a screening test will be available. It may be that it will be found to be the mammalian malaria, suitable for large scale testing and investigation, which has been so much desired.

FIGURE VI

Drug	P. gallinaceum	P. berghei
48-210	4.3	4.7
Mepacrine	2.0	7.8
Pamaquine	18.0	<2
Chloroquine	13.	13
Chlorguanide = Paludrine	12.	4.6

Goodwin, L. G., Nature, 164, 1133 (1949)

The elaboration of the lead provided by the antimalarial activity of 2,4-diamino-5-p-chlorophenoxypyrimidine and its 6-methyl derivative has followed more or less straightforward lines. The biological work is still rather fragmentary and it would be premature to attempt to correlate biological activity and chemical activity on a comprehensive scale. However, a few points can be illustrated.

In the first place, not all of the substances of the diaminopyrimidine spectrum of inhibitors possess antimalarial activity, but many of the more potent compounds have some degree of antimalarial activity. This is true of diaminopteridines[6] which lie at the antifolic end of the spectrum and of diaminopurine which lies at the opposite end. These have in common the diaminopyrimidine structure and some sort of substituent at the 5-position of the pyrimidine ring. A number of subseries are under investigation, and some of these have shown compounds of very high activities. The 5-aryloxy-2,4-diaminopyrimidines[7] are used for purposes of illustration. (Figure VII). In the first place, the unsubstituted phenoxy derivative is not very active, and has activities of the same order of magnitude on the two test organisms. Substitution of the aromatic nucleus by halogen in the para position increases the activity somewhat but the activity against P. berghei is enhanced more than that against P. gallinaceum. The meta chloro derivative is even more active against P. gallinaceum but considerably less active against P. berghei.

FIGURE VII

5-PHENOXY-2,4-DIAMINOPYRIMIDINES

Activity and Substitution in the Aromatic Nucleus

Substituent of Phenyl	6 Substituent	Quinine Equivalent	
		P. gallinaceum	P. berghei
none	H	0.5	0.3
p-Cl	H	0.8	1.0
m-Cl	H	1.1	0.5
none	CH_3	0.5	0.1
p-Cl	CH_3	4.5	4.7
m-Cl	CH_3	6.8	0.5
p-OCH_3	H	>0.5	0.0

The addition of a methyl group in the 6-position of the pyrimidine diminishes the activity against Plasmodium berghei when the benzene ring is unsubstituted but increases the activity of the p-chlorophenoxy compound by a factor of about 5. The corresponding meta derivative is even more active against P. gallinaceum but of relatively low potency against P. berghei. One might guess from this that the electron distribution in the aromatic nucleus influences the activity markedly, and that P. berghei is more sensitive to these effects than P. gallinaceum. This supposition is borne out by all the results through several series of compounds and in general the activity against P. berghei is enhanced by electron attractive groupings in the para position. One further illustration of this may be found on the final line of this slide. The p-methoxy derivative, containing an electron donor group, retains activity against P. gallinaceum but is essentially inactive against P. berghei. The effect of the alkyl group in the 6-position is somewhat unpredictable. Moreover, the basicity of the whole molecule, as measured by the pKa of the monohydrochloride, seems to be of secondary importance. Undoubtedly, steric factors are involved which may be unravelled by further work.

Finally, I should like to return to the question, which was implied in the beginning, of the mechanism of action of these substances. Is their antimalarial action an expression of antifolic acid activity? First, some indirect evidence may be derived by comparing antifolic activity in the Lactobacillus casei system with antimalarial activity (Figure VIII). The next slide shows the antifolic acid activities of some of the diaminopyrimidines and their antimalarial activities, with paludrine as a reference compound. The activity in the L. casei system is given as the amount causing 50 per cent inhibition in the usual folic acid growth medium and the figures given, therefore, are inversely proportional to the activity. It is seen that all these pyrimidine derivatives are much more active than paludrine against L. casei but less active than paludrine against malaria. Furthermore, some related pyrimidines, kindly furnished by Professor Todd, show no activity in the L. casei system but have appreciable activities as antimalarials. Thus it would appear that if the antimalarial activity is related to the antifolic activity the nature of this relationship is somewhat obscure. Nevertheless the antimalarial activity of the pyrimidines is at least partially reversed by folic acid in vivo and potentiated by sulfonamides, just as is that of chlorguanide. (We are indebted to Dr. Joseph Greenberg for this information). Furthermore, Dr. Greenberg and his colleagues, Miss Taylor and Dr. Josephson, have found the pyrimidines to be relatively inactive in vitro. In this respect also they resemble chlorguanide and differ from other antimalarials. These facts would seem to suggest the same mode of action for the

pyrimidines as for chlorguanide. A direct test of this possibility on a chlorguanide-resistent strain of Plasmodium gallinaceum (again by Dr. Greenberg and associates) revealed that no cross resistance developed. Thus their action appears to be similar, but not identical to that of chlorguanide.

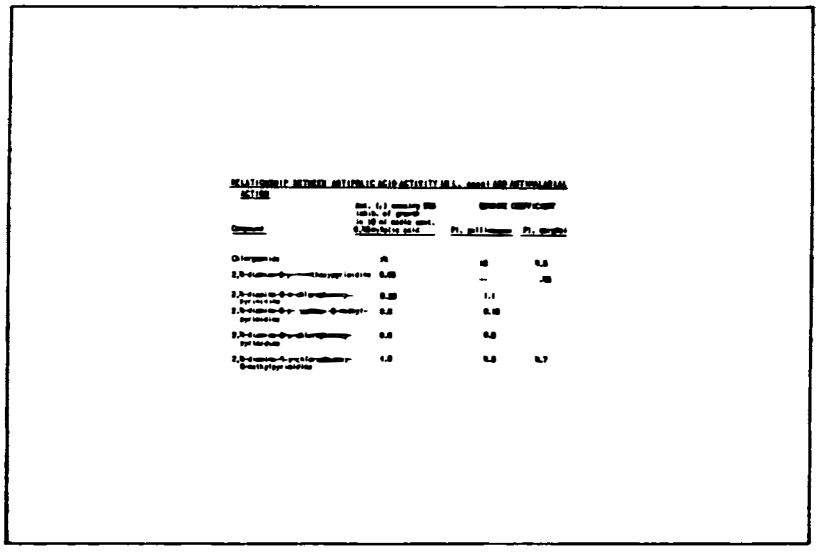

Figure VIII

Before concluding, I should point out that no work with our pyrimidines in the human malarias has been reported yet, and very little work with sporozoite-transmitted infections has been carried out. Some compounds with extremely high potencies, by present-day standards, have been found, but high potency in a substance is no guarantee that it will have activity on the exoerythrocytic forms of the parasites, and no real advance would have been made in the absence of this type of activity.

The outlines of a pattern relating to biological activity and chemical structure in the pyrimidine series are beginning to appear but considerably more work will be required before the whole picture can be filled in.

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RELATION OF STRUCTURE OF DIPHENYL COMPOUNDS TO FUNGITOXICITY

by

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INTRODUCTION

The literature of microbiology is full of data on the toxicity of benzene derivatives. By contrast diphenyl has been neglected. Nevertheless, diphenyl seems to be an architecturally significant nucleus in fungicides. During a span of 12 years we have worked sporadically on diphenyl derivatives. The time seems ripe to exhume the data from the catacombs of the literature and our file drawers, to correlate it, and to work it into as reasonable a set of conclusions as possible.

Bateman and Henningsen in 1923[4] demonstrated that diphenyl retarded somewhat the growth of Fomes annosus in culture. In 1938 work in our laboratory began with the finding that bis (4-aminophenyl) methane was fungitoxic. A few bits of data were published in 1945[13]. The latest development is the demonstration of the practical significance for plant disease control of bis (2-hydroxy-5-chlorophenyl) sulfide[14]. We have investigated approximately one hundred forty analogues. The action of eighty-six of these will be discussed below.

In the interim, Goodavage[8] disclosed the mildew proofing action on cotton cloth of bis (2-hydroxy-5-chlorophenyl) methane and of bis (2-hydroxy-3,4,5-trichlorophenyl) methane. Marsh and co-workers[17] have published excellent papers on these compounds and their relatives.

Technique Used

Since results depend upon techniques used, these will be indicated briefly. The details have been printed elsewhere[13].

What is called fungitoxicity often depends upon the method of assaying a test compound. Briefly, we may say that two basic techniques are employed; (1) spores suspended in distilled water are germinated overnight on dried deposits of the test chemical, (2) the fungus is grown for several days on treated food. Some investigators, notably Goldsworthy and co-workers,[9,10] modify technique (1) by placing the non-germinated spores on a clean food supply and note recovery.

Results from the three methods vary. Diphenyl itself, for example, is a good inhibitor of growth - a poor inhibitor of spore germination. In visualizing the effect of test chemicals we should remember that a germinating spore carries its own rations. It merely needs to mobilize them quickly to form a short germ tube. A test chemical can only prevent this mobilization and it must penetrate and act within a very few hours if it is to be effective.

On the other hand, a growing hypha must obtain its nourishment from the ambient medium A test chemical has additional possibilities in this case. It can alter the food outside the spore as well as inside. It can prevent the food from entering.

Goldsworthy's technique permits the test compound to be removed from the spore by the media.

In our experiments, fungitoxicity of the test compound has been assayed by the spore germination method. The two test organisms were Stemphylium (formerly Macrosporium) sarcinaeforme and Sclerotinia fructicola. These are very common test fungi.

Two techniques have been used for applying the test compounds; (A) a solution in a suitable solvent is applied with a graduated one milliliter syringe to the flat bottom of a cylindrical depression in a thick glass slide (cavity slide), (B) a suspension is applied by spraying to a cellulose-nitrate coated glass slide.

Multiple doses are used normally differing by a factor of 10 for technique A and a factor of the square root of 2 for technique B.

Spores of the test fungi are added to the dried deposits. Results for technique A are recorded as the least dose that prevents germination. Results for technique B are recorded as percentage of spores not germinating - usually without correction for the usual one or two percent of natural mortality.

Criteria of Action

Data on spore inhibition for technique B are plotted against dose, using a logarithmic-probability grid, and a curve is fitted by inspection in accordance with the suggestion of Wilcoxon and McCallan[18]. Such a curve has two qualities - position and slope. Position indicates potency of the test substance. It is usually expressed as ED 50, i. e., the effective dose for 50 per cent inhibition. In other words, compounds can be compared in terms of the amount required for a given response.

Slope is calculated as the change in probit mortality with unit change in log. dose. Hence, the larger the number, the steeper the slope.

Slope may be expressed by the well-known function of $\frac{\Delta x}{\Delta y}$. Dr. A. E. Dimond of this laboratory has suggested that slope can be derived from the dosage-response curve as plotted on log. -probit paper. The formula simply is:

$$\text{Slope} = \frac{1}{\log \text{ED } 84 - \log \text{ED } 50}$$

Slope is usually accepted as an indication of mode of action. If compounds show different slopes, they presumably differ in the manner in which they inhibit spore germination. Of course, similar slopes need not necessarily indicate similar modes of toxic action.

In the tables included herewith the test compounds are listed in terms of both slope and ED 50 and ED 100 expressed in micrograms and molality per cm^2. It must be remembered, of course, that an ungerminated spore is not necessarily dead. Possibly, the toxicant could be removed by Goldsworthy's[10] technique or otherwise and the spore would recover.

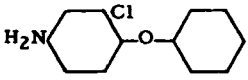
Toxicity at 1000 micrograms per cm^2 is considered essentially non-toxic. A compound must be toxic in the range of 1 to 10 micrograms per cm^2 before it can be considered highly toxic.

The compounds were used as received from the manufacturer or from Eastman Kodak Company. In general, they were laboratory samples and presumably they were reasonably pure.

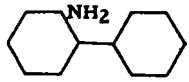
Data are presented in Tables 1 to 6. Table 1 comprises the names of the test compounds arranged alphabetically and the corresponding structural formulae. The toxicity data are given in Tables 2 to 6.

TABLE 1

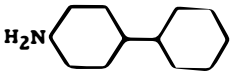
CHEMICAL NAMES AND STRUCTURES OF TESTED DIPHENYL DERIVATIVES



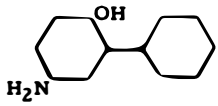
4-Amino-2-chloro diphenyl ether



2-Amino diphenyl

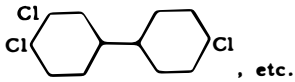


4-Amino diphenyl



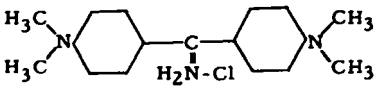
4-Amino-2-phenyl phenol

TABLE 1 (Cont.)

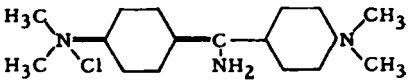


, etc.

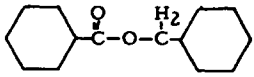
Arochlor 1248
Mixture of trichlorodiphenyls, position of chlorines uncertain



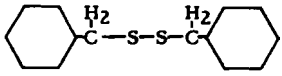
Auramine base hydrochloride



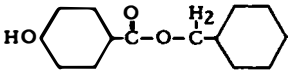
Auramine O



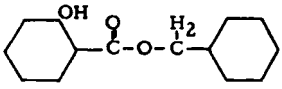
Benzyl benzoate



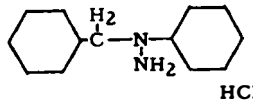
Benzyl disulfide



Benzyl-4-hydroxy benzoate

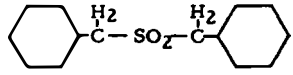


Benzyl-2-hydroxy benzoate

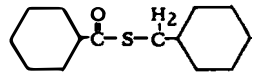


HCl

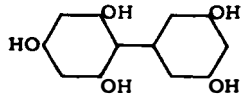
α-Benzyl-α-phenyl hydrazine hydrochloride



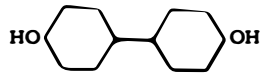
Benzyl sulfone



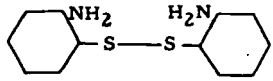
Benzyl thiobenzoate



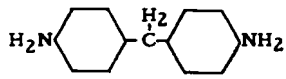
2, 3', 4, 5', 6-Biphenylpentol



4, 4'-Bisphenol

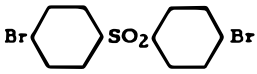


Bis(2-aminophenyl) disulfide

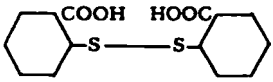


Bis(4-aminophenyl)methane

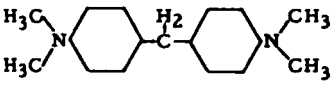
TABLE 1 (Cont.)



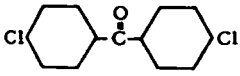
Bis(4-bromophenyl) sulfone



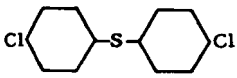
Bis(2-carboxyphenyl)disulfide



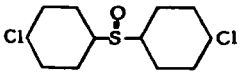
Bis (4-dimethyl aminophenyl) methane



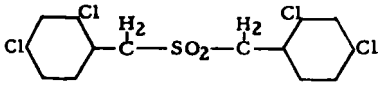
Bis (4-chlorophenyl) phenone



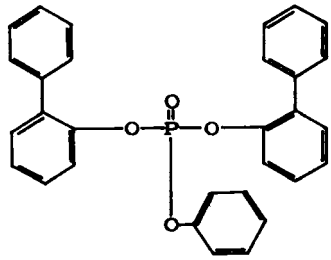
Bis (4-chlorophenyl) sulfide



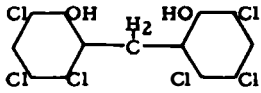
Bis (4-chlorophenyl) sulfoxide



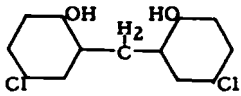
Bis (2,4-chlorobenzyl) sulfone



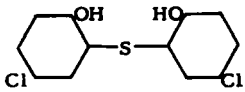
Bis (2-diphenyl) monophenyl phosphate



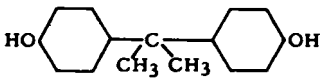
Bis (2-hydroxy-3,5,6-trichlorophenyl) methane



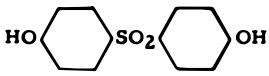
Bis (2-hydroxy-5-chlorophenyl) methane



Bis (2-hydroxy-5-chlorophenyl) sulfide

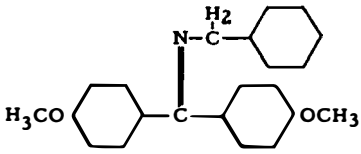


Bis (4-hydroxyphenyl) isopropane

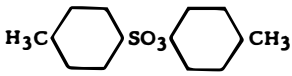


Bis (4-hydroxy phenyl) sulfone

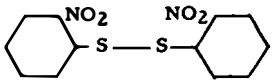
TABLE 1 (Cont.)



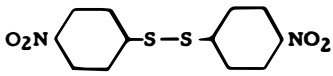
Bis (4-methoxyphenyl) benzylimido methane



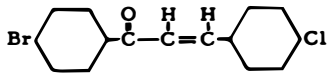
Bis (4-methyl phenyl) sulfonic acid



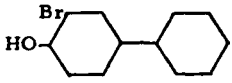
Bis (2-nitrophenyl) disulfide



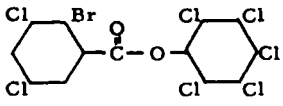
Bis (4-nitrophenyl) disulfide



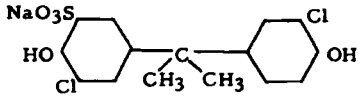
4-Bromo-4'-chloro chalcone



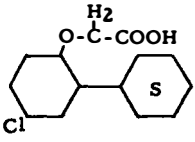
2-Bromo-4-phenyl phenol



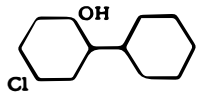
2-Bromo-3,5-dichloro-pentachlorophenyl benzoate



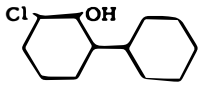
6-Chloro-4-(3-chloro-4-hydroxy-α,α-dimethylbenzyl)-1-phenol-2-sodium sulfonate



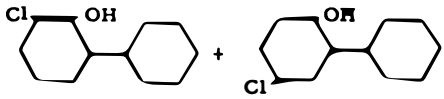
4-Chloro-2-cyclohexylphenoxy acetic acid



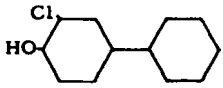
4-Chloro-2-phenyl phenol



6-Chloro-2-phenyl phenol

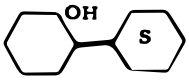


6-Chloro-2-phenyl phenol
4-Chloro-2-phenyl phenol
} mixture

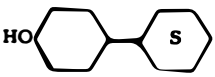


2-Chloro-4-phenyl phenol

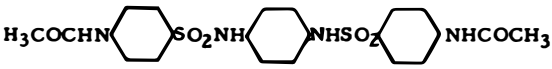
TABLE 1 (Cont.)



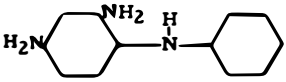
2-Cyclohexylphenol



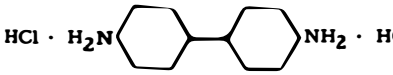
4-Cyclohexylphenol



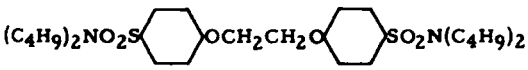
$N^4, N^{4'}$-Diacetyl-$N^1, N^{1'}$-4-phenylenebis sulfanilamide



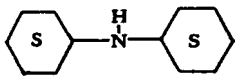
2,4-Diamino diphenyl amine



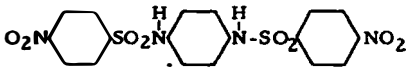
4,4'-Diamino diphenyl dihydrochloride



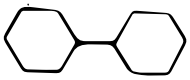
N,N'-Dibutyl-4,4'-ethylenedioxybis benzenesulfonamide



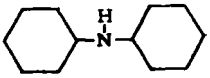
Dicyclohexyl amine



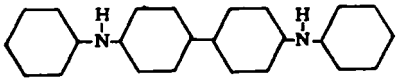
4,4'-Dinitro-N,N'-4-phenylenebis benzenesulfonamide



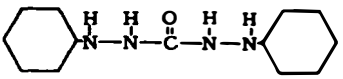
Diphenyl



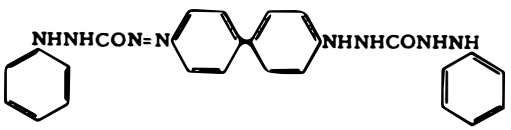
Diphenyl amine



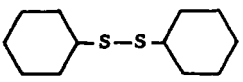
Diphenyl benzidine



s-Diphenyl carbazide

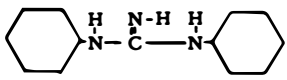


s-Diphenyl carbazone

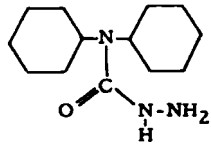


Diphenyl disulfide

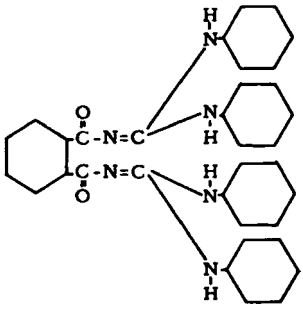
TABLE 1 (Cont.)



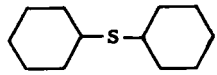
Diphenyl guanidine



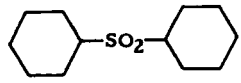
4,4-Diphenyl semicarbazide



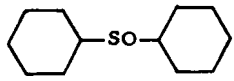
Diphenyl guanidine phthalate



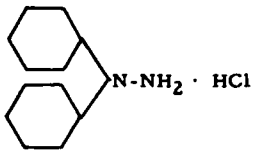
Diphenyl sulfide



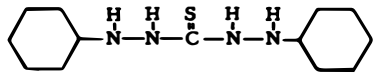
Diphenyl sulfone



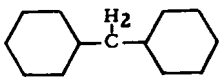
Diphenyl sulfoxide



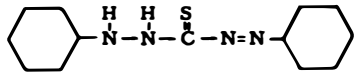
as-Diphenyl hydrazine hydrochloride



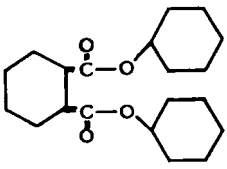
Diphenyl thiocarbazide



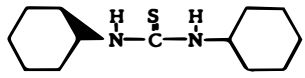
Diphenyl methane



Diphenyl thiocarbazone

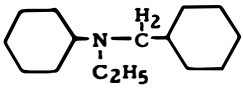


Diphenyl phthalate

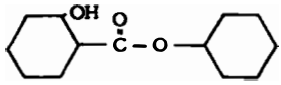


Diphenyl thiourea

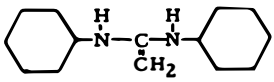
TABLE 1 (Cont.)



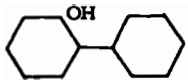
Ethylbenzyl aniline



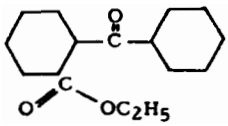
Phenyl-2-hydroxybenzoate



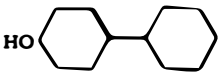
Ethylidene aniline



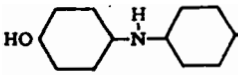
2-Phenyl phenol



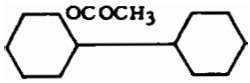
Ethyl-2-benzoyl benzoate



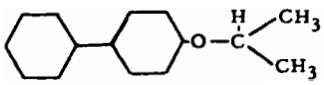
4-Phenyl phenol



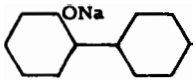
4-Hydroxy diphenyl amine



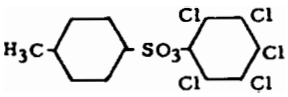
2-Phenyl phenol acetate



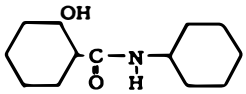
Isopropyl diphenyl ether



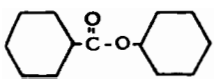
2-Phenyl sodium phenate



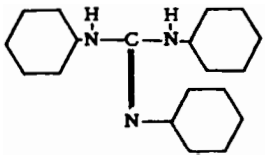
Pentachlorophenyl-4-toluenesulfonic acid



Salicylanilide

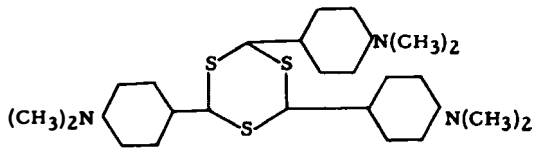


Phenyl benzoate



Triphenyl guanidine

TABLE 1 (Cont.)



2,4,6-Tris (4-dimethyl amino phenyl)-s-trithiane

EXPERIMENTAL

We introduced this paper with the statement that diphenyl seems to be an architecturally significant nucleus for a fungicide. Diphenyl itself is a curious compound. It is a very weak inhibitor of spore germination. In fact, it is adjudged ineffective for the spores of our two test fungi. It is a moderate to good inhibitor of mycelial growth, however, as Bateman and Henningsen[4] first reported and as Heiberg and Ramsey[11] have confirmed. Heiberg and Ramsey demonstrated diphenyl to be a vapor-phase fungistat, which appeared to act chiefly on the plasma-membrane. The apparent hiatus between spore germination and mycelial inhibition data may perhaps be explained on the basis of "availability"[13]. Diphenyl may enter the protoplast so slowly that there is time for a germ tube to develop before the fungistat is sufficiently concentrated within the fungus to produce its effect. This growth of 50 to 100μ of mycelium would not be apparent when measuring mycelial colonies macroscopically.

Diphenyl itself is simply two benzene rings joined end to end. Sometimes the two rings are joined through bridges of various types. We have studied the fungitoxic effect of different bridges and also the effect of hydroxyl, carboxyl, amino, and halogen substitutions.

Effect of Bridges

The effect of bridges will be discussed first. Data are given in Table 2 and Table 3. Some of these bridges are simple; others are very complex. In general, bridges by themselves added no toxicity to diphenyl. The following bridged compounds were non-toxic:

TABLE 2

LIST OF NON-TOXIC BRIDGED COMPOUNDS

Benzyl disulfide	Diphenyl phthalate
Dicyclohexylamine	4,4-Diphenyl semicarbazide
Diphenyl amine	Ethyl benzyl aniline
Diphenyl benzidine	Ethylidene aniline
s-Diphenyl carbazide	Bis (2-diphenyl) monophenyl phosphate
Diphenyl guanidine phthalate	Triphenyl guanidine
Diphenyl methane	

TABLE 3

TOXICITY DATA FOR DIPHENYL DERIVATIVES WITH -CO-O- AND RELATED BRIDGES

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Benzyl benzoate	5/6/42	5/6/42	Not Toxic	<1130	-	-	-	-	-	-
	5/24/50	5/24/50	Not Toxic	113						
Benzyl thiobenzoate	3/30/50	3/30/50	Not Toxic	Not Toxic	-	-	-	-	-	-
	5/24/50	5/24/50	Not Toxic	Not Toxic						
Benzyl-4-hydroxy-benzoate	9/8/42	9/8/42	Not Toxic	-	-	6.2	-	2.72	-	9.40
Benzyl-2-hydroxy-benzoate	1/28/43	1/28/43	Not Toxic	<1130	-	-	-	-	-	-
	5/17/50	5/17/50	Not Toxic	Not Toxic	-	-	-	-	-	-
Bis (4-chlorophenyl) phenone	11/16/42	11/16/42	Not Toxic	<1130	-	-	-	-	-	-
Diphenyl phthalate	5/20/42	5/20/42	Not Toxic	Not Toxic	-	-	-	-	-	-
Ethyl-2-benzoyl benzoate	5/15/42	5/15/42	Not Toxic	Not Toxic	-	-	-	-	-	-
Phenyl benzoate	4/18/44	4/18/44	-	-	38.7	18.4	19.5	9.29	-	3.08
	5/4/50	5/4/50	1130	113	-	-	-	-	-	-
2-Bromo-3,5, dichloro-pentachlorophenyl benzoate	9/7/49	5/1/50	Not Toxic	113	-	-	-	-	-	-
	5/4/50	5/4/50	Not Toxic	113	-	-	-	-	-	-
Phenyl-2-hydroxy benzoate	5/22/42	5/22/42	Not Toxic	<1130	-	-	-	-	-	-
	5/17/50	5/17/50	Not Toxic	113	-	-	-	-	-	-
Salicylanilide	2/16/38'	-	-	-	5.6	-	2.6	-	2.40	-
	5/16/50	5/16/50	113	11.3	-	-	-	-	-	-
4-Bromo-4-chloro-chalcone	10/5/48	10/6/48	Not Toxic	113	-	-	-	-	-	-

* Test fungi. St means Stemphylium, Sc means Sclerotinia.

The bridge seemed to have importance in a few cases. The bridge seemed to be more important in the toxicity of the compounds to Sclerotinia than to Stemphylium. For example, Stemphylium was inhibited by very few bridged compounds with otherwise unsubstituted rings. The most effective of these was diphenyl guanidine, ø - NHCNHNH - ø. This has an exposed =NH group joined by its double bond to the central carbon in the chain. Its action will be discussed below in the section on $-NH_2$.

Sclerotinia, on the other hand, responded to several bridged compounds with unsubstituted rings. Compounds with sulfide or sulfoxide bridges were more effective against Sclerotinia than Stemphylium. Compounds with sulfone bridges were not toxic to either.

Effect of -CO-O- and Related Bridges

Sclerotinia also reacted to nearly all compounds with a -CO-O- bridge between the rings, but Stemphylium did not. (Data in Table 3). This was the situation with benzyl benzoate, benzyl-4-hydroxy benzoate, benzyl-2-hydroxy benzoate (benzyl salicylate), phenyl benzoate, 2-bromo-3,5-dichloropentachlorophenyl benzoate and phenyl-2-hydroxy benzoate (phenyl salicylate). Benzylthiobenzoate was not toxic to either organism. Clayton et al.[5] report that benzyl salicylate is able to prevent the downy mildew disease of tobacco, although it was not very effective in inhibiting spore germination. The -CO- bridge in bis (4-chlorophenyl) phenone and in 4-bromo-4'-chloro chalcone was also toxic to Sclerotinia but not to Stemphylium. If, however, the two chlorines are removed from bis (4-chlorophenyl) phenone and $-COOC_2H_5$ is put on in the 2-position, it is toxic to neither (ethyl 2-benzoylbenzoate). Goldsworthy and Gertler[10] report a -CO- bridged compound that is non-fungicidal, bis (4-aminophenyl) ketone. This must be viewed in the light of their technique, however. They put treated spores on agar.

There is one other interesting bridge effect. As just noted, phenyl-2-hydroxybenzoate (phenyl salicylate) is toxic to Sclerotinia only. Salicylanilide is toxic to both organisms. The compounds are alike except for the bridge. In the former the bridge is -CO-O-. In the latter the bridge is -CO-NH-. The action of this compound will be discussed below under the section on -OH attached to the bridge.

Effect of Substituting -OH

Ever since Lord Lister and carbolic acid, we have known that the addition of an -OH to benzene induces toxicity to microorganisms. Likewise the addition of an -OH to diphenyl will increase fungitoxicity.

We have studied more -OH compounds than any other type. For convenience these will be divided on the basis of substitution on one ring or on both in the 4- and 2- positions or on the bridge. Data are given in Table 4.

The well-known effect of -OH substitution on fungitoxicity shows up. Some other less well-known relations also appear. Among other things, fungitoxic -OH compounds in general have very flat dosage-response curves. They range from about 3.0 to 6.0.

Effect of -OH in the 4-Position

4-Phenyl phenol is an easy point of departure. It may be visualized as having an -OH on one end of the diphenyl long axis. 4-Phenyl phenol is moderately toxic to Stemphylium, quite toxic to Sclerotinia. We tested two similar compounds in which the phenyl portion of the molecule had been changed: 4-cyclohexyl phenol and benzyl-4-hydroxy benzoate. The toxicity to Stemphylium was completely knocked out and the toxicity to Sclerotinia was greatly reduced by both changes. These two substitutions steepened the dosage-response curve for Sclerotinia which suggests that the mode of action was also changed. 4-Hydroxydiphenyl amine was moderately toxic to Stemphylium.

Appraisal of changes in ED 50 must be cautious, however, if the slope is changed simultaneously.[13]

Effect of -OH in the 2-Position

Passing to 2-phenyl phenol, we have a compound with the -OH along the side of the

TABLE 4

TOXICITY DATA FOR DIPHENYL DERIVATIVES WITH -OH SUBSTITUTIONS

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides				Slope of Toxicity Curve	
					Micrograms per cm^2		Molarity x 10^{-8} per cm^2			
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for a single -OH in the 4-position										
4-Cyclohexyl phenol	12/16/42	12/16/42	Not Toxic	-	Not Toxic	7.9	Not Toxic	4.48	-	4.80
	12/23/42	12/23/42	Not Toxic	-	Not Toxic	4.0	Not Toxic	2.27	-	4.22
4-Phenyl phenol	12/16/42	12/16/42	-	-	7.1	0.7	4.17	0.41	2.82	4.05
2-Chloro-4-phenyl phenol	1/17/43	1/17/43	-	-	8.2	1.5	4.01	0.73	3.19	5.22
2-Bromo-4-phenyl phenol	6/1/43	4/19/44	-	-	34.4	5.4	13.81	2.17	-	7.00
4-Hydroxydiphenyl amine	5/4/38	-	113	-	-	-	-	-	-	-
Benzyl-4-hydroxy benzoate	9/8/42	9/8/42	Not Toxic	-	Not Toxic	6.2	Not Toxic	2.72	-	9.40
Data for a single -OH in the 2-position										
2-Cyclohexyl phenol	12/16/42	12/16/42	-	-	6.2	0.6	3.52	0.34	1.45	3.19
4-Chloro-2-cyclohexyl phenoxy acetic acid	1/24/50	1/25/50	11.3	11.3	-	-	-	-	-	-
2-Phenyl phenol	12/16/42	12/16/42	-	-	Weak	4.5	Weak	2.65	-	3.80
2-Phenyl phenol acetate	4/1/48	11/4/48	1130	113	-	-	-	-	-	-
2-Phenyl sodium phenate	2/23/42	2/23/42	Not Toxic	-	-	2.2	-	1.14	-	2.40
4-Amino-2-phenyl phenol	8/3/49	7/26/49	1130	11.3						
4-Chloro-2-phenyl phenol	9/7/49	9/7/49	113	1.13						
	5/19/50	5/19/50			-	3.2	-	1.56	-	8.30

TABLE 4 (Cont.)

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for a single -OH in the 2-position (Cont.)										
6-Chloro-2-phenyl phenol	6/17/43	4/19/44	-	-	5.1	3.7	2.49	1.80	4.40	5.50
Mixture of 4- and 6-chloro-2-phenyl phenol	12/28/42	12/28/42	Not Toxic	-	-	11.2	-	5.47	-	4.22
Benzyl-2-hydroxybenzoate	1/28/43 5/17/50	1/28/43 5/17/50	Not Toxic Not Toxic	1130 Not Toxic	-	-	-	-	-	-
Phenyl-2-hydroxy benzoate	5/22/42 5/17/50	5/22/42 5/17/50	Not Toxic Not Toxic	1130 113	-	-	-	-	-	-
Salicylanilide	12/16/38 1/28/42 5/16/50	- 1/28/42 5/16/50	- 1130 113	- 1130 11.3	5.6 -	- -	2.6 -	- -	2.40 -	- -
Data for -OH on each ring in 4-position										
4,4'-Bisphenol	5/4/50 5/20/50	5/4/50 5/20/50	113 -	1.13 -	- -	- 1.2	- -	- 0.64	- -	- 6.00
Bis(4-hydroxyphenyl)iso-propane	12/23/42 5/4/50	12/23/42 5/4/50	Not Toxic Not Toxic	- 113	- -	11.2 -	- -	4.91 -	- -	4.22 -
6-Chloro-4-(3-chloro-4-hydroxy-2,2-dimethyl-benzyl)-1-phenol-2-sodium sulfonate	2/10/49	2/10/49	Not Toxic	Not Toxic	-	-	-	-	-	-
Bis(4-hydroxyphenyl)sulfone	11/23/48	9/21/48	Not Toxic	Not Toxic	-	-	-	-	-	-

TABLE 4 (Cont.)

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for -OH on each ring in 2-position										
Bis(2-hydroxy-5-chloro-phenyl)sulfide	11/19/43	11/19/43	Not Toxic	-	-	4.8	-	1.67	-	5.70
	-	4/25/44	-	-	-	0.2	-	0.07	-	4.22
		1/10/48	-	-	-	0.3	-	0.10	-	6.60
Bis(2-hydroxy-5-chloro-phenyl)methane	8/26/42	-	-	-	9.3	-	3.46	-	1.56	-
	8/19/42	-	-	-	7.8	-	2.90	-	-	-
	5/18/50	5/18/50	-	-	5.0	0.27	1.85	0.10	5.70	3.2
Bis(2-hydroxy-3,5,6-tri-chlorophenyl)methane	8/26/42	-	-	-	7.9	-	1.94	-	1.56	-
	8/19/42	-	-	-	7.8	-	1.92	-	-	-
Data for multiple -OH groups										
2,3',4,5',6-Biphenylpentol	10/5/48	10/6/48	1130	Not Toxic	-	-	-	-	-	-

* Test fungi. St means Stemphylium, Sc means Sclerotinia.

diphenyl long axis and near the junction of the two rings. This change in position of the OH from that in 4-phenyl phenol weakens the fungitoxic action and may possibly flatten the slope.

In this case we have examples also of tampering with the -OH- free ring. The insertion of a $-CO-O-CH_2-$ bridge and even a -CO-O- bridge (benzyl-2-hydroxy-benzoate and phenyl-2-hydroxy benzoate) reduced the potency for 2-phenyl phenol just as much as for 4-phenyl phenol. This effect is particularly striking in view of the fact discussed below that $-SO_2-$ bridge also reduced the potency of a dihydroxy compound. Using cyclohexane for the second ring gave a strikingly opposite effect for 2-phenyl phenol than for 4-phenyl phenol. It increased rather than reduced the potency of 2-phenyl phenol to both organisms and flattened the slope. Since all four tests involved were made on the same day, this can hardly be a result of biological variation. This obviously would warrant further study.

Although the data on halogenation of 2-phenyl phenol are incomplete, the evidence is that 6-chloro-2-phenyl phenol and 4-chloro-2-phenyl phenol are more fungitoxic and have steeper slopes than 2-phenyl phenol alone. It seems significant that halogenation of both 4-phenyl phenol and 2-phenyl phenol should steepen the slope of the dosage-response curve.

Effect of -OH on Both Rings

We have tested several compounds with -OH on both rings. These are often called biphenols or bisphenols. We tested four bisphenols with the -OH in the 4-position and three with the -OH in the 2-position. The most striking result is that of the seven compounds only three are toxic to Stemphylium. Of course, only a few of the phenyl phenol series were toxic to Stemphylium.

Sclerotinia was more sensitive than Stemphylium to the bisphenols just as it was more sensitive to the phenyl phenols. In the case of 4,4'-bisphenol it was some hundredfold more sensitive. The insertion of an isopropane bridge seems to quench what little toxicity that 4,4'-bisphenol shows toward Stemphylium, and it reduces the toxicity to Sclerotinia. The insertion of a sulfone ($-SO_2-$) bridge quenches the toxicity to both. It seems significant that the insertion of a -CO-O- bridge reduces or even quenches the toxicity of 2-phenyl phenol and 4-phenyl phenol.

The interest in one compound of the 4,4'-bisphenol series equals the length of its name, 6-chloro-4-(3-chloro-4-hydroxy-α,α-dimethylbenzyl)-1-phenol-2-sodium sulfonate. It is non-toxic to Sclerotinia. The problem is why? Of course the compound has so many substituents that one cannot be positive which one is basically responsible. This compound may be considered as a chlorinated 4,4'-bisphenol compound with an isopropane bridge and a sodium sulfonate group in the 2-position to the hydroxyl on one ring. For the following reasons, we must conclude, tentatively at least, that the sulfonate group is mainly responsible for quenching the toxicity: (a) although an isopropane bridge reduced toxicity of 4,4'-bisphenol to Sclerotinia, it did not quench it; (b) chlorination has not quenched toxicity elsewhere; (c) since chlorination in the 5-position does not quench the toxicity of -OH in the 2-position, it presumably does not do so for -OH in the 4-position.

We have then two cases in which a sulfone grouping quenches the toxicity - in the bridge of bis (4-hydroxy phenyl) sulfone and on the ring in 6-chloro-4-(3-chloro-4-hydroxy-α,α-dimethyl-benzyl)-1-phenol-2-sodium sulfonate. This brings to mind the report by Horsfall and Rich[15] who found that a sulfonic acid in the 5-position quenches the fungitoxicity of 8-quinolinol. Here is a good case for further research.

It is of further interest to note that although slope data are meager, the slope of the Sclerotinia curve for bis (4-hydroxyphenyl) isopropane is 4.22, very close to that for 4-phenyl phenol. Apparently, this suggests that the -OH group inhibits the fungus by the same mechanism whether it rides on one ring or on both.

There is a slight further confirmation of the effect of halogenation on slope already noted under the phenyl phenols. The slope of bis (2-hydroxy-5-chlorophenyl) sulfide for Sclerotinia is steeper than that for bis (4-hydroxyphenyl) isopropane. Although again one cannot ascribe this for sure to the chlorine, this is reasonable because of the effect of chlorine to steepen the slope of the monohydroxy diphenyls.

One compound demonstrates the significance of the -OH in the bisphenols. Arndt[11] showed that bis (2-hydroxy-5-chlorophenyl) methane was effective as a curative for cottonseed that is infected with Colletotrichum. His data reveal that replacing the -OH groups with acetoxy groups quenched the toxicity.

Effect of Multiple -OH Substitutions

The effect of multiple -OH substitutions is interesting. We have one compound with five -OH groups, 2, 3', 4, 5', 6-biphenylpentol. It was essentially non-toxic to both test organisms. It is of interest here to look at the molar toxicities of one, two, and five -OH groups on a diphenyl nucleus. The molar ED 50 values were 0.41, 4.91, and infinity (not toxic) for 4-phenyl phenol, bis (4-hydroxy phenyl) isopropane, and 2, 3', 4, 5', 6 biphenylpentol; respectively. Horsfall[13] reports data showing that adding extra -OH units to phenol, phenyl phenol, and naphthol usually reduces the fungitoxicity.

Effect of -OH Attached to the Bridge

Dion and Lord[6] show that the addition of an -OH to the methane bridge of bis (4-dimethyl-aminophenyl) methane lends toxicity to this compound.

At this point we must refer back to the interesting anomaly between salicylanilide and phenyl-2-hydroxy benzoate mentioned earlier. The bridges are -CO-NH- and -CO-O-, respectively. The former is toxic to Stemphylium, the latter is not. We offer the following tentative explanation to explain it: we assume that the double bond to the carbon in the bridge of salicylanilide can resonate between the oxygen and nitrogen to give an equilibrium $-\overset{OH}{\overset{|}{C}} = N \rightleftharpoons -\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{N}}-$. This should give an -OH compound which then should have the flat slope characteristics of -OH compounds. Salicylanilide has a very flat slope. Finally, such resonance cannot occur in a -CO-O- bridge because no mobile hydrogen is available.

Effect of Substituting $-NH_2$

$-NH_2$, like -OH, lends toxicity to the diphenyl nucleus. Of course, $-NH_2$, like -OH, may be substituted on the ring or on the bridge. Data are given in Table 5. Fungitoxic $-NH_2$ compounds in general have steep dosage-response curves. They range from about 5.0 to 9.0.

Effect of $-NH_2$ on One Ring

Here also it is of interest to contrast the two rings of diphenyl with the single ring of benzene. The addition of an amine group to diphenyl enables it to prevent spore germination. The addition of an amine group to benzene (to form aniline) does not[13].

This result can be stated in other words. Benzene does not prevent spore germination. It cannot be made to do so by the addition of a ring (to form diphenyl) or the addition of an $-NH_2$ group (to form aniline). It can be made to prevent spore germination, however, by the addition of both a ring and an $-NH_2$ group to form aminodiphenyl. How can this be?

Since we are studying structure, we can make substitutions on the ring or on the $-NH_2$ group to see which is the more important. The $-NH_2$ is the simpler. Therefore, diphenyl amine is of interest because in it the second ring merely replaces one of the hydrogens of the amine. Except for the loss of one hydrogen atom, diphenyl amine has the same molecular weight as aminodiphenyl, but diphenyl amine is not fungitoxic. If, however, amino groups are added to diphenyl amine to form 2, 4-diaminodiphenyl amine, the toxicity is restored. Goldsworthy et al.[9] first reported the fungitoxicity of this compound. Barry et al.[2] showed that 2-aminodiphenyl amine was toxic to tuberculosis bacteria.

For these data we can devise one conclusion. Toxicity appears to require 2-rings plus a primary amino group. Of the two, the amino group seems to be the more important because (a) an amino group makes a diphenyl nucleus toxic, (b) substitution on the amino group quenches the toxicity.

Without a more detailed study, we cannot be sure about the comparative toxicities of the 2-substitution, the 4-substitution and the 2, 4-substitutions except to say that the double

substitution is probably not more toxic than a single substitution. The 4-substitution is probably a little more toxic than the 2-. The slopes of the three compounds are probably identical, suggesting that they inhibit the spores by the same mechanism.

Effect of $-NH_2$ on Both Rings

We obtained data on several compounds that show the effect of making a substitution of an amino group on each ring. All were toxic unless the amino hydrogen was substituted. Everitt and Sullivan[7] showed that bis (2-aminophenyl)sulfide was quite toxic in cultures of fungi that attack human skin, and we show that the same compound with a disulfide bridge was toxic to our spores. Benzidine (HCl) may be considered as the basic compound for 4, 4'-substitution. It is 4, 4'-diaminodiphenyl. Its toxicity curve has about the same slope as 4-aminodiphenyl which suggests that the two compounds kill by the same technique. The toxicity of the benzidine may be blocked by substitution of a benzene ring for one hydrogen in each amine group. (See diphenyl benzidine). The hydrogens are similarly blocked in s-diphenyl carbazone and the toxicity is blocked for Sclerotinia. s-Diphenyl carbazone, however, has a double bonded nitrogen and a carbonyl group to account for the toxicity to Stemphylium which it exhibits.

Another 4,4'-diamino substitution occurs in bis (4-aminophenyl) methane. This compound is very close to 4,4'-diaminodiphenyl in toxicity and slope, which suggests also that the methane bridge plays no role in the toxicity. The toxicity of this compound also was quenched by substituting both hydrogens with methyl groups as in bis (4-dimethylaminophenyl) methane.

2,4,6 Tris (4-dimethylaminophenyl)s-trithiane is a basically similar compound to bis (4-dimethylaminophenyl) methane and it is also non-toxic, presumably because the amino hydrogens have been substituted.

Goldsworthy and Gertler[10] have shown that bis (4-aminophenyl) ketone is non-fungitoxic. This is another case where oxygen attached by a double bond to the bridge is associated with low toxicity.

Effect of $-NH_2$ Attached to the Bridge

The $-NH_2$ group adds toxicity when added to the bridge as well as when added to the ring, just as in the case of -OH. as-Diphenyl hydrazine (HCl) is such a compound which consists of an $-NH_2$ group added to the nitrogen bridge of diphenyl amine. Thus, diphenyl amine is made toxic. α-Benzyl α-phenyl hydrazine is also toxic. This differs only in having an extra $-CH_2-$ in the bridge.

Of course, phenyl hydrazine is also fungitoxic. One can consider that this is aniline with an $-NH_2$ group attached to the amine group. Horsfall[13] has suggested that, through the Hinsberg reaction, phenyl hydrazine binds up the glucose in the spore thus depriving it of energy. Presumably, these two diphenyl hydrazines act also to deprive the spore of energy.

The addition of an $-NH_2$ group to a methane bridge also imparts toxicity just as the addition to an amine bridge. We have said above that bis (4-dimethylaminophenyl) methane was not toxic. If an $-NH_2$ group is added to the methane bridge to form auramine (HCl) toxicity is added. In fact, the toxicity and slope are almost precisely identical with that of the bis (4-aminophenyl) methane before it is substituted with methyl groups. Dion and Lord[6] obtained toxicity to Fusarium by adding a hydroxyl group to the methane bridge to form bis (4-dimethylaminophenyl) methanol. In fact, this compound was more toxic in their tests than the $-NH_2$ analogue.

If now the primary amine on the methane bridge is reduced to a secondary amine, as in bis (4-methoxyphenyl) benzylimido methane, the toxicity is blocked again.

From all this study of $-NH_2$, we can conclude that toxicity of an amino diphenyl compound depends upon the action of a primary amine. It matters not whether the primary amine is attached to the ring or to the bridge. Another point is that the primary amine should be in connection with two rings. One ring is not enough.

The biologist wonders if the mode of toxicity is the same when the compound is activated by $-NH_2$ as it is by -OH. This may be studied by comparing the slopes of the dosage-response curves. Perusal of the data in Table 2 shows that the amine substituents give steeper slopes in

TABLE 5

TOXICITY DATA FOR DIPHENYL DERIVATIVES WITH -NH_2 SUBSTITUTIONS

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for -NH_2 on one ring only										
4-Amino diphenyl	4/13/43	11/29/43	-	-	4.0	2.9	2.36	1.71	5.50	6.60
	-	12/7/43	-	-	-	1.2	-	0.71	-	4.60
	-	12/10/43	-	-	-	1.3	-	0.77	-	5.00
4-Amino-2-chloro-diphenyl ether	4/20/50	4/20/50	113	11.3	-	-	-	-	-	-
4-Amino-2-phenyl phenol	8/3/49	7/26/49	1130	11.3	-	-	-	-	-	-
2-Amino diphenyl	4/13/43	4/13/43	Not Toxic	-	-	2.5	-	1.48	-	5.00
	-	11/26/43	-	-	-	3.4	-	2.01	-	9.40
	-	11/29/43	-	-	-	12.4	-	7.34	-	6.60
	-	12/7/43	-	-	-	2.9	-	1.72	-	8.80
	-	12/10/43	-	-	-	3.6	-	2.13	-	8.30
	5/18/50	5/18/50	113	11.3	-	-	-	-	-	-
2,4-Diaminodiphenyl amine	9/26/42	-	-	-	0.5	-	0.25	-	7.40	-
	11/27/42	11/27/42	-	-	6.2	1.0	3.12	0.50	7.40	-
Data for -NH_2 on both rings										
Bis(2-aminophenyl) disulfide	4/28/48	11/16/48	1.13	1.13	-	-	-	-	-	-
4,4-Diamino diphenyl di-hydrochloride	10/2/42	-	-	-	3.1	-	1.21	-	5.00	-
	10/7/42	-	-	-	3.4	-	1.32	-	6.00	-
(Benzidine base dihydro-chloride)	11/4/43	-	-	-	5.3	-	2.06	-	6.00	-
N,N'-Diphenyl benzidine	1/8/45	1/8/45	Not Toxic	Not Toxic	-	-	-	-	-	-

TABLE 5 (Cont.)

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides				Slope of Toxicity Curve	
					Micrograms per cm^2		Molarity x 10^{-8} per cm^2			
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for $-NH_2$ on both rings (Cont.)										
s-Diphenyl carbazone	2/16/45	2/16/45	<1130	Not Toxic	-	-	-	-	-	-
	5/18/50	5/18/50	1130	113	-	-	-	-	-	-
Bis (4-aminophenyl) methane	11/4/43	-	-	-	1.9	-	0.96	-	7.40	-
Bis (4-dimethylaminophenyl) methane	2/16/45	2/16/45	Not Toxic	Not Toxic	-	-	-	-	-	-
2,4,6-Tris (4-dimethyl-aminophenyl) s-trithiane	11/16/49	11/16/49	Not Toxic	Not Toxic	-	-	-	-	-	-
Data for =NH- and -NH- in the bridge**										
α-Benzyl-α-phenylhydrazine HCl	5/17/44	5/17/44	-	-	6.2	<2.0	2.64	-	10.0	-
$N^4, N^{4'}$-Diacetyl-N', N'-4-phenylenebis sulfanilamide	10/10/47	10/10/47	1230	Not Toxic	-	-	-	-	-	-
2,4-Diaminodiphenyl amine	9/26/42	-	-	-	0.5	-	0.25	-	7.40	-
4,4'-Dinitro-N,N'-4-phenyl-enebis benzene sulfonamide	9/9/48	9/21/48	11.3	Not Toxic	-	-	-	-	-	-
Diphenyl carbazide	5/23/50	5/23/50	Not Toxic	1130	-	-	-	-	-	-
s-Diphenyl carbazone	5/18/50	5/18/50	1130	113	-	-	-	-	-	-
	5/23/50	5/23/50	650	1130	-	-	-	-	-	-
Diphenyl guanidine	11/4/43	-	-	-	10.9	-	5.17	-	7.40	-
Diphenyl hydrazine HCl	5/17/50	5/17/50	113	113	-	-	-	-	-	-

TABLE 5 (Cont.)

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for =N- and -NH- in the bridge (Cont.)**										
Diphenyl thiocarbazide	5/18/50	5/18/50	Not Toxic	113	-	-	-	-	-	-
	5/23/50	5/23/50	Not Toxic	300	-	-	-	-	-	-
Diphenyl thiocarbazone	5/17/50	5/17/50	1130	1130	-	-	-	-	-	-
	5/23/50	5/23/50	Not Toxic	70	-	-	-	-	-	-
4-Hydroxy diphenyl amine	5/4/38	-	113	-	-	-	-	-	-	-
Salicylanilide	2/16/38	-	-	-	5.6	-	2.6	-	2.40	-
Data for $-NH_2$ attached to the bridge										
Auramine base hydrochloride	5/27/42	-	-	-	2.2	-	1.72	-	10.8	-
	1/5/43	1/5/43	-	-	3.1	6.8	1.02	2.10	8.0	1.63
	5/16/50	5/16/50	113	11.3	-	-	-	-	-	-
Auramine O	5/25/43	5/25/43	<1130	<1130	-	-	-	-	-	-
	5/17/50	5/17/50	11.3	113	-	-	-	-	-	-
As-Diphenyl hydrazine hydrochloride	2/16/45	2/16/45	<1130	<1130	-	-	-	-	-	-
α-Benzyl-α-phenyl hydrazine hydrochloride	5/17/44	5/17/44	-	-	6.2	<2.0	2.64	-	10.0	-
Diphenyl guanidine	11/4/43	-	-	-	10.9	-	5.17	-	7.40	-
Triphenyl guanidine	4/29/42	4/29/42	Not Toxic	Not Toxic	-	-	-	-	-	-
Diphenyl guanidine phthalate	5/15/42	5/15/42	Not Toxic	Not Toxic	-	-	-	-	-	-

TABLE 5 (Cont.)

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for $-NH_2$ attached to the bridge (Cont.)										
4,4-Diphenyl semicarbazide	12/16/45	12/16/45	Not Toxic	Not Toxic	-	-	-	-	-	-
Bis (4-methoxyphenyl) benzyl-imido methane	2/16/45	2/16/45	Not Toxic	Not Toxic	-	-	-	-	-	-

* Test fungi. St means Stemphylium, Sc means Sclerotinia.

** The following compounds with =N- or -NH- in the bridge were not toxic to the spores of the two test fungi: Dicyclo hexyl amine, diphenyl amine, diphenyl benzidine, diphenyl guanidine phthalate, diphenyl semi-carbazide, diphenyl thiourea, ethylbenzyl aniline, ethylidene aniline, triphenyl guanidine.

all cases than the hydroxyl substituents. The best example perhaps is to be found in the comparison of 2-phenyl phenol and 2-aminodiphenyl. The slope of the dosage-response curves of the amine compound is 7.80 and the slope of the hydroxyl curve is 3.80, much flatter.

We interpret this to mean that the two types of compounds act on the fungus at different loci and hence the mode of action is probably different.

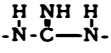
Effect of =N- and -NH- in the Bridge

We tested twenty-one compounds with =N- and -NH- in the bridge. Of this number, nine were recorded as non-toxic to the spores of the test organisms. With only a single exception, the nitrogen in these non-toxic compounds occurred either in a secondary or in a tertiary amine The exception was 4,4-diphenyl semicarbazide. In this compound the $-NH_2$ occurs at the end of a short chain attached to an amine bridge, but this chain also has a -C=O- grouping which is associated in several compounds with low toxicity. Except for the carbonyl group, diphenyl semicarbazide is not too dissimilar to as-diphenyl hydrazine which is fungitoxic.

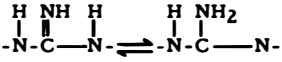
Of the twelve toxic compounds with nitrogen in the bridge, we have already discussed the toxic action of the primary amines on the two hydrazines and 2,4-diaminodiphenyl amine. We have discussed the hydroxyl of 4-hydroxyl diphenyl amine and salicylanilide. The $N^4,N^{4'}$-diacetyl compound is only very weakly fungitoxic; the toxicity of the 4,4'-dinitro compound is probably due to the $-NO_2$ group. This accounts for the toxicity of seven of the twelve.

The eighth compound that we might discuss is diphenyl guanidine. It appears to be a key compound. It has no toxic substituents on the ring. It has a secondary amine attached to the bridge, but most available evidence indicates that secondary amines are not very fungitoxic.

We offer the following explanation which parallels that for salicylanilide already discussed. The bridge is as follows:



Presumably the double bond can resonate as follows:



If so, a primary amine exists at equilibrium, and it can be fungitoxic. If it is, it should show a steep dosage-response curve and it does.

The resonance discussed can be prevented by substituting to give triphenyl guanidine or diphenyl guanidine phthalate. If the resonance theory is sound, these compounds should be non-toxic and they are non-toxic.

This leaves only the carbazide-carbazone, thiocarbazide-thiocarbazone series to be discussed. These are -CO- and -CS- analogues and should not be fungitoxic to Stemphylium and only weakly toxic to Sclerotinia if the quenching action of -CO- groups is general. The facts are in good agreement. This series is not fungitoxic at all or only negligibly fungitoxic to Stemphylium, and they are only weakly toxic to Sclerotinia.

Effect of Sulfur

As we have already suggested in the section on bridges, the introduction of sulfur into a diphenyl molecule imparts fungitoxicity. We have studied twenty-four sulfur-containing compounds. Data are given in Table 6. The sulfur in all cases was either a part of a bridge or attached to the bridge. Fourteen of the compounds were more or less toxic to Sclerotinia. None was toxic to Stemphylium unless the compound contained a toxic grouping other than the sulfur. Presumably, this is because of the well-known fact that Sclerotinia is sensitive to sulfur. Stemphylium is not.

Five types of sulfur-containing bridges were tested - sulfide, disulfide, sulfoxide, sulfone, and sulfate. No compound with a sulfate bridge was toxic unless it contained some toxic substituent on the ring. Also the fact is noteworthy that no sulfone of the five tested was toxic to either

organism. Goldsworthy and Gertler[10] have shown the non-fungitoxic quality of the sulfone bridge.

How about sulfides and sulfoxide? Data of Marsh et al[17] show that the sulfide compound is more toxic than sulfoxide and sulfone. Combining their data and ours, it appears that the order of fungicidal value is -S- > -SO- > $-SO_2-$. If so, the more oxidized the sulfur, the less its fungitoxicity. This suggests that the sulfide compound must compete unfavorably with the fungus as an oxygen consumer and thus reduce its respiration. This needs testing.

The disulfide appears to be the most toxic of the unsubstituted diphenyl compounds with sulfur in the bridge. Also, it provides the nucleus for several interesting substitutions. If the compound is changed from an -S-S- bridge to a $-CH_2-S-S-CH_2-$ bridge, the toxicity disappears, reason unknown.

Several 2,2'-substitutions on diphenyl disulfide were studied. -COOH and $-NO_2$ in the 2-position quench or seriously hamper the toxicity of the sulfur in the bridge. On the other hand, $-NH_2$ in the same position provides one of the most, perhaps the most, fungitoxic compound in the whole series here reported. In fact, it is the only compound in the sulfur series that is significantly toxic to Stemphylium. Goldsworthy and Gertler[10] reported the fungitoxicity of bis (4-aminophenyl) sulfide.

The effect of bis (2-aminophenyl) disulfide is not particularly unexpected. It possesses $-NH_2$ groups that are known by other evidence to lend toxicity to both organisms. The difficulty is with the analogues that contain active carboxyl and nitro groups. Normally they lend toxicity if anything, not take it away. In fact, bis (4-nitrophenyl) disulfide is fungitoxic.

Perhaps, the explanation is as follows. The oxidized carboxyl and nitro groups compete with the reduced sulfur so that neither can inhibit spores. If the nitro group is removed spatially to the far-end of the molecule, the effect is not noticeable.

Effect of Substituting Halogen

Chlorination of diphenyl has not improved its fungitoxicity because Arochlor, a commercial mixture of chlorinated diphenyls, was no more toxic in our tests than diphenyl itself. Bis (4-chlorophenyl) phenone also was non-toxic to Stemphylium but slightly toxic to Sclerotinia. We have already shown that diphenyl sulfone was non-toxic. The addition of bromine in the 4-position on each ring of diphenyl sulfone did not make it toxic. Chlorination of diphenyl sulfide to form bis (4-chlorophenyl) sulfide reduced its toxicity. The same was true of the chlorination of diphenyl sulfoxide to form bis (4-chlorophenyl) sulfoxide. Horsfall[13] has reported that DDT is non-fungitoxic. This is bis (4-chlorophenyl) trichloroethane.

Chlorination or bromination seemed to reduce the fungitoxicity of phenyl phenol. A mixture of 4-chloro-2-phenyl phenol and 6-chloro-2-phenyl phenol was less toxic than 2-phenyl phenol. 2-Chloro-4-phenyl phenol was less toxic than 4-phenyl phenol and 2-bromo-4-phenyl phenol was even less toxic than 2-chloro-4-phenyl phenol.

This latter evidence that a bromine compound is less potent than its chlorine analogue is confirmed by the fact that bis (2-hydroxy-5-bromophenyl) sulfide is very much less toxic than bis (2-hydroxy-5-chlorophenyl) sulfide to both Chaetomium and Aspergillus (see Marsh et al[17]). From the evidence of Marsh et al it is clear that the result is not due merely to the larger atomic size of bromine.

Hatfield[11] made a study of the effect of chlorinating phenyl phenols on toxicity to Fomes annosus in culture. He found that 2-chloro-orthophenyl phenol was slightly more toxic than orthophenyl phenol. 4-Chloro-orthophenyl phenol was less toxic and 2,4-dichloro-orthophenyl phenol was still less toxic. These compounds are practical wood preservatives.

The only case so far found where chlorination increases toxicity is bis (2-hydroxyphenyl) methane. Marsh, Butler, and Clark[17] say that chlorination in the 5 and 5' positions increases fungitoxicity of that compound. On the other hand, their data show that in the case of a similar bisphenol with a sulfur bridge, the 5,5'-dichloro substitution was less toxic to Chaetomium tha· the 5,5'-dimethyl compound.

TABLE 6

TOXICITY DATA FOR DIPHENYL DERIVATIVES CONTAINING SULFUR

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data on -S- in the bridge										
Diphenyl sulfide	5/9/50	5/9/50	Not Toxic	113	-	-	-	-	-	-
Bis(2-hydroxy-5-chlorophenyl) sulfide	11/19/43	11/19/43	Not Toxic	-	-	4.8	-	1.67	-	5.70
	4/25/44	-	-	-	-	0.2	-	0.07	-	4.22
	1/10/48	-	-	-	-	0.3	-	0.10	-	6.60
Bis(4-chlorophenyl) sulfide	5/9/50	5/9/50	Not Toxic	Not Toxic	-	-	-	-	-	-
Data for -S-S- in the bridge										
Diphenyl disulfide	2/3/43	2/3/43	Not Toxic	1130	-	-	-	-	-	-
	5/4/50	5/4/50	Not Toxic	11.3	-	-	-	-	-	-
Benzyl disulfide	2/3/43	2/3/43	Not Toxic	Not Toxic	-	-	-	-	-	-
	5/18/50	5/18/50	Not Toxic	Not Toxic	-	-	-	-	-	-
Bis(2-carboxyphenyl) disulfide	12/30/48	11/13/48	1130	1130	-	-	-	-	-	-
	5/4/50	5/4/50	1130	1130	-	-	-	-	-	-
Bis(2-aminophenyl) disulfide	4/28/48	11/16/48	1.13	1.13	-	-	-	-	-	-
Bis(2-nitrophenyl) disulfide	2/3/43	2/3/43	Not Toxic	Not Toxic	-	-	-	-	-	-
	5/23/50	5/23/50	Not Toxic	Not Toxic	-	-	-	-	-	-
Bis(4-nitrophenyl) disulfide	2/3/43	2/3/43	Not Toxic	1130	-	-	-	-	-	-
	5/23/50	5/23/50	Not Toxic	11.3	-	-	-	-	-	-

TABLE 6 (Cont.)

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for =S=O in the bridge										
Diphenyl sulfoxide	11/26/43	11/26/43	Not Toxic	-	-	7.8	-	3.86	-	-
	5/9/50	5/9/50	1130	113	-	-	-	-	-	-
Bis(4-chlorophenyl) sulfoxide	5/9/50	5/9/50	Not Toxic	1130	-	-	-	-	-	-
Data for $=SO_2$ in the bridge										
Diphenyl sulfone	2/3/43	2/3/43	Not Toxic	Not Toxic						
	5/9/50	5/9/50	Not Toxic	Not Toxic						
Bis(4-bromophenyl)sulfone	2/3/43	2/3/43	Not Toxic	Not Toxic						
Bis(4-hydroxyphenyl)sulfone	11/23/48	9/21/48	Not Toxic	Not Toxic						
Benzyl sulfone	5/9/50	5/9/50	Not Toxic	Not Toxic						
Bis(2,4-dichlorobenzyl)sulfone	1/19/50	1/19/50	Not Toxic	Not Toxic						
Data for $=SO_3$ in the bridge										
Bis(4-methylphenyl)sulfonic acid	1/10/50	1/10/50	Not Toxic	Not Toxic						
Pentachlorophenyl-4-toluene-sulfonic acid	12/30/49	12/30/49	113	1130						
$N^4, N^{4'}$-Diacetyl-N', N'-phenylenebis sulfanilamide	10/10/47	10/10/47	1130	Not Toxic						
N, N'-Dibutyl-4, 4'-ethylene-dioxybis benzene sulfonamide	9/8/48	9/23/48	Not Toxic	Not Toxic						
4, 4'-Dinitro-N, N'-4-phenylenebis benzene sulfonamide	9/9/48	9/21/48	11.3	Not Toxic						

TABLE 6 (Cont.)

Compound	Date of Test		ED 100 - Cavity Slides Micrograms per cm^2		ED 50 - Sprayed Slides Micrograms per cm^2		ED 50 - Sprayed Slides Molarity x 10^{-8} per cm^2		Slope of Toxicity Curve	
	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*	St*	Sc*
Data for =C=S and =C=O in the bridge										
s-Diphenyl thiocarbazide	5/18/50	5/18/50	Not Toxic	<113						
s-Diphenyl carbazide	2/16/45	2/16/45	Not Toxic	Not Toxic						
	5/17/50	5/17/50	Not Toxic	Not Toxic						
4,4-Diphenyl semicarbazide	2/16/45	2/16/45	Not Toxic	Not Toxic						
s-Diphenyl thiocarbazone	11/16/43	11/16/43	Not Toxic	<1130						
	5/17/50	5/17/50	1130	1130						
s-Diphenyl carbazone	2/16/45	2/16/45	<1130	Not Toxic						
	5/18/50	5/18/50	1130	113						
Miscellaneous Sulfur Derivatives										
2,4,6-Tris(4-dimethyl-aminophenyl)-s-trithiane	1/16/49	11/13/48	Not Toxic	Not Toxic						

* Test fungi. St means Stemphylium, Sc means Sclerotinia.

As reported in the section on -OH derivatives, halogenation steepened the slope of the dosage-response of both 4-phenyl phenol and 2-phenyl phenol. Chlorination appeared to steepen the slope of hydroxyphenyl sulfide.

Effect of Position of Substituent on the Ring

The comparative ranking of 2 and 4 -OH substituents are as follows: 2-cyclohexyl phenol > 4-cyclohexyl phenol, 4-phenyl phenol > 2-phenyl phenol, 2-chloro-4-phenyl phenol > 4-chloro-2-phenyl phenol, benzyl-4-hydroxybenzoate > benzyl-2-hydroxybenzoate.

In the case of amino substituents 4-amino diphenyl > 2-amino diphenyl and bis (4-nitrophenyl) disulfide > bis (2-nitrophenyl) disulfide. Out of the six comparisons the 4-position was more active than the 2-position in five cases.

Apparently, slope was not influenced by the position of the substituent group. Presumably then position has no influence on mode of reactivity merely, but on the amount of activity.

Effect of -CH=CH- Bonding

We have a little evidence on the reactivity of the -CH=CH- bond. Marsh et al[17] have clearly shown that if a bisphenol has a -CH=CH- bridge it is more fungitoxic than if it has a $-CH_2-$ bridge. Likewise, auramine O is more toxic than auramine itself. The chief difference is that one ring of the former is quinoid with opposite double bonds.

We have some data on cyclohexyl phenol versus phenyl phenol. In the case of the ortho position, the unsaturated phenyl ring was less toxic to both organisms than the saturated cyclohexyl ring. On the other hand, the reverse was true for both organisms for the para position. There can be little doubt as to the accuracy of the result because all our comparisons were made on the same day. Of course, it is possible that impurities could account for it, but it seems unlikely.

Synergism Between Diphenyl and Acenaphthene

Acenaphthene is no more toxic to our spores than diphenyl. If acenaphthene is added to diphenyl, however, the mixture is fungitoxic as Bateman[3] first showed. This is a strange phenomenon, the data for which appear in the graph in Figure 1.

It was of some interest to ascertain the proportion of acenaphthene and diphenyl to give maximum fungitoxicity. Two tests were made and both agreed that 75 percent by weight of diphenyl and 25 percent of acenaphthene gave maximum response against Stemphylium. Sclerotinia was not tested.

Synergism is not uncommon between two toxicants or between a toxicant and a non-toxicant. It is a great rarity between two non-toxicants. Of course, probably neither compound is as bland as water, and if so, we can only say that synergism of a higher order is displayed by two weak toxicants.

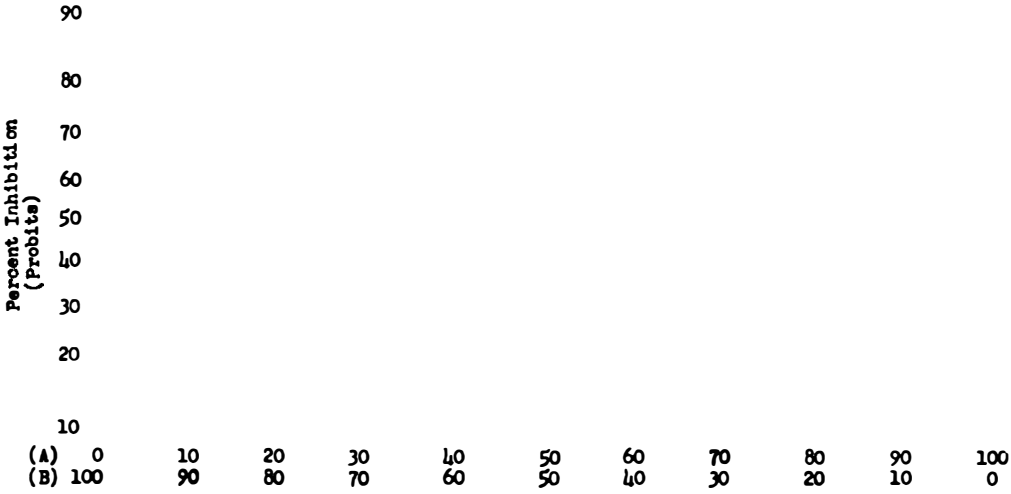
It is difficult to visualize how acenaphthene and diphenyl could react to produce a new toxic molecule. We can speculate that each acts on a different locus in the cell. If only one is applied and only one locus is affected, perhaps the organism can function by a different route. If, however, both are blocked, it cannot continue the struggle. Obviously, the problem needs additional research.

DISCUSSION

We should not close out this paper without some discussion of the biological implications of the data. Some of these have been scattered along in the text. More effort should be given to correlating the work on physiology of living fungi with the work on the physiology of dying fungi.

Various possibilities exist for accounting for the fungitoxicity of various chemicals.

Figure 1. Synergism between diphenyl and acenaphthene.
Depression slide test using Stemphylium sarcinaeforme.



(A) = Percent of Diphenyl in mixture.
(B) = Percent of Acenaphthene in mixture.
Total concentration for each mixture was 625 ppm.

Permeation

Most compounds presumably must permeate the cell wall before they can do damage to the spore.

One is struck in checking over the data by the fact that effective compounds can be considered as having lipophilic and hydrophilic components, polar and non-polar groups. This point is being discussed also in a collateral paper on nitrogen heterocycles[15].

The non-polar group in many cases must have about twelve carbon atoms as we have discussed elsewhere[13]. For example, in this series, $-NH_2$ on one ring is not sufficient. It must have two rings - twelve carbon atoms.

The classical theory, of course, is that of Overton-Meyer. They postulated many years ago that the semi-permeable membrane around the cell is composed of a fat and water emulsion. Hence, a compound that penetrates readily should be soluble in both. Of course, if the fat solubility is too high, the compound may not be water-soluble enough to react once inside the cell.

Analogues of Cell Constituents

None of these compounds seems sufficiently similar to be acting as a deleterious analogue of a vitamin, sugar, or amino acid in the cell. Of course, phenylalanine, thyroxine, and iodogorgoic acid are ring-containing amino acids. These amino acids do not contain double rings, however.

Reaction with Cell Constituents

As mentioned earlier the diphenylhydrazine compounds may be interfering with glucose utilization by reacting to form glucoseazone by the Hinsberg reaction. The diphenylhydrazine compounds may react with ketone groupings in the cell as well.

According to Eastman's reagent catalogue bis (4-aminophenyl) methane is useful for reacting with aliphatic acids. Since several of these occur in cells perhaps this is the mechanism of its action. Similarly, 2-hydroxydiphenyl is said to react with lactates and pyruvates and 4-hydroxydiphenyl is said to react with lactates.

Interference with Energy Systems

Since benzidine reacts with phosphates, one is tempted to suggest that it and perhaps some of its relatives may interfere with the phosphorylation enzymes that are involved in sugar metabolism.

Of course, energy is developed in the spore by a series of oxidation reactions. One finds himself tempted to speculate on the position that fungicides occupy in the oxidation-reduction system of the fungus cell. Little, that is concrete, has yet come from such speculations.

It seems reasonably clear that the more reduced the sulfur, the more fungitoxic are the diphenyl compounds with sulfur bridges. If so, we deduce that the reduced sulfur must compete for oxygen with the spore and thus interfere with its respiration. This deduction is confirmed somewhat by the fact that carboxyl, carbonyl, and nitro groups seem to quench toxicity that seems due to reduced sulfur or to $-NH_2$. This needs studying with a respirometer.

One of the characteristics of diphenyl is that the two rings tend to rotate about the point of juncture. Dion and Lord[6] pointed out that this could be stabilized with a second bridge. In general, they found that if the second bridge contained nitrogen or oxygen, toxicity is only slightly reduced. If the second bridge contains sulfur, their data show a striking reduction in potency. In our studies p-hydroxydiphenyl was toxic, but 3-hydroxyphenothiazine was not toxic.

SUMMARY

1. Diphenyl seems to offer an interesting architectural nucleus for making fungitoxic compounds.

2. The technique of testing was to apply the test compound to spores in the absence of extraneous matter like agar or nutrients. The test organisms were Stemphylium sarcinaeforme and Sclerotinia fructicola.

3. Toxicity was measured as percentage of non-germinated spores. When dose is plotted against spore inhibition on a logarithmic-probability grid, a linear curve is usually generated. Such a curve has two characters that are useful - position and slope. Position is expressed as ED 50 - the effective dose for 50 percent response. This is a measure of potency. Slope is a measure of mechanism. If two compounds have different slopes, we assume that they are acting by different mechanisms.

4. Unsubstituted rings show very limited fungitoxicity.

5. If the two rings are separated by bridges, toxicity is occasionally enhanced. A -CO-O- or an -S- bridge may lend toxicity to Sclerotinia not to Stemphylium.

6. If -OH is added to one of the rings, to both, or to a bridge, toxicity is added. The slope of the dosage-response curve of -OH compounds is usually flat.

7. As the number of -OH groups in the molecule increases the toxicity falls.

8. Likewise, if $-NH_2$ is added to one of the rings, to both, or to the bridge, toxicity is added. The slope of the dosage-response curve of $-NH_2$ compounds is usually steep.

9. An increase in the number of $-NH_2$ groups in the molecule does not increase the toxicity and they do not change the slope of the curve which presumably means that they do not change the mode of biological action.

10. Slope data suggest that -OH and $-NH_2$ substitutions inhibit spores by different mechanisms.

11. Fungitoxicity of amino diphenyl compounds seems to depend upon the presence of a primary amine. If the primary amine is converted to a secondary or tertiary amine by replacing the hydrogens, toxicity disappears.

12. Moreover, a primary amine attached to benzene is not toxic. It must be attached to diphenyl. In other words, toxicity rests on a molecule as big as diphenyl plus a primary amine. The data available suggest that the primary amine is the toxic grouping; the rings serve as vehicles to carry it into the spore.

13. In the diphenyl compounds, as in many others, the addition of sulfur adds toxicity to Sclerotinia not to Stemphylium. The former, of course, is sulfur-sensitive. The latter is not.

14. Halogenation of non-toxic diphenyl compounds did not make them toxic. Halogenation of toxic phenolic types of compounds in general reduced their toxicity to spores. Hatfield[11] reported that chlorination and phenyl phenols increased toxicity to growing mycelia. We had no halogenated amino derivatives for study.

15. Out of six comparisons made 4-substitution imparted more fungitoxicity than 2-substitution in five cases. On the other hand both substitutions gave the same slope, which suggests that they inhibited the fungus by similar mechanisms.

16. Compounds with double bonds in the bridge or in substitutions seemed to be more fungitoxic than others.

17. There is a curious case of synergism between diphenyl and acenaphthene. Neither inhibits spores alone. A mixture inhibits the spores very well indeed. Nothing is known about the mechanism.

18. Some possible modes of action on the fungus are discussed. Some of the compounds may react with cell constituents, as for example, diphenylhydrazine with glucose. Diphenyl benzidine may react with essential metals. Most of the active compounds contain replaceable hydrogen. They lose their toxicity if they lose their replaceable hydrogen.

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DISCUSSION

DR. LOUIS F. FIESER (Harvard University, Cambridge, Massachusetts): I want to compliment Dr. Horsfall on a very interesting paper and, particularly, on the application of exact methods for analysis of the bio-assay results. Use of dose-response curves and attention to both the LD50 and the slope of the curve is, I think, of distinct advantage.

It would seem to me that a further advance in correlating the data might be to use not the toxicity on the weight basis, but to compare molar toxicities. That might iron out some of the results which appear a little irregular on a purely empirical basis; that is, comparing a bromo compound with a chloro compound. On a weight basis, the chloro compound may seem a good bit more active, but on a molar basis they come out to be about the same.

As an organic chemist, I want to say I was a little bit unhappy about the discussion of structure with relationship to biological activity. The compounds discussed are all regarded as diphenyls; as a matter of fact, that is the term used in the title of the paper. Yet, to an organic chemist, diphenyl is a compound with two rings joined together; if the rings are separated by CH_2, you have diphenylmethane, which is something quite different. Separation of the rings by the group NH gives diphenylamine. Diphenylmethane and diphenylamine are not diphenyl compounds. To compare the effect of substituents in one series, where the two rings are joined together, with that in another series, where they are separate is, I think, taking a little bit of liberty with organic chemistry.

However, I must say that organic chemists or anybody else who has tried to work out relationships between structure and biological activity have not progressed very far, so that this is merely a personal comment on the paper; and I say that, if Dr. Horsfall can disregard some of the principles which appeal to a chemist, and still make some sense out of the correlation, I wish to congratulate him.

DR. HORSFALL: Mr. Chairman, that is an excellent comment, with respect to comparing diphenyl with diphenylmethane.

I realize that diphenylamine is not diphenylmethane, and that diphenylmethane is not diphenyl. I realize that a chemist would not compare directly an amino substitution on diphenylmethane with an amino substitution on diphenyl.

Back in the nervous part of my speech, before I got going, I neglected to say that we made an extensive study of the effect of bridges on toxicity. In general the bridges had little influence on the toxicity. Hence we tried to compare substituents irrespective of the bridge concerned.

DR. RICH: These were all converted on a molar basis, weren't they?

DR. HORSFALL: Oh, yes; in the manuscript which will be printed, the data are all expressed on a molar basis.

DR. McKEEN CATTELL (Cornell University Medical College, New York, New York): I fully agree with what Dr. Horsfall has said regarding the differences in slope of the dosage-response curve as indicating a qualitatively different action. There is, however, a consideration which, it seems to me, needs emphasis. We are concerned about the fundamental action of these compounds in the final solution. It is the goal of the pharmacologist to determine what reaction these compounds influence.

The differences in slope may well be related to subsidiary factors, such as the retention of the compound in the body, the speed with which it is broken down and its absorption; factors could conceivably throw us off the track in our reasoning in relation to the actual mechanisms of action. I am wondering whether, in these closely related diphenyl compounds when there was an ammonium or a hydroxyl substitution, if there might not have been an influence on the stability of the molecule, or a difference in its absorption or penetration.

DR. HORSFALL: I dare say you have a point there, Dr. Cattell. We have made an extended study of what slope means in these compounds (not these compounds exclusively, but compounds in general). We plant pathologists are apt to spray surfaces to protect them against disease. We have the problem of what we call coverage; it is analogous to the greased pig problem, whether it is better to put on concentrated mixtures thinly spread, or weak mixtures with a lot of water. In other words, if you are permitted in the country fair to have two thousand pounds of human flesh to catch the pig, is it better to have ten 200-pounders or twenty 100-pounders? You are more apt to catch him with twenty 100-pounders, but you are more apt to hold him when you catch him, with ten 200-pounders.

That makes a difference in slope in the response curve. The ten 200-pounders come out with a flatter slope than the twenty 100-pounders.

That is a case of distribution, you see; in your case, it is excretion from the body. It is admitted that slope does differ with other factors than mere mechanism of action but, if the slopes are different, it shows us things about the compounds which we would not have known if we had not had slope; therefore, it makes it a worth-while point for advance of further research.

EFFECT OF STRUCTURAL CHANGES IN PLANT INSECTICIDES AND RELATED SYNTHETIC COMPOUNDS ON THEIR TOXICITY TO INSECTS

by

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The spectacular results obtained in recent years in the development of new synthetic-organic insecticides and the wide publicity accorded them have tended to obscure the value of the insecticides derived from plants. Valuable as the chlorinated hydrocarbon and organic phosphorus-containing insecticides are, they have not completely displaced the naturally occurring organic plant products. Pyrethrum, the rotenone-containing plants derris and cube, and nicotine from tobacco, which are the best known plant insecticides, are still used in sizable quantitites. A few others, such as ryania, quassia, sabadilla, and hellebore, find utilization for specific purposes and in limited localities. Others, such as Heliopsis, Spilanthes, and pellitory are still in the experimental stages of investigation.

Although the insecticides derived from plants do not have the long residual action of some of the synthetic organic insecticides, they possess other characteristics that merit their use in controlling agricultural and household insects. In contrast to the chlorinated hydrocarbon insecticides rotenone and pyrethrum present no health hazard from spray residues. Nicotine, although a toxic alkaloid, is very soluble in water and hence, when employed against aphids, mealy bugs, and thrips, is readily removed and therefore presents little or no spray-residue problem.

Pyrethrum can be used safely in food establishments, households and dairy barns without concern for toxic residues. Moreover, its rapid paralytic, or knockdown, effect is especially important in the control of disease-carrying insects.

Derris and cube are still the only effective insecticides for controlling cattle grubs, a pest that annually causes losses of one hundred million dollars to our livestock industry.

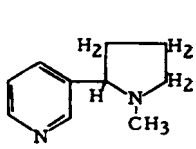
Plants have been used as insecticides for several hundred years, but chemical investigations of their active principles are relatively recent. Nicotine was first isolated from tobacco in 1828,[68] but sixty-five years elapsed before its structural formula was established.[67] Rotenone, the principal insecticidal constituent of derris and cube, has been known since 1892,[18] but it was not until 1932 that its structural formula was determined.[58,59] Structural formulas for the pyrethrins, the insecticidal constituents of pyrethrum, were first proposed in 1924,[80] but not until 1946 were the detailed structures of these complicated esters established with certainty.[60]

Nicotine, rotenone, and the pyrethrins all have complicated structural formulas, and a comparison of them reveals no common grouping of atoms to which insecticidal action might be ascribed. Numerous derivatives of each of these compounds and closely related products have been prepared and tested for insecticidal properties, but only a few are equal to the natural products.

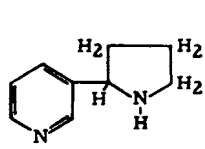
TOBACCO

Nicotine and Related Alkaloids

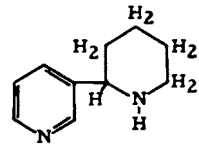
The earlier efforts to correlate the chemical structure of plant insecticides with insect toxicity were devoted chiefly to derivatives and analogs of nicotine, partly because its less complicated structure, compared to the pyrethrins and rotenone, facilitated the syntheses of related compounds. Moreover, the availability of two closely related alkaloids, nornicotine[61] and anabasine,[64] made further comparisons possible. Nicotine (I) is 1-methyl-2-(3-pyridyl)-pyrrolidine, nornicotine (II) is 2-(3-pyridyl)pyrrolidine, and anabasine (III) is 2,3'-pyridyl piperidine.



I



II



III

All three occur naturally in levorotatory form.

It is natural to inquire whether insect toxicity is due to the molecule as a whole or to some special grouping of its component parts. The point of attachment of the two rings, optical activity, and other questions present themselves. When tested against Aphis rumicis L. natural levo-nicotine and levo-anabasine were much more toxic than the dextro forms; on the other hand, the natural levo-nornicotine was only slightly more toxic to this insect than the dextro isomer.

Both dl-nicotine (I) and dl-nornicotine (II) were much more toxic to Aphis rumicis than the corresponding alpha forms, 1-methyl-2-(2-pyridyl)pyrrolidine and 2-(2-pyridyl)pyrrolidine. Tests were also made of dipyridyls,[73,76] dipiperidyls,[77,79] pyridyl piperidines,[78,79] and pyridyl pyrrolidines,[9,72] as well as of simpler derivatives of pyridine[52] and pyrrolidine.[53] With the exception of neonicotine (III),[78] all were distinctly inferior to the three natural alkaloids. Neonicotine, obtained as one of the reaction products of pyridine and sodium, is the optically inactive form of anabasine.

Table I summarizes some of the entomological results obtained with nicotine and the more important related compounds. The data were taken from a table compiled by Metcalf.[63] The toxicity values were obtained from articles by several different authors and therefore represent order of magnitude of relative toxicity rather than precise comparisons.

TABLE 1

RELATIVE TOXICITY OF NICOTINE
AND ITS DERIVATIVES AND ANALOGS TO APHIS RUMICIS

Compound	Median lethal concentration
Levo-nicotine	1
Dextro-nicotine	5
dl-Nicotine	2
dl-α-Nicotine	31
Levo-nornicotine	0.5
Dextro-nornicotine	0.7
dl-Nornicotine	1

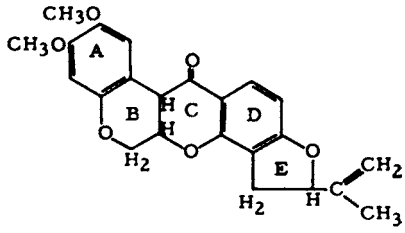
TABLE 1 (Cont.)

Compound	Median lethal concentration
dl-α-Nornicotine	31
Levo-anabasine	0.1
dl-Anabasine (neonicotine)	5
2,3'-Dipyridyl	100
3,2'-Pyridyl-piperidine	50
2,3'-Dipiperidyl	100
2-p-Tolyl pyrrolidine	50
Pyrrolidine	20
Pyridine	125
Piperidine	25

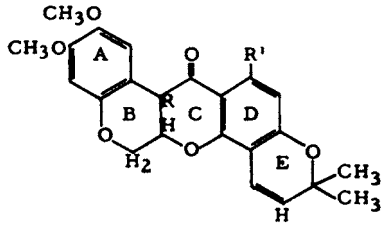
DERRIS AND CUBE

Rotenone and Rotenoids

These compounds are the principal insecticidal constituents of a number of leguminous fish-poison plants, of which derris and cube are the most important commercially. In the roots of these two plants the rotenone content usually constitutes one-third to one-half of the total ether extractives, the remainder being rotenoids. Rotenoids is the name given to a group of compounds closely related structurally to rotenone. Rotenone[58] has the structural formula (IV), and the principal rotenoids - deguelin,[5] tephrosin,[6,39] and toxicarol[7] - the formula (V).



IV



V

R = H, R' = H Deguelin
R = OH, R' = H Tephrosin
R = H, R' = OH Toxicarol

In rotenone ring E is a substituted dihydrofuran ring, whereas in the three rotenoids it is a substituted pyran. The chemistry of the rotenoids has been summarized by Haller et al.[33]

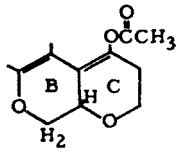
Numerous derivatives of rotenone were obtained in the course of determining its structure. Some of them were tested by Gersdorff,[21,23,24,25] using goldfish as the test animal in a procedure developed by him.[20] The results obtained are summarized in Table 2.

TABLE 2

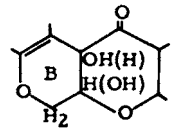
RELATIVE TOXICITY OF ROTENONE
AND SOME OF ITS DERIVATIVES TO GOLDFISH

Compound	Approximate Relative Toxicity
Rotenone	1
Isorotenone	0.23
Dihydrorotenone	1.4
Acetyl rotenone	0.55
Acetyl dihydrorotenone	0.5
Rotenolone	0.1
Acetyl rotenolone	0.11
Dihydrorotenolone	0.2
Acetyl dihydrorotenolone	0.13

Dihydrorotenone and the other dihydro derivatives differ from the parent compound in that the side chain of ring E (formula IV) has been saturated. In isorotenone the double bond of the side chain has been shifted into the five-membered ring. Acetyl rotenone is an enol acetate (VI), and rotenolone is a hydroxyrotenone, as shown in VII.



VI



VII

With the exception of dihydrorotenone, which was about 1.4 times as toxic to goldfish as rotenone, all the derivatives were much less toxic than the parent compound. However, dihydrorotenone is no more toxic to insects than rotenone and is less toxic to some. Gersdorff[25] points out that "each change in chemical constitution effects a characteristic change in toxicity independent of the effect of any other change."

Gersdorff[22] also compared three rotenoids with rotenone in toxicity to goldfish. In these tests all three rotenoids were optically inactive isomers and the rotenone was optically active. At the time optically active rotenoids were not available and rotenone has not yet been obtained in inactive form. The results are as follows:

TABLE 3

RELATIVE TOXICITY OF ROTENONE
AND SOME ROTENOIDS TO GOLDFISH

Compound	Approximate Relative Toxicity
Rotenone	1.0
Deguelin	0.56
Tephrosin	0.23
Toxicarol	0.65

Rotenone and the rotenoids contain asymmetric carbon atoms and optically active isomers of all except deguelin have been isolated in crystalline form from the plant extracts. Evidence has been presented[34] that deguelin occurs in the plants as a levo-rotatory isomer, but all attempts to obtain it in crystalline form have resulted in failure. The relative toxicity of rotenone and the optically active and inactive forms of three rotenoids against several insects are summarized in Table 4.

TABLE 4

RELATIVE TOXICITY OF ROTENONE
AND RELATED COMPOUNDS TO INSECTS

Compound	Approximate Relative Toxicity	Test Insect and Reference
Rotenone	100	
Dihydrorotenone (active)	70	House fly (Musca domestica L.) (82)
	>30	Silkworm (Bombyx mori L.) (75)

TABLE 4 (Cont.)

Compound	Approximate Relative Toxicity	Test Insect and Reference
Deguelin (inactive)	10	Bean aphid (Aphis rumicis L.) (12)
	10	House fly (82)
	30	Silkworm (75)
Deguelin concentrate (active)	50	House fly (82)
Dihydrodeguelin (active)	50	House fly (82)
Dihydrodeguelin (inactive)	13	House fly (82)
Tephrosin (inactive)	2	Bean aphid (12)
	10	Silkworm (75)
Toxicarol (active)	7	Bean aphid (12)
Toxicarol (inactive)	<1	Bean aphid (12)
Dehydrorotenone	0	Imported cabbage worm (Ascia rapae L.) (13)

With mosquito larvae as the test insects, Fink and Haller[17] determined the relative toxicity of rotenone, deguelin, and the optically active and inactive forms of isorotenone and dihydrodeguelin. The results are illustrated graphically in Figure 1. As in the tests by Gersdorff[25] with goldfish, the optically active compounds were more toxic than the corresponding optically inactive compounds at all concentrations tested.

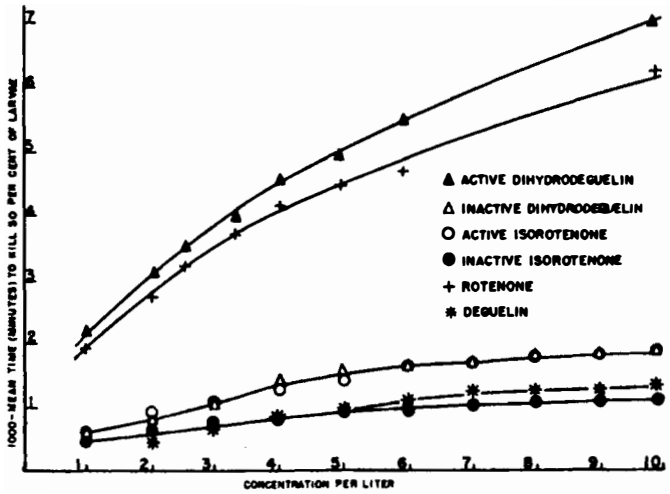


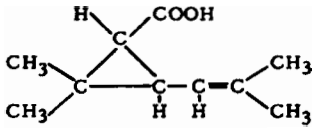
Fig. 1 Relative toxicity of rotenone, deguelin, and the optically active and inactive forms of isorotenone and dihydrodeguelin

It will be noted that in this series of compounds, as with nicotine and related compounds, optical activity plays an important role in insecticidal action.

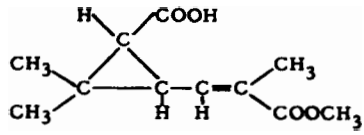
PYRETHRUM

Pyrethrins and Cinerins

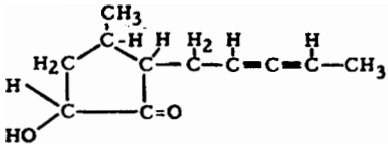
The insecticidal action of pyrethrum is due to a mixture of several closely related esters. From their classical pioneering studies Staudinger and Ruzicka[80] concluded that two compounds, designated pyrethrin I and pyrethrin II, were the principal active ingredients. However, studies by LaForge and Barthel[54,55,56] have demonstrated the presence of two other insecticidal esters, which have been designated cinerin I and cinerin II. Both the pyrethrins and the cinerins are high-boiling oils, and all four compounds are closely related in chemical structure. Pyrethrin I and cinerin I are esters of chrysanthemum monocarboxylic acid (VIII) and pyrethrin II and cinerin II are esters of chrysanthemum dicarboxylic acid monomethyl ester (IX).



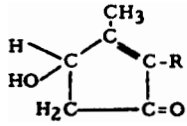
VIII



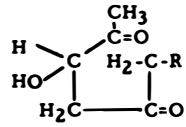
IX



X



XI



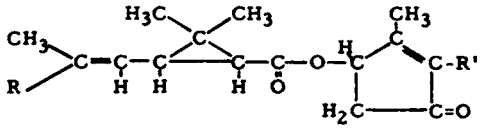
XII

XIa R = $-C(H_2)-C(H)=C(H)-C(H)=CH_2$

XIb R = $-C(H_2)-C(H)=C(H)-CH_3$

The alcoholic component of both pyrethrins is known as pyrethrolone and that of the cinerins as cinerolone. The structural formula originally proposed for pyrethrolone is given in X. More recent studies by LaForge and his associates in this country and Gillam and West in England have necessitated a revision and modification of formula X to that given in XIa. The studies leading to this structure, which is in accord with all the known facts, are excellently summarized by Harper.[40]

The structural formula of cinerolone is given in XIb. Pyrethrolone and cinerolone differ only in that the side chain of the former is a doubly unsaturated five-carbon straight chain while that of cinerolone is a singly unsaturated four-carbon chain. The accepted structural formulas for the pyrethrins and the cinerins are shown in XIII.



XIII

Pyrethrin I R = CH_3
Pyrethrin II R = CH_3OOC
} R' = $-CH_2-CH=CH-CH=CH_2$

Cinerin I R = CH_3
Cinerin III R = CH_3OOC
} R' = $-CH_2-CH=CH-CH_3$

Comparative toxicities of the pyrethrins and the cinerins to house flies[26] are shown in Table 5.

Each compound was obtained by esterification of the appropriate naturally occurring dextrorotatory keto alcohol with the proper naturally occurring dextro acid. The esters obtained from the optically inactive keto alcohols and the optically active acids did not differ in toxicity from the compounds with both components optically active[26]. Also with cinerin I, in which the side chain of the keto alcohol has been shown to have a cis configuration,[10] no difference in toxicity was noted when the side chain had a trans configuration.[27]

TABLE 5

RELATIVE TOXICITY OF PYRETHRINS AND CINERINS TO HOUSE FLIES

Compound	Relative Toxicity
Pyrethrin I	1
Pyrethrin II	0.25
Cinerin I	0.69
Cinerin II	0.17

Both the keto alcohols and the acids of the pyrethrins are unsaturated. Staudinger and Ruzicka[31] have shown that most of the insecticidal action of the pyrethrins is destroyed by minor changes in their molecules. Thus, the catalytic hydrogenation of a crude pyrethrin concentrate yielded a product that was insecticidally inactive. In these experiments the cockroach, probably Blattella germanica L., was used as the test insect. Similar results were obtained by Haller and Sullivan[38] in experiments with house flies. They found that on mild catalytic hydrogenation of of pyrethrum concentrates, containing 63.5 per cent of pyrethrin I, 6.5 per cent of pyrethrin II, and the remainder inert constituents, not only the toxicity but also the knockdown was for the greater part destroyed. A pyrethrum concentrate containing 83.7 per cent of pyrethrin II and 5.4 per cent of pyrethrin I with the remainder inert, although having good knockdown properties, caused so little mortality before hydrogenation that a marked reduction would not normally be

expected. More recent data[26] on the effect of hydrogenation of the double bonds in pyrethrin I and cinerin I are given in Table 6.

TABLE 6

RELATIVE TOXICITY OF SOME HYDROGENATED DERIVATIVES OF THE PYRETHRINS AND CINERINS TO HOUSE FLIES

Compound	Relative Toxicity
Pyrethrin I	1
Tetrahydropyrethrin I	0.06
Isodihydropyrethrin I	0.5
Cinerin I	0.69
Dihydrocinerin I	0.08
Isodihydrocinerin I	0.35

Tetrahydropyrethrin I was prepared by catalytic hydrogenation of natural pyrethrin I. All attempts to prepare a dihydro compound have thus far failed.[35] Hydrogenation of the side chain not only reduces toxicity considerably but also destroys the characteristic knockdown effect of the pyrethrins. The dihydrocinerin I was obtained from optically inactive keto alcohol and the optically active dextro acid.[57] In the preparation of the isodihydro compounds both the keto alcohols and dihydro acid were optically active.

SYNTHETIC PYRETHRIN-LIKE ESTERS

The destruction of most of the toxicity of both the pyrethrins and the cinerins on saturation of the side chains in the keto alcohol portions of their molecules made it of interest to determine the effect of other changes in the side chains, such as changes in length, branching of the chain, position of the unsaturated bond, and stereochemical effects. Methods for the preparation of such compounds have recently been described. The excellent method developed by Schechter et al.[74] for the synthesis of cyclopentenolones makes possible the preparation of a wide variety of keto alcohols analagous to cinerolone. The keto alcohols on esterification with chrysanthemum monocarboxylic acid, for which an improved method of preparation has recently been published[4], yield the suitably substituted esters.

Six substituted cyclopentenolones were prepared. They are represented by the general formula XI, in which R indicates different side chains, which are shown in Table 7. These keto alcohols were esterified with the natural d-trans-chrysanthemum monocarboxylic acid. Two of the keto alcohols were also esterified with the synthetic racemic (dl) cis and trans forms of this acid. Thus there were ten compounds closely related to cinerin I available for comparison of

toxicity.[27] Their comparative toxicities to house flies are given in Table 7.

TABLE 7

RELATIVE TOXICITY OF ANALOGS OF CINERIN I TO HOUSE FLIES

No.	Cyclopentenolone Side Chain	Chrysanthemum Monocarboxylic Acid	Relative Toxicity
1	$-CH_2CH=CHCH=CH_2$ (Pyrethrolone)	d-trans- (natural)	1
2	$-CH_2CH=CHCH_3$ (Cinerolone)	"	0.7
3	$-CH_2CH=CHCH_3$	"	0.7
4	$-CH_2CH=CH_2$	"	3.3
5	$-CH_2C(CH_3)=CH_2$	"	1.7
6	$-CH_2CH_2CH=CH_2$	"	0.3
7	$-CH_2CH=C(CH_3)_2$	"	0.1
8	$-CH_2CH_2CH_2CH_3$	"	0.08
9	$-CH_2CH=CHCH_3$	dl-cis-	0.2
10	$-CH_2CH=CHCH_3$	dl-trans-	0.2
11	$-CH_2CH=CH_2$	dl-cis-	.9
12	$-CH_2CH=CH_2$	dl-trans-	.9

Compounds 2 and 3, which differ only in that the side chain in 2 has a cis configuration while that of 3 is trans, are about equally toxic. Compound 4 when esterified with the natural dextro acid is more than three times as toxic as compounds 11 and 12 obtained from the optically inactive acids.

Esterification of the synthetic trans cinerolone with the optically inactive acids (compounds 9 and 10) also caused reductions in mortality to about one-third of the values obtained when natural acid is used. A mixture of compounds 11 and 12 will shortly be commercially available.

By the synthesis developed by Schechter et al.[74] it is also possible to prepare a cyclopentenolone in which the methyl group in the three position has been replaced with other groups. The substitution of the phenyl group for the methyl group in the allyl ester decreased the toxicity to one-fifteenth that of the allyl homolog of cinerin I.[27] Gersdorff has also tested on house flies an uncyclized compound (formula XII where R = allyl) esterified with natural chrysanthemum acid. It was less than one-seventieth as toxic as the corresponding cyclized compound (compound 4 in Table 7).

In addition to the esters mentioned above, the synthesis developed by Schechter et al.[74] opened up a whole new field for further investigation. Many other modifications can be made in the keto-alcohol portion of the molecule and such alcohols can then be esterified not only with chrysanthemum monocarboxylic acid, but with other substituted cyclopropanecarboxylic acids or with entirely different types of acids.

Studies in which the acid component of pyrethrin I and cinerin I, chrysanthemum monocarboxylic acid (VIII), has been esterified with alcohols other than pyrethrolone or cinerolone have also been reported. Staudinger and Ruzicka[81] tested the reaction products of chrysanthemum acid chloride with various alcohols and phenols against cockroaches. In none of the experiments was the reaction product isolated, the assumption being that the reaction had proceeded with the formation of the desired ester. None of the products tested was sufficiently toxic to warrant further studies. Harvill[44] esterified the acid with an homologous series of eighteen aliphatic alcohols ranging from ethyl to cetyl and tested them against aphids and cockroaches. The lauryl, myristyl, and cetyl esters showed some toxicity to the aphids, but none of the esters had the pyrethrin-like action on cockroaches. With the improved method of obtaining chrysanthemum acid[4] now available, other esters are worthy of further detailed studies.

SYNERGISTS

A recent development in the utilization of pyrethrum which is of considerable practical importance is the discovery that certain synthetic organic compounds can replace part of the pyrethrins without reducing either their paralytic or their killing power. In many instances an increased effectiveness is obtained with the combination. Compounds of this type have been loosely referred to as synergists, activators, and intensifiers, and the belief has developed that all these compounds are effective only in the presence of pyrethrins, that all are equally useful with pyrethrum, and that all are nontoxic to warm-blooded animals. These assumptions are not valid. As with other synthetic organic compounds, the toxicity of these products to a wide variety of insect pests, as well as their effect on warm-blooded animals, must be ascertained before their general value in economic entomology can be stated.

One of the first compounds shown to increase the effectiveness of the pyrethrins is N-isobutylundecyleneamide (XIV).[88]

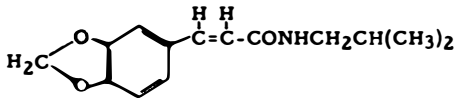
$$CH_2{=}CH(CH_2)_8CONHCH_2CH(CH_3)_2$$

XIV

When tested in kerosene solution as a spray against house flies, the amide alone was of little value. A spray containing 40 mg. of pyrethrins and 420 mg. of the amide per 100 ml. was superior to the standard spray solution containing 100 mg. of pyrethrins per 100 ml.

An investigation of the effect of N-isobutylundecyleneamide and the pyrethrins on house flies has been made by Hartzell and Scudder.[42] In histopathological studies they found that each chemical shows rather distinct and characteristic effects upon the central nervous system and associated tissues of the adult house fly. Pyrethrum has a widespread clumping effect on the chromatin of the cell nuclei, while the amide seems to cause a chromatolysis or dissolution of the chromatin. A combination of the two products shows a histological picture that is a summation of the effects of each.

Another unsaturated isobutylamide that increases the effect of the pyrethrins is fagaramide (N-isobutyl-3,4-methylenedioxycinnamamide) (XV), occurring in the root bark of Zanthoxylum senegalense DC. and Z. macrophyllum Oliver.[31,86]



XV

Fagaramide, at 2 mg. per ml. plus 0.5 mg. pyrethrins per ml., killed as many house flies as a solution containing twice as much pyrethrins but without the fagaramide.[29]

Several insecticidal N-isobutylamides of aliphatic unsaturated acids have been isolated from plant materials - namely, spilanthol (N-isobutyl-4,6-decadienamide) (XVI) from the flower heads of Spilanthes oleraceae Jacquin[1,19] and S. acmella Murr.,[30] pellitorine (N-isobutyl-2,6-decadienamide) (XVII) from the roots of Anacyclus pyrethrum DC.,[32,48] N-isobutyl-2,6,8-decatrienamide (XVIII) from the roots of Heliopsis longipes (A. Gray) Blake,[51] herculin (N-isobutyl-2,8-dodecadienamide) (XIX) from the bark of Zanthoxylum clavaherculis L.,[47] and scabrin [(N-isobutyl-2,4,8,10,14-octadecapentaenamide) (XXa) or (N-isobutyl-2,4,8,12,14-octadecapentaenamide) (XXb)].[49]

$CH_3(CH_2)_2CH{=}CHCH{=}CH(CH_2)_2COR$ XVI

$CH_3(CH_2)_2CH{=}CH(CH_2)_2CH{=}CHCOR$ XVII

$CH_3CH{=}CHCH{=}CH(CH_2)_2CH{=}CHCOR$ XVIII

$CH_3(CH_2)_2CH{=}CH(CH_2)_4CH{=}CHCOR$ XIX

$CH_3(CH_2)_2CH{=}CH(CH_2)_2CH{=}CHCH{=}CH(CH_2)_2CH{=}CHCH{=}CHCOR$ XXa

$CH_3(CH_2)_2CH{=}CHCH{=}CH(CH_2)_2CH{=}CH(CH_2)_2CH{=}CHCH{=}CHCOR$ XXb

$CH_3(CH_2)_4CH{=}CHCH{=}CHCOR$ XXI

$CH_3(CH_2)_8CH{=}CHCOR$ XXII

where R is $NHCH_2CH(CH_3)_2$

Compound XVI has been reported[65,66] to be an effective mosquito larvicide. Compound XVII, which differs from XVI only in the position of one of the double bonds, shows toxicity to house flies somewhat greater than one-half that of pyrethrins.[48] Compound XVIII, having one double bond more than XVI and XVII, is somewhat more toxic than pyrethrins to house flies.[51] Compound XIX has approximately the same order of toxicity to houseflies as the pyrethrins.[47] Scabrin (XXa or b) is appreciably more toxic than the pyrethrins to house flies.[49] Compounds XVI to XX all show the rapid paralytic or knockdown action characteristic of the pyrethrins. However, saturation of the double bonds gives compounds that are completely devoid of insecticidal activity, although N-isobutyllauramide (hydrogenated XIX) shows some synergism with pyrethrins.

Geometrical configuration about the double bonds plays a large part in the toxicity of the unsaturated isobutylamides. For example, the cis-trans and trans-trans isomers of compound XVII were synthesized and found to be nontoxic to house flies.[11,50]

N-Isobutyl-2,4-decadienamide (XXI), differing from XVI and XVII only in the position of one double bond, and N-isobutyl-2-dodecenamide (XXII), differing from XIX only by the lack of a double bond in position 8, were both synthesized. They showed rapid paralytic or knockdown

action but gave very low mortality of house flies.

Another group of compounds that have been found to be especially useful with pyrethrum are certain piperonyl or methylene dioxyphenyl derivatives. Interest in this class of compounds resulted in a large measure from an observation of Eagleson[16] in the course of testing pyrethrum solutions in admixture with a number of vegetable and fish oils against house flies. Eagleson found that sesame oil, to the exclusion of all other oils, markedly increased the effectiveness of the pyrethrins. That the increase in toxicity was due to a synergistic or activator effect, and not to the addition of another insecticide, was shown by the failure of sesame oil alone to kill flies. By means of a hypnotic-dose technique developed by him, Eagleson[15] followed the recovery of house flies that had been sprayed with a pyrethrum insecticide to which various percentages of sesame oil had been added. The results, given in time-torpor curves, are shown in Figure 2.

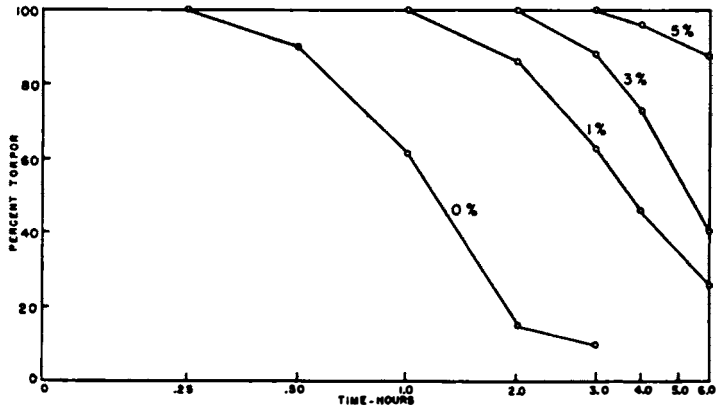


Fig. 2 Recovery of house flies sprayed with pyrethrum insecticide to which various percentages of sesame oil had been added

Each point on the curves represents the mean torpor, at successive intervals after spraying, for five replications on approximately fifty-five flies. With the pyrethrum solutions to which no sesame oil had been added, 92 per cent of the flies recovered. When 5 per cent of sesame oil was added to the pyrethrum solution only 12 per cent of the flies sprayed recovered. By his method Eagleson[14] had previously shown that few flies that are still affected six hours after the spraying ever recover.

At the suggestion of Eagleson a chemical study of sesame oil was undertaken by Haller and co-workers.[37] Sesame oil was separated into four fractions by means of high-vacuum distillation. Each fraction was separately added to pyrethrum extract in refined kerosene and tested against house flies by the turntable method.[3] The results are shown in Table 8. From the combined first and second fractions a crystalline solid was isolated and shown to be sesamin. When it was added to pyrethrins in a refined kerosene-acetone mixture, the effectiveness against flies was greatly increased (Table 9). (Ten per cent of acetone in the kerosene is necessary to dissolve the sesamin.) It was not possible to obtain from the noncrystalline active fraction any crystalline compound other than sesamin.

TABLE 8

EFFECTIVENESS AGAINST HOUSE FLIES OF VARIOUS FRACTIONS OF SESAME OIL, WITH AND WITHOUT PYRETHRUM, IN REFINED KEROSENE

(2 tests with about 150 flies each; concn. of pyrethrins 1 mg., and of sesame oil and its fractions 10 mg./cc.)

Material	Knockdown in 10 min., %	Mortality in 48 hr., %
Sesame oil	0	2
Pyrethrins	99	21
Pyrethrins + sesame oil	100	57
Pyrethrins + fraction I	100	100
Pyrethrins + fraction II	100	91
Pyrethrins + fraction III	100	21
Pyrethrins + fraction IV	100	29

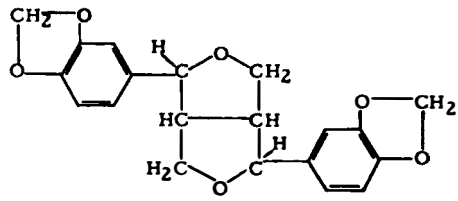
TABLE 9

EFFECTIVENESS AGAINST HOUSE FLIES OF FRACTIONS OF SESAME OIL, WITH AND WITHOUT PYRETHRUM, IN REFINED KEROSENE PLUS 10% OF ACETONE

(2 tests with 150 flies each; concn. of pyrethrins 1 mg., and of sesame oil fractions 2.5 mg./cc.)

Material	Knockdown in 10 Min., %	Mortality in 24 hr., %
Pyrethrins	100	20
Sesamin (crystalline fraction)	0	5
Pyrethrins + sesamin (crystalline fraction)	100	85
Pyrethrins + noncrystalline residue	100	89

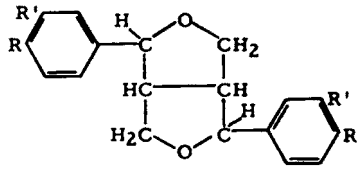
Sesamin has the following structural formula:



It is a bicyclodihydrofuran substituted symmetrically with two methylenedioxyphenyl groups.[2,8] It has four asymmetric carbon atoms, and natural sesamin is dextrorotatory.

Hartzell and Wexler[43] have studied the histological effects of pyrethrum and sesamin on the central nervous system and muscles of the house fly. Flies rendered moribund by pyrethrum showed clear spaces in the brain tissue and dissolution of the fiber tracts, and sesamin caused vacuolation around the large nerve cells. When sesamin and pyrethrum were combined, not only were the nerve fibers destroyed but the larger nerve cells were highly vacuolated until almost complete lysis of the tissue resulted. An excellent summary of the physiological studies of pyrethrum and the various activators or synergists that have been studied is given by Metcalf.[63]

A number of plant materials have been shown to contain compounds related to sesamin.[36] Among these compounds are asarinin, found in various oriental plants and in the bark of American prickly-ash; pinoresinol, a constituent of the exudate of spruce and related species; and eudesamin, a constituent of kino gum from eucalyptus. Their relation to sesamin is shown in the formula XXIII.



XXIII

R,R' = O_2CH_2 (methylenedioxy) for sesamin and asarinin
R = OH and R' = OCH_3 for pinoresinol
R,R' = OCH_3 for eudesamin

Asarinin is levorotatory and is the optical antipode of isosesamin, which is obtained on treatment of sesamin with alcoholic hydrochloric acid. As some of these compounds were available, they were tested for their synergistic effect with the pyrethrins. The diacetyl derivative of pinoresinol was also included. Isosesamin and asarinin were as effective as sesamin, but pinoresinol dimethylether, the optical antipode of eudesamin, was without appreciable synergistic action, as were pinoresinol itself and its diacetyl derivative. The results are summarized in Table 10.

TABLE 10

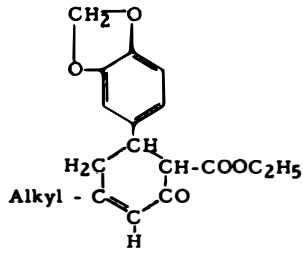
EFFECT OF SESAMIN AND RELATED COMPOUNDS ON THE INSECTICIDAL ACTION OF PYRETHRINS AGAINST HOUSE FLIES

(3 tests using about 150 flies per test; solvent, refined kerosene plus 10% of acetone where needed to increase solubility)

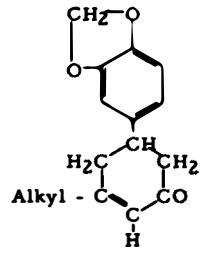
No.	Material	Concentration %	Average Mortality After 24 hrs. %
	Sesamin and its isomers:		
1	Sesamin	0.2	4
2	Sesamin + pyrethrins	0.2 + 0.05	84
3	Isosesamin	0.2	5
4	Isosesamin + pyrethrins	0.2 + 0.05	87
5	Asarinin	0.2	14
6	Asarinin + pyrethrins	0.2 + 0.05	88
7	Pyrethrins (control)	0.05	25
	Pinoresinol and derivatives:		
8	Pinoresinol	0.18	1
9	Pinoresinol + pyrethrins	0.18 + 0.05	12
10	Dimethyl pinoresinol	0.2	1
11	Dimethyl pinoresinol + pyrethrins	0.2 + 0.05	17
12	Diacetyl pinoresinol	0.03	2
13	Diacetyl pinoresinol + pyrethrins	0.03 + 0.05	11
14	Pyrethrins (control)	0.05	19

From these findings it was concluded that the methylene dioxyphenyl grouping was an important one for compounds useful as synergists with pyrethrum. They have led to the preparation and testing of a large number of different types of compounds containing the grouping. Many of the compounds that have been prepared are derived from safrole because it is the most ready source of the methylene dioxyphenyl group. Workers at the Boyce Thompson Institute have been testing products obtained by the addition of various aldehydes,[70] mercaptans,[69] maleic acid esters,[83] and various other compounds,[85] to the double bond of safrole and isosafrole. Derivatives of piperonal,[71,83] piperonylic acid,[28] piperine and related compounds[41,45,84] have also been tested. From the research in this field three products - piperonyl cyclonene, piperonyl butoxide, and n-propyl isome - have been developed that have found commercial acceptance. Technical piperonyl cyclonene obtained on condensation of alkyl-3,4-methylene dioxystyryl ketones with ethyl acetoacetate[46,87] contains 80 per cent of a mixture of compounds XXIV and

XXV in addition to higher molecular condensation products.

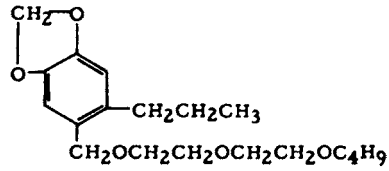


XXIV



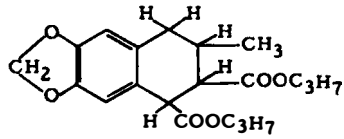
XXV

Piperonyl butoxide[62, 87] is obtained as a technical product containing 80 per cent of XXVI.



XXVI

n-Propyl isome,[83] prepared by a Diels-Alder type condensation of isosafrole with n-propyl maleate, has the structure shown in XXVII.



XXVII

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DISCUSSION

DR. D. F. MARSH (University of West Virginia, Morgantown, West Virginia): I think since the time of Cushny's work with the cocaines and epinephrine, pharmacologists in general have been interested in the very intriguing problem of the effect which optical isomerism may have on the biological activity. I do not mean by this that I think the biological activity is the direct result of rotation of plane polarized light; it is just that these are two interesting properties which are possessed by the same structures in the molecule.

Perhaps I misunderstood the data on the slide but, if the data is as I understood it, it has some very interesting aspects in relation to this problem of synergism or potentiation or antagonism, or whatever you call it.

In regard to the alkyl analogs of cinerin, the activity of the d-material was given as 3.3, I believe.

If you take one part of the d-isomer and one part of the talc or other inactive material, presumably the activity of that mixture would be about 1.7. According to the data given on the slide, the dl-mixture was given as having an activity of only 0.9.

I presume that this might be due to the technique of testing. Perhaps Dr. Haller will elaborate on this although it is quite a wide margin.

I wonder if it could mean that the l-isomer is acting not only in a noncontributory manner, but is acting, shall we say, to protect the house fly, as it were, against the d-isomer. If that is the case, does it mean, then, that we have here a sort of p-aminobenzoic acid - sulfanilamide mutual antagonism between the two?

There was another example of this which happened to strike my eye earlier. Perhaps, again, I misunderstood the data but levo anabasine was listed as having a toxicity of 10.1. If it does, then the dl-material was given as requiring five times as much to be toxic.

In this case, the d-anabasine is removing the activity of about twenty-four times as much levo anabasine, if this is the true situation. The dl-mixture ought to be about 0.2 instead of being 'way up there at 5.

I do not know; maybe there is a very simple explanation of this but, in any event, the figures are very intriguing from a pharmacological view.

DR. HALLER: Thank you, Dr. Marsh.

Perhaps I did not make it clear enough that the figures I gave are to indicate order of magnitude, and they were not all done in the same laboratory at the same time.

If we take anabasine, for example, and I could cite some of the other figures, too, you will find the same thing - where you may actually appear to have antagonism. I do not believe that is so. You will recall that I said we need more work in this field, because too much of it has been done in different laboratories. My slides are a compilation of results from different places, trying to bring out the effect of optical activity and the effect of configuration rather than the precise data. I sometimes feel it might be well worth while, from the standpoint of studies in this field, to start from scratch, making these compounds in pure form, and repeating some of that early work. That is no reflection upon the early work at all. We are going ahead with the studies of the pyrethrin-like esters and I must say that we have a man in our organization, Gersdorff, who has really done some very careful work in making the comparisons of which you speak, not only in this field, with house flies, but the earlier work which I showed, with rotenone on goldfish. Some of his papers are well worth studying, for any of you who may be specifically interested.

I notice Mr. Schechter coming up. I might say to you that he has done some very fine synthetic work on the pyrethrins and cinerins working with Dr. LaForge.

MR. SCHECHTER: Thank you, Dr. Haller. I have some later information on this particular point which might shed a little more light on the question Dr. Marsh raised.

We have recently tested the allyl-substituted cyclopentenolone esterified with the l-isomer of chrysanthemum monocarboxylic acid and find that the ester has only about one-fiftieth of the activity of the ester with the d-form of the acid: that is, it is relatively nontoxic.

In this series of tests, the ester with the dl-acid (which is the ester in commercial use) has a relative toxicity of about 1.5 or 1.6, as I remember it. This fits in closely with the fact that the ester with the l-acid is relatively nontoxic since the ester with the d-acid has the highest toxicity and the combination appears to be approximately half as toxic as the ester with the d-acid.

In the case of the individual dl-cis and dl-trans acids, we had only very small amounts available which were sent to us by Dr. Harper of Kings' College, England. We had so little that we could not check on their purity so that the figures obtained with esters of these acids might perhaps be due to the lower degree of purity of the acids employed. This should be checked up more closely and we may do so in the future.

DR. ALFRED BURGER (University of Virginia, University, Virginia): I was particularly intrigued with Dr. Haller's remarks, that the pyrethrins cause such rapid paralysis. These compounds are alpha-beta unsaturated ketones, and they may have some relation to those antibiotics which, as Geiger and Conn have pointed out, add mercaptan groups. Even though the reaction of mercapto groups with enzyme systems does not explain all the antibiotic properties of a compound, one could reason that if the pyrethrins would be capable of adding to sulfhydryl groups, the deactivation of many enzyme systems in the insect might be due to this reaction. A thought coming to mind in seeing the formula of sesamin is the possible overlapping with that of biotin.

Biotin has a thiophane ring condensed with a ureyl group and carries a butyric acid side chain. The formula of sesamin with its condensed hydrofuran rings conveys a similar sterical picture at least of one portion of the molecule.

I would like to ask Dr. Haller whether anybody has ever tested biotin antagonists, of which there are quite a few, as insecticides.

DR. HALLER: So far as I know they have not been tested.

DR. BURGER: The second point concerns the toxicity of nicotine and anabasine. In these compounds, Dr. Haller has pointed out the insecticidal toxicity drops every time you remove the pyrrolidine or the piperidine portion from the beta to the alpha position.

In 1942, Nieman and Hays of the California Institute of Technology, pointed out that, if you put onto a pyridine ring an ethylamine side chain in the alpha position, you get histaminic activity. They related it at that time with possible chelation of the two nitrogen atoms in the ionic form of the compound.

If you write the molecule of histamine or anabasine in the ionic form, you cannot possibly construct such a chelated formula but, if you construct the alpha isomer of nicotine and anabasine then, particularly in the case of anabasine where there is no N-methyl group to introduce any steric factors, you have the possibility of constructing a chelated ring.

I want to ask Dr. Haller whether anybody has ever considered that nicotine toxicity to insects might be in any way connected with histamine-type activity.

DR. HALLER: Here, again, the question, so far as I can answer it, is no. I do not know of anything which has been done along those lines, Dr. Burger. Probably that is one of the biggest weaknesses we have in our work, in that, we are working with exceedingly minute organisms, and it is not easy to bring out some of these points you have mentioned. I do not know specifically that anyone has given that particular angle or problem any thought, nor that tests of that sort have been made. A big field is there, as I said.

THE EFFECT OF DRUGS ON PHYSIOLOGICALLY ACTIVE THIOL SYSTEMS

by

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There are at the present time many compounds of widely different chemical structures that are being introduced into living animals to produce rather specific biological effects, therapeutic or otherwise, and that on more or less solid grounds are believed to produce their effects by inhibiting or in some other way regulating the activity of thiol systems. It is the purpose of this survey to bring together in one discussion work that has been going on in several fields as widely separated as mercurial diuretics and fungal antibiotics. A presentation will first be made of the kinds of chemical reaction known to occur between the type compounds used and simple thiols. This will be followed by an examination of the basis in each field for ascribing the drug action to thiols and, as far as possible, an assessment of the present validity of this basis. It seems particularly appropriate to attempt this in a symposium on Chemical-Biological Correlation since there are present so many with detailed experience in particular special fields that we need to touch and so there is every opportunity for a lively discussion on this subject which has many controversial aspects.

Since it is easier to classify the drugs used than the effects aimed at, or we might say the biological targets, we will arrange the subsequent discussion along the lines of the chemical types of the compounds. In Table 1 are listed the type compounds to which discussion will be limited together with the specific targets or the general effects they are aimed at. There is little at the start that can be said about any common features of the targets, except that they are all living, metabolizing cells or systems of cells. But chemically there is a common feature to all of the drugs. They all could react with simple thiol compounds. This fact, that a large number of drugs of varied chemical structure could react with thiol compounds has led to a tendency to group these compounds together on the basis of this common property. They are sometimes called thiol reagents. Bacq[5] has gone so far as to call them thioloprives. While there may be something to say for the introduction of such a new term, the word will not be used in this review because it is felt that it could have a tendency to narrow the possibilities of the mechanisms of drug action considered. To cite a single specific instance, in his analysis of the action of vesicants, Bacq has not left room for the important possibilities suggested by the subsequent work of Pirie[101] in which is presented evidence for the action of mustard gas on collagen, a protein containing no thiols at all.

TABLE 1

Drug	Target of Effect
Hg^{++} ; $S_4O_6^{=}$	Proximal tubule of kidney
As^{+++}; As^{+++++}	Trypanosomes, spirochetes
Sb^{+++} ; Sb^{+++++}	Schistosomes, Leishmanias, Filarias
Arsenyl halides as lewisite	Pyruvate oxidase, vesication
Alkyl halides as; iodoacetate, sulfur mustards, nitrogen mustards	Vesication, lacrimation, Lundsgaard effect
Unsaturated carbonyls	Antibiotic
Disulfide; sulfoxide-sulfide	Antibiotic
Quinones	Antibiotic, spermicide
Naphthoquinones	Antiplasmodic, antibiotics, clot-mechanism
Alloxan	β-Cells of the pancreas

Before beginning the chemical-biological correlation it will be best to survey carefully the chemical base. This seems particularly desirable since there are several kinds of reaction known to occur easily with simple thiol-disulfide systems, such as cysteine-cystine, and which could conceivably also occur in more complex biological systems but which have never been described or considered in such material. The compounds listed in Table 1 can be separated into two groups depending on their action with thiols; some act as oxidants, some act to form a covalent bond with an electron pair of the sulfur atom of the thiol. The oxidant action is most simply represented as a complete removal of an electron pair from a thiol molecule or ion as:

$$R\text{-}S\text{-}H \rightleftharpoons \overset{\oplus}{H} + R\text{-}\overset{\ominus}{S}\text{-} \rightleftharpoons R\text{-}\overset{\oplus}{S} + 2\epsilon \qquad (I)$$

This is immediately followed by condensation of the positive ion formed with a remaining negative mercaptide ion because of the tendency of the latter with its electron-rich sulfur nucleus to share an electron pair with an electron-poor atomic nucleus:

$$R\text{-}\overset{\ominus}{S}\text{-} + R\text{-}\overset{\oplus}{S} \longrightarrow R\text{-}S\text{-}S\text{-}R \qquad (II)$$

In this way a disulfide is formed as a secondary product of the primary oxidation reaction. A mechanism such as this might explain the great difficulty which has always been encountered in trying to measure oxidation-reduction potentials of thiol-disulfide systems. It has long been known that the disulfide exerts no effect on these potentials. If the reaction of equation (II) is only very slightly reversible then the disulfide might exert little effect on reaction (I). It will also be shown later that when disulfides do dissociate they seem to do so by a mechanism different from the reverse of (II) and representable by the first of equations (XII). The oxidants of Table 1 that can bring about the reaction (I) are pentavalent arsenic and pentavalent antimony. Chloropicrin has also been reported sometimes to oxidize thiols smoothly and completely to disulfides though the reduction product simultaneously formed from the chloropicrin is not known.[33,67] Hellstrom[63] has made some interesting observations on oxidations brought about by certain halogenated acids according to one or the other of the following:

$$\begin{aligned} &2\ HOOC\text{-}CH_2\text{-}SH + X\text{-}CH_2\text{-}COOH \longrightarrow (HOOC\text{-}CH_2\text{-}S)_2 + CH_3\text{-}COOH + HX \\ &2\ HOOC\text{-}CH_2\text{-}SH + 2\ X\text{-}CH_2\text{-}COOH \longrightarrow (HOOC\text{-}CH_2S)_2 + 2\ CH_3\text{-}COOH + X_2 \end{aligned} \qquad (III)$$

These reactions were studied mainly in acid solutions and there is no data on how far into the neutral range they may persist. This particular type of oxidation has not been reported for any biochemical system, nor has it even been found to occur in the physiological pH range.

Among the oxidants that can convert thiols to disulfides there is one group of special interest and that is compounds that themselves have the disulfide structure. These include not only the organic disulfides but also the inorganic salt, sodium tetrathionate, which has a disulfide structure. The special interest attaching to organic disulfides as oxidants is that they are not certainly known to oxidize any organic compounds other than thiols and therefore their reduction in an organic system can be considered a test for the presence of thiols. This oxidation presumably occurs in two separate and successive steps as follows:

$$R\text{-}S\text{-}S\text{-}R + R'\text{-}S\text{-}H \rightleftharpoons R\text{-}S\text{-}H + R\text{-}S\text{-}S\text{-}R' + R'\text{-}S\text{-}H \rightleftharpoons R\text{-}S\text{-}H + R'\text{-}S\text{-}S\text{-}R' \qquad (IV)$$

Mixed disulfides, the product of the intermediate step, are more commonly known among aromatic disulfides and even these are made by reactions other than reaction (IV). There is another aspect of this disulfide-thiol interaction of interest and that is the apparently parallel reaction between thiols and the sulfoxide-sulfides, or as they are sometimes called, alkyl thiosulfinates.[128]

$$R\text{-}S\text{-}SO\text{-}R + R'\text{-}S\text{-}H \longrightarrow R\text{-}S\text{-}S\text{-}R' + R\text{-}SOH \qquad (V)$$

Finally there is an interesting analog of reaction (IV). This is the reduction of disulfides by bisulfite according to the reaction investigated by Clarke;[28]

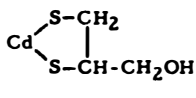
$$R\text{-}S\text{-}S\text{-}R + Na\text{-}SO_2\text{-}ONa \longrightarrow R\text{-}S\text{-}S\,O_2\text{-}ONa + R\text{-}S\text{-}Na \qquad (VI)$$

But this reaction differs from that of equation (IV) in that even with excess sulfite the analog of the second half of reaction (IV) does not occur.

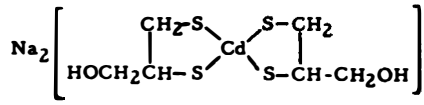
All the other compounds listed in Table 1 undergo the second type of reaction with thiols, that is the formation of a covalent link between the electron-rich sulfur nucleus of the thiol and an electron-poor nucleus of the compound. This is another aspect of the same tendency already illustrated in equation (II), of a mercaptan to share one of its free electron pairs with an electrophilic atomic nucleus. Among such atomic nuclei are the heavy metal ions which form very slightly dissociated mercaptides according to the type equation;

$$2\ R\text{-}S\text{-}H + Hg^{++} \rightleftharpoons R\text{-}S\text{-}Hg\text{-}S\text{-}R + 2\ H^{+} \qquad (VII)$$

Trivalent arsenic and antimony, bivalent cadmium and lead, monovalent gold and silver readily form analogous mercaptides. Though all reactions of the type illustrated for mercury in equation (VII) are reversible, the equilibria are usually displaced far to the right. Such a simple mercaptide is not the only type of compound formed between thiol and metal. Some metals can, under favorable conditions, form chelate complex compounds when there is near to the thiol group another group also capable of complex formation. The simplest example of this is illustrated by compounds of the type formed from BAL and cadmium chloride. These were studies by Gilman and coworkers[50] and are of several forms. Mixing cadmium chloride and BAL in neutral or acid solution precipitates the white compound (VIII). At alkaline reaction and with excess thiol a water soluble cadmium complex is formed to which should probably be assigned the structure (IX). Conversion of (VIII) to (IX) occurs at pH 7 to 8. Analogous compounds are

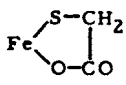


(VIII)

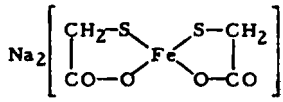


(IX)

formed with mercury. Such metal complex compounds are quite stable and are similar to the simple mercaptides of mercury, arsenic and antimony in being only very slightly dissociated. Complexes of the type (IX) have the coordinating metal in the tetracovalent condition, they are stable anionic complexes. That all four sulfur atoms are coordinated with the metal nucleus is shown by the acid strength of the complex which is such that at pH 8 the anion exists as a disodium salt whereas mercaptans are not completely ionized until a pH of about 11 is reached. The low dissociation of such complex ions into metal ion and BAL in neutral to alkaline solution is shown by the fact that on addition of sulfide ion no heavy metal sulfides precipitate. The conversion of the water insoluble complex (VIII) to the water soluble complex (IX) is completely analogous to the changes occurring among complexes formed between the divalent metal ions, iron or cobalt, and the thiol acids, cysteine or thioglycollic acid.[114,115] These complexes must be made in the complete absence of air. At pH 4 to 6 they precipitate as crystalline complexes of the form (X) while in the presence of excess thiol acid and at pH 8 to 10, water soluble complexes of the form (XI) are produced which can be separated in crystalline condition by addition of alcohol. Compounds of the type (XI) are the primary stage involved in the iron



(X)



(XI)

catalyzed oxidation of thiol acids by oxygen. Such complexes may also be involved in the oxidation of protein thiols even when oxidation is carried out by oxidants other than air. This is indicated by the work of Anson[2] who showed that oxidation of denatured egg albumin thiols by ferricyanide is inhibited by cyanide. Both the complex types represented by (VIII) or (X) and (IX) or (XI) are of interest in considerations of the action of drugs in tissues since they could be formed in the physiological pH range.

Besides these reactions between metal ions and thiols there is another kind that can occur between some metal ions and disulfides. This has been described in a series of studies by several workers for the metals silver and mercury.[105,127] It is an oxidation-reduction reaction in which part of the disulfide molecule is reduced to thiol and part is oxidized to a higher level. At first this higher level was thought to be a sulfonic acid but the most recent work of Lavine[74] has supplied reasonable evidence that the higher level is a sulfinic acid, at least for the case of the disulfide cystine. The reaction written in two ways to account for these two opinions would be;

$$2\ R\text{-}S\text{-}S\text{-}R + 2\ H_2O \longrightarrow 3\ R\text{-}S\text{-}H + R\text{-}S\text{-}O_2H$$
$$3\ R\text{-}S\text{-}S\text{-}R + 3\ H_2O \longrightarrow 5\ R\text{-}S\text{-}H + R\text{-}S\text{-}O_3H \qquad \text{(XII)}$$

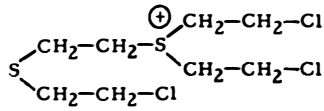
Though this reaction has been studied mainly in acid solution this is primarily for reasons of experimental convenience. There is no reason to think it can not also occur at neutral pH range. The function of the metal ion appears to be to upset equilibria and to drive the reaction toward the right by removal of the thiol as insoluble mercaptide. Whether the formation of a soluble complex with low dissociation would be equally effective in driving the reaction forward has never been studied. There is still uncertainty regarding many phases of the reaction; it is conceivable that with different disulfides or in different pH ranges or with different metals, one or the other of reactions (XII) would predominate. Though the main driving mechanism of the reaction seems to be the formation of the slightly dissociated mercaptide, insolubility of the metal sulfinate or sulfonate would also help to determine its direction. There has been no study of possible application of this reaction to such disulfide containing proteins as keratin or insulin. Furthermore, if the driving mechanism of the reaction is the formation of slightly dissociated metal mercaptides then it could be expected that trivalent arsenic and antimony compounds might bring about similar changes. Such possibilities have never been described but would be rather easy to test.

One of the commonest types of nuclear condensation of a thiol compound is with alkyl halides. This reaction is probably preceded or at least assisted by ionization of the halogen;

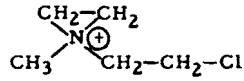
$$R\text{-}CH_2\text{-}Cl \longrightarrow Cl^- + R\text{-}CH_2^+ + R\text{-}S^- \longrightarrow R\text{-}CH_2\text{-}S\text{-}R \qquad \text{(XIII)}$$

This is really similar to the nuclear condensations already described as for example in (II) where condensation of thiol occurs with an ozidized, electron-poor sulfur nucleus or in (VII) where it occurs with a metal ion. In (XIII) the condensation occurs with an electron-poor carbon atom and yields a thio ether. This is the type of condensation that takes place when alkyl halides such as chloracetate, mustard gas or bromacetophenone react with thiols. The speed of such reactions varies enormously with both the alkyl halide and the thiol undergoing reaction. The possibility of alternative oxidative reactions that can occur under certain conditions with particular halogen acids has already been mentioned in equation (III). The extent and limitations of such oxidative reactions has not yet been explored. The reactions of mustards with thiols was first investigated by Helfrich and Reid[61] and appeared to be simply of the type (XIII). More recently, as a result of intensive study of both sulfur and nitrogen mustards, the reaction has been found to be far

more complex. Here it can be only briefly noted that while both sulfur and nitrogen mustards appear to react in aqueous media by intermediate formation of sulfonium and ammonium compounds respectively there is a fundamental difference. The sulfur mustards[132] appear to react by way of intermediate forms such as (XIV) while the nitrogen mustards[57] are believed to

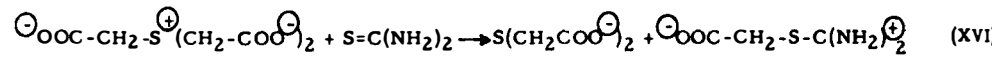


(XIV)



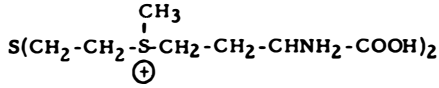
(XV)

react by way of intermediates of the type (XV). In each case the conclusions are based on studies of the kinetics of the reactions and on the isolation of intermediate compounds or their derivatives. The unexpectedly high reactivity of both sulfur and nitrogen mustards is presumably related to the formation of the positive onium ions which would be expected to have a greater tendency to act as alkylating agents because of a greater tendency to yield positively charged alkyl ions than the simple alkyl halides of reaction (XIII). The alkylating action of a sulfonium compound can easily be shown to occur by warming thetine and thiourea in neutral aqueous solution. The following reaction occurs;[124]



(XVI)

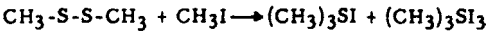
These considerations suggest another kind of reaction than can take place between sulfur compounds and active alkyl halides and that has not often been considered in biochemical studies. This is the interaction of the alkyl halides with thioethers, either such as are formed as a result of reaction (XIII) or such naturally occurring ones as methionine. This reaction, which leads to the production of sulfonium salts, has been studied for the particular case of the action of mustard gas on methionine, yielding the disulfonium ion;[131]



(XVII)

Such sulfonium salts could themselves again act as alkylating agents, transferring one of the combined alkyl groups to some other nucleus capable of attaching an alkyl group as in (XVI).

As with the action of mercury salts, it has, in the past, been generally assumed that alkyl halides react only with thiols. More recently the possibility of reaction with thioethers has been considered. That alkyl halides could also react with disulfides has scarcely ever been considered. Yet it was long ago shown[133] that disulfides can also react with alkyl halides according to the equation;



(XVIII)

Though this reaction was carried out at higher temperatures it is altogether likely that with more reactive alkyl halides, such as the mustards, a similar reaction could take place under physiological conditions.

The formation of sulfides according to equation (XIII) has usually been considered to be practically irreversible but recently Peters and Wakelin[99] have described a procedure in which a thio ether in the presence of a mercury or silver salt at acid reaction undergoes a scission such that on subsequently making the solution alkaline a positive nitroprusside test can be shown. The reaction was studied in particular for the case represented in (XIX);

$$SO_2(CH_2\text{-}CH_2\text{-}S\text{-}CH_2CHNH_2COOH)_2 \longrightarrow HS\text{-}CH_2\text{-}CHNH_2COOH + HO\text{-}CH_2\text{-}CH_2\text{-}SO_2\text{-}CH_2CH_2\text{-}S\text{-}CH_2\text{-}CHNH_2\text{-}COOH \quad \text{(XIX)}$$

To what extent this reaction is related to the particular structure shown is not at present apparent. It seems likely that the presence of the thioether in a position β with respect to a sulfone might make it especially likely to undergo a hydrolytic reaction. Of the two thioether groups present only one is split. The reaction may be related to a hydrolytic process long ago studied by Otto in which disulfones are split in alkali;

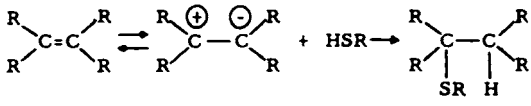
$$R\text{-}SO_2\text{-}CH_2\text{-}CH_2\text{-}SO_2\text{-}R \longrightarrow R\text{-}SOOH + R\text{-}SO_2\text{-}CH_2\text{-}CH_2\text{-}OH \quad \text{(XX)}$$

This splitting of a thioether with a silver or mercury ion according to (XIX) is also reminiscent of the similar splitting of disulfides by the same metal ions.

It must also always be kept in mind that these alkyl halides can alkylate atomic nuclei other than sulfur nuclei.[87,118] Frequently such alkylations at nitrogen or oxygen nuclei are slower, but just as the speed of sulfur alkylation can vary greatly as a result of nearby structural features, so it may be expected that alkylation of other nuclei will also be greatly influenced by neighboring structural elements.

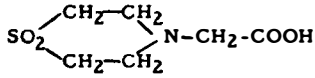
We know that protein structures and protein denaturation have great effects on alkylation speeds though we have as yet only vaguely formulated theories as to why this is so. Rosner[110] showed that iodoacetate continues to react with denatured egg albumin long after the reaction with thiol groups has been completed. Anson and Stanley[3] showed that tobacco mosaic virus could be almost completely inactivated by iodoacetamide under conditions such that few, if any, thiols react. Two amino acid residues that would appear most likely to be involved in such a reaction through their side chains are lysine and arginine.

The next type of reaction we will consider is that in which the thiol attaches itself to an ethylenic structure. Such a structure has, among its resonance forms, one in which there is an electron-poor carbon nucleus and this will be the one to which the thiol sulfur will attach itself;



(XXI)

This reaction, as most of the preceding, involves attachment of the electron-rich sulfur nucleus to an electron-poor nucleus. The process has often been studied from the point of view of Markownikoff's rule.[30] These reactions are generally not readily reversible. The reaction of some mustards with thiols has been thought to go through a stage in which the alkylhalide of the mustard is first converted to an alkene.[8] Divinyl sulfone in some respects reacts to give the same products as mustard sulfone. For instance with glycine both sulfones yield the same sulfonazane;[18]

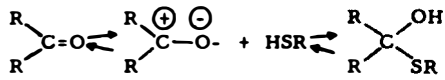


(XXII)

The reaction of both sulfones with thiosulfate, an inorganic thiol, led to the conclusion that mustard sulfone is first converted to divinyl sulfone and so both sulfones finally yield the same thiosulfonate;[130]

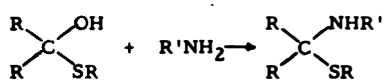
$$SO_2(CH_2\text{-}CH_2Cl)_2 \longrightarrow SO_2(CH{=}CH_2)_2 \longrightarrow SO_2(CH_2CH_2\text{-}S\text{-}SO_2\text{-}ONa)_2 \qquad \text{(XXIII)}$$

Somewhat similar to the addition of thiols to alkenes is the addition of thiols to carbonyl groups;[116,117]



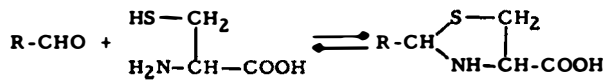
(XXIV)

Though reaction (XXIV) is quite analogous to reaction (XXI) yet there is an important difference in degree of stability of the products. Reaction (XXIV) is far more readily reversible, so much so that the products often give positive nitroprusside reactions and always react with iodine to regenerate the carbonyl compound and produce the disulfide of the thiol. The reaction with iodine is so rapid that titration to a sharp end-point can be performed just as with free thiol. Generally reaction (XXIV) occurs only with more reactive carbonyl compounds such as occur in aldehydes but reactive ketonic carbonyl groups such as that of pyruvic acid also form such addition compounds which can in most cases be called semi-mercaptals. Acetylation of the hydroxyl group generally stabilizes the products. Such acetylated semi-mercaptals no longer give a nitroprusside test, even at pH 10. They are only very slowly oxidized by iodine and cannot be titrated to a sharp end-point. An interesting property of the semi-mercaptals formed in reaction (XXIV) is the ease with which they react with amino compounds;[119]



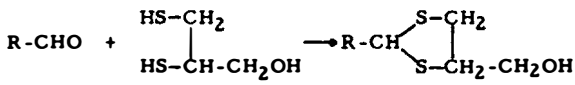
(XXV)

In the particular case of cysteine the thiol and amino groups are in the same molecule and appropriately spaced so thiazolidine compounds result;[108,117]



(XXVI)

Such thiazolidines also dissociate sufficiently so that on treatment with iodine they yield cystine and the original aldehyde. On treatment in neutral aqueous solution with iodoacetate they yield S-carboxymethyl cysteine. Acetylation of these thiazolidines, which occurs at the nitrogen atom, stabilizes them so that the products are only very slowly oxidized by iodine to yield cystine. Such thiazolidines can also be formed from the half hidden aldehydes of reducing sugars,[120] but curiously pyruvic acid seems to react with cysteine to produce only a semi-mercaptal and not a thiazolidine derivative. Lieberman[80] has found that 3-ketosteroids also yield such thiazolidines with cysteine.[88] Application of these reactions to protein thiols have been made in the study of keratine. Related to the formation of semi-mercaptals and thiazolidines is the reaction in which aldehydes give cyclic mercaptals directly with BAL;[96]



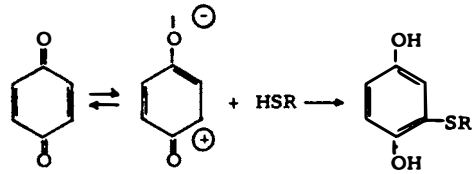
(XXVII)

Though there are no drugs in use which are thought to act as a result of reactions of the form of either (XXI) or (XXIV), these reactions have been discussed at length because of the fact that some antibiotics have structures combining both the ethylene linkage and the carbonyl group, thus uniting the possibilities of both reactions (XXI) and (XXIV) in one molecule. Addition of thiol to such a system of double bonds in conjugate position has been studied for several cases.[17,90] The condensation of thioglycollate and benzalacetophenone gives a β-keto-sulfide;

$$C_6H_5\text{-}CH{=}CH\text{-}CO\text{-}C_6H_5 \longrightarrow C_6H_5\text{-}\underset{\displaystyle S\text{-}CH_2\text{-}COOH}{\underset{|}{CH}}\text{-}CH_2\text{-}CO\text{-}C_6H_5 \qquad \text{(XXVIII)}$$

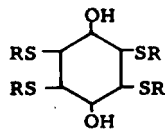
Such thioethers seem to dissociate reversibly far more readily than those of equation (XXI) but do so only at rather alkaline reaction. The thioether of (XXVIII) has a carbonyl group in the same position relative to the sulfur that in (XIX) is occupied by a sulfone group. This condition may be the cause of the more ready dissociation of these thioethers.

The quinone molecule is one that in the smallest possible compass combines just about all the possibilities for reaction with thiols so far discussed. Quinones are strong oxidants; they could be imagined to react with thiols by oxidizing them to disulfide; they have reactive ethylenic links and also reactive carbonyl groups and so might possibly react in accordance with equations (XXI) or (XXIV); they furthermore contain two separate and complete α,β unsaturated carbonyl groupings and so might be considered along with the antibiotics of this general structure. It is best, however, to consider quinone as a special system, among whose resonance isomers is one, with an electron-poor nucleus, which can attach to itself the electron-rich mercaptide ion;

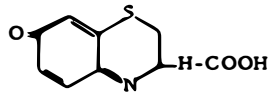


(XXIX)

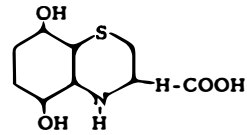
The resulting substituted hydroquinone has been isolated in several cases.[129] In the presence of remaining quinone this monosubstituted hydroquinone can itself become oxidized to a quinone and if there is more thiol present the whole cycle of reactions can recur leading to a disubstituted hydroquinone. In fact continued repitition of this whole sequence will yield, if the supply of thiol holds out, a tetra-alkylthio-substituted hydroquinone and such a compound with the structure (XXX) has been shown to be formed in good yield in the reaction between quinone and thioglycollate.[121] The reaction of quinone with cysteine is far more complex but by working in quite dilute solution Kuhn and Beinert[72] have isolated a crystalline product to which they ascribe the structure (XXXI). No proof is offered for this structure and another possibility that would need to be



(XXX)



(XXXI)



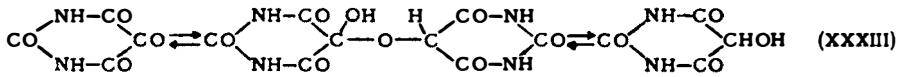
(XXXII)

considered is the structure (XXXII). In view of the high tendency of quinones to undergo nuclear condensation not only with thiols but also with amines this structure might seem even more probable. However formula (XXXII) contains two hydrogen and one oxygen atom more than formula (XXXI) and the analytical data fits (XXXI) better. Substitution reactions similar to (XXIX) have also been found to occur between thiols and naphthoquinones. However if the quinone used has all available positions adjacent to the quinone carbonyl groups substituted then these condensation reactions cannot occur. Such completely substituted quinones or naphthoquinones could however still react as oxidants of thiols.

Equation (XXIX) shows the condensation of an electron-rich thiol with a quinone. This type of condensation can also occur with great ease with other electron-rich nuclei, such as the nitrogen of amines. Amino-substituted hydroquinones have long been known to be formed by the action of many kinds of amines, even tertiary amines such as pyridine, with unsubstituted quinones.[10] It is probably such condensations that account for the tanning properties of quinone

which could condense with the free basic side chains of the lysine or the arginine residues of collagen.

There remains to be discussed the complex chemistry of alloxan, but here we are on far less certain ground, for the chemistry of alloxan is even less well understood than that of quinones. Alloxan, like quinone, could be imagined to react with thiols either because of its properties as an oxidant or because it has an active carbonyl group. On reduction alloxan goes through two separate steps, absorbing a single electron at each step. The first step, which can be brought about by hydrogen sulfide at room temperature, yields the bimolecular form alloxantin and the second step, which can be brought about by the same reductant at elevated temperature, yields dialuric acid:



(XXXIII)

On the other hand alloxan has one carbonyl group located between two adjacent carbonyl groups, a structure which also occurs in mesoxalic acid and ninhydrin. This structure is known to have a particularly marked tendency to add molecules such as water and alcohols. In conformity with this, alloxan is known to form stable addition compounds with water, alcohol and phenols. Addition of thiols has not been studied. In the cold alloxan reacts with sodium bisulfite to produce a normal bisulfite-carbonyl addition compound. At higher temperatures this breaks up to give the reduction product alloxantin. It is not possible at present to make a decision between the two simple and obvious reactions that could occur between alloxan and thiols; reduction of alloxan to dialuric acid and oxidation of thiol to disulfide or condensation of the two compounds to a semi-mercaptol. Other reactions, in addition to these two, could only be imagined.

This finishes the survey of the known chemical reactions that could occur between the drugs of Table 1 and thiols, or their close relatives, the disulfides. It might be well to summarize this briefly as regards specificity. The oxidants, as oxidants, appear to be fairly specific as a group; disulfide, sulfide-sulfoxide, tetrathionate, pentavalent arsenic and antimony do not oxidize any other known tissue components nearly as rapidly as they oxidize thiols. The work of Purr[107] suggests an exception; the possibility that a mixture of ferrous iron and ascorbate may be oxidized by disulfide. Also rather specific for thiols are the trivalent arsenic and antimony compounds, including lewisite. These react very easily with thiols and with little else except hydroxy acids and phenolic compounds. Mercury raises the interesting possibility of being able to react with either thiols or disulfides. It is also known to form complexes with amino compounds. With the alkyl halides the variety of possible reactions suddenly becomes quite broad and includes any electrophilic nucleus. The unsaturated carbonyl compounds and the quinones also can react with a great variety of different molecular types besides thiols and do not even show any great preference for thiols. Of alloxan we can only say at present that it reacts with many kinds of molecules.

With this background of the possible ways thiols or disulfides could react with the compounds listed in Table 1 we are in a better position to examine some of the drugs used and to judge the value of explanations offered for their mode of action. There remains however one important feature to keep in mind. All the preceding disucussion has concerned the interaction of simple thiol compounds with the reagents listed in Table 1. In the biological problems we must face however, the thiols are for the most part protein thiols and the reactivity or accessibility to reagents of protein thiols is known to be greatly dependent on the state of the protein. For those proteins which can exist in either a native or a denatured state, the thiols may be partly or entirely unreactive to many thiol reagents when the protein is in the native state.

It seems appropriate to begin the examination of drugs that could conceivably have some action on a physiological thiol system with those that contain mercury. The older name of the thiol group is mercaptan, a word apparently synthesized from a Latin phrase to record the general property of the thiol compounds to capture or combine with mercury. But though this property of mercury to combine tightly with thiols has been known since the dawn of chemistry, it is only within the last few years that thought and experiment have been directed to an examination of the possibility of explaining the action of the extensively used mercurial diuretics in terms of the combination of mercury and physiologically active thiols. Handley and LaForge[59] first

showed that the diurectic action of both organic mercurials, mercuhydrin and mersalyl, and of inorganic mercury compounds is prevented or abolished by BAL, thioglycollate and glutathione. Cysteine in contrast had little effect in preventing mercury diuresis. In dogs the administration of BAL caused an abrupt drop in urine output to pre-mercurial level. Sussman and Schack[137] came to substantially the same results using mercuhydrin and further pointed out that BAL inhibition of diuresis might be due to formation of a stable mercury-thiol complex and not to an antidiuretic action of BAL inself. Farah and Maresh[35] thought these effects of reversing mercur ial diuresis with thiols suggested the possibility that the mechanism of action of mercurial diuretics is an inactivation of thiol enzymes responsible directly or indirectly for the resorption of salt and water from the lumen of the kidney tubules. In addition to their diuretic effect, mercurials were known to have another important though far less marked physiological effect. The use of mercurials as diuretics was known to be associated with occasional toxic effects on the heart and in animals such toxic effects had been measured. DeGraff and Lehman[32] had shown a protective effect against this cardiotoxicity of mercurials following administration of sodium thiosulfate. Lehman[78] related the cardiotoxicity of mercurials to the degree of instability of the complexes, that is the ease with which such complexes dissociated, presumably to yield mercuric ion. Long and Farah[81,82] had shown that the monothiols, cysteine, glutathione and thioglycollate, markedly reduce the cardiac toxicity of mersalyl. They further found that BAL could suppress completely both the cardiotoxic and the diuretic actions of mersalyl. In the reversal of either the cardiotoxic action of this mercurial or its acute toxicity by thiols, BAL was, in each case, effective in about a fifth of the equivalent amounts of cysteine or glutathione. Farah and Maresh[35] found it possible to produce an important differential effect. By the use of the monothiols, cysteine and glutathione, they could abolish the cardiac toxicity of mersalyl without reducing its diuretic action. All of this work was consistent with the idea that both the diuretic effect and the cardiotoxic action of mercurials was due to inactivation or disturbance, by the drug, of physiologically essential thiol systems. But the finding of Farah and Maresh, besides its obvious clinical importance, raised new ideas concerning the mechanism of mercurial action. If monothiols could compete successfully with the cardiac system that was poisoned by the mercury but could not compete with the renal system, it would appear as if these two physiological systems exerted quite different degrees of mercury binding. The renal system seemed to bind the mercury in a manner far less readily reversible. This could be interpreted as an application, at a biological level, of phenomena already described at a biochemical level; that is that inhibition of some enzymes by arsenic or mercury could be reversed by monothiols or dithiols while inhibition of other enzymes by mercury could not be reversed by monothiols but only by dithiols. This reasoning had been the starting point for the extensive research programs centering about BAL. Stocken and Thompson[135] have discussed many of the phenomena leading to the conclusions that the arsenic acceptor of many living cells can form more stable compounds with arsenic than any simple thiols so far investigated. Barron and his co-workers[12] in a study of a great variety of enzymes, found that inhibition by lewisite or by other arsenicals could be more effectively reversed by BAL than by glutathione. If inhibition by mercury is due to combination with thiol enzymes it would seem quite natural to expect for mercury a similar more complete reversal of effect with BAL than with a monothiol, provided ring compounds can be formed. Yet the work of Thompson and Whittaker[140] does not bear this out for the case of pyruvate-oxidase inhibited by mercury or by antimony. In both cases inhibition is reversed by monothiols and by BAL, yet there is not the same strikingly greater effect by dithiols. Barron and Kalnitsky[11] studied the reversal of mercury inhibition of succinoxidase brought about by a variety of thiols and also found the contrast in efficiency of dithiol and monothiol less pronounced than in the case of reversal of lewisite inhibition. In fact for mercury inhibition they found 1, 3 dimercaptopropanol far more effective than BAL in reactivating succinoxidase. The effect of thiols in reversing inhibition of enzyme action by heavy metals is thought to be a reflection of the competition of thiol and enzyme for the metal. The existence of such a competitive effect by no means proves that the enzyme combines with the metal through thiols of its own. If the enzyme-metal compound dissociates rather readily, then monothiols as glutathione and cysteine suffice to take the metal away from the enzyme. In the case of more tightly bound enzyme-metal compounds monothiols are ineffective metal removers and only BAL type dithiols are effective. But both among dithiols and among monothiols there is a great variation in ability to reverse mercury or arsenic inhibition of enzyme activity. Thiols cannot be put in a linear sequence with regard to effectiveness in reversing metal inhibition, nor can it even be said that all dithiols are more effective inhibition reversers than all monothiols. With organic mercurials the possibility of formation of five membered rings, such as lewisite can form with BAL, may not even exist.

The work so far reported strongly suggests the possibility that mercurial diuretics bring about their action by inhibiting physiologically essential thiols of the kidney tubule but can not be regarded as proof. There is nowhere yet any direct proof that mercury reacts with any physiologically functional kidney thiols. All that is proved by reactivation experiments with monothiols and dithiols is that whatever it is that mercury does can be reversed more or less effectively by these agents. The possibility of mercury inhibition by complex formation with amide, amino or heterocyclic nitrogen compounds must still be recognized and would be just as consistent with all inhibition reversal experiments. The use of theophylline with mercurials is probably itself an expression of the combination of mercury with an amide. Yet this thiol theory of the action of mercurial diuretics is a good working theory because it will suggest the further experiments needed to establish or to disqualify itself. It can also be of value as a guide to identify the enzyme systems of the kidney tubule responsible for salt and water resorption and to lead to an understanding of this process.

This thiol theory of mercurial diuresis has gotten valuable support from another line of work, that on the nephrotoxic action of tetrathionate. The tetrathionate ion has already been described as a member of a class of oxidants, the disulfides, which are rather specific for thiols. That tetrathionate can also react with protein thiols has been shown by the work of Anson[2] on denatured egg albumin. In this protein all the thiols are exposed. On the other hand unrease contains both exposed and hidden thiols and only the latter condition its activity.[38,62] Since tetrathionate does not inactivate this enzyme Fischer and Goffart[39] conclude that tetrathionate reacts only with exposed thiols. Gilman and his co-workers showed that tetrathionate produces discrete lesions of cells of the proximal tubules of the kidney with nuclear degeneration within a half hour after injection. They also showed that in rabbit and dog, tetrathionate is rapidly reduced to thiosulfate. After confirming the ability of this inorganic disulfide to oxidize organic thiols such as BAL, cysteine and glutathione, they showed that cysteine or thiosorbitol protect rabbits against the nephrotoxic action of tetrathionate. In searching for a mechanism, the conclusion was reached that the effect of tetrathionate is extracellular. This was based on the fact that tetrathionate appears to be distributed in extracellular fluid. Goffart and Fischer[56] carried this idea further and concluded that the attack of tetrathionate is not on protein thiols of the kidney at all but on glutathione. This conclusion was based on an estimate of the thiols of kidney tissue. The amount of Prussian blue deposited in kidney tissue after treatment with ferricyanide followed by ferric salt was estimated and it was confirmed that kidney tissue is rich in thiols. After intravenous lethal doses of tetrathionate the kidney slices still show as strong a Prussian blue deposit as normal kidney. On the other hand estimation of the glutathione content of rabbit kidney showed a disappearance of 80 percent of the reduced glutathione after injection of a lethal dose of tetrathionate. They conclude, as did Gilman and his co-workers, that the tetrathionate acts on kidney cells by removing diffusible glutathione rather than by penetrating these cells itself. Philips et al.[100] studied the effect of tetrathionate on succinoxidase, a typical thiol enzyme, taking material from several sources. Tetrathionate is a powerful inhibitor of the succinic dehydrogenase component of succinic oxidase. On other grounds succinic dehydrogenase is known to be strongly inhibited by thiol reagents.[13] An assay was then made of the activity of the succinoxidase system of rabbit kidney cortex 60 minutes after an intravenous nephrotoxic dose of tetrathionate. This assay showed that no specific inactivation of succinic dehydrogenase in vivo had occurred as a result of tetrathionate injection. So it was concluded that inactivation of some other thiol enzymes of kidney cortex must be more directly concerned with the nephrotoxic action of tetrathionate. Clinical evidence[15] shows some diminution of tubular resorptive capacity after tetrathionate administration indicating a similarity of the action of this drug in man and laboratory animals.

There are now known several types of compounds that are nephrotoxic and that have in common a high degree of reactivity with respect to thiols. Of these, mercurials could act by mercaptide or complex formation, tetrathionate by oxidation. The convergence of the results of work with mercury compounds and tetrathionate gives weight to the theory that a thiol system is involved in their physiological action. The nephrotoxic effects of cadmium and alloxan point in the same direction. Gilman[50] finds that the water soluble cadmium-BAL complex exerts a marked nephrotoxicity. It is for this reason that prophylactic administration of BAL to rabbits with intravenous lethal doses of cadmium chloride did not save the animals from fatal renal insufficiency. The higher renal toxicity of cadmium-BAL complex than of simple cadmium ion is ascribed to its ability to penetrate cells of the renal tubules where liberation of cadmium by metabolic transformation of the complex follows. Nephrotoxic action of antimonials will be mentioned later.

Closely associated with the effect on the kidney of the drugs described is their effect on the heart. This has been observed in the clinical use of mercurials in cases of cardiac insufficiency. The effect of mercurials on the heart can occur by various mechanisms. Mercuric chloride removes the inhibitory effect on the rhythmical contractions of heart muscle due to the action of the vagus nerve; this effect is restored after washing the heart with cysteine.[70] At another level of action, Bailey and Perry[7] found actin-myosin interaction to depend on the thiols of myosin. These thiols are also concerned in ATPase activity of myosin. A variety of thiol reagents, including iodosobenzoate, hydrogen peroxide or iodine, iodoacetamide or chlormercuribenzoate, reduce both enzyme activity and ability to form actomyosin at about the same rate. The groups concerned are mainly, but not entirely, those giving the nitroprusside test and are accessible to oxidants. Godeaux[52] found that the supercontraction of myosin fibers in a solution of ATP containing potassium chloride and magnesium chloride was inhibited completely and instantly by mercuric chloride; the supercontraction reaction was also inhibited more or less rapidly by hydrogen peroxide, potassium iodate, chloropicrin, mustard gas, chloracetone and iodoacetamide. He furthermore believed the order in which these compounds arranged themselves with regard to this action on myosin fibers to be identical with the order of their action on protein thiols. While chloropicrin blocks thiols completely and inhibits myosin supercontraction completely, monochloracetone can block only 60 percent of the protein thiols and only at high concentration (.1 M) does it inhibit superprecipitation completely.[53,54] This slower acting vesicant he also found, could block 40 percent of the myosin thiols without having any effect on superprecipitation, but when it blocked the next 20 percent of the myosin thiols the superprecipitation was completely inhibited. Under these conditions the hidden thiols have not yet reacted. Polis and Meyerhof[102] point out that iodoacetate attacks only superficially located thiols while mercury compounds also attack less accessible thiols. They show that low concentrations of iodoacetamide, iodoacetate, iodosobenzoate and phenylmercuric hydroxide activate ATPase by 30-80 percent. Glutathione antagonized both activating and inhibiting influences. Fredericq[42] finds that chloropicrin, while reacting to destroy all the thiols of native myosin, does so in two distinct stages, the first is an almost instantaneous reaction, the second is a gradual one.

Another aspect of the action of mercurial compounds supposed to be related to thiols is their use as antiseptics and parasiticidal agents. Though this bactericidal effect of mercuric chloride is antagonized by thiols[36] it cannot be reversed by thiols.[139] That mercury compounds can also inhibit enzymes supposed not to require thiols has raised suspicions as to whether in its antibacterial action mercury really attacks essential thiols.

The next drugs of Table 1 include the trivalent and pentavalent arsenicals. This is the group of drugs most thoroughly explored with regard to action on thiol systems. The arsenicals are the drugs which, together with the disulfides, are probably most nearly specific in reacting readily with thiols and not with any other known tissue components. Since the aspects of arsenicals in their role as therapeutic agents for the treatment of infections with spirochaetes or trypanosomes has already been analyzed by Eagle and Doak it will not be discussed here. The aspects of arsenicals related to the vesicant action of lewisite will be discussed later in connection with the halogenated vesicants.

It is curious that though the arsenicals constitute the group of drugs whose mode of action has longest been suspected to be related to thiols, and have been most thoroughly studied in this connection, the antimonials have scarcely received any attention at all from this point of view and generally have been far less studied in the laboratory. Even the preparation of new compounds has lagged far behind the more impressive array of available arsenicals. Yet clinically antimonials have been used almost as long as arsenicals. Disregarding its more ancient uses, antimony in the form of tartar emetic came into clinical use in 1908 in the treatment of trypanosomiasis, in 1913 in leishmaniasis and in 1915 in schistosomiases. Certainly it was natural, because of the early promise of arsenicals and because of the close chemical similarity of arsenic and antimony, to test antimonials for anti-parasitic properties. Though antimonials have been used in combatting a number of disease conditions, it is principally in schistosomiasis, filariasis and some forms of leishmania infections, particularly kala-azar, that they have come to be most widely used. Because so much less work has been done with the mechanism of action of antimonials in biological systems and even on isolated enzyme systems we can only guess at its mode of action by analogy, based on the similarity of chemical reactions of antimony compounds to those of arsenic compounds. Certainly in simple chemical systems we know that pentavalent antimonials are as readily reduced by thiols to the trivalent conditions as are the pentavalent arsenicals. We also know that trivalent antimony forms mercaptides at least as readily as the trivalent arsenic compounds. That the formation of such mercaptides also extends to protein

thiols appears probable from the claims of several patents in which antimony is reported to form combinations with the products resulting from partial hydrolysis, reduction and dialysis of keratins.[136,144] The function of the partial hydrolysis is merely to render the kerateine water soluble. Even before the advent of BAL the protective effect of monothiols on antimonial toxicity to mice had been noted by Launoy.[73] More extended studies by Chen and Geiling[26] showed that cysteine not only reduced the toxicity to mice of trivalent antimonials but also reduced their toxicity to trypanosomes. Studies of the effect of BAL on antimony toxicity has led to contradictory results. Braun et al.[19] found BAL to decrease the toxicity of tartar emetic, fuadin and neostam, but Gammill et al.[44] while finding that BAL decreased the toxicity of tartar emetic, reported that it increased the toxicity of fuadin and neostam. Sandground[111] has reported findings of considerable interest on the detoxication to rats of the pentavalent antimonial stibosan with p-aminobenzoic acid. At the same time p-aminobenzoate did not interfere with the trypanocidal potency of stibosan. This was an extension of previous work on similar detoxication of pentavalent arsenicals. Subsequently Sandground[112] showed that a variety of substituted benzoates also provided great protection to rats against carbarsone and arsanilic acid. Williamson and Lourie[143] found that p-aminobenzoate antagonizes the trypanocidal activity of tryparsamide. McChesney et al.[86] broadened the field of protective agents still further when they reported that ascorbate and some of its analogs protected rats against toxic effects of neoarsphenamine. All these detoxication studies have been made in vivo. Whether they have any relation to thiols is not at present known. They can serve as leads for future work and in the meantime may give rise to some caution against too ready acceptance of the thiol theory of antimonial and arsenical drug action.

A question that must constantly recur with reference to the action of drugs presumed to operate by affecting a thiol system is that if one such drug produces a certain physiological effect, why do not all the others produce the same effect, at least in some measure? If mercurials damage kidney tissue and more specifically the tubules, why do not antimonials also do so? Franz[41] found that one of the earliest changes, observable within a few hours after administration of tartar emetic intravenously to rabbits, was a swelling and degeneration of kidney epithelium. Harris[60] found the most conspicious injury produced in rats given intravenous lethal doses of tryparsamide or stibosan was a necrosis of the convoluted tubules. p-Aminobenzoate given intravenously or orally to such poisoned rats resulted in a marked decrease in such lesions.

As regards the direct action of antimonials on the parasites against which they are used, we have such data as that of Gellhorn and Van Dyke[49] on the accumulation of antimony in those organs as the spleen and liver in which the leishmania parasites congregate in hamsters. Some interesting findings were made in a screening study of drug effects on experimental infections of Schistosoma mansoni in mice.[122] It was shown that whereas almost any antimony compound had some effect on the parasitic worms, arsenic and bismuth compounds were completely without effect. A single mercurial had a weak effect in ridding the mice of these parasites. The specificity of antimony in this case, though arsenic, bismuth and mercury as well as antimony all react similarly with thiols, is not explained. Furthermore though all antimonials showed some effect in disturbing the worms or actually killing them their efficacy varied widely. It seemed that fat soluble antimonials were more effective and those tried were mostly of the type $Sb(SR)_3$ in which antimony is already combined with thiol. Finally, one of the most efficacious drugs found[123] was the antimony complex of BAL, a complex in which antimony might be expected to be tightly enough bound so that it could not be toxic to either host or parasite. This drug is given by mouth and actually is scarcely toxic to the mouse but appears highly effective in ridding the mouse of the schistosome worms. The schistosome worm is a more highly organized animal than the bacteria or protozoa. It has a cuticle and a digestive tract and it is likely that the drug reaches the worm through the digestive tract. The worm lives in the bloodstream of its host; in the adult stage it lives in the veins of the mesenteries. It would seem that the most plausible path of the drug fed to the mouse would be to penetrate the intestine, enter the bloodstream, be ingested by the worm and penetrate its intestine. The visible effect of the drug is a degeneration of the sexual organs of the parasite. If antimonials are toxic to animals because of a thiol interaction it might be expected that they would be toxic to all animals. Yet when given to a host carrying parasites it is sometimes possible to kill the parasite without killing the host, that is, the therapeutic index of the drug is greater than one. If BAL forms a very firm complex with antimony and detoxifies the antimony with respect to the host, it might be thought that BAL would also detoxify the antimony with respect to the parasite. This is not always the case. We already know that though BAL can detoxify arsenic, antimony and mercury compounds in man, it intensifies the toxicity of lead and seems to cause no great change in the toxicity of cadmium.[83] BAL seems incapable of releasing cadmium from lung tissue.[125] The mapharside-BAL compound is more toxic to rats than

mapharside itself,[95] yet the presence of excess BAL reverses these relative toxicities. Phenylthioarsenites were found to have about the same toxicity as the parent phenylarsenoxides to amebic parasites, but to have a considerably reduced toxicity to their hosts.[1] With respect to schistosome worms Bueding[21] has found that BAL increases the effect of mercury in inhibiting glycolysis, that is BAL intensifies the toxicity of mercury to the worm. It seems clear that we are in no position to make useful predictions concerning the effect of heavy metals combined with BAL in animals. Each species and each metal must be tested empirically and there is reasonable hope that useful combinations can be found to detroy parasites, whether bacterial, protozoal or helminthic, and at the same time to be relatively harmless to the host, whether man or beast.

The next group of drugs to consider is that of the alkyl halides, including the war gases. The target of these drugs is perhaps best defined by their descriptive classification as lacrimators, sternutators, lung irritants and vesicants but though these are the immediate and obvious effects most of the compounds have potentialities for slower, far more generalized systemic toxicity, both cytotoxic and nucleotoxic. This group also includes arsenicals of the lewisite type and the muscle poison, iodoacetate, which Lundsgaard brought into prominence. All the members of this group are either alkyl halides or arsenyl halides. They are all capable of reaction with thiols in such a way as to attach directly to the sulfur the alkyl group as in (XIII) or the arsenyl group in a manner analogous to equation (VII). The products are called in the first case thioethers, in the second arsenic mercaptides or thioarsenites. It has already been pointed out that different reaction mechanisms are known to occur, as for the sulfur and nitrogen mustards, (XIV, XV, XXIII); and that even totally different end products may result, as shown in equations (III). There is no question that all these compounds could react with thiols, there is even not much doubt that in biological systems they do react with thiols. However, whether their main action in biological systems, that is whether the action that produces the observed biological effect is directly due to interference with physiologically functioning thiols is not at all clear. Chemically the group is characterized as a highly electrophilic group. Many of the compounds are capable of reacting with electron rich atomic nuclei other than thiol sulfur. Of particular importance would be nitrogen in amino, imino or heterocyclic form. For instance the tertiary amino nitrogen of pyridine reacts easily with iodoacetate and so do other tertiary amines. In the reduction of the pyridine nucleotides during their natural functioning cycle the quaternary pyridinium salt is reduced to a tertiary amino compound and in this condition could possibly react with the active alkylating agents of this group. This has never been shown to occur. The arsenic compounds of this group, compounds of the lewisite type, are on the other hand probably far more specific for either thiol or hydroxy compounds since arsenic seems to have little affinity for nitrogen and few compounds are known with an arsenic-nitrogen link. The action of lewisite on kerateine investigated by Stocken and Thompson[134] led to the conclusion that only the thiols of the protein react with lewisite. On the other hand it has already been pointed out that another vesicant, mustard gas itself, readily combines with collagen, a protein containing no thiols, yielding a sulfur containing product. Mustard in aqueous solution has been shown to react with carboxylate ions to yield esters.[89] Binet and Wellers[14] found the action of mustard gas on yeast and dog skin did not affect the thiol content and conclude that the toxicity of mustard is not due to reaction with tissue thiols. The amount of mustard sulfur combining with proteins may be ten times the equivalent of the thiols that react.[9] Goffart[55] studied the effect of a variety of vesicants on skin and concluded that while chloropicrin, chloracetophenone and iodoacetamide react rapidly with skin thiols, mustard does not act in the same way. The effect of mustard on yeast growth can not be interpreted simply as a binding of glutathione.[69] In a study of the effect of lacrimators, Mackworth[85] found them to react far more rapidly with protein thiols than with simple thiols and made the interesting observation that denatured enzymes containing no demonstrable thiol could protect active enzymes against inhibition by lacrimators. Yet under the same conditions, denatured egg albumin, known to contain active thiol, afforded no protection. Peters and Wakelin[98] showed that mustard gas does not abolish fixed thiols of muscle though mustard sulfone and phenyldichloroarsine readily do so. Examining the reaction of mustard with kerateine they find only a quarter of the combined mustard to be bound to thiol. Thus mustard differs from lewisite which seems to attack only thiols. These same authors[97] point out that the toxic dose of mustard gas in vivo is of a far lower order of magnitude than the concentration required to inhibit enzyme systems. They found N,N-diethyldithiocarbamate, which might have been expected to decrease the inhibition of the pyruvate oxidase system by mustard, actually increased the inhibition. That the potentiating effect of the dithiocarbamate might be due to formation of the compound $(C_2H_5)_2N\text{-}CS\text{-}S(CH_2)_2S(CH_2)_2Cl$ seemed possible from the high toxicity of this compound to pyruvate oxidase. A similar report of increase in toxicity of mustard is that of Kucharik and Telbisz[71], that a mixture of mustard and glutathione is more toxic to paramecia than mustard alone. It is findings such as these that emphasize the objections to the

simple concept that all potential thiol reagents really produce their biological effects by reaction with physiologically essential thiols. Certainly it cannot be said that the drugs of this group produce their effects by similar mechanisms or even that they all react with the same targets. Yet there is a curious similarity in their immediate effects which permits grouping them as vesicants.

Perhaps even more complicated than the vesicants and their relatives are the antibiotics. Among these compounds are found a wide variety of structures. Of these, two types have been listed in Table 1 as potentially capable of reacting with thiols and in fact known to react with the simpler ones. These are the α,β or β,γ unsaturated carbonyl compounds on the one hand and the disulfides or alkylthiosulfinates on the other hand. The curious property that many antibiotics have of becoming inactivated on incubation with simple thiols, even when there is no obvious possibility of interaction known, was first pointed out by Cavillito and Bailey.[23] Geiger and Conn[47] first pointed out a structural feature of some antibiotics, the unsaturated ketones, that could account not only for the inactivation of these antibiotics by cysteine, but for the antibacterial action itself. This idea was worked out in particular for clavacin and penicillic acid and served as a guide in a search for other antibiotics among relevant organic compounds and led to the finding of some acrylophenones and dibenzoylethylenes with marked antibacterial activity.[46] Such antibacterial unsaturated ketones when mixed in excess with thiols abolish the nitroprusside reaction of the thiols and when mixed with excess thiol lose their antibacterial properties. These unsaturated ketones were further found to have an inactivating effect on enzymes known to have essential thiols. Cavallito and Haskell[25] extended this structural idea to include α,β and β,γ unsaturated lactones and for the α,β,γ -angelica lactones isolated products of reaction with cysteine and related aminothiols. Broderson and Kjaer[20] made an extensive study of unsaturated lactones and found only a few to have antibacterial properties. They concluded that this structure by itself was not sufficient to give a compound antibacterial properties. Rinderknecht et al.[109] in general supported the results of Geiger and presented additional data along the same line. Cavallito[22] made a study of thiol structure as related to inactivation of a number of different antibiotics and found two main groups of antibiotics; one composed of such substances as gliotoxin or diallyl sulfoxidesulfide, readily inactivated by any of a half dozen thiols tried; and a second group, including penicillin and streptomycin which was rapidly inactivated only by cysteine or a derivative of cysteine having both thiol and amino group free. On the basis of this difference in specificity to inactivation by thiols he suggested the interesting idea that these latter antibiotics might block the growth of a polypeptide chain by blocking the free cysteinyl end when this was formed at the end of such a chain. Bailey and Cavallito[6] made an important distinction among those antibiotics inactivatable by incubation with cysteine, dividing them into two distinct groups on the basis of whether the bacteriostasis they produce is, or is not, reversed by addition of cysteine. Those in which this bacteriostasis is readily reversed by cysteine are characterized as showing little specificity to inactivation by thiols; those in which bacteriostasis is not reversed by cysteine are more specific and inactivated only by cysteine or close analogs. This classification is identical with that just described based on the specificity of the thiol inhibitor. Thus a reasonable case has been made that the antibiotics with unsaturated ketone or unsaturated lactone structure could exert their action through non-specific binding of essential thiols, reversible by addition of cysteine. For the antibiotics gliotoxin and allylsulfoxidesulfide the picture is somewhat similar though in this case the reaction with thiols, while also non-specific, is of the thiol-disulfide oxidation-reduction type.[24]

There remain the curious antibiotics penicillin and streptomycin. Both are inactivated by incubation with cysteine and both are rather specific in not being readily inactivated by other thiols. In the case of streptomycin the inactivation by cysteine might be due to thiazolidine formation with the free carbonyl group of the biose component since streptomycin is also inactivated by carbonyl reagents such as hydroxylamine and semicarbazide. Furthermore dihydrostreptomycin is not inactivated by cysteine. Several workers[16,48,141] have shown that streptomycin is inactivated by a large number of reducing agents and even loses activity in the presence of bacteria under reducing conditions. From existing data no clear case can be made that streptomycin exerts its antibacterial effect by reaction with essential bacterial thiols though the possibility is still open.

With the penicillins the relation to thiols is quite different. Penicillin can undergo no obvious reaction with thiols and it is in fact not rapidly inactivated by thiols generally but only by cysteine and cysteinyl glycine. The structure of penicillin has been shown to be sufficiently similar to that of glutathione[37] to suggest the possibility of an anti-essential-metabolite function.

Pratt and Dufrenoy[103,104] have also suggested a thiol involvement of penicillin action but along totally different lines. The argument rests on observations of patterns produced by redox potential indicators flooded over penicillin assay plates. The pattern with 2,6-dichlorphenol-indophenol is as follows: the zone of inhibited growth is stained blue; the uninhibited areas are faintly blue and between these two areas is a narrow colorless ring that indicates a zone of enhanced growth. The colors of these zones is interpreted as being related to their glutathione contents in the sense that the less the color the higher the glutathione content and the higher the glutathione content the more active the growth. Then in the central zone of dark blue color there is no glutathione and no growth, in the outer areas of faint blue color there is a certain amount of glutathione associated with normal growth and in the narrow zone between these there is no blue color at all showing a high glutathione concentration and greatly enhanced growth. These authors then show that flooding plates with formaldehyde, which is assumed to block the glutathione thiols, results in the formerly narrow colorless ring of high glutathione content now staining deep blue showing the glutathione to have been blocked. These indications that penicillin inhibition is correlated with disappearance of bacterial glutathione is then linked with the observation of Gale and Taylor[43] that penicillin inhibits the uptake of glutamic acid by Staph. aureus. The possibility is suggested that penicillin prevents synthesis of the essential glutathione in the cell by preventing the entrance into the cell of a necessary component of the glutathione. Another curious feature of penicillin is that its antibacterial action is enhanced both in vitro and in vivo by the presence of low concentrations of cobalt salts. In line with the suggestion that the action of penicillin may lie in blocking glutathione formation by keeping glutamic acid out of the cell, it would seem reasonable to interpret the enhancing effect of cobalt by a related mechanism as inhibiting glutathione formation by removal of cysteine in the form of the highly stable complexes it is known to form with cobalt ions. Another suggestion for the mechanism of the inhibition of penicillin action by cysteine is that of a chain reaction of penicillin destruction initiated by cysteine.[126] This is based on the decreasing ratio of cysteine required to antagonize increasing concentrations of penicillin.

Quinones, both benzoquinones and naphthoquinones, are known to have a strong antibacterial effect.[4,91] In addition to this they have been recognized as the most effective compounds for killing spermatozoa[58] and the cercarial stage of schistosome parasites.[113] Some naphthoquinones have highly fungicidal properties.[138] Finally some naphthoquinones have a chemotherapeutic as well as a prophylactic effect in Plasmodium infections in ducks and chickens.[27] The mechanism of the antibacterial action of quinones has been considered from the point of view of their high oxidation potential,[92] as well as from the point of view of their general reactivity.[58] The ease with which unsubstituted or partially substituted quinones react with simple thiols has already been discussed. In addition they condense rapidly with a variety of amines, including even cyclic tertiary amines such as pyridine. Colwell and McCall[29] have considered it probable that the antibacterial and antifungal action of napthoquinones is due to reaction with thiols. Foote and co-workers[40] have tried to relate the inhibition of spore germination of some fungi with the inhibition of carboxylase activity by a number of naphthoquinones. Carboxylase is a thiol enzyme. They went even further and, from the failure of prolonged incubation of naphthoquinones with carboxylase to cause any more inhibition than when these components were freshly mixed, concluded that the naphthoquinones are able to react with and to inhibit only the functioning enzyme and not the resting enzyme. They tried to give this idea some plausibility by pointing out that sluggishly reacting thiols of the enzyme might become available or reactive only during the functioning of the enzyme. The explanation of the antibacterial action of quinones as due to a condensation with essential thiols has been criticized by pointing out that many completely substituted quinones, such as spinulosin, are quite potent antibiotics. It is probable that such completely substituted quinones cannot condense with thiols by reactions of the form (XXIX). Halogen substituted quinones, however, seem to have a reactivity toward thiols similar to alkyl halides and far greater than aryl halides. For instance, chloranil reacts as readily with thioglycollate as quinone itself and yields identically the same product.[121] The mechanism of the reaction of thiols with halogenated quinones is altogether different from the reaction with unsubstituted quinones. In the former case no hydroquinone is produced, in the latter hydroquinone may be produced. It is conceivable that other sufficiently negative groups might be displaced just as are halogens from substituted quinones. Certainly the fungicidal properties of the 2,3-dichlor 1,4-naphthoquinone[138] could be due to thiol interaction, by replacement of halogen by thiol. Hoffman-Ostenhof[65] has studied the inhibiting effect of a variety of quinones on known thiol enzymes such as urease and papain. He found a close parallelism in the effect of many quinones on these enzymes and thought that consequently the mechanism of quinone inhibition for these enzymes is probably similar. He further concluded that there was no such parallel between the inhibition of these enzymes by quinones and the antibacterial potency of the

same quinones, since many of the most potent antibiotics had little or no inhibiting effect on the enzymes. His conclusion is that the antibiotic action of quinones is probably complex. Geiger[45] has made some observations that may help to unravel some of this complexity. In the first place he finds a difference in the requirements of gram-negative and gram-positive bacteria as regards the structure of quinones that will inhibit their growth. Whereas gram-positive organisms were stopped by all quinones tried, the gram-negative organisms were inhibited only by quinones with at least one unsubstituted ortho position. In the second place the antibacterial activity of quinones can be blocked by thiols only in the case of the gram-negative bacteria and not with gram-positive bacteria. These findings become of even greater interest when considered together with the work of Henry, Stacey and Teece[64] on the isolation of a thiol containing nucleoprotein from gram-positive bacteria with specific gram staining properties. Such a nucleoprotein could not be isolated from gram-negative bacteria. Of great interest is the observation that under the influence of bacteriostatic concentrations of penicillin, cells of Staph. aureus gradually lose their positive reaction to the Gram stain.[34]

Another interesting physiological mechanism relating quinones and thiols was suggested in detail by Lyons.[84] He formulated a theory for the mode of action of Vitamin K as a functional part of prothrombin. Fibrinogen, he thought, had hidden thiols which were unmasked by one component of prothrombin only to be converted immediately to the disulfide of fibrin by an oxidation by the naphthoquinone component of prothrombin. One piece of evidence for this scheme was the claim to have found naphthoquinone in prothrombin by a colorimetric test. Dam[31] was unable to confirm this. Yet some sort of thiol mechanism is implied in reports[68] of the inhibition of fibrinogen coagulation by iodoacetamide.

The last of the drugs of Table 1 to discuss is alloxan. This compound long known and studied has sprung into prominence during the last few years because with it a diabetic condition can be produced in experimental animals. Injection intravenously of proper doses of alloxan into several species of laboratory animals leads to rapid and selective necrosis of the β-cells of the islets of Langerhans.[93] Previous administration of glutathione or cysteine completely blocks this necrotizing effect of alloxan on the β-cells and also prevents development of diabetes.[75] That thiols protect animals against development of diabetes only if administered immediately before or at the same time as the alloxan led to the opinion that the effect produced by alloxan is due to combination with thiols rather than to oxidation of them to disulfides.[77] The opinion that glutathione combines with alloxan was further supported by the appearance of an additional maximum in the absorption spectrum at 305 μμ on mixing glutathione and alloxan. Yet it was also shown[94] that glutathione can reduce alloxan to dialuric acid. The fact is we do not have clear data at present as to which reaction takes place or, if both take place, which predominates. Alloxan, like ninhydrin which it resembles structurally, also reacts with amino acids having a free amino group.[79] Purr[106] claims alloxan converts protein thiol to disulfide without affecting other protein groups. Hopkins and co-workers[66] found alloxan at low concentration could inhibit succinic dehydrogenase. Hexosediphosphatase inhibition by alloxan could not be reversed by cysteine, suggesting that the reaction is not an oxidative one.[142]

As with many other thiol reagents already discussed there is no question that alloxan can and does react with tissue thiols. The critical question that is not yet sufficiently answered is this; does the diabetes produced in animals by alloxan result from reaction with tissue thiols? Lazarow[76] gives a degree of plausibility to an affirmative answer with the following observations. Since β-cells synthesize insulin, a disulfide protein, they may be expected to have a higher redox potential than other tissues. This appears to be so from results of staining with Janus green. Since the β-cells have a weaker reducing potential they probably contain less thiol, either as cell protein or as reduced glutathione. This would make them less capable of destroying alloxan than other cells with a greater thiol reserve. Or looking at the same matter in another way, the synthesis of insulin, containing 12 percent cystine, by the β-cells is likely to compete with glutathione synthesis, so these cells are apt to be left with only a small glutathione reserve to protect them from alloxan.

Summarizing the broad survey attempted, we find few sharp conclusions. Arsenic compounds, whether trivalent or pentavalent, are known to react with no tissue components other than thiols and the evidence that they do in fact react with tissue thiols is quite good. Of antimonials we can say almost the same although some antimony compounds can also react with hydroxy acids. If there is less evidence that they do react with tissue thiols this is largely because much less work has been done with antimonials. Mercury compounds can equally readily

react with thiols and in many cases are known to react with tissue thiols. But since mercurials could also react with disulfides and a great variety of amino compounds we are here on less certain ground. Tetrathionate and the disulfide antibiotics are not certainly known to react with anything but thiols. With the alkyl halides, unsaturated carbonyl antibiotics, quinones and alloxan we are on altogether uncertain ground and no generalization is at present likely to be good except that they are all capable of reacting with thiols. In some individual cases there is a fair amount of evidence that they do produce their effects by reaction with tissue thiols. In a few cases, notably the sulfur and nitrogen mustards, there is good evidence that at least some of their effects are unrelated to thiols. For most of these compounds the great need is for more evidence.

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DISCUSSION

DR. HARRIS L. FRIEDMAN (Lakeside Laboratories, Milwaukee, Wisconsin): I would like to make a few comments, very briefly since the time is short, as to the role of thiol groups and the effects of mercurials. We have been very interested in this, as have many others. There are many conflicting results which need to be explained if the mechanism by inhibition of thiol group is correct. Handley[1] of Baylor has recently shown that mercurial diuretics do inhibit the succinic dehydrogenase enzyme which occurs in the kidney, and there is enough mercury ion in the kidney to account for the inhibition; but still, as Dr. Schubert has pointed out, it cannot be deduced that this is what actually happens.

Recently, also, Ruskin[2] and co-workers have shown that vitamin C can detoxify the action of a mercurial on the heart just as well as BAL or thioglycolic acid. I would like to ask Dr. Schubert, in this case, how that can be tied in with the sulfhydryl groups.

[1] C. A. Handley, Fed. Proc. 8, 299 (1949)

[2] A. Ruskin, J. E. Johnson, Proc. Soc. Exp. Biol. Med., 72, 577 (1949)

DR. SCHUBERT: I am glad to hear about that ascorbic acid effect. The only answer I can give is another case, in which antimony compounds have been detoxified also with ascorbic acid and, in fact, with a large number of substitute benzoic acids.

Ascorbic acid, incidentally, could react with thiols. I once studied that a little bit, and the only thing I could find in that particular case was that oxidized ascorbic acid very readily oxidized thiols. Reduced, on the other hand, it is not very likely to interact with thiols, but it could interact with disulfides, and there is some work by Purr in which he tries to show that disulfides are reduced by a mixture of ferrous iron and ascorbic acid. That is the only interaction I know of between those two.

Of course, the other thing which is known is that thiols generally protect ascorbic acid against oxidation. That is a common effect of many of these so-called sluggishly acting reductants. You get the same sort of thing with hydroquinone. It can also inhibit oxidation of sulfur compounds.

DR. F. S. PHILIPS (Sloan-Kettering Institute, New York, New York): I was pleased to hear Dr. Schubert's discussion, because I have felt for some time that there are many questions to be answered before one can attribute the mechanism of action of a compound to sulfhydryl inactivation. Everything Dr. Schubert has said has been explicit, but I should like to emphasize some other points of view than those developed in his presentation.

The case of the nitrogen and sulfur mustards should be emphasized since there is an interest in determining the common factors in radiation poisoning, and the action of the β-chloralkylamines and sulfides. Biologically, the physical agents, i. e. penetrating radiations, and the above chemical agents have many properties in common. There is a growing belief among some workers that radiant energy may cause cellular damage through reaction, which involve sulfhydryl groups. It would, therefore, be of importance if it could be established that the physiological mechanism of action of mustards also involves reaction with sulfhydryl groups. However, it should be stressed that mustards probably do not act biologically through inactivation of sulfhydryl groups. I take as authority for this statement, Dr. Schubert himself, and among others, R. A. Peters, who has stated quite definitely that, though mustards may react with simple sulfhydryl derivatives their reaction with sulfhydryl in complex compounds, such as proteins, is not very striking, and much less than that which occurs with carboxyl, amino, or phosphoryl groups.

There is an aspect to the understanding of the properties of agents which react with sulfhydryl groups, which is often not considered by investigators who hypothecate sulfhydryl inactivation as a mechanism of biological action. I believe Dr. Schubert raised the same point.

One may well ask why, for example, alloxan should be the only sulfhydryl reactive reagent which damages the β-cells of the pancreas, whereas other sulfhydryl oxidizing agents do not damage this particular cell; indeed, very few other agents have been found to affect the β-cell. Moreover, alloxan has as another major site of action the proximal tubule of the kidney. It is likely that both the proximal tubule of the kidney and the β-cell of the pancreas are of about equal sensitivity to the agent. Nevertheless, tetrathionate, a powerful sulfhydryl oxidant, does not touch the β-cell of the pancreas while it causes extensive, alloxan-like damage in proximal tubules.

Other examples of the role of cellular distribution in the mechanism of action of sulfhydryl reactive agents may be stated. When ionic cadmium is injected into an animal, its actions on the proximal tubule of the kidney are not striking; its toxicity would suggest other sites of action which, at present, are not well understood. Nevertheless, if cadmium is introduced in the form of a cadmiumdibal complex, it exerts a selective toxic action in this region of the kidney.

One might also ask why iodo acetate and a number of other "sulfhydryl inhibitors" do not attack this particular region of the kidney to the same extent.

It appears, then, that an important feature in understanding the mechanism of action of sulfhydryl reactive agents concerns not merely their affect on enzymes in cells after having entered certain cells, but also the factors which determine specificity of cellular distribution and cellular sites of action.

DR. SCHUBERT: I will just take a second; I think there are some other cases.

I think the discussion Dr. Philips gave is very much to the point, and I am quite in agreement with everything he said. I think there are two things which need to be considered. One, of course, is the thing which was discussed yesterday, which is the matter of cell permeability, and this is the thing which Gilman discusses in connection with the high toxicity of cadmium-BAL complexes, and the fact that BAL does not protect against cadmium poisoning.

I would just like to point out one additional example of that character, and that is that, among antimony compounds, we found one of the most effective for getting rid of the Schistosoma worm was the antimony-BAL compound itself. If BAL is given with antimony, and can take the antimony away from enzyme systems, you are going to have a little difficulty explaining why that should be better for killing schistosome worms than a compound in which you did not have the BAL attached to the antimony at all.

DR. CHALMERS L. GEMMILL (University of Virginia, Charlottesville, Virginia): I would just like to ask Dr. Schubert if he could give us some help with the recent observation1/ we have made. The inhibition of invertase by mercuric chloride, can be reversed with BAL or increased with cysteine; in other words, you can work in both ways depending on whether a dithiol or a monothiol is used.

I would also like to state that ascorbic acid has been described as a reactivator of the mercuric chloride inhibited invertase system.

1/ Gemmill, C. L. and E. Bowman, J. Pharm. Exp. Therap. In press, (1950).

DR. SCHUBERT: I do not believe I can say anything very intelligent about that case; I did not know about that one, either. I am glad to know about it, but that is about all I can say. I think, of course, that invertase is one of the enzymes which, presumably, contain no essential thiols.

Just by way of emphasizing this point that these metals can combine with groups other than thiols, antimony, as far as we now know, combines only with sulfur compounds; yet, recently, we had occasion, when working with collagen, to find that collagen is able to attach to itself at neutral reaction quite large amounts of antimony. So, possibly, invertase can combine, through groups other than thiol groups, with the mercury. Certainly it is likely that occurs, but why you should get the activation with the glutathione and not with BAL that is not clear.

INFLUENCE OF ISOSTERIC REPLACEMENTS UPON BIOLOGICAL ACTIVITY

by

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PART 1

INTRODUCTION

To the synthetic organic chemist interested in medicinal chemistry, every physiologically active compound of known structure is a challenge - a challenge either to better it, or perhaps merely to equal it. For it must be remembered that even the most innocuous drug is not tolerated by some people.

There are numerous ways of attacking such a problem, and this audience is certainly familiar with them. One of the methods which has been used frequently, very often with success, is that of isosteric replacement. The examples of this type of replacement in the literature are very numerous, and the fruitful results in the fields of sulfonamides, antimetabolites, and antihistamines are well known.

The concept of isosterism, first introduced by Langmuir,[13] has been changed over the years by the work of many others. It will be the object of this paper to survey the history of isosterism, to classify the varieties of isosteric replacements which are recorded in the literature, and to note the influence of these replacements on the biological activity of compounds. We shall then be able to see if any general relationships apply, and what conclusions may be drawn from such data.

PART 2

THEORETICAL

Langmuir in 1919[13] pointed out the remarkably similar physical properties of carbon dioxide and nitrous oxide. He deduced from the octet theory that the number and arrangement of electrons in these molecules are the same. Compounds showing such relationship to one another were termed isosteric compounds or isosteres. These terms were not restricted to compounds, but were extended to groups of atoms which hold pairs of electrons in common (termed by Langmuir comolecules). Comolecules were likewise considered isosteric if they contained the same number of atoms and possessed the same number and arrangement of electrons.

Langmuir predicted twenty-one types of isosteric groups of which only a few will be mentioned here:

CHART 1

Langmuir Type	
2	$O^{=}$, F^{-}, Ne, Na^{+}, Mg^{++}, Al^{+++}
3	$S^{=}$, Cl^{-}, A, K^{+}, Ca^{++}
5	Br^{-}, Kr, Rb^{+}, Sr^{++}
8	N_2, CO, CN^{-}
9	CH_4, NH_4^{+}
10	CO_2, N_2O, N_3^{-}, CNO^{-}

Langmuir postulated that when isosteric comolecules are also isoelectric, that is when they have the same total charge, all their physical properties should be closely similar. Only three such pairs occurred in Langmuir's tables:

N_2 and CO; CO_2 and N_2O; N_3^{-} and NCO^{-}

However, no direct comparison can be made of the physical properties of isosteres having different electrical charges.

Even though the classes are distinct from one another, it was demonstrated that comparisons of comolecules in different groups could still be made. If any two substances are very much alike in physical properties, then any isoelectric isosteres of these substances should show close relationships with one another. Thus in types 3 and 8, argon and nitrogen resemble each other closely, therefore chloride ion (isosteric with argon) should resemble the cyanide ion (isosteric with nitrogen). Likewise the similarity between K^{+} and NH_4^{+} can be derived from argon and methane. It is to be emphasized that in Langmuir's terminology K^{+} and NH_4^{+} are not isosteres of one another.

Whereas Langmuir compared only physical properties, Seifriz[17] showed in 1948 that CO_2 and N_2O are both reversibly anesthetic to the slime mold Physarum polycephalum.

In 1921, W. Huckel[11] pointed out that the imino group (=NH) in homopolar compounds corresponds to the oxygen atom and that the $-NH_2$ and -OH groups correspond to the F atom.

Somewhat later (1925) Grimm[8] very markedly extended the concept of isosterism. In place of Langmuir's term "isosteric comolecule", Grimm preferred the term pseudoatom. His hydride displacement law states:

> "Atoms anywhere up to four places in the periodic system before an inert gas change their properties by uniting with 1 to 4 hydrogen atoms, in such manner that the resulting combinations behave like pseudoatoms, which are similar to elements in the groups 1 to 4 places, respectively, to their right."

Grimm showed this relationship by the following chart:

CHART 2

	Group	4	5	6	7	0	1
Number of	0	C	N	O	F	Ne	Na
Hydrogens	1		CH	NH	OH	FH	
	2			CH_2	NH_2	OH_2	FH_2^+
	3				CH_3	NH_3	OH_3^+
	4					CH_4	NH_4^+

Beginning in 1932, Professor Erlenmeyer[5] at the University of Basel in Switzerland has published a series of papers on isosteric compounds. He has given great impetus to the modern concept of isosterism in organic chemistry, particularly in relation to biological activity. Erlenmeyer accepted Grimm's classification and has broadened it even further. His definition of isostere is:

> "Atoms, ions, or molecules in which the peripheral layers of electrons can be considered to be identical are termed isosteres."

By Erlenmeyer's definition all elements in the same group of the periodic table are isosteric so long as they have the same number of electrons in their outermost shell. In a unique application of this concept, Erlenmeyer in 1933[6] considered S and CH=CH in an aromatic nucleus to be isosteric by counting only the "peripheral" electrons in the C=C pseudoatom (whether it be written -CH=CH- or =CH-CH=).

In 1946, Mentzer[14] demonstrated that, in certain circumstances, the group $-CH_2-CH_2-$ could be replaced by -CO-O- with no change in biological activity of the parent compound. He did not term these isosteric pairs.

Some authors, as Mentzer[14] and Erlenmeyer[3] use the term potential-cycle or pseudocycle to bring out the steric relationships between the ring and opened form of physiologically active molecules.

Occasionally the term "isolog" is used by some authors (as Fieser[7] in the United States and Steinkopf[18] in Europe) where "isostere" is usually designated. Isologous compounds, however, need not be isosteric.

It is obvious from this brief survey that the term "isosteric" has varied in meaning with different writers - from a narrow to a very broad concept. In this discussion we shall accept the term in its broadest meaning and study the influence of isosteric replacements on the biological activity of molecules. We shall not consider physical properties, although it is not implied that such properties as mixed-crystal formation are not of great significance for isosteric relationships.

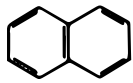
We shall term compounds "bio-isosteric" if they fit the broadest definition for isosteres and have the same type of biological activity.

The biological equivalence of isosteric groups receives support from immunological studies. Landsteiner[12] was able to prepare artificial antigen-antibody systems by coupling diazotized aromatic amines with proteins, and injecting these protein complexes into animals to form antibodies. He discovered that these antibodies have the specific power of combining with the group attached by the azo linkage. This group, which is of known structure, he termed the haptenic group. The specificity of the antibody in any serum could be tested, by means of the precipitin reaction, with related complex proteins. In general, the combining power is highly,

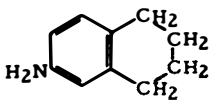
but not completely, specific.

Erlenmeyer,[4,19] using Landsteiner's method, demonstrated the serological similarity of several isosteric atoms and pseudoatoms. The following are illustrations of several types of isosteric replacement, where the resulting compounds give cross-reactions:

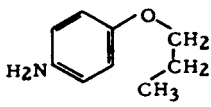
CHART 3

SEROLOGICAL SPECIFICITY	
C_6H_5-O-C_6H_4-N=N-Protein C_6H_5-NH-C_6H_4-Protein C_6H_5-CH_2-C_6H_4-Protein	-C_6H_4-PO_3H_2 -C_6H_4-AsO_3H_2 not -C_6H_4-SbO_3H_2
-C_6H_4-SO_3H -C_6H_4-SeO_3H not -C_6H_4-SO_2H	 -CO-NH-C_6H_4-  -CO-NH-C_6H_4- not  -CO-NH-C_6H_4-

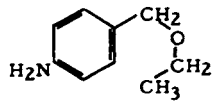
I



II



III



I and II +++

I and III ±

II and III ++

From Erlenmeyer[4,19]

While this work is not necessarily foolproof, and indeed has been criticized by Heidelberger[10] as requiring more and better support, it is in good agreement with Pauling's views.

Pauling[15] has also studied serological reactions to provide information about the molecular structure and configuration of simple substances. He compared the process of antibody formation to the production of a replica by pressing a plastic material against a mold and permitting it to harden. The polypeptide chain, with its power of assuming alternative configurations, is the plastic material, and the surface of the antigen serves as the die or mold. The complementariness of antigen and antibody includes not only surface configuration, but also juxtaposition of special combining groups, such as a negatively charged group in the antibody with a positively charged group in the antigen, and a hydrogen-bond-forming group carrying the proton with a similar group presenting an electron pair. Thus, isosteric replacements in an antigen which do not affect the shape or polarity of the molecule should not interfere with its reaction with the antibody.

Pauling has extended this concept of spatial surface configuration to include biological specificity in general.

Deductions from serological reactions are limited because strongly polar groups have predominant effects. However, this type of study should show when parts of a molecule are simple "space fillers", that is occupy specified geometrical bulk.

Since the discovery that the antagonism of the sulfonamides to p-amino benzoic acid is an antimetabolite effect, due to the close similarity of structure, isosteric replacements in other essential nutrients have yielded many compounds of interest. This field has been thoroughly reviewed in the literature,[16] and is the subject of a separate part of the symposium. We shall merely mention some of the types of isosteric replacement in the numerous antimetabolites which have been made, and only a few specific examples will be given in this paper:

CHART 4

Essential Nutrient	Atom or Group Replaced	Replacement
Riboflavin	2-CH_3	2-Cl
Thymine	-CH_3	-OH, -Br, or -NH_2
Mesoinositol	6-OH	6-Cl
Thymine, lysine, folic acid	-NH_2	-OH
Folic acid	-OH	-NH_2
p-Amino benzoic acid, glutamic acid	-COOH	-$CONH_2$
p-Amino benzoic acid, niacin	-COOH	-$COCH_3$
Arginine	-O-	-CH_2-
Uracil, thymine, niacin amide	-O-	-S-
Methionine	-S-	-O-, or -CH_2-
Purines	-CH=	-N=
Phenyl alanine, p-aminobenzoic acid	benzene	pyridine

CHART 4 (Cont.)

Essential Nutrient	Atom or Group Replaced	Replacement
Phenyl alanine	benzene	thiophene, furan, pyrrole
Niacin	pyridine	thiazole
Thiamine	thiazole	pyridine
Methionine	-S-	-CH=CH-
Valine, niacin, pantothenic acid, aspartic acid, oxybiotin, heteroauxin	-COOH	$-SO_3H$
p-Amino benzoic acid	-COOH	$-AsO_3H_2$
p-Amino benzoic acid	-COOH	$-PO_3H_2$

The isosteric compound formed may have either the same activity as the original, or more usually it may have an antagonistic effect. In either case, it is proof that isosteric replacement gives compounds acting by the same mechanism, that they are truly bio-isosteric.

Ideally, to make comparisons between structure and biological activity, two criteria are necessary: (1) Substances compared must act by the same mechanism and (2) The structure involved in the test should be the structure of the compound under study. However, in practice, for many types of biological activity only in vivo tests can be used, and even when using in vitro tests, we cannot be sure that the above criteria apply. In so far as possible, examples have been chosen which are based on in vitro activity, and mainly those using an isolated tissue or micro-organism. It is not claimed that the in vitro tests will necessarily correlate with in vivo or clinical studies; nonetheless the data obtained may be a useful guide for further work and may be adaptable to other series of compounds.

Activities found in one screening test need not parallel the relative activities of the compounds in another test. Since data in the literature are usually lacking for tests other than those in which the authors were most interested, it is seldom possible to make such alternate lists.

Biological activities, as absorption, distribution, conjugation (detoxification), taste, odor, side effects of drugs, will not be discussed.

PART 3

TABLES OF DATA

In order to classify the data for presentation, the following chart is pertinent for organic compounds.

CHART 5

Class 4	Class 3	Class 2	Class 1	
	Sb	Te	I	
	As	Se	Br	
	P	S	Cl	
C	N	O	F	Ne
N^+ P^+ S^+	-CH-	-NH- -PH-	-OH -SH	
		$-CH_2-$	$-NH_2$ $-PH_2$	
			$-CH_3$	

Table modified from Grimm[8]

We have designated these four types from the number of covalent bonds. Above are the elements of the same periodic group, below are the isosteric hydrides. The following tables will show the effect of isosteric replacement on biological activity within each type. The -S- and -C=C-, and other special cases do not fit into this chart and will be treated separately.

Discussion of Tables

Class 1 - Halogens and Hydrides (OH, NH_2, CH_3)

Tables 1-4 list examples of comparisons of Class 1 of the chart previously shown. Table 1 contains examples of multiple comparisons, Table 2 compares halogens only, Table 3 compares halogens with hydrides, Table 4 compares hydride with hydride. An attempt has been made to select examples from fields of current interest.

Table 1. If one were to judge results of isosteric replacements from Table 1, it would be difficult to arouse enthusiasm. The most unusual case is the replacement of the chain -OH of epinephrine by NH_2; the activities of other members of this series should be of great theoretical interest.

Table 2. In general the results are what would be expected, activities usually showing a gradient with the molecular weights. There are exceptions, mostly with the extreme members F and I.

Tables 3 and 4. These again show unpredictability of response. The α-hydroxy-β-phenylethylamine examples have been amply discussed by Hartung.[9]

General Conclusions to Class 1. It is not possible to predict when members of this class will be bio-isosteric; in most instances they will not be. Very often activity is specific to one member which would be called, in Ehrlich's terminology, an anchoring group. Differences in activities may be attributable to differences in polarity of the groups, to solubility differences, or to chemical reactivity. The most likely pairs of bio-isosteres are:

halogen and CH_3

halogen and OH,

the most unlikely pairs are:

OH and NH_2.

Class 2 - (S, O, NH, CH_2)

Tables 5 - 7 inclusive list examples of Class 2 replacements. For convenience, the examples have been divided into structural types: ether, ketone, and ester.

Table 5: Ether Type. These show a better probability of bio-isosterism than Class 1 types. The member which fits in the least is -NH. Sulfur is not always bio-isosteric with oxygen, in fact surprisingly less than might be anticipated. Probably polarity differences play a predominant role.

Table 6: Ketone Type. The most interesting examples are probably the thio-barbiturates. In general, these types have restricted comparability.

Table 7: Ester Type. Many amides and thioesters related to the local anesthetic and antispasmodic esters are known, but practically none have come into use. More thought should be given to the replacement of ester oxygen by the CH_2 group.

General Conclusions to Class 2. Isosteric replacement in this group has better promise of usefulness than in Class 1. While methoxy and ethyl often do not show similarity, in other cases interchanging O and CH_2 yields compounds of similar activity. Here, as in Class 1, polarities probably play a dominant role.

Class 3 - Tertiary N and Tertiary C

Tables 8 and 9 list examples of Class 3. Most of the known examples of this class occur in the aromatic ring systems (Table 8). No attempt has been made to list the numerous examples in well-known fields as the sulfonamides and antihistamines but a few are given to refresh memories. Commercially this has been the most valuable application of bio-isosterism.

Table 9 contains non-aromatic examples. Because of polarity differences the aliphatic types can seldom be expected to display bio-isosterism, but it is a more likely assumption that diphenylamine and benzhydryl derivatives would show such similarity. More examples are desirable.

While tertiary P, As and Sb theoretically are electronically isosteric with N and CH, practically, except between As and Sb, they show little bio-isosterism.

Class 4 - Quaternary C, N, S, Etc. (Table 10)

The spatial tetrahedral geometry and the positive charge are of paramount importance for this class. In general, a quaternary carbon, because it lacks a charge, is not interchangeable with quaternary nitrogen.

The Special Classes

Table 11 - Aromatic C=C and O, S, NH. Following Erlenmeyer, it is generally agreed that the pairs, benzene and thiophene, and pyridine and thiazole, are isosteric. Furan and pyrrole differ markedly from benzene in both physical and chemical properties. Indeed, Bradlow, Vanderwerf and Kleinberg[2] in a brief discussion of the concept of isosterism, state that "Proponents of the principle of isosterism do not point out the fact that by definition pyrrole is also isosteric with benzene, thiophene and furan." There are sufficient examples, however, to indicate that these rings may be bio-isosteric although the furan and pyrrole compounds are usually weaker in activity than those containing benzene and thiophene. The activity of furan isosteres in the antihistamine field indicates that such replacements cannot be ignored.

The latter part of the table contains comparisons of an ethylenic bridge between two aromatic rings with S, O and NH. Not enough examples are available to draw conclusions, but this should be an interesting replacement type.

Table 12 - Carbonyl and Sulfone (or Sulfoxide). The structural relationship between p-aminobenzoic acid and sulfanilamide has been emphasized by Bell and Roblin[1] in explaining the mode of action of sulfonamides. The exchange of -COOH for $-SO_3H$ in many metabolites to produce antagonistic substances has shown this to be a general phenomenon. Therefore, a comparison has been made in this table of compounds with carbonyl and sulfone groups. The sulfoxide has been added since spatially it more nearly resembles the carbonyl than does the sulfone grouping. Electronically neither the sulfoxide nor the sulfone group is isosteric with the carbonyl group. The ionic bond of the sulfur groups further emphasizes the difference.

The table does not indicate any striking resemblances except for the amidone type example.

Table 13 - $-CO-O-$ and $-CH_2-CH_2-$. These groups are not electronically isosteric; it is most likely that they owe their activity to similar spatial fixation (as discussed in the following type).

Table 14 - Spatial Fixation by Ring or Double Bond. That three dimensional spatial characteristics play a highly important role in biological activity is known to everyone. The vastly different activities often noted in optical or cis-trans isomers must be considered in any attempt to explain the mechanism of biological activity. When two molecules are almost identical spatially, that is, are superimposable in three dimensions, we may expect similar activity provided the polarities are situated in corresponding parts of the molecules.

Table 14 lists examples of ring and open forms. In many cases there is striking agreement; often however, one form is completely inactive. Where agreement between activities is found, it is interesting to assume that the open and closed forms can be superimposed in three dimensions.

Benadryl is an interesting example. When the rings are forced into the planar form of the fluorene ring, activity is lost; one might speculate therefore that in the "active" form of Benadryl the two benzene rings do not lie in the same plane. From the example of Trasentin and Pavatrine the opposite appears to be the case.

Table 15.- Polarity Shift, Exo-Endo Cyclic. This and the following table are offered in an attempt to systematize data scattered throughout the literature. This table demonstrates the effect of moving a polarity from without to within a ring, the shift being to the adjacent position. The probability is that the polarity of the atom must remain quite similar in order to retain the same activity.

Table 16 - Reversed Adjacent Polarity. This table illustrates the effect of reversing adjacent polar groups. Many instances are of great interest; this is a transformation to be kept in mind in seeking new compounds.

CONCLUSIONS

We have seen that similarity in biological action need not result from isosteric replacement - isosteres need not be bioisosteres. This is not surprising in view of the complexity of the simplest living systems.

As we all know, simple isosteric replacements often give compounds of interest and value. In addition, there are two important types of information to be learned from such replacements. One is that we discover which groups cannot be eliminated in order to retain the desired activity (i.e., the anchoring groups); the other is that we learn which parts of the molecule are important because of their bulk space characteristics. These facts enable a more intelligent approach to the synthesis of new compounds.

The similarity of biological activity in so many instances, and the successful results already achieved through isosteric replacements, show that this is a type of variation which the synthetic chemist must keep in mind. If chemical reactivity and polarities are considered, the predictability of bio-isosteric replacement is quite high.

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EXPLANATION OF TABLES

In the first column the structural formulas of the compounds under consideration are written with the variable isosteric group represented as X.

In the second column the biological list is stated (e.g. anti-tubercular, narcotic, anesthetic, etc.) together with other pertinent data necessary - as to whether in vitro or in vivo, what organ, organism, or animal was used, what challenging agent, if any, was used, and in what terms the activity data are expressed with a designation of the reference compound if such was used. Below, in parenthesis, the references to the literature are given. This list is found immediately following the tables.

The remaining column headings usually designate the atom or group represented by X. In cases where this is not so, the heading is self evident. The data in these columns are the activities of the compounds in the terms used by the original workers; this varies with different authors from quantitative figures, a system of plusses, to mere statements of activity or non-activity.

TABLE 1

CLASS 1

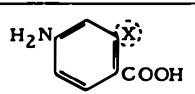
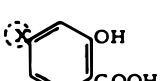
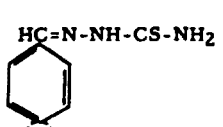
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Anti-tubercular In vitro Salicylate No. (16)	F < 1 Cl 6 Br 7 I 20	< 1	< 1	27
	Anti-tubercular % curative in guinea pigs (111)	Cl 19%	16%	-	inactive
	Anti-tubercular In vitro (94)	Cl inactive	most active	inactive	weak
	Anti-tubercular In vitro Molar conc. for stasis (72)	Cl 1/500	1/1,000	1/40,000	
	Anti-tubercular In vivo (mouse) (74)	Cl slight	definite	slight	

TABLE 1 (Cont.)

CLASS 1

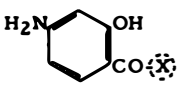
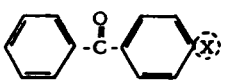
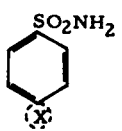
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Phenol Coefficient vs. B. Thyphosus Phenol = 1 (140)	F 1 Cl 3.9 Br 5.4	12	?	2.5
	Anti-tubercular In vitro Molar Conc. for Stasis (72)		1/40,000	1/80	1/17
	Anti-tubercular In vitro Stasis - mg % (57)	Cl 2.5	10		2
	Antibacterial (120)	inactive	inactive	active	inactive

TABLE 1 (Cont.)

CLASS 1

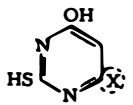
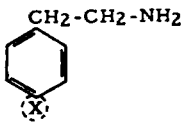
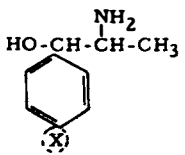
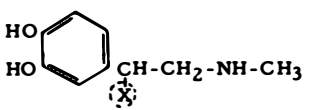
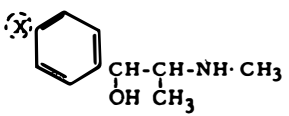
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Antithyroid (109, 138)		inactive	weak	potent
	Pressor Activity Epinephrine = 1 (66, 103)	F or Cl "weak"	1/70		"weak"
	Pressor Activity (66, 143, 64, 81, 67)	F, Cl "possess pressor activity"	1/50 epi	? activity unpublished	1/200 epi
	Pressor Activity (18, 152, 93)	Cl - known ?	OH - epi SH - "epi-like"	1/10 epi	?
	Pressor Ephedrine = 1 (66)		< 1	2	< 1

TABLE 1 (Cont.)

CLASS 1

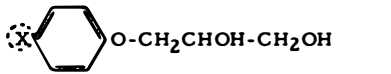
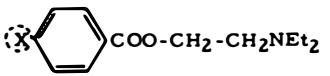
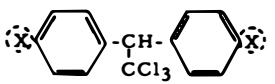
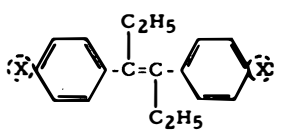
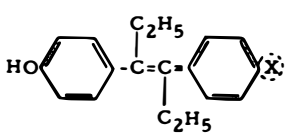
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Muscle relaxant ø-O-CH_2-CHOH-CH_2OH = 1 (99)	Cl 2.1		0.2	1.2
	Local anesthetic Procaine = Standard (27, 128)	F comparable	comparable	(standard)	
	Insecticidal vs. Lice Standard = DDT (113)	F 1/4 DDT Cl DDT Br 1/2 DDT	inactive	inactive?	1/5 DDT
	Estrogenic (135)	Br very weak	potent 0.4 gamma	very weak	inactive?
	Estrogenic (135)	Br 100 gamma	0.4 gamma	7.5 gamma	

TABLE 1 (Cont.)

CLASS 1

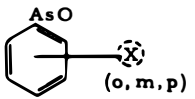
Compound	Biological Test (Reference)		Halogen	-OH	$-NH_2$	$-CH_3$
	vs. T. pallidum		Cl			
	in vitro	ortho	83	84	88	84
		meta	110	79	104	97
	Ø AsO = 100	para	85	72	83	102
	(38)					

TABLE 2

CLASS 1

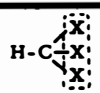
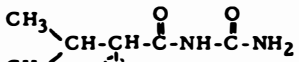
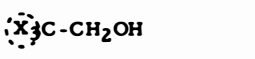
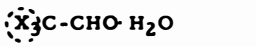
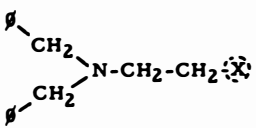
Compound	Biological Test (Reference)	I	Br	Cl	F
	Narcosis (56, 70)	narcotic	narcotic	narcotic	inactive
	Hypnosis (56)	inactive	hypnotic	hypnotic	
 	Narcosis (91)		narcotic (2 x Cl cmpd) inactive	narcotic narcotic	
	Adrenergic blocking (119)		active (same as Cl)	active	
	vs. Vaccinia virus (144)			active	inactive

TABLE 2 (Cont.)

CLASS 1

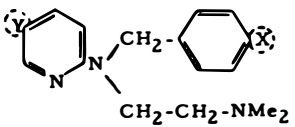
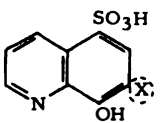
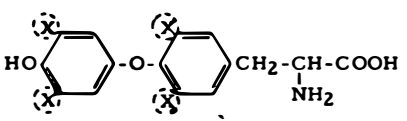
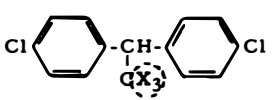
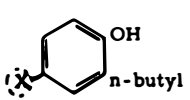
Compound	Biological Test (Reference)	I	Br	Cl	F
	Antihistamine X PBZ = 1 Y (147)	- 1/2 -	1 1/2	2 1/2	3
	Amebic Dysentery (149)	active	inactive	inactive	
	vs. Myxedema in humans (95)	1	1/17	1/250	
	Insecticidal (113)		good	excellent	weak
	Phenol coefficient vs. S. aureus (140)		313	257	60

TABLE 3

CLASS 1

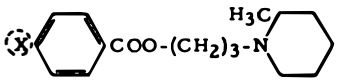
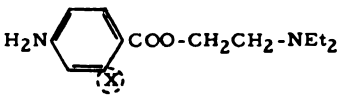
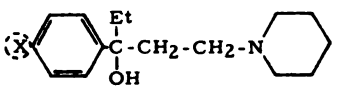
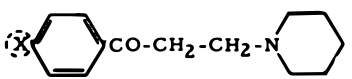
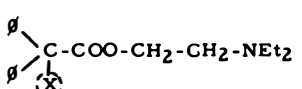
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Local anesthesia minutes (107)	12	15	10			?	9
	Local anesthesia Procaine = 1 (84)			2				4
	Antispasmodic vs. Furmethide Trasentin = ++ (37)		+++	+++				+++
	Ditto (37)		+	0				+
	Antispasmodic vs. acetylcholine, Ba, or histamine (92)			+++		+++		

TABLE 3 (Cont.)

CLASS 1

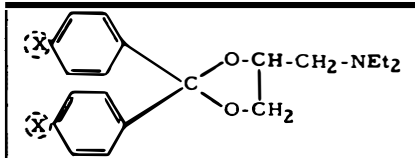
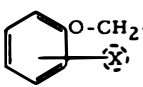
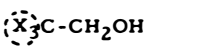
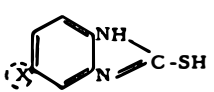
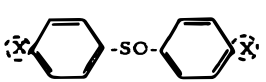
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Antispasmodic vs. acetylcholine, Ba (19)			+				++
	Muscle relaxant Ø-O-CH_2-CHOH-CH_2OH = 1 ortho meta para (9,99)		1.3	1.7 2.4 2.1				2.2 2.1 1.2
	Narcotic (91,101)		strong-est	strong				very weak
	Antithyroid Thiouracil = 100 (26)	11	27	44				12
	Insecticidal vs. clothes moth (88)		effective	effective				inactive

TABLE 3 (Cont.)

CLASS 1

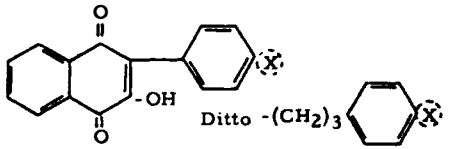
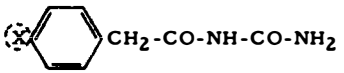
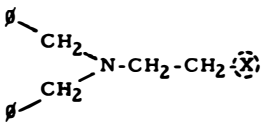
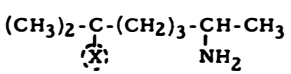
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Antimalarial in ducks ED_{95} in mg/kg (54)	>40 28	29 19	45 20	>60 60			73 15
	Anticonvulsant vs. electroshock (136)			±				++
	Adrenergic blocking (119)		active	active		inactive		
	Cardiac action (100)			+		+		
Vitamin D 3-substituent	(10)		inactive	inactive		active		

TABLE 3 (Cont.)

CLASS 1

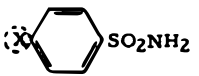
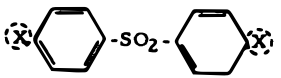
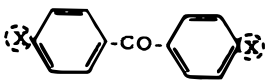
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	(88)			insect-icidal			bacter-icidal	
	(88)			ditto			ditto	
	(88)			ditto			ditto (weak)	

TABLE 4

CLASS 1

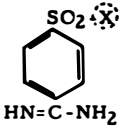
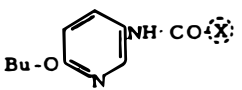
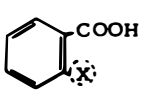
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	(36)			Active vs. bacteria (both have same effect on blood pressure and respiration)	Active vs. rickettsia
	Antitubercular in vitro vs. 607 stasis in mg % (58)			16	1/16
$H_2N\text{-}CS\text{-}NH\text{-}X$	Antithyroid (7)			+	++
$Me_3\overset{+}{N}\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CO\text{-}X$	Parasympathomimetic (117,20)			active	active
	vs. E. coli in vitro Sulfadiazine = 1 (8)			1/125	1/9
	Analgesic (56)		active	inactive	-

TABLE 4 (Cont.)

CLASS I

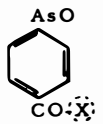
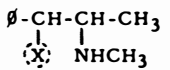
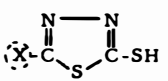
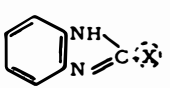
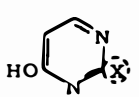
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	vs. T. pallidum in vitro ØAsO = 100 (40)		7	45	
	Pressor CNS stimulation (93)		1 ++	1/3 +	
	Antithyroid Thiouracil = 100 (26, 108)	7		156	
	Ditto	116		6	
	Antithyroid (7)	active	inactive		

TABLE 4 (Cont.)

CLASS 1

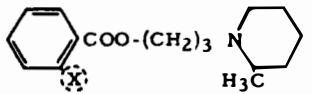
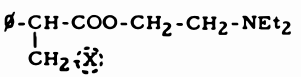
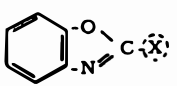
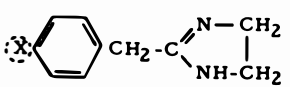
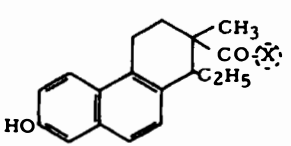
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	Local anesthesia (107)	inactive	active		
	Antispasmodic vs. Acetylcholine Atropine = 1 (21, 63)		1/7		1/10
	Anticonvulsant (25)		inactive		++++
	Effect on blood pressure (130)		pressor		depressor
	Estrogenic (115)		active		inactive

TABLE 4 (Cont.)

CLASS 1

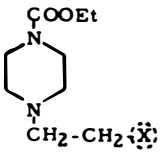
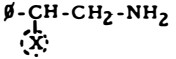
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	vs. Filariasis (139)		inactive		active
	Pressor (66)		1		1

TABLE 5

CLASS 2

Type R-X-R

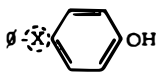
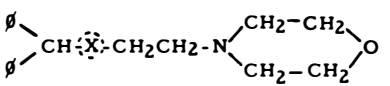
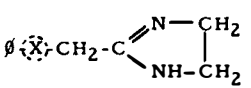
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Phenol Coefficient vs. Staph. aureus (140)	S 100 Se 100	40	10	100
	Antitubercular Salicylate No. (16)		40	2	160
	Antihistamine (17, 97, 127)	weak ?	active	very weak	active
	Blood pressure effect (130)		pressor	pressor	depressor
	Trypanocidal (6)	±	+++	+	++

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

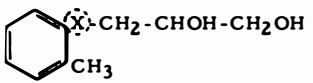
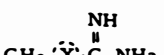
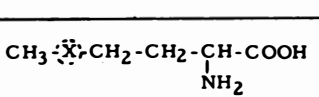
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Muscle relaxant Ø-O-CH_2-CHOH-CH_2OH = 1 (99)	1.3	2.2	0.3	
	Pressor effect (51)	++	+	+	+
	Narcosis (56)	inactive	narcotic	inactive ?	narcotic
	Local anesthesia (75,118)	inactive	active	active	
	Lipotropic activity (133)	active	active		

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

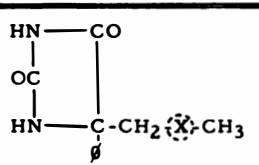
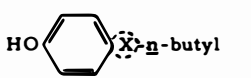
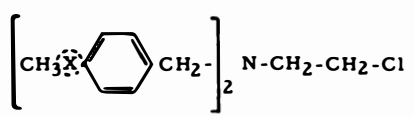
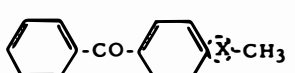
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Antivonvulsant vs. electroshock (98)	+++	+++		++++
	Estrogenic (135, 106)		100 mg.		100 mg.
	Phenol coefficient vs. Staph. aureus (140)	60	9		20
	Adrenergic blocking (119)		++		inactive
	Antitubercular stasis in mg % (57)		1.7		20

TABLE 5 (Cont.)

CLASS 2

Type R-X-P

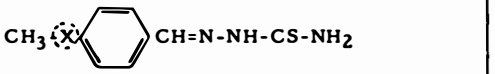
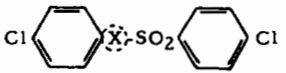
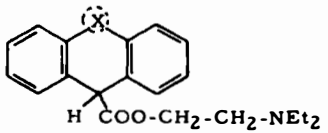
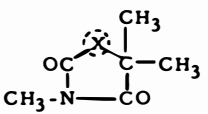
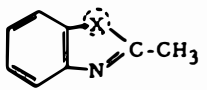
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Antitubercular in mice (74)	slight	active	slight	
	Insecticidal (88)		active	inert	
	Antispasmodic vs. acetylcholine Atropine = 1 vs. Ba Papaverine = 1 (22)	1/45 3/4	1/3 3	1/21 3/4	1/35 1.5
	Anticonvulsant (136)	inactive	active	inactive	
	Anticonvulsant (25)	very little	active		

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

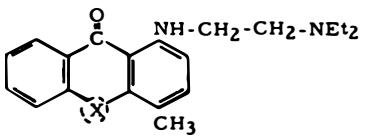
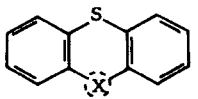
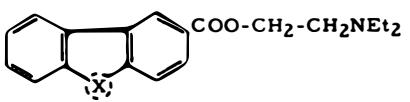
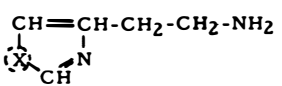
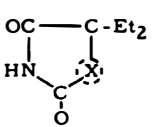
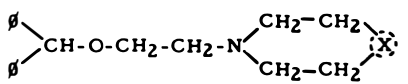
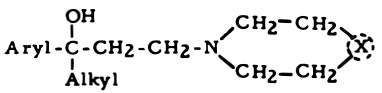
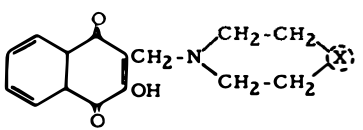
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Schistosomiasis in humans in mice (12)	 active active	 inactive active		
	Insecticidal (113)		active	active	
	Local anesthesia rabbit cornea (23)	 +	 ++	 +++	
	Effect on blood pressure (49)	 depressor		 depressor	
	Narcotic (47)	++	+		

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

Compound	Biological Test (Reference)	S	O	NH	CH_2
	Antihistamine Benadryl = 1 (97)	weak ?	1/2	inactive	1
	Antispasmodic (37)		+		++
	Antimalarial in ducks (54)		inactive		active

Aromatic rings are treated under "Special Cases".

TABLE 6

CLASS 2

Type $R-\overset{\overset{X}{\|}}{C}-R$

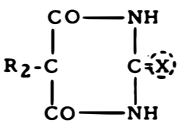
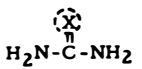
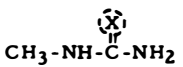
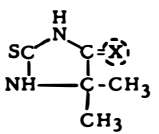
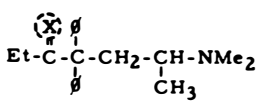
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Hypnotic effect duration (32, 141, 142, 56, 116)	 ++ +	 + ++	 inactive	 weak
	Diuretic (96)	1	1	(toxic)	
	Ditto (96)		0.8	13.4	
	Anticonvulsant % protection from metrazol (68)	 100%	 44%	 0	
	Analgesic mg/kg (29)		12.5	12.5	

TABLE 6 (Cont.)

CLASS 2

Type R-C(=X)-R

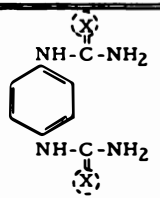
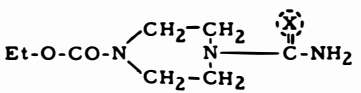
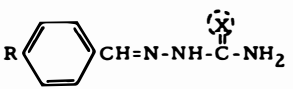
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Trypanosomiasis (71)		inactive	active	
	Filariasis (86)		inactive	active	
	Antitubercular in mice (74)	active	inactive	inactive	

TABLE 7

CLASS 2

Type R-CO-X-R

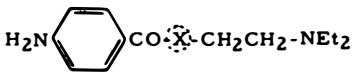
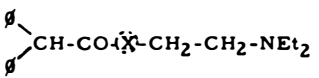
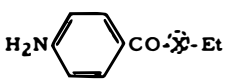
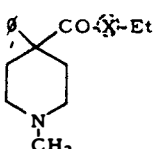
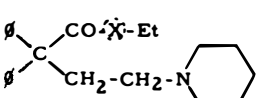
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Local anesthesia (118, 65)	active	active	weak?	
	Antispasmodic vs. acetylcholine Trasentin = 1 (21, 110, 124)	4	1	7/10	
	Antitubercular salicylate no. (16)		40	1	80
	Analgesic (84)		1		1
	Analgesic (84)		1		1/5

TABLE 8

CLASS 3

Aromatic Rings

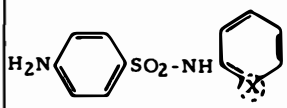
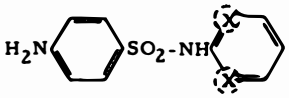
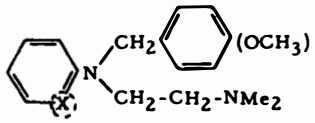
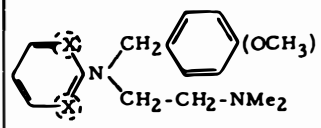
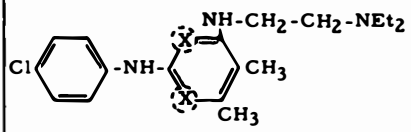
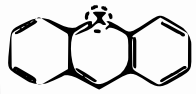
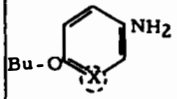
Compound	Biological Test (Reference)	=CH-	=N-
	Antibacterial (8, 120)	moderate	active
	Ditto	moderate	very active
	Antihistamine (148, 78, 80, 59)	active	active
	Ditto	active	active
	Antimalarial (102)	inactive	active
	Antispasmodic vs. acetylcholine Atropine = 100 (92)	15	5
	Antitubercular vs. 607 mg. % stasis (52)	1/16	1/32

TABLE 8 (Cont.)

CLASS 3

Aromatic Rings

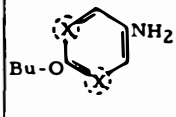
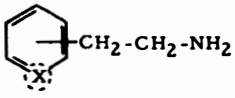
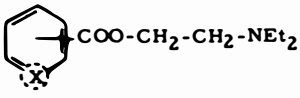
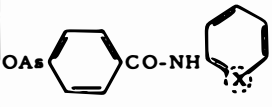
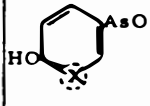
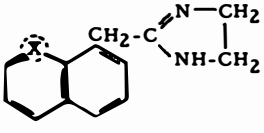
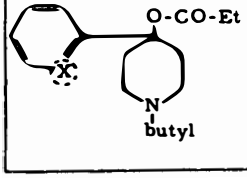
Compound	Biological Test (Reference)	=CH-	=N-
	Antitubercular vs. 607 mg. % stasis (52)	1/16	1/16
	Effect on blood pressure (66, 77, 60)	pressor	α depressor β pressor γ pressor (weak)
	Local anesthesia (30)	active	α inactive β active (weak) γ inactive
	vs. T. pallidum in vitro ØAsO = 100 (41)	97	74
	Syphilis (56, 123)	active	active
	Effect on blood pressure (130)	pressor	depressor
	Analgesic (125)	strong	weak

TABLE 8 (Cont.)

CLASS 3

Aromatic Rings

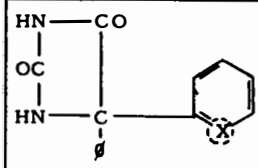
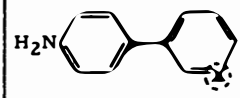
Compound	Biological Test (Reference)	=CH-	=N-
	Anticonvulsant vs. electroshock (98)	++++	++++
	Antitubercular salicylate no. (44)	1600	600

TABLE 9

CLASS 3

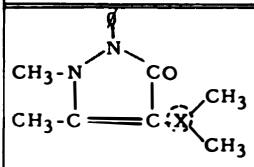
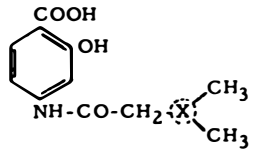
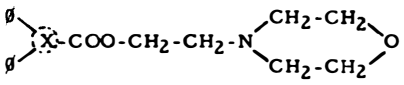
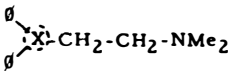
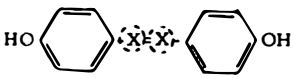
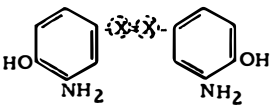
Compound	Biological Test (Reference)	=CH-	=N
	Antipyretic (50, 121)	+	+
	Antitubercular stasis at mol. conc. (72)	1/1200	1/160
	Antispasmodic intestinal strip Papaverine = 100 (28)	75	30
	Antihistamine Benadryl = 1 (126, 104)	1	1.25
	Estrogenic (131, 135)	10 mg.	10 mg.
	Syphilis (149)	Sb unstable	As Salvarsan

TABLE 10

CLASS 4

Compound	Biological Test (Reference)	C	N	S	P	As	Sb
$Me_{3,4}\overset{+}{X}$	Muscarine-like (depressor) (76)		+++	++	++	+	+
$Me_{2,3}\overset{+}{X}\text{-}CH_2\text{-}CH_2\text{-}OAc$	Depressor Acetylcholine = 100% (151, 122)		100	10	10	2	
$Me_3\overset{+}{X}\text{-}CH_2\text{-}CH_2\text{-}OH$	Lipotropic activity Methyl donor (150)		+ yes			+ no	
$Me_3\overset{+}{X}\text{-}CH_2\text{-}CH_2\text{-}OAc$	Rate of hydrolysis by cholinesterase (2)	fast	fast				

TABLE 11

SPECIAL CLASS

Aromatic C=C, S

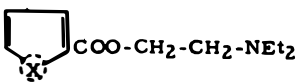
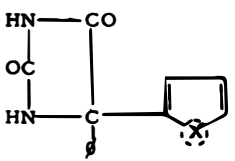
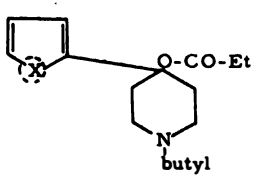
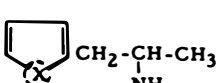
Compound	Biological Test (Reference)	-HC=CH-	S	O	NH
	Local anesthesia Cocaine = 10 (62)	4	1	trace	2
	Anticonvulsant (98)	++++	++++		+
	Analgesic (125)	strong	weak	weak	
	Pressor in dogs stimulatory in humans (4)	++ +	++ 0	+ 0	
	Anticonvulsant Electroshock Metrazole (136)	 +++ ++	 + 0	 0 0	

TABLE 11 (Cont.)

SPECIAL CLASS

Aromatic C = C, S

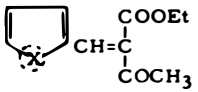
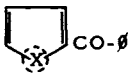
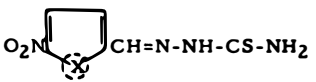
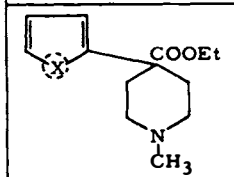
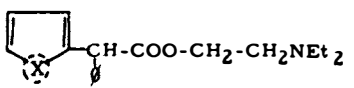
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Insecticidal vs. clothes moth (88)	excellent		excellent	
	Antitubercular mg. % stasis (57)	5		10	
	Antitubercular in mice (74)	active		inactive	
	Analgesic (13)	++		+	
	Antispasmodic vs. acetylcholine Atropine = 1 (15, 53)	1/40	1/20		

TABLE II (Cont.)

SPECIAL CLASSES

Aromatic C= C, S

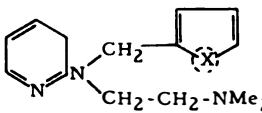
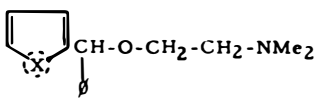
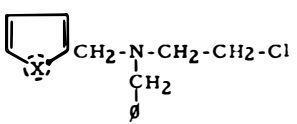
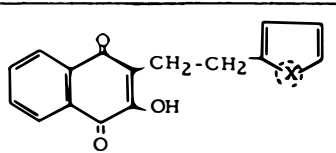
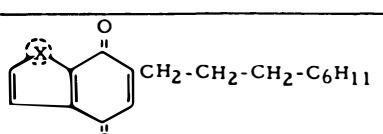
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Antihistamine, ileum PBZ = 1 (104, 80, 89, 11)	1	1	1	
	Ditto Benadryl = 1 (97, 104, 11)	1	1/4	ca. 2	
	Adrenergic blocking (119)	++	++		
	Antimalarial in ducks ED_{95} in mg/kg (54)	>100	85		
	Ditto (54)	20	weak		

TABLE 11 (Cont.)

SPECIAL CLASSES

Aromatic C = C, S

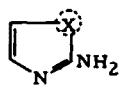
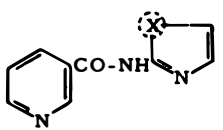
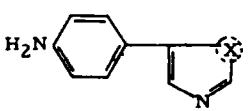
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Antithyroid (132)	0	++		
	Antitubercular ++ corresponds to 50% of streptomycin activity (87)	0	++		
	Analeptic (48)	+	+		
	Antitubercular salicylate No. (44)	600	2,000		
	Antibacterial (120)	inactive ?	weak		

TABLE 11 (Cont.)

SPECIAL CLASSES

Aromatic C = C, S

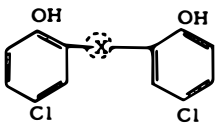
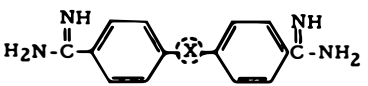
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Fungicidal (105)	++	++		
	Trypanocidal mice (6)	+++	+	+++	++
	Estrogenic dose in mg. (135, 106)	10		100	

TABLE 12

SPECIAL CLASS

CO, SO, SO_2

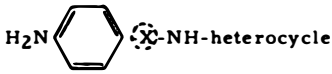
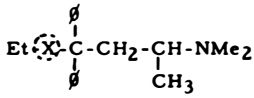
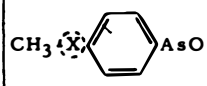
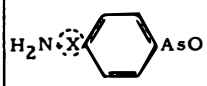
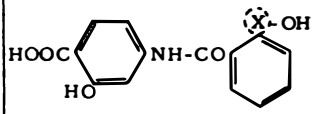
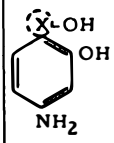
Compound	Biological Test (Reference)	CO	SO	SO_2
	Insecticidal vs. clothes moth (88)	inactive	active	active
	Antibacterial vs. pneumococcus (120)	inactive	weak	active
	Antibacterial (83, 120)	inactive		active
	Analgesic Amidone = 100 (42)	100		120
	vs. T. pallidum ØAsO = 100 (39)	42		49
 HO" " Me_2N" "	vs. T. pallidum ØAsO = 100 (41)	45 7 8		29 3 112
	Antitubercular in vitro (72)	active		inactive
	Ditto (94)	active		inactive

TABLE 13

SPECIAL CLASS

-COO-, CH_2-CH_2

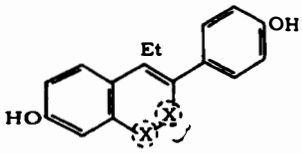
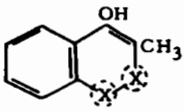
Compound	Biological Test (Reference)	-CO-O	-CH_2-CH_2-
	Estrogenic (112)	100 gamma 	100 gamma
	Vitamin K activity (112)	active 	active

TABLE 14

SPECIAL CLASS

Spatial Fixation by Ring or Double Bond

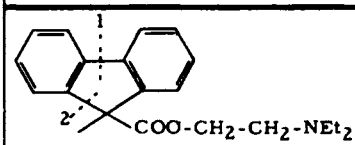
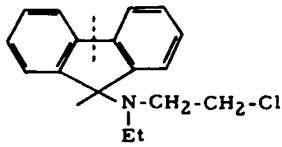
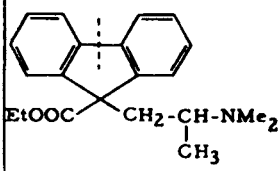
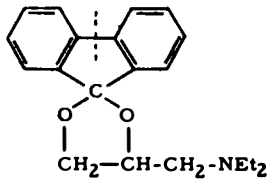
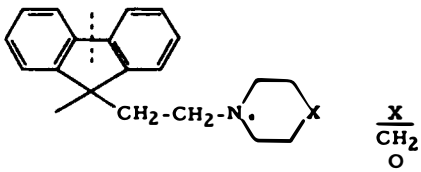
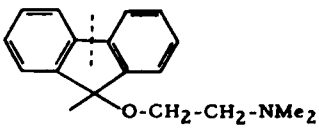
Compound	Biological Test (Reference)	Open Form	Ring Form
	Antispasmodic vs. acetylcholine Atropine = 100 (22,92)	1 2.3 2 0.7	14
	Adrenergic blocking (119)	++	+++
	Analgesia (134)	strong	weak
	Antispasmodic vs. acetylcholine (19)	active	weak
	Antispasmodic vs. acetylcholine, Ba, histamine (84,17)	active	less active
	Antihistamine (126)	active	inactive

TABLE 14 (Cont.)

SPECIAL CLASS

Spatial Fixation by Ring or Double Bond

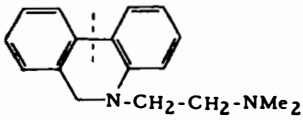
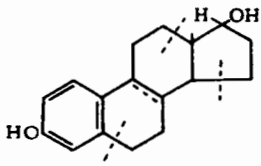
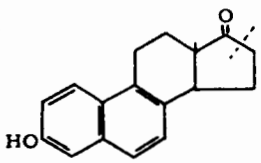
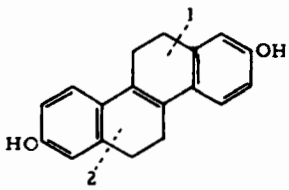
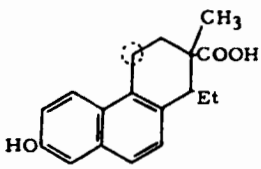
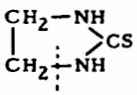
Compound	Biological Test (Reference)	Open Form	Ring Form
	Antihistamine (79)	active	inactive
	Estrogenic (31)	1/40,000 of stilbestrol	active
	Estrogenic in gammas (115)	0.4	1
	Estrogenic (135)	1 ++ 1 and 2 +++	+
	Estrogenic in gammas (115)	0.5	0.4
	Antithyroid Thiouracil = 100 (26)	35	63

TABLE 14 (Cont.)

SPECIAL CLASSES

Spatial Fixation by Ring or Double Bond

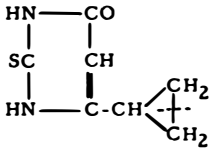
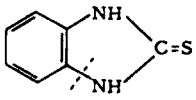
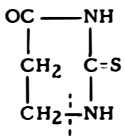
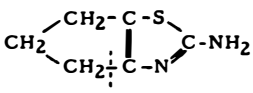
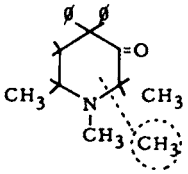
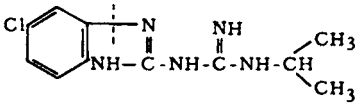
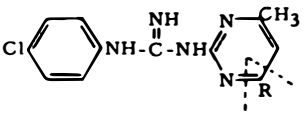
Compound	Biological Test (Reference)	Open Form	Ring Form
	Antithyroid Thiouracil = 100 (137)	100	100
	Antithyroid Thiouracil = 100 (109)	14	116
	Ditto (109)	14	10
	Effect on blood pressure (45)	depressor	pressor
	Analgesic (14)	active (Amidone)	inactive
	Antimalarial (1)	active (Paludrine)	inactive
	Antimalarial (34)	+++	++

TABLE 14 (Cont.)

SPECIAL CLASS

Spatial Fixation by Ring or Double Bond

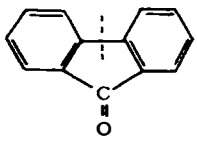
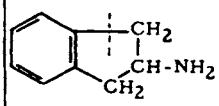
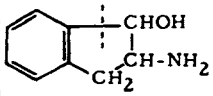
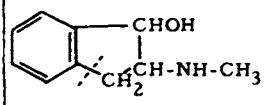
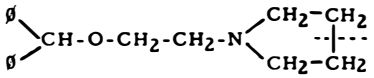
Compound	Biological Test (Reference)	Open Form	Ring Form
	Antitubercular (57)	active	inactive
	Sympathomimetic Vasoconstrictor Pressor (66)	+ weak	+ weak
	Ditto Vasoconstrictor Pressor (66)	+ +	+ weak
	Bronchodilator (69)	good	good
	Antihistamine Benadryl = 1 (153)	0.1	2

TABLE 15

SPECIAL CLASS

Polarity Shift

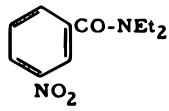
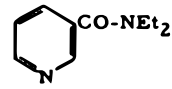
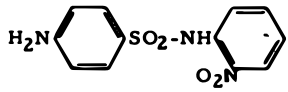
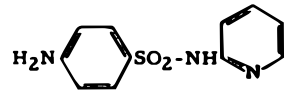
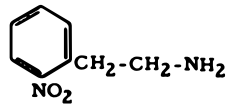
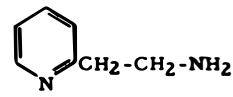
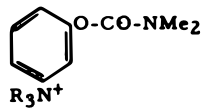
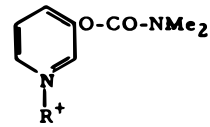
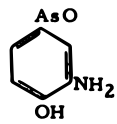
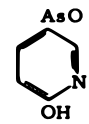
TEST	Exocyclic	Endocyclic
Analeptic activity (46)	 Active	 Active
vs. Hem. strep. in mice (43)	 15% greater activity than Sulfanilamide	 (Sulfapyridine)
Histamine-like activity (90)	 None (pressor compound)	 Weak (0.02 x histamine)
Parasympathomimetic (154, 73, 3)	 Active	 Active
vs. Syphilis (123)	 Active	 Active

TABLE 15 (Cont.)

SPECIAL CLASS

Polarity Shift

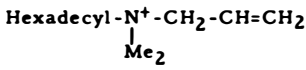
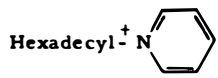
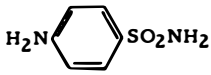
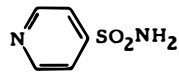
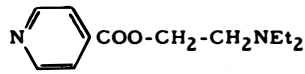
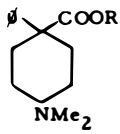
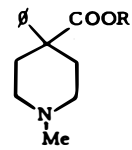
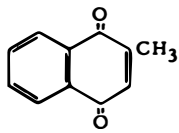
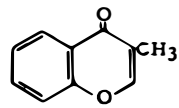
TEST	Exocyclic	Endocyclic
Antibacterial vs. Staph. aureus		
(146)	1/25,000	1/25,000
Antibacterial		
(145)	Active	Inactive?
Local anesthetic		
(30)	Active	Inactive
Analgesic		
(129)	Weak	Strong
Vit. K. activity		
(114)	Potent	Weak, but active

TABLE 16

SPECIAL CLASS

Reversed Polarity

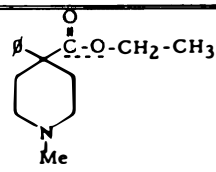
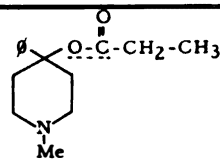
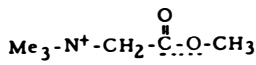
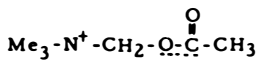
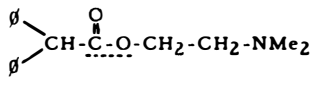
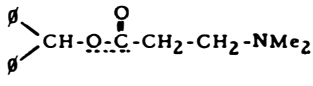
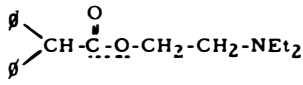
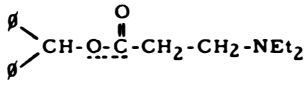
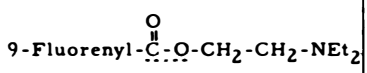
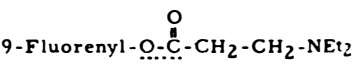
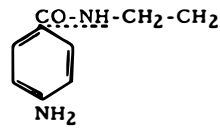
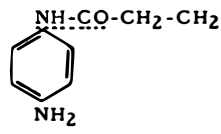
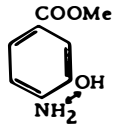
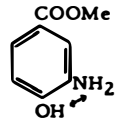
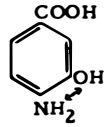
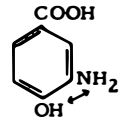
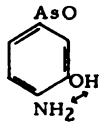
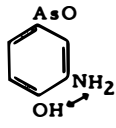
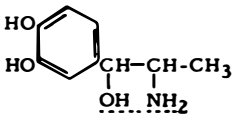
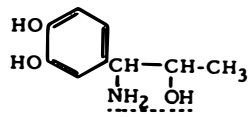
TEST		
Analgesic (82, 55)	 Demerol	 30 x Demerol
Choline-like (Muscarinic) Acetylcholine = +++ (33)	 (Betaine ester) ++	 (Acetyl - formocholine) ++
Antispasmodic vs. Histamine vs. Acetylcholine (5)	 very weak moderate	 very weak moderate
Antispasmodic (35)	 (Trasentin)	 Activity?
Ditto (35)	 (Pavatrine)	 Activity?
Local anesthetic (61)	 weak?	 Activity?

TABLE 16 (Cont.)

SPECIAL CLASS

Reversed Polarity

TEST		
Local anesthetic (56)	 (Orthoform) Active	 (Orthoform new) Active
Antitubercular molar conc. for stasis (72)	 1/200	 1/200
vs. T. pallidum in vitro ØAsO = 100 (40)	 40	 38
Pressor (85,66)	 1/12 epi	 Inactive

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DISCUSSION

DR. D. W. WOOLLEY (Rockefeller Institute for Medical Research, New York, New York): This question of isosterism is one I have given considerable thought to, and I would like to tell you how I look at the matter. As Dr. Friedman pointed out, Erlenmeyer gave great impetus to this study, with investigations in the early thirties. He came against a blank wall, though, when the isostere of thiamine was made; that is, a thiazole ring in the vitamin was replaced by a pyridine ring and, contrary to what had been expected (that is, that it would have thiamine activity), it was an exceedingly powerful antagonist; it had the exactly opposite effect from thiamine. This was one of the serious challenges to the idea of isosteric replacement giving rise to biological materials of the same potency, and it still is.

The confusion which seems to reign in the field, and to which Dr. Friedman referred, resides partly in this fact. If in a drug, one makes isosteric replacements, such as the exchange of a pyridine ring for a thiazole, one may obtain a compound of similar qualitative action, although in all respects the two may not be identical. Actual experience showed that drugs of similar activity could be found by making such isosteric changes in existing drugs. This may have been because various members of a series of antimetabolites were being made. If, however, the limit is exceeded and the metabolite rather than the analog is made, an entirely different situation arises.

Among pharmacologists and others as well, hormones have been considered as drugs. Choline, for instance, for years, was thought of as just a synthetic drug, until it was isolated from tissues and gradually shown to be an essential metabolite. The case of adrenalin sometimes can be viewed in the same way. The confusion of drugs with metabolites was therefore profound with the hormones. With the vitamins, however, the matter can be seen more clearly. Because the vitamins are essentially nontoxic, enormous doses can be given to normal animals with no detectable effect. This is not true of many of the hormones.

If in a vitamin various isosteric replacements are introduced, an antagonist, frequently a very powerful one, which has exactly the opposite effect to that of the vitamin, may result. The same is true with many amino acids. With this in mind, we can regard some of the groups of isosteric drugs as being mere families of antimetabolites. If the real metabolites to which they may be related are tested, then there should be no surprise if their activity is quite the opposite of the series to which they are isosteric.

I think, in coming down to the practical matter of thinking about synthetizing useful agents, we must never lose sight of this fact that, if we start with the biologically active compound (that is, the metabolite), it is an entirely different problem than if we start with the product in the laboratory; to wit, an already existing analog. Isosteric replacement may have quite opposite effects in these two situations.

DR. FRIEDMAN: This may be an erroneous way of looking at it, but I always consider that an antimetabolite really has the same mechanism of action as the metabolite. Thinking in a pictorial manner, both the metabolite and its isostere start with the same mode of action: the isostere is hooked into the system in the same place as the metabolite would have been. But the rest of the mechanism beyond this stage is blocked when the antimetabolite is present and thus growth, or whatever consequence is involved, is prevented.

PANEL DISCUSSION

ON

ANTIMETABOLITES, CARCINOGENESIS AND CANCER CHEMOTHERAPY

M. J. Shear, Moderator

National Cancer Institute
National Institutes of Health
Bethesda, Maryland

ANTIMETABOLITES

D. W. Woolley
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New York, New York

Mr. Chairman, I feel I have used up my five minutes already. To keep within the time limit, I think we can dispense with most of the slides. I believe it is needless to say to you all that antimetabolites exist, or to show you the structure of sulfanilamide, p-aminobenzoic acid, and so forth; I believe you all know those. The basic phenomenon, it should be recalled, is that, if one takes a vitamin - it has been done with all the water-soluble ones and some of the fat-soluble ones, too - or an amino acid, and in some cases a hormone or a purine, any one of a number of metabolically essential compounds, and if one modifies its structure in some small way, replacing an atom by some other atom or replacing a group by some other group, one frequently obtains a substance which produces, in various kinds of living things, the signs of deficiency of that metabolite.

Frequently, one can overcome the toxicity attendant on such a demonstration, and nullify the effect by giving the structurally related metabolite. This has been done with bacteria and with viruses; it has been done in animals; it has even been done, in a few cases, in man.

This idea arose some fifteeen or twenty years ago among enzymologists, and received great impetus by the discovery that a clinically useful agent, sulfanilamide, was of this type: that is, its action was overcome by a structurally related, metabolically important substance, p-aminobenzoic acid. Many people thought it would be easy to reverse the idea and make new bactericidal and therapeutic agents, by alteration of the structure of other metabolites. In general, this has not proven to be so. There have been far more failures than successes. One just cannot select at random some amino acid or some vitamin and alter its structure in such a way as to produce an anti-metabolite, and thus arrive at a fairly useful therapeutic agent.

One of the problems before us this afternoon, is how to arrive at therapeutically active agents. It has therefore seemed to me that one of the ways we should approach this problem is to try to understand selective toxicity.

As you know, sulfanilamide derivatives are essentially nontoxic to higher animals, yet are quite harmful to many pathogenic bacteria. I think we should inquire why this is so, because I believe any attempt, other than those based on chance, to make therapeutic agents will have to delve into the reasons why such selective effects are obtained. In the case of the sulfanilamide drugs, something is known as to why they have this selective effect. Why don't they kill animals as well as bacteria? From the work of Woods and from that of Miller, it is clear that p-aminobenzoic acid is used as a substrate from which, by a series of reactions, the living cell synthesizes folic acid. Practically all of the pathogenic bacteria carry out this reaction very well; that is, they produce their own p-aminobenzoic acid, and they metabolize that further and produce folic acid. Higher animals, however, apparently do not do this, at least not at a rate sufficiently fast to meet their needs.

Woods has also shown that sulfanilamide inhibits this enzymic synthesis of folic acid from p-aminobenzoic acid.

One cannot inhibit the synthetic reaction in animals, because they do not possess it; hence, they go largely unharmed, but the bacteria do possess this reaction; they depend on it for multiplication and, when the reaction is inhibited, their growth (multiplication) is inhibited.

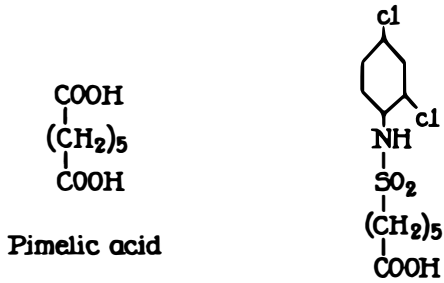
So I think the basis of selectivity, one of the reasons why the sulfonamide drugs are selective, is the difference in their nutritional requirements for folic acid.

We can test this hypothesis by applying it to some other metabolic system.

If we choose a vitamin for which we know a biological precursor, then we can make an analog of that precursor and should be able to show that it is poisonous to those species which synthesize the vitamin, and not poisonous to those which depend on their diets and do not synthesize it.

If I might have the last three slides, please.

We chose biotin because it is a vitamin which is required by animals, and not required by certain pathogenic bacteria, such as the tubercle bacillus.



Slide 1

It fits our needs beautifully. We also chose it because it had previously been known that pimelic acid was probably a precursor from which it was formed in vivo. Therefore, the problem was to make analogs of pimelic acid. This could easily be done. Because of the experience accumulated over the past decade, a rather good guess can be made as to how the structure of a metabolite should be changed in order to form an anti-metabolite. The compound shown in the first slide was the first one tried; it is the dichloro-sulfanilide corresponding to pimelic acid, and it has the selective activity envisioned for it.

Parenthetically, if one wishes to correlate activity and structure, this analog presents an interesting case. If this compound had been found accidentally and its activity had been observed, without knowledge of how it was brought about, the ring system would have attracted attention, because rings are rather attractive to manufacturers of drugs. The alkyl side chain might have been pictured as something attached to make the compound soluble, or otherwise to improve its properties. Actually, however, it is quite the other way. The ring exists as part of the structural alteration which makes this compound an antimetabolite. The structural analogy lies in the side chain. Among the antihistamines, this same consideration can sometimes be seen. For example, in phenergan, the phenothiazine ring is part of the structural alteration of histamine; the part which bears the structural analogy is the side chain.

In the case of the pimelic acid analog, it was found that, by moving the halogens to different positions around the ring or by using bromine atoms instead of chlorines, activity could be markedly enhanced or diminished.

The second slide will show some of the effects of this analog on Bacillus tenuis. The addition of the sulfanilide inhibited growth, and the effect was overcome by pimelic acid or biotin.

Inhibition of Growth of B. tenuis Cultured in Flasks by ε-(2,4-Dichlorosulfanilido)-caproic Acid and Its Reversal by Pimelic Acid or Biotin

Analogue	Biotin	Pimelic acid	Turbidity
γ per cc.	γ per cc.	γ per cc	
0	0	0	70
20	0	0	60
50	0	0	82
100	0	0	98
100	0.0005	0	66
100	0	3	70

Slide 2

The antagonism with pimelic acid was competitive whereas that with biotin was noncompetitive. If an amount of biotin which would meet the needs of growth was present, then no amount of the analog was poisonous until a very high concentration was reached (2 mg. per cc.).

As predicted, this compound was nonpoisonous to mice, a species which requires a dietary source of biotin; 10 mg. per day injected into a mouse caused no detectable effect.

In the third slide a number of micro-organisms are shown along with their susceptibility to this analog. Except for E. coli, which does not fit the prediction, the ones which require biotin are not affected by the analog; the ones which make their own are.

Correlation of Toxicity of ε-(2,4-Dichlorosulfanilido)-caproic Acid with Nutritional Needs for Biotin

Organism	Analogue needed for half maximal inhibition	Nutritional requirement for biotin
	γ per cc.	
Bacillus tenuis	58*	Not required, but stimulatory under certain conditions
Acetobacter suboxydans	350	Not required, but somewhat stimulatory
Mycobacterium tuberculosis H37Rv	20†	Not required
Escherichia coli	No effect at 1000	" "
" " *biotinless*	" " " 1000	Required
Proteus strain 4	" " " 1000	"
Staphylococcus aureus	" " " 300	"
Lactobacillus casei	" " " 500	"
" *arabinosus*	" " " 500	"
Leuconostoc mesenteroides	" " " 500	"
Hemolytic streptococcus H69D	" " " 500	"
Saccharomyces cerevisiae	" " " 1000	"

* When cultured in test-tubes, a value of 20 was uniformly found.
† Incubation time 4 days.

Slide 3

This is a laboratory model to think about in trying to visualize one way in which selectively active agents can be made. This is undoubtedly not the only basis on which to proceed; it is merely one.

CHEMICAL-BIOLOGICAL CORRELATION IN THE FIELD OF CARCINOGENESIS

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The Weizmann Institute of Science
Rehovoth, Israel

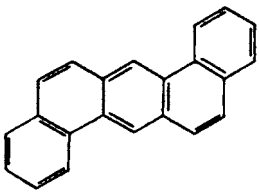
Chemical carcinogenesis became an established science when Kennaway, Cook and their associates, isolated and identified 3,4-benzpyrene as a potent carcinogenic constituent of coal-tar.[9] Previous tests of a wide range of compounds had already demonstrated that tumors could be artificially induced in mice by repeated applications to the skin of the synthetic 5-ring polycyclic hydrocarbon, 1,2,5,6-dibenzanthracene, though no such action was obtained with anthracene, phenanthrene, carbazole, acridine, chrysene, pyrene, picene, perylene, and many other hydrocarbons and related compounds.

Here, then, was a promising field for the study of the relation between chemical structure and biological activity of a unique kind; and both Kennaway and Cook, in England, and Fieser and Shear, in the United States, were quick to realize the potentialities of this new approach. In less than a decade, prior to the outbreak of World War II, several hundred new compounds were synthesized and tested for carcinogenic activity, including interesting series of homologues, and related derivatives, of certain key compounds, carefully chosen with an eye to the elucidation of the biochemical mechanism of carcinogenesis.

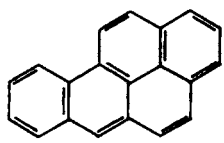
In the short time available to me here, I shall only be able to pick out one or two illustrative examples from this important branch of carcinogenesis, and to deal equally briefly with other important groups of carcinogens of different chemical structures and biological properties, in order that I may devote a little time to a consideration of postulated mechanisms of action and chemical-biological correlations, and to conclusions that may be drawn about the validity of such associations.

Of the fifteen possible 5-ring polycyclic hydrocarbons, twelve were already known at the time these early investigations were in progress, and of these, only two - 1,2,5,6-dibenzanthracene and 3,4-benzpyrene - had been found to be carcinogenic.[2] These two compounds could be considered structurally related, in the sense that the 1,2-benzanthracene configuration was present in both. Since 1,2-benzanthracene itself displayed little or no carcinogenic activity, it seemed reasonable to extend the study to substituted derivatives of this compound.

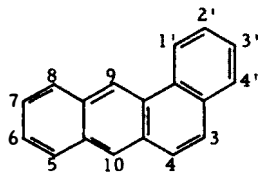
The accumulated data from these studies have been tabulated by Hartwell,[17] and the extensive literature on the subject ably reviewed by Cook and Kennaway,[10,11,12] Fieser,[14,15] and more recently by Badger.[1] The more important conclusions derived from this work may briefly be summarized as follows:



1,2,5,6-dibenzanthracene



3,4-benzpyrene



1,2-benzanthracene

(1) Carcinogenic activity was conferred on the 1,2-benzanthracene molecule by methyl substitution in positions 5, 9 and 10, and somewhat less so in positions 6, 3 and 4, with no activity when substitution was in any other position.

(2) Di- and trimethyl substitution conferred even greater carcinogenic activity, provided again that these occupied the above-mentioned 'active' positions. (Certain anomalous results were obtained, as, for instance, the absence of carcinogenic action by 3,9-dimethyl-1,2-benzanthracene.)

(3) Ethyl and propyl substitutions were also effective, but increasing the length of the side chain beyond a point, led to diminution, and finally to extinction, of carcinogenic activity.

(4) Polar substituents were, on the whole, unfavorable for carcinogenic activity.

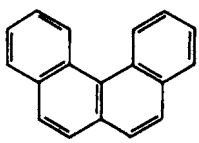
(5) Hydrogenation of any part of the aromatic ring structure usually led to loss of activity, though there were a few exceptions to this.

(6) Substitution of a thiophene or pyridine nucleus for one of the benzene rings of a carcinogenic hydrocarbon, did not seem to interfere with carcinogenic activity, though an oxygen-containing ring destroyed activity.

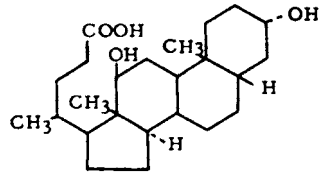
The results, so far, were encouraging, in favor of a close correlation between chemical structure and carcinogenic activity. There were, however, already indications at an early stage, that the 1,2-benzanthracene structure was not an essential prerequisite for carcinogenic activity, since 3,4-benzphenanthrene was found to be carcinogenic.

When Lacassagne[24] demonstrated that mammary tumors could be induced in mice by injections of the ovarian hormone, estrone, both sterol chemistry and endocrinology came within the ambit of experimental carcinogenesis.

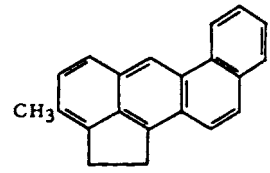
On the basis of, what then seemed, a plausible hypothesis[21,2] - that the body might, under certain conditions, convert sterols into carcinogenic polycyclic hydrocarbons - attempts were made to carry out this transformation synthetically. By cyclization of the side chain, followed by dehydrogenation, bile acids were successfully converted into 20-methylcholanthrene, which was subsequently shown to be highly carcinogenic.



3,4-benzphenanthrene



desoxycholic acid

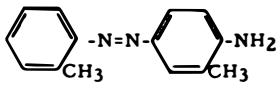


20-methylcholanthrene

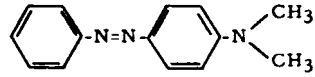
The idea that estrone might act by preliminary conversion into a methylcholanthrene-type of carcinogen, became somewhat weakened when it was found that mammary tumors could also be induced by stilbestrol and other synthetic estrogens of simpler structure. It seems possible, moreover, that the function of estrone in mammary tumor production might be merely to prepare the tissue for subsequent action by a viral or other factor, rather than that it was itself a straightforward carcinogen. At the same time, it is now known that there are sterols in the body that possess weak, though definite carcinogenic activity.[19]

The concept of a correlation between chemical structure and carcinogenic activity was faced with a more serious difficulty when Yoshida[35] demonstrated that liver tumors could be induced in rats and mice by feeding or injections of an azo dye - 2-amino-5-azotoluene.

Kinosita[22] extended this observation to other azo compounds, and showed that p-dimethylamino-azobenzene was even more potent in producing liver tumors in the rat. The work has been followed up by several investigators, using a large group of azo compounds, including isomers, analogues, and split products of those previously shown to be carcinogenic. (The early literature is well reviewed by Shear,[31] while reviews of subsequent work are presented by Kirby,[23] Orr,[29] Cook,[8] Miller and Miller,[25] and others.)



2-amino-5-azotoluene



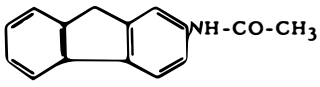
p-dimethylaminoazobenzene

Within the group of azo compounds, as in the case of polycyclic hydrocarbons, it is possible to discern a certain correlation between structure and biological activity. Starting with p-dimethylaminoazobenzene, carcinogenic activity is maintained when one of the two methyl substituents is replaced by hydrogen, or by an ethyl group, but is entirely lost when both methyl groups are replaced by ethyl or other groups. Additional methyl groups in other parts of the molecule also appear to influence carcinogenic activity according to a pattern, though one which is not easy to interpret.

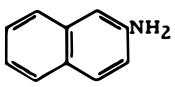
The azo group appears to be important for carcinogenesis, in so far as all the reduced products tested proved to be inactive. (Yet, it was recently found that p-dimethylaminostilbene - where the azo group is replaced by a -CH=CH- group - is also carcinogenic.[16])

Related to the azo group of carcinogens are certain amino compounds, such as β-naphthyl-amine, responsible for tumor production in the urinary bladder,[20] and amino- and acetylamino-fluorene, which induce a variety of tumor types, including those of the liver, breast, external auditory canal, etc.,[14] when these compounds are incorporated in the diet.

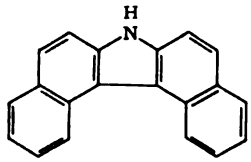
Attempts have been made to find a structural link between these carcinogens of somewhat 'simple' structure and those belonging to polycyclic hydrocarbons, on similar lines to the suggested relation between sterols and polycyclic hydrocarbons. An interesting example of such a postulated conversion is from two molecules of β-naphthylamine to 3,4,5,6-dibenzcarbazole, the latter being known to be carcinogenic.[6,8]



Acetylaminofluorene



β-naphthylamine



3,4,5,6-dibenzcarbazole

There is, as yet, no experimental evidence in support of this hypothesis; the arguments in its favor are: (1) that some of these postulated conversions in vivo can be carried out synthetically, and (2) that the pattern of carcinogenic action by these relatively simple compounds differs from that of polycyclic hydrocarbons, in that in the case of these 'simple carcinogens', tumors do not usually arise at the site of application or injection, but appear in certain specific organs, the distribution being different for the different compounds. This might be expected to occur if the 'simple' carcinogens were really precursors of carcinogens, and that tumors only arose in those organs in which the chemical conversion, from precursor to carcinogen, took place.

The little that is known about the metabolism of carcinogens in the body, is insufficient to confirm or contradict the above hypothesis. (See review by Boyland and Weigert. [7]) Polycyclic hydrocarbons are oxidized in the body to phenolic derivatives, presumably through an intermediate stage of dihydrodihydroxy- derivative, and there is more recent evidence [18] that the oxidation can go further in the body, leading to the break-up of the molecule. The phenolic derivatives, so far isolated, possess little or no carcinogenic activity.

More is known about the metabolism of azo carcinogens. (See Miller and Miller. [25]) Such compounds tend to be demethylated, and also undergo cleavage at the azo linkage. Except for the first stage (demethylation to the monomethyl compound), metabolic changes in the compounds lead to loss of carcinogenic activity.

It will be noted that, so far, it is possible, by fanciful speculation, to correlate chemical structure and carcinogenic activity. But this breaks down completely when one passes on to other types of carcinogens, such as urethane (ethylcarbamate), which produces tumors of the lung, [27] and carbon tetrachloride, which produces tumors of the liver; [13] to say nothing of the claims in the literature of skin tumor production by painting with HCl or NaOH, [26] or sarcomas by injection of glucose, [28] or even by implantation of bakelite disks subcutaneously. [33]

An attempt has been made, in recent years, to find a common feature among carcinogenic compounds, based on physio-chemical criteria. French scientists [30] have been trying to correlate carcinogenic potency of hydrocarbons and related compounds with the electron density at the K region of the molecule. The comparisons are based on quantitative evaluations of the electron densities, and on a quantitative grading of the respective carcinogenic potencies. This, at once, raises the important issue, as to whether carcinogenic potency can be considered as an absolute value.

It will be recalled that the early studies on carcinogenesis were restricted to skin painting in mice, by the English group, and to subcutaneous injections in mice, by the American group. Though the results, in most cases, were remarkably similar by the two methods of assay, this was not so in every case. For instance, 10-methyl-1,2-benzanthracene was found to be highly potent by the subcutaneous route, but relatively weak when tested on the skin. [32] Several other such examples were noted, and in the case of some of the weaker carcinogens, some were active only on the skin, and others, only subcutaneously.

Such differences became very much more accentuated when these studies were extended to other tissues, and to other species of animals. For instance, the mouse and the rabbit behave very differently in their response to carcinogenesis according to the carcinogen used. Tar readily induces skin tumors in both species; but benzpyrene, one of the constituents of tar, is strongly carcinogenic to the skin of the mouse, but only weakly so to the skin of the rabbit. A benzpyrene-free fraction of tar has recently been isolated from tar which behaves in the opposite manner to benzpyrene: it is very strongly carcinogenic to the skin of the rabbit, but not to the skin of the mouse. [4] Several other examples may be quoted, such as the fact that β-naphthylamine produces bladder tumors in the dogs but not in the rabbit; or the fact that 2-amino-5-azotoluene produces liver tumors in mice even more effectively than in rats, whereas p-dimethylaminoazobenzene is very active in rats but hardly at all in mice. Perhaps the most striking example is the behavior of 9,10-dimethyl-1,2-benzanthracene, which is the most potent carcinogen for skin in rabbits and mice, and very active for sarcoma production when injected subcutaneously in mice, but entirely non-carcinogenic by that route in the rabbit.

What, then, is meant by the carcinogenic potency of a compound? Should 9,10-dimethyl-1,2-benzanthracene be considered the most potent of all carcinogens, because it undoubtedly is so when tested on the rabbit's skin, or should it be deemed non-carcinogenic, which it is when tested in the same animal by the subcutaneous route?

Our present-day concept of carcinogenic potency is based on very limited, and arbitrarily chosen, conditions of assay, and there is no doubt that, by extending the tests to other tissues and other species of animals, a very different picture would be obtained.

The problem is even more complex, since it is now known that carcinogenesis is not a single biological process, but comprises at least two independent phases - initiating action and promoting action. [5] Some agents can induce promoting action without being capable of

inducing the initiating process; and it is conceivable that there are other agents that produce the opposite effect. Carcinogens, as we know them, produce both effects, but we cannot say yet whether a relatively weak (over-all) carcinogenic effect is due to deficiency of either one or other, of both effects.

In the light of all this, one cannot avoid the conclusion that the search for a close correlation between chemical structure and carcinogenic activity may be founded on an unjustifiable premise. It is tempting for the scientist to look for correlations, since without them, mere accumulation of data lead to confusion and chaos; but he must always beware of seeking relationships that do not, in fact, exist. A whiff of chloroform and a blow on the head with a stick both produce unconsciousness. That does not mean that there is any relationship between the chemical constitution of chloroform and the wood from which the stick is composed.

It is implicit in the argument of a chemical-biological correlation that the chemical agent produces the biological effect by a one-stage process. Where there are many stages, and particularly where some involve alternative routes, no absolute correlation can be expected.

Perhaps the most important outcome of the work on chemical carcinogenesis, and of the failure to find a close over-all correlation between chemical structure and carcinogenic activity, is the indication it affords that carcinogenesis is a more complex phenomenon than has hitherto been imagined.

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A CONSIDERATION OF CHEMICAL-BIOLOGICAL CORRELATION IN EXPERIMENTAL CANCER CHEMOTHERAPY

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In a brief introduction to a discussion on aspects of chemical-biological correlation in experimental cancer chemotherapy, there is time to do little more than highlight some of the observations in the hope that others will be stimulated to discuss more extensively the questions presented. When the symposium was being arranged, we expressed doubt that experimental cancer chemotherapy would at this time be a suitable part of a program on chemical-biological correlation; however, it may be worthwhile to review the present status of the field even though from published data only a few generalizations may be made with respect to correlations of chemical structure with antitumor activity.

The introductory remarks have been collected under the title "A Consideration of Chemical-Biological Correlation in Experimental Cancer Chemotherapy", rather than a more imposing one such as "The Correlation of Anti-Tumor activity with Chemical Structure". This choice of title reflects the present status of experimental chemotherapy. While it has advanced considerably during the past fifteen years, there still is a paucity of active compounds and a complete lack of adequately effective substances. With only a few exceptions,[32,6,35,43] there is an acute lack of collected data of a uniform nature on sufficiently large series of compounds to supply the basis for correlations. Many of the existing data have not been adequately covered in publications and may offer unknown possibilities for correlation. In the evaluation of data from various sources it must be recognized that the results from one search for anti-tumor activity cannot *a priori* be combined with data from another study in which different experimental tumors, different test conditions or differences in both have been employed. This would appear to be emphasizing the obvious but experience has indicated the need to point out the possible differences in effects observed with different tumors. Table 1 shows the differences in inhibition of the development of certain tumors by several compounds of various types. Table 2 illustrates differences in the response of several strains of mouse leukemia to selected materials.

Many of the general factors concerned with any chemotherapy study apply equally well to experimental cancer chemotherapy studies.[22,42,37] Thus, the choice of the tumor and test conditions in a chemotherapy screening study may be made in such a manner as to permit the discovery of activity with many materials or none at all. This is suggested by the data in Table 1 and by other observations. The rat tumors, for example, have appeared more susceptible to the inhibiting action of the nitrogen mustards than have the most susceptible mouse tumors in the spectrum of tumors examined.[49] If the ultimate goal of an experimental cancer chemotherapy program is the selection of materials with an adequate, differential adverse effect upon abnormal tissue under conditions of practical use, the ideal procedure would appear to be that which would reveal leads for selection of compounds for study in addition to pointing out the more effective compounds worthy of clinical trial. A program of this nature need not exclude the intelligent employment of *in vitro* techniques, such as tissue culture, which yield data within the limitations of the procedure.

It is appropriate that the discussion on experimental cancer chemotherapy be held jointly with those on carcinogenesis and anti-metabolites. Inhibition of the growth of tumors by carcinogenic substances has been noted and proposed as a possible basis for the therapy of cancer.[18,2] The category of antimetabolites includes many compounds of diverse structures which are considered to act by blocking the utilization of the structurally related normal metabolites.[52] This represents one of the most interesting and fruitful approaches in cancer chemotherapy studies, as illustrated by recent studies with desoxypyridoxine,[44]

TABLE 1

DIFFERENCES IN THE INHIBITION OF VARIOUS TUMORS BY SELECTED COMPOUNDS

Compound Name	Dose mg/kg/day	Mouse Tumors					Rat Tumors		
		Sarcoma 180	EO 771	HP Melanoma	Wagner Osteogenic Sarcoma	Patterson Lympho-Sarcoma	Sarcoma 39	F-J Carcinoma	Walker 256
8-Azaguanine	75	-	++	-	-	-	-	-	-
2,4,6-Tris(ethylenimino)-s-triazine	0.25	±	-	-	+	-	+++	+++	+++
Aminopterin	0.25	++	+	±	-	++	+++	-	+++
Methoxypyridoxyl bis(β-chlor-ethyl)amine	5.0	±	±	-	++	±	+++	+++	+++

Grading of Tumor Inhibition:

- \- Growth more than 3/4 the average diameter of the controls
- ± Growth from 1/2 to 3/4 the average diameter of the controls
- \+ Growth from 1/4 to 1/2 the average diameter of the controls
- ++ No growth or growth to 1/4 average diameter of the controls
- +++ Destruction of tumor

TABLE 2

CHEMOTHERAPEUTIC ACTIVITY OF COMPOUNDS

AGAINST DIFFERENT STRAINS OF MOUSE LEUKEMIA

Compound	Representative per cent prolongation in survival time for leukemia strains			
	Ak 1394	Ak 4	F T_{13} line 15	F T_8 line 291
Methyl bis(β-chlor-ethyl)amine	100	40	-	-
Amethopterin	20	150	-	-
Urethane	100	20	100-200	25-50
Potassium arsenite	60	0	300-350	10-40
Benzene	100	0	40-60	0-25

Data derived from Burchenal[4,5,6] and from Kirschbaum.[24]

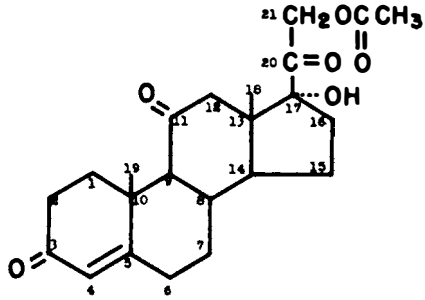
anti-folic acids,[36,10,5,28,31,48,39,27] anti-riboflavins,[45] and the anti-purines, 2,6-diamino-purine,[4] and 8-azaguanine.[23,40,14,47,26] As might have been anticipated, the usefulness of this approach has been limited in one or more instances by the metabolic requirements of the normal cells.[30,51,11,8] Analogs of folic acid, particularly the 4-amino-analogs, have been of interest for studies of their ability to act as antagonists of folic acid in bacteria,[12] and animals.[11,13,29,51,30] Some of the resulting information led to study of these compounds against leukemia,[10,5,36] and solid tumor in animals.[28,31,48,39] We have not observed, as yet, any published correlations of anti-bacterial with anti-tumor activity.

It is to be expected that the anti-metabolite approach will be extended to include other anti-vitamins and anti-metabolites. This is being done in the case of anti-amino acids, with analogs of carbohydrate metabolism and with purines, pyrimidines, and pteridines for possible interference with nucleic acid metabolism.[38] In the latter category, weak tumor inhibitions have been observed which suggest some correlations.[41]

A few general conclusions on anti-tumor activity of other classes of compounds may be drawn from published data. The nitrogen mustards have been studied extensively in leukemia[7] and solid tumors.[16,19,38] It has become evident that most of the nitrogen mustards with two β-chlorethyl groups have been effective, whereas those with only a single group have been inactive. The presence of two reactive groups in compounds such as the nitrogen mustards and other alkylating agents has been postulated as a requirement for anti-tumor activity based upon the mechanism of chromosome damage;[15,19] however, there is evidence that this postulate may not be well founded.[1] In contrast to the general effectiveness of the bis-β-chlorethyl amines against susceptible tumors are the data on carbamates. Against Sarcoma 180[3] and mouse leukemia[6,35] the activity of urethane has been unique among the carbamates and thiocarbamates thus far tested. With respect to inhibition of another tumor, Walker carcinosarcoma 256,[20] and to other biological effects, such as hypnosis,[17] carcinogenesis,[25] leukopenic action,[34] and mitotic inhibition,[9] the carbamates are more generally active.

Preliminary reports of the extent of activity have been made by Shear and his associates[21,33] for various groups of compounds, including arsenicals, acridines, diphenyl ethyl amines, and podophyllin and colchicine derivatives. Further reports on correlations in these studies may be made in this symposium.

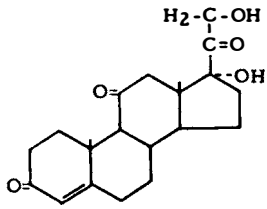
Recently we have had an opportunity to see relationships in the structure of certain steroids and their ability to inhibit the development of transplanted mouse lymphosarcomas.[50] Figure 1 shows the structure of cortisone acetate with the system of numbering indicated for



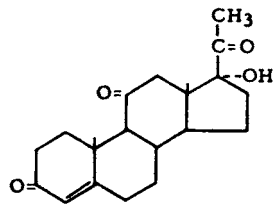
COMPOUND E

Figure 1 11-Dehydro-17-hydroxycorticosterone acetate

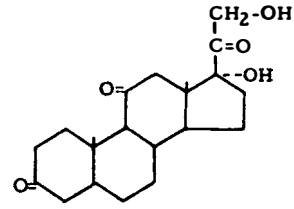
reference in the use of Tables 3 and 4. Table 3 shows the structural relationships of the three steroids, compounds E, F, and A, thus far found active against the lymphosarcoma. Several inactive steroids have been included and 21-desoxycortisone, which remains questionable because limited results available indicate activity with ten times the dose level of cortisone acetate.[53,46] Some of the formulas of the compounds are presented in Figure 2. Table 4 shows a number of steroids which have been tested at five to ten times the dose level of cortisone without apparent effect upon the lymphosarcoma. In addition to the other variations in structure, it is important to note that all of the compounds lack the 11-oxygen function. Thus far the steroids which have inhibited the mouse lymphosarcoma have possessed in common an 11-oxygen group, a 20-keto group and a 3-keto group with the Δ^4 unsaturation. The 21-hydroxy group is important from the quantitative aspect, if it is not essential.



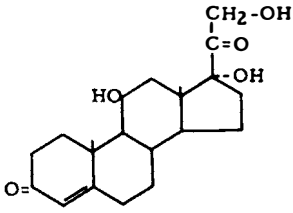
DEHYDRO E
Active



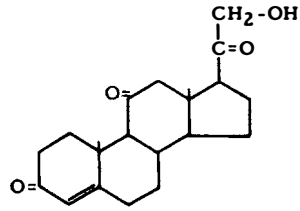
21-DESOXY E
? Active



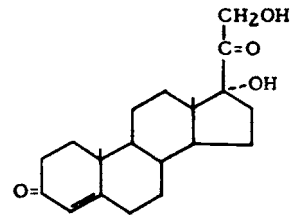
DEHYDRO E
Inactive



COMPOUND F
Active



COMPOUND A
Active



COMPOUND S
Inactive

Figure 2

Many of the points have been referred to briefly because of the limitations in time and with the understanding that some of the investigators, who have made contributions in this field, are here to present their viewpoints. It is to be hoped also that some of the panel members will be able to present from their unpublished data correlations in areas of interest which we have not discussed. Although the data at hand may permit only a few limited correlations of structure with anti-tumor activity, it can be anticipated that the increasing efforts devoted to experimental cancer chemotherapy studies will provide within the next few years chemical-biological correlations as important as those discussed in other meetings of this symposium.

TABLE 3

STRUCTURAL RELATIONSHIPS OF A FEW STEROIDS

WITH RESPECT TO INHIBITION OF MOUSE LYMPHOSARCOMA

11-Keto	11-OH	Δ^4-3-Keto	20 Keto	3-OH	17-OH	21 OH		Activity
+	-	+	+	-	+	+	Cortisone	+
-	+	+	+	-	+	+	Compound F	+
+	-	+	+	-	-	+	Compound A (17-Desoxy-cortisone)	+
+	-	+	+	-	+	-	21-Desoxycortisone	?
+	-	-	+	-	+	+	Dihydrocortisone	-
+	-	-	+	+	-	-	11-Keto pregnanolone	-
-	-	+	+	-	+	+	Compound S (11-Desoxy-cortisone)	-
-	-	+	+	-	-	+	Desoxycorticosterone (11,17-Desoxycortisone)	-

TABLE 4

ASPECTS OF STRUCTURE OF STEROIDS INACTIVE AGAINST MOUSE LYMPHOSARCOMA

11-Oxy	Δ^4-3-Keto	20-Keto	3-OH	17-OH	20-OH	21-OH	
-	+	-	-	+	-	-	Testosterone
-	+	+	-	-	-	-	Progesterone
-	-	+	+	-	-	-	Δ^5-Pregnenolone
-	-	+	+	-	-	-	$\Delta^{5,16}$-Pregnenolone
-	+	+	-	-	-	+	Desoxycorticosterone
-	+	+	-	-	-	+	21-OH-Pregnenolone
-	+	+	-	+	-	-	17α OH-Progesterone (Δ^4)
-	+Δ^1	+	-	+	-	-	17α OH-Progesterone (Δ^1)
-	+	+	-	+	-	-	17β OH-Progesterone
-	-	+	+	+	-	-	17α OH-Allopregnanolone (Cmpd. L)
-	+	+	-	+	-	+	17α,21-OH-Progesterone (Cmpd. S)
-	+	-	-	+	+	+	17α-Triolone
-	+	-	-	+	+	+	17β-Triolone

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ANTIMETABOLITES

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Mr. Chairman, Ladies and Gentlemen:

I should like to speak on the application of bacterial mutants to several problems that concern antimetabolites. In the first place, certain antimetabolites have given rise to the concept of competitive inhibition which has played such an important role in pharmacology that it might be called the cornerstone of much of the work being considered here today. We propose to extend that concept to the normal physiology of the growing cell, using it as the basis of an integrative mechanism for which we believe adequate, though indirect, evidence is now available. After discussing this topic, a moment will be spent in comparing the results provided by inhibition analysis and by mutants in two types of studies: those designed to determine paths of biosynthesis and those designed to determine the site of action of an inhibitor. Finally, possible application of mutants to the development of new chemotherapeutics will be noted.

I am sure you are all familiar with the work which was started in 1941 when Beadle and Tatum isolated various Neurospora mutants, each of which lacked the activity of the enzyme of one essential biosynthetic reaction. These mutants grow only if supplied with the product of the blocked reaction, or a derivative of that product. These organisms are elegant tools for metabolic study, as it is possible to eliminate, with complete specificity, any one of a variety of enzymes. Similar biochemical mutants can be obtained with bacteria.

Our interest in this field arose rather accidentally through an interest in chemotherapy which led to the development of an efficient method of mutant isolation, based on a unique property of penicillin: namely, penicillin sterilizes bacteria only when they are growing. The colon bacillus grows on a minimal medium containing only glucose and salts. It, therefore, synthesizes all its amino acids and other components from these materials. The mutants, however, by definition, are cells which cannot grow on this medium. When a large population of bacteria containing a few mutants is exposed to penicillin in minimal medium, the predominant parent cells grow, and are promptly sterilized by the penicillin. The mutants, on the other hand, do not grow; hence they survive. With this simple trick a wide variety of interesting mutants were soon isolated. These have been employed in studies of certain paths of biosynthesis which will be briefly described in order to illustrate the techniques involved.

It is known that in a variety of cells, from bacteria to mammalian liver, ornithine is converted to citrulline which in turn is converted to arginine. We have E. coli mutants blocked before each of these three compounds. In this series, as in many others, a mutant, trying vainly to synthesize the required compound, accumulates in the medium the precursor of the blocked reaction, normally present only in traces. The technique of syntrophism (cross-feeding) can be used to permit very simple demonstration, without any elaborate biochemical techniques, of precursor accumulation. The arginine-requiring mutant spills out its precursor, citrulline; by diffusion through the agar, this gives rise to a gradient of stimulation of the neighboring streaks which can grow on this compound. In turn, the citrulline-requiring mutant feeds the ornithineless mutant. These observations are illustrated in the slides.

This technique is now being used to analyze unknown paths of biosynthesis. What concerns us at present, however, is the broader problem of trying to find how the various single reactions in the growing cell are so beautifully integrated. E. coli is exceedingly economical in its pattern of synthesis; the normal cell excretes no significant amount of any amino acid. This fact was easy to ascertain by the use of appropriate mutants. The cell therefore synthesizes these components in exact proportion to their relative requirements, and hence must possess efficient

regulatory mechanisms. A possible clue to their nature arose when we observed that a block in synthesis of one amino acid causes excretion not only of a precursor, as was illustrated above, but often also of another amino acid, usually one with a close structural relationship. It seemed likely that an analysis of this breakdown in the regulatory mechanism would throw light on the nature of the mechanism, for medical investigators well know that physiological mechanisms are often first recognized when distorted by pathological conditions. An analysis was consequently carried out - in a much less logical and sequential fashion, I fear, than will be conveyed in the following account.

The key was furnished by a phenomenon discovered by Bonner in a mutant of Neurospora requiring a closely related pair of amino acids, isoleucine and valine. This double requirement results from a single genetic block. It could be shown that the block interrupts only isoleucine synthesis, but as a result a precursor accumulates which competitively inhibits an enzyme concerned with a similar precursor of valine. The α-keto analogue of isoleucine was originally thought to be the inhibitory precursor, but Adelberg and Tatum subsequently showed that the mutant with the double requirement accumulates primarily the α,β-dihydroxy acid rather than the keto acid. The principle of internal inhibition, however, remains unchanged by this later development.

We have here an example of an internal or physiological block secondary to a genetic block. What is the significance of this internal inhibition for the cell? A rather teleological attitude toward general physiology gives rise to the following considerations. If an excess of a given metabolite can completely block a reaction, then the normal amount of that metabolite might govern that reaction, and complete absence might permit excessive synthesis by removal of the physiological brake.

This hypothesis can be tested. We fortunately have not only E. coli mutants of the type described above, with a double requirement for isoleucine and valine; there is also one blocked later in isoleucine synthesis, which requires only this amino acid, and another blocked very early, which responds to either the 4-carbon compound α-aminobutyric acid (AAB) or the 6-carbon isoleucine. According to the notion outlined above, the mutant which is blocked very early and can respond to AAB should fail to make the normal governor; hence, it should excrete valine. Furthermore, there is a second, more critical test of this hypothesis. An excess of AAB should restore to the system the governing intermediate which is absent on account of the genetic block, and hence should abolish valine excretion. Similarly, if the biosynthetic reactions are reversible, an excess of isoleucine should have the same effect.

The culture plates illustrated on the slides show that these expectations are fulfilled. The AAB-requiring mutant, growing to a limited extent on a small amount of isoleucine or of AAB, feeds a valineless mutant heavily. Furthermore, an excess of either isoleucine or AAB completely abolishes excretion of valine. The control tests required for these conclusions have been carried out: it has been shown that the compound excreted is valine, and it is abolition of excretion, and not of response, which is caused by the excess of isoleucine or AAB.

This rather complicated explanation of amino acid excretion might seem to rest on a wobbly foundation, were it to rely only on this pair of mutants. Identical behavior, however, has been observed with another pair of related amino acids, tyrosine and phenylalanine. Several phenylalanine-requiring mutants excrete tyrosine; reciprocally, tyrosineless T9 excretes phenylalanine, but T8, another tyrosineless mutant with a different genetic block, fails to excrete phenylalanine. We would postulate that a metabolite in the sequence of synthesis of either compound is a governor of the other sequence. Excretion would be explained by an early block, which prevents formation of the governing compound. On the other hand, the tyrosine mutant which fails to excrete phenylalanine could be explained by a later block, which does not interfere with the production of this governing compound. Just as in the previous series, an excess of phenylalanine completely abolishes excretion of tyrosine, and an excess of phenyl-pyruvic acid, a known precursor, has the same effect. Similarly with the tyrosineless mutant, an excess of its growth requirement completely abolishes excretion of phenylalanine. In all these cases the compounds excreted have been identified by paper chromatography as well as microbiological response, and appropriate controls have shown that the excess of the growth requirement abolishes excretion rather than response.

While other theories might explain the fact of excretion, it would be difficult to explain in any other way its abolition by excess of the growth factor. Though the evidence for this theory is indirect, its consistency with the varied data is impressive. The cell is not a bag of independent enzymes, but rather appears to control, in at least some cases, not only the relative number of molecules of each enzyme per cell, but also the activity per enzyme molecule. This idea, though hardly novel, is not frequently stressed. To it we have added a specific control mechanism which involves a dual role for certain metabolites: substrate for one enzyme and governor of another. This concept is an extension to physiology of the principle of competitive inhibition, so well established in pharmacology. Such integrative mechanisms are surely not restricted to bacteria but undoubtedly enter into normal and disturbed growth regulation in animal and plant cells, including differentiation, regeneration, and neoplasia.

And now we might discuss briefly the use of mutants in connection with studies employing growth inhibitors. Following the development of the concept of competitive inhibition of essential metabolites by structural analogues, growth inhibitors have been rather widely used in recent years to analyze paths of biosynthesis. This methodology, called inhibition analysis, involves the assumption that a competitive antagonist is a precursor, and a non-competitive antagonist is a product, of the affected reaction. This assumption is not always safe; Hitchings has shown that this interpretation of certain data would lead to the absurd conclusion that bromouracil is a normal metabolite.

We have several examples that further illustrate the dangers inherent in inhibition analysis. Beerstecher and Shive, using this approach with E. coli, concluded that tryptophan can be converted to phenylalanine, and the latter is a precursor of tyrosine; furthermore, phenylpyruvic acid did not act as a precursor of phenylalanine. All three of these conclusions are in conflict with those derived from studies on mutants. For in addition to the strains noted above, we have mutants blocked early in aromatic synthesis which require all three aromatic amino acids; others, blocked even earlier, require in addition PABA and, in some cases, a previously unknown bacterial vitamin. These observations, supplemented by others, conclusively demonstrate that these three amino acids are derived from a common precursor; no one is a normal precursor of any other. Furthermore, the absolute requirement of these strains for all three amino acids implies that these compounds are not interconvertible in the mutants even by reversal of normal biosynthetic paths; one would, therefore, not expect them to be interconvertible in the parent wild type either, since the genetic blocks in these mutants do not lie in the path of the potential reversed syntheses. Finally, phenylpyruvic acid is used practically as well as phenylalanine by all of these strains.

As another example, in our laboratory Dr. Werner Maas, using both inhibition analysis and mutants, has shown that D-serine inhibits the growth of a wild type strain of E. coli by interfering with the conversion of β-alanine to pantothenate; the inhibition is overcome competitively by the former compound, and non-competitively by the latter. Glycine also overcomes the inhibition competitively, and would, therefore, be interpreted, according to the principles of inhibition analysis, as a precursor of β-alanine. However, glycine cannot satisfy the growth requirement of a mutant blocked in the synthesis of β-alanine, and yet it does reverse the inhibitory effect of D-serine on the growth of this mutant. It is clear that glycine does not act simply by promoting the synthesis of β-alanine; a more subtle mechanism must be present. These several conflicts suggest that in some cases inhibition antagonisms involve more complex mechanisms than those that have been considered in studying paths of biosynthesis by this approach.

The above discussion has been concerned with the limitations of growth inhibitors, compared with mutants, in analyzing paths of biosynthesis. In addition, mutants can throw light on the mode of action of inhibitors themselves. As was stated above, and will be documented elsewhere, the use of mutants has considerably strengthened the evidence for the site of action of D-serine. Furthermore, the combined study of bacterial mutants and growth inhibitors leads naturally to an investigation of the biochemical mechanism underlying drug resistance. The D-serine-pantothenate system appears in some ways to be more suitable for an attack on this problem than the clinically effective chemotherapeutics. The compounds involved possess simple chemical structures; the metabolic path involved is better understood than most others; the affected reaction can be studied with non-growing cells; mutants with a high degree of resistance to D-serine can be obtained easily. Preliminary experiments have ruled out several mechanisms that have been widely assumed for drug resistance: it does not involve an increase in the production of either pantothenate or β-alanine, nor does it involve formation of pantothenate via

an alternate metabolic pathway bypassing β-alanine. Among the remaining possibilities, D-serine may be prevented from reaching its site of action, or the previously sensitive enzyme may have been changed, qualitatively or quantitatively, in such a manner that it can now function adequately in the presence of the inhibitor. This problem is under investigation.

Finally, mutants can be useful in the development of new chemotherapeutics. The systematic synthesis of metabolite analogues has provided a host of cytotoxic agents that are effective in vitro, but has been disappointing in its failure to yield chemotherapeutics that are useful in vivo. It is now well recognized that this failure can be accounted for by the requirement of the animal host for the very metabolites that have served as models. This situation arises from the fact that the known bacterial metabolites are nearly all mammalian metabolites as well. But this fact does not depend solely on the unity of biochemistry in nature; it depends also on the unity of purpose of biochemists. In general, microbial nutrition has served as a handmaiden for investigators primarily interested in mammalian metabolism. But metabolites peculiar to microorganisms do exist. The success of sulfonamides depends on the fact that PABA as such is not used as a metabolite by animals, whereas the PABA-containing folic acid of the animal is not used, at least when supplied externally, by many bacteria. It follows that we should search further for specifically microbial metabolites.

For some time it has been clear that mutants might be used in the search not only for precursors of known metabolites, but also for new growth factors. We have recently been studying a mutant blocked early in aromatic synthesis, which requires tyrosine, phenylalanine, tryptophan, and PABA; for rapid growth it requires further a fifth substance, present, like PABA, in the filtrate of the wild type E. coli. This substance has been tentatively identified as a simple aromatic compound, active in trace amounts. From the point of view of this audience, it is particularly significant that this substance, like PABA, is present in relatively large amounts in yeast extract but not in liver extract. It therefore offers real promise as a model for chemotherapeutic synthesis.

CARCINOGENESIS

L. F. Fieser
Harvard University
Cambridge, Massachusetts

It is a pleasure to see here so many of the people with whom I collaborated in the work you mention, including Dr. Shear, Dr. John Wood, Dr. Arnold Seligman, Dr. Fred Novello; I guess Dr. Konrad Dobriner can be included as well.

I would not attempt to try to find a common factor between a blow on the head and chloroform, as suggested so aptly by Dr. Berenblum. As a matter of fact, I think it is asking a little bit too much to try to find a common factor between carcinogenetic hydrocarbons, the azo dyes, the estrogenic substances, and so on. It seems to me, if one could understand a little about the way in which any series of compounds of these different groups functions, we would be well ahead.

At the time I stopped work on the problem of the carcinogenic hydrocarbons, it seemed to me that the very pronounced chemical activity exhibited by some of the most potent ones was probably concerned in the reaction of carcinogenesis, as some of the hydrocarbons exhibit chemical activity of a specific type which far surpasses that of related, less carcinogenic hydrocarbons.

Then, in a summarizing lecture at the University of Pennsylvania celebration, I postulated that the carcinogen reacts with sulfhydryl groups of a protein, and that this is the reaction which is responsible for initiation of malignancy. That was a nice guess at the time, but I am sure it has been ruled out by now, because the hydrocarbons just do not react with proteins in a chemical way.

Careful examination of the postulated correlation between structure and chemical reactivity or carcinogenic activity shows that there are rough parallelisms but that no exact relationship exists; any attempt at exact relationship seems to fall down. So chemical reactivity is not quite the whole story.

As I see it now, a hydrocarbon - I will stick to hydrocarbons - to be a potent carcinogen, has to have various properties. One property is highly developed chemical reactivity of a certain type; probably another factor is the shape of the molecule. It looks as though the hydrocarbon has to have a fairly flat surface in order to be effective. A methyl group attached to the benzanthracene nucleus may permit carcinogenic activity, whereas the homolog with an ethyl or propyl or butyl group begins to lose that property. That may be because only the methyl group can stick out flush with the flat surface of the ring system.

Then, perhaps, the over-all size is important. You could pick out a hydrocarbon such as aceanthrene, which has the same kind of chemical reactivity as methylcholanthrene but still lacks carcinogenic potency. It may be that the molecule is a little too small to give the right distribution characteristics.

I am impressed with the idea that, if we are ever going to understand the relationship between chemical structure and any kind of biological activity, we are going to have to take into account another factor, and that probably is the distribution characterisistic of the molecule, distribution particularly between lipophilic and hydrophilic phases. A compound which has all the other properties required but does not have the right distribution characteristics may be outside the field of biologically active compounds.

What kind of interaction with a cell constituent involved in gene determination could occur with a hydrocarbon of the right chemical activity, shape, size and distribution characteristics? I do not think it is a chemical reaction, as I said before. The nearest guess at present is that it

is a process of adsorption, or of some form of complex formation, and the requirement for specific chemical reactivity is merely to give the molecule sufficient pep so that it can form suitable complexes. It seems to me possible that the phenomenon of carcinogenesis may have some relationship to the ability of the carcinogenic hydrocarbons to form complexes with polynitro compounds. The trinitrobenzene derivatives of methylcholanthrene, benzpyrene, and other particularly potent carcinogens are deeply colored, whereas complexes of less reactive hydrocarbons are less highly colored. The color probably is just one indication of stability of the complex and of the reactivity of the components. Possibly forces similar to those involved in the formation of these polynitro complexes are involved in carcinogenesis.

So, as a way of moving ahead in the future, it seems to me - just a guess at the moment - that any way of setting up experimental methods of detecting or measuring complex formation or adsorbability of the agents might shed new light on the general problem.

CANCER CHEMOTHERAPY

Howard E. Skipper
Southern Research Institute
Birmingham, Alabama

I have been told that, after about two minutes, Dr. Shear will lower the boom, so I am certainly going to get through with my short discussion of carbamates in chemotherapy of leukemia within that time.

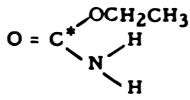
Our work with carbamates has shown several things with regard to the relation between structure and biological activity (anti-leukemic activity). Certainly it is mostly negative in this respect since we have found that ethyl carbamate (urethan) is the only one of a rather large series of this type compound which is very effective in prolonging life span in mouse leukemia.

However, when one compares the anti-leukemic activity and the carcinogenic activity of carbamates (work by Dr. Larson of the National Cancer Institute), we see a very nice correlation; ethyl carbamate is the most active by far in either of these respects.

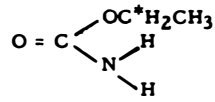
There seems to be little correlation between leukopenic activity (ability to depress the total white blood count) of the carbamates in normal mice and anti-leukemic activity of this series. Many carbamates have been found to be leukopenic while urethan is the anti-leukemic carbamate.

There does seem to be a very good correlation between leukopenic action in mice and mitotic poisoning activity of carbamates in such organisms as the sea urchin egg (work by Dr. Ivor Cornman, George Washington University).

We have been most interested in trying to find out why urethan is so specific with regards to anti-leukemic activity. In certain mechanism studies we have labeled the carbonyl and the methylene carbon atoms of the urethan molecule:



Carbonyl-labeled urethan



Methylene-labeled urethan

and have compared the distribution of the active atoms in normal and neoplastic mice as well as excretion and exhalation. It appears quite clear that urethan is broken down in the body to carbon dioxide, ethyl alcohol, and ammonia. We have found that cancerous mice do not degrade the molecule nearly so rapidly as do normal mice.

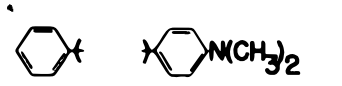
In an isolated system, living sperm from the sea cucumber, which is largely living desoxyribose nucleoprotein, Dr. Cornman and I have observed that carbon 14 from carbonyl-labeled and methylene-labeled urethan are fixed to a higher degree than labeled CO_2 or methylene-labeled ethyl alcohol, the respective labeled hydrolysis products of the two labeled urethans. This is not the case in sea urchin eggs where the cell is fairly low in nucleoprotein. We think that this result may be suggestive of the mechanism of the carcinogenic and anti-leukemic actions of urethan.

CARCINOGENESIS

H. P. Rusch
University of Wisconsin
Madison, Wisconsin

I have a few slides which illustrate some of the relationships between the chemical structures of certain compounds and their carcinogenic activities. These studies have been carried out by Drs. J. A. and E. C. Miller of our department, and the data are published in Cancer Research, 5, 227; 9, 504, 652 and J. Exp. Med., 87, 139.

First, I will mention some studies on certain aminoazo dyes. These compounds induce liver tumors when they are fed to rats for several months. As shown by Haddow and Kon, replacement of the azo linkage by an ethylene linkage to give 4-dimethylaminostilbene results in a carcinogen which is, as far as the liver is concerned, a slower acting carcinogen than the aminoazo dyes.



	relative activity
$-N=N-$	6
$-CH=CH-$	weak (Haddow and Kon)
$-CH=N-$	0
$-N=CH-$	0
$-\overset{\overset{O}{\|}}{C}-NH-$	0

Slide 1

Substitution of -CH=N-, -N=CH-, or -C-NH- for the azo linkage results in inactive compounds.
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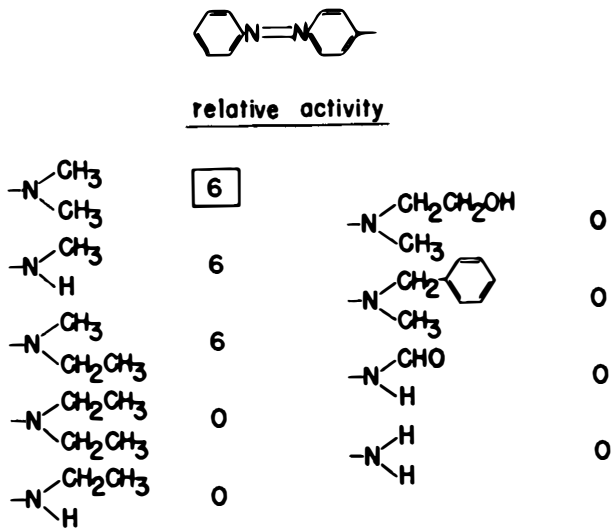
O

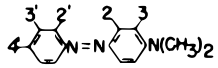
The second slide illustrates the effect of alterations in the substituents on the amino group on the potency of the aminoazo dyes. 4-Dimethylaminoazobenzene is assigned an activity of six, and 4-monomethylaminoazobenzene with only one methyl substituent and 4-ethylmethylaminoazobenzene with one ethyl and one methyl substituent also have activities of six. Thus, in order to



Slide 2

be carcinogenic the compounds must have at least one N-methyl group. However, as illustrated by the non-carcinogenic dyes 4-β-hydroxyethylmethyl- and 4-benzylmethylaminoazobenzene, the second substituent on the amino group can also affect the potency of the compound.

Introduction of substituents into the rings also strongly affects the carcinogenic activities of the aminoazo dyes. Either a hydroxy or trifluoromethyl group in any of the prime positions abolishes the activity of 4-dimethylaminoazobenzene. With other substituents the activity of the compound depends on the position of the group. Thus the 3'-methyl derivative of 4-dimethylaminoazobenzene is about twice as active as the parent compound while the 2'-methyl derivative is only one-third to one-half as active and the 4'-methyl derivative is very weak. 2- and 3-methyl-4-dimethylaminoazobenzene are both inactive under our conditions. Similarly, introduction of a chloro or nitro group in the 3' position results in a more active compound than if the same group is inserted into the 2' or 4' position. On the other hand, compounds containing fluorine in any one of the prime positions are more active than the unsubstituted dye. More recent work has shown that the 2-fluoro and 2',4'-difluoro derivatives of 4-dimethylaminoazobenzene are also about twice as active as 4-dimethylaminoazobenzene. Since substituents in the 2, 2', or 4' positions should hinder a benzidine rearrangement of the corresponding hydrazo derivatives, the high activities of these fluorinated derivatives largely eliminate the possibility that such a rearrangement is involved in the mechanism by which these dyes induce the carcinogenic process; such a theory was proposed several years ago by certain British experimenters.



Relative Activities (unsubstituted dye = 6)

position	substituent HO-	CH_3-	NO_2-	Cl-	F-	CF_3-
4'	0	<1	0	1-2	10	0
3'	0	10-12	5	5-6	10	0
2'	0	2-3	3	2	7	0
2	0	0				
3		0				

Slide 3

Recent studies on the effects of various alterations in the structure of 2-acetylaminofluorene on its carcinogenic activity are presented in the fourth slide. Replacing the $-CH_2-$bridge in 2-acetylaminofluorene by -S- as in 3-acetylaminodibenzothiophene did not alter the carcino-

TABLE I
THE CARCINOGENIC ACTIVITIES OF ANALOGS OF 2-ACETYLAMINOFLUORENE
WITH THE GENERAL FORMULA

TUMOR SITE	MOS	COMPOUND FED (1.6E GM./KG./DAY IN BASAL DIET) 2-ACETYLAMINO-FLUORENE	2-ACETYLAMINO-DIBENZOTHIOPHENE	2-ACETYLAMINO-DIBENZOTHIOPHENE 5-OXIDE	2-ACETYLAMINO-DIBENZOFURAN
		2-	3-	2-	3-	2-	3-	2-	3-
		NUMBER OF RATS							
SURVIVAL*	4	9/10	10/10	9/10	8/10	7/7	7/7	6/7	7/7
	4	6	0	5	0	1	0	0	0
MAMMARY GLAND	6	6	0	6	0	1	0	2	0
	8	7	0	6	0	1	0	5	0
	4	1	0	0	0	0	0	0	0
EAR DUCT	6	2	3	3	3	1	0	0	1
	8	4	6	3	6	2	0	2	1
	6	0	1	0	0	0	0	0	0
LIVER	8	0	7	0	0	0	0	0	0
	4	3(3)	10(10)	3(3)‡	8(8)	6(6)	7(7)	6(6)	7(7)
ANY†	6	2(2)	7(7)	1(1)	5(5)	5(2)	7(7)	4(4)	6(6)
	8	0	0	1(0)	2(0)	4(0)	6(6)‡	1(1)	6(5)

*NUMBER LIVING AT 6 MONTHS OVER NUMBER AT START

†TOTAL NUMBER OF TUMOR-FREE RATS (NUMBER OF TUMOR-FREE RATS STILL ALIVE)

‡ONE RAT, NOT INCLUDED HERE, DEVELOPED A TUMOR AT ANOTHER SITE (SEE TEXT)

Slide 4

genicity of the molecule for either mammary gland or ear duct tissue. Substitution of -S- for the

$-CH_2$ as in 3-acetylaminodibenzothiophene-5-oxide greatly lowered the activity towards these two tissues, while insertion of an -O- bridge as in 3-acetylaminodibenzofuran only partially diminished the activity of the molecule in these respects. Unlike 2-acetylaminofluorene, however, none of these three compounds had any carcinogenic activity towards the rat liver.

Further studies will be necessary to determine what features these various carcinogenic processes have in common and how these relatively small variations in structure interfere with or promote the reactions which lead to tumor formation.

CANCER CHEMOTHERAPY

R. B. Angier
Lederle Laboratories
American Cyanamid Company
New York, New York

I feel, somehow, out of place up here, being an organic chemist, but I presume that Dr. Farber wanted to make it official, to get these formulas on the screen so that everybody would know what he is talking about.

A rather large number of analogs of folic acid have been prepared. The basic structure we have up here, which includes the pteridine nucleus, the methylene linkage to the benzoic acid portion of the molecule and the PABA residue. We see there are four different substituents on here. These four different groups have been changed in a number of ways to produce the various analogs which are being described. I do not expect Dr. Farber will talk about all of these but, in order to cover the ones he will mention, I am putting these all on. Number one is merely a change of the glutamic acid to aspartic acid on the end of the molecule. Following, is the dl aspartic acid derivative. Of course, in all other cases, the other substituents are the ones you will find in folic acid itself.

This is your 9-methyl pteroyl glutamic acid, in which you have a methyl group on the bridge carbon atom. The antagonist activities are shown here, also. We will pass them by.

These involve some of the more potent antagonists which have been discussed. In aminopterin we see merely the change of the R-group in the 4-position to an amino group. That comes under the class of isosteres, which were discussed earlier. This compound becomes, then, an extremely potent antagonist, microbiologically as well as in the animal.

Amino anfol, has the 4-amine but, in the place of the glutamic acid out here, it has the aspartic acid.

A-methopterin which is even more potent than is aminopterin, has an amino group in the 4-position, replacing the OH and a methyl group on the N^{10} position.

A-ninopterin has two methyl groups, one on the 9-position and one on the N^{10} position.

Adenopterin also has an amino group in the R-position, and two methyl groups, one in the 9- and one in the 10-position. Aminoteropterin has three glutamic acids out here, instead of just one. They are linked in the gamma position; each linkage is gamma. Also aminoteropterin has an amine in the 4-position.

Amino alanfol has an amino group in the 4-position while the glutamic acid residue of folic acid has been replaced by dl-alanine.

And lastly, amino treofol has an amino group in the 4-position while the glutamic acid residue has been replaced by dl-threonine.

CANCER CHEMOTHERAPY

Sidney Farber
Children's Hospital
Boston, Massachusetts

Mr. Moderator, one comment, first, about the chemical structures which Dr. Angier just showed you. Our studies were made, first, with two folic acid antagonists, without the amino in the 4-position. They were made by the American Cyanamid Co. group of chemists; and both had anti-leukemic activity, but the activity was weak, and no prolonged or real remissions were attained. There is no close correlation whatsoever between the action on mouse leukemia and the action on man. All of the excellent results have been attained with compounds with the amino in the 4-position, and every compound with the amino in the 4-position has been toxic.

I think we can state, first of all, that the initial impression we had concerning a correlation between toxicity and carcinolytic action does not obtain. The same mechanism may be working in both instances, but it is perfectly possible to obtain clinical results without bringing about any important toxic changes in the patient.

To summarize clinical results very briefly, our group has had the opportunity to treat and care for some two hundred children with acute leukemia in the last three and one-half years, with the various folic acid antagonists. An over-all remission rate of approximately 50 per cent was obtained for this group. In one instance with the 4-aminopteropterin, to which Dr. Angier referred, the remission rate has reached 66 per cent for a period of time.

Prolongation of life up to twenty-two months has been achieved. In all instances, after remissions have been attained, resistance to the treatment has occurred eventually and, in all instances, death has occurred. As the moderator has pointed out, no cure has, therefore, been attained in acute leukemia. We have seen, however, for the first time, a carcinolytic effect which has been produced by no other series of compounds. This holds true for aminopterin, for the 9-methyl, the 10-methyl, the 9,10-dimethyl, the 4-aminoteropterin and for other compounds to which reference has been made. No one is really that much better than another in this series, so that no strong preference can be stated at this time.

It is of considerable interest that, when empirical trial was made with the action of these compounds on other solid tumors in man, a carcinolytic or carcinostatis action was demonstrated in a number of totally unrelated forms of cancer, cancers unrelated to leukemia, such as neuroblastoma, carcinoma of the breast, or carcinoma of the prostate, with metastatic lesions elsewhere, and so on. In all instances, after a period of excellent tumor effects, varying from three months to, in some instances, twelve months, the "resistance" occurred and, in every instance, in every patient observed long enough to permit this statement, death has occurred.

I think, certainly, this is the most effective group of compounds in acute leukemia. They are of great interest in other forms of cancer. The problems which the workers concerned with the further study of these compounds would like to have answered are, first, the nature of the toxic changes; second, the mechanism of their carcinolytic and carcinostatic action.

Finally, there is the actual mechanism of resistance of the cancer cell which has once responded. There is considerable information coming from different laboratories which bears upon this subject. Eventual solution of that problem, however, will be necessary before further progress can be reported in the treatment of patients with incurable cancer with these antifolic compounds. The use of these compounds in conjunction with, or following, ACTH, cortisone, and related compounds has been of great interest. There is an effect which can be produced by the two in series or at the same time which, in some instances, cannot be produced by the antifolic compounds alone.

One last comment, if I may make it, Mr. Moderator. When one begins to work with patients with cancer incurable by surgery or radiation, the patient is included in the research team, as is the mouse. Since the purpose of cancer chemotherapy is the cure of cancer in man, it is essential that the splendid work going on with other agents, must be accompanied by actual observation on the living human being with cancer which is incurable by present methods of therapy.

A final point in this discussion: If that is done, then the finest medical and surgical care available must be given to the patient with incurable cancer before chemotherapy is instituted, and chemotherapy must be stopped if, at any time, other forms of treatment promise more for the patient than the form of trial therapy which has been instituted.

ANTIMETABOLITES AS CHEMOTHERAPEUTIC AGENTS

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There are two contrasting ways in which one may go about the preparation of a new chemotherapeutic agent. One is the purely empirical approach - the testing of a wide variety of chemical substances, the selection of a few which have a selective effect on the undesirable organism or tissue, followed by further testing of variants of the selected molecule. The other approach, the deductive or logical way, stems from previous knowledge of a difference in metabolism between the host and the parasitic tissues upon which one might capitalize in a variety of ways. Of the two, the empirical, inductive way, has been much the more productive and has yielded nearly all the chemotherapeutic agents which are available. This does not mean that the deductive method may not yet yield an abundance of new drugs; it means simply that insufficient knowledge of intermediary metabolism has been available or that insufficient thought has been applied to the problem of selection of metabolic differences worthy of exploration.

Antimetabolite studies, which have as their aim the production of chemotherapeutic agents, might be thought of as one way in which one might capitalize on a metabolic difference between host and parasite. Thus, had one known of the bacterial requirements for p-aminobenzoic acid, and had the absence of a human requirement for this growth factor been clearly demonstrated, sulfanilamide might have followed as a logical consequence of this knowledge, rather than as an empirical discovery. However, much the greater part of the work in the antimetabolite field has been concerned with the demonstration of the phenomenon itself. For this purpose, the most suitable metabolites are those which are used by a wide variety of living organisms. Pyrithiamine, for example, has the characteristics of a competitive inhibitor of thiamine in a bacterium, and produces symptoms in the mouse of thiamine deficiency which can be reversed with thiamine. One is convinced, therefore, that such a substance acts by competing with the metabolite for some cell surface, probably an enzyme system.

Information of this sort does not, in general, lead to new chemotherapeutic agents, for thiamine appears to be required by all living cells and the requirements probably do not differ quantitatively by a large ratio. What is needed for the production of new chemotherapeutic agents in a logical way is knowledge either of a large quantitative difference in the requirement for the metabolite, or, much better, the existence of a qualitative difference between host and parasite in the performance of some metabolic task. This kind of knowledge is available only rarely. To some extent it may be gained through the antimetabolite study itself.

Our approach to new chemotherapeutic agents has been via the biochemistry of nucleic acids. It was felt that quite generally there must be a differential requirement between host and parasitic tissues for the precursors of nucleic acids, since the rapid growth of the parasitic tissue must involve a rapid synthesis of nucleic acids. So the possibility exists that chemotherapeutic agents for a variety of disease processes might arise on this purely differential rate basis. There were also reasons for believing that qualitative differences in the biosynthesis of nucleic acids do exist. When our work was begun it was known only that the white rat does not use guanine or uracil as precursors of its nucleic acid; however, it was known that certain bacteria and molds do require one or the other or both of these substances. Such observations offered the promise that antimetabolites of the natural purines and pyrimidines might selectively block pathways which are essential to certain organisms and not to others.

In the cancer field, this approach has not yet produced a cure but it has given a few leads which may be regarded as hopeful. The activities of the antifolics and of diaminopurine probably are to be regarded as the result of quantitative differences between normal and neoplastic tissues in the need for certain precursors of nucleic acids, but the mode of action of both these substances requires clarification. Despite inferences to the contrary, it appears improbable that any real

qualitative differences in nucleic acid synthesis between normal and neoplastic tissues have yet been found. The activity of 8-azaguanine on certain tumors is still somewhat of a mystery. The utilization of guanine is certainly not the common denominator of neoplastic growth which it was hoped it might be, for many tumors fail to respond to the analogue. Moreover, studies by Dr. George B. Brown with isotopically labeled guanine have shown no clear-cut difference in the use of guanine by tissues of the host and the neoplasm. On the other hand, this substance, the antifolics, diaminopurine and the other purines and pyrimidines which have a selective inhibitory effect on neoplastic tissues should be regarded as leads which may be elaborated by further studies and modifications of the molecules; these may eventually result in chemotherapeutic agents of real value.

One of the axioms of antimetabolite-metabolite studies is that maximal activity of the structural analogue is produced with a minimum number of changes of the metabolite molecule - the change of one grouping usually giving a stronger antimetabolite than an alteration of two groups or atoms and so on. This has a considerable validity with respect to antimetabolite studies per se but could be very misleading if applied to the problems of chemotherapy. For purposes of illustration, one may return to the example of the sulfonamides. The molecule representing a minimal change from p-aminobenzoic acid would be sulfanilic acid. If one stopped there he would not have a very effective chemotherapeutic agent. If he continued to work he might soon recognize the importance of the pKa's of the metabolite and antimetabolite and thus come to sulfanilamide. This substance is a useable chemotherapeutic agent but has several undesirable side effects. In other words, it becomes involved, unexpectedly, in the host's metabolism in a number of ways. In actual practice, of course, given the lead provided by the empirical discovery of the action of sulfanilamide, the chemists proceeded to modify that molecule in a variety of ways. As work progressed some of the patterns of chemical-biological correlation became apparent, and in turn could be related to certain physical properties of the molecules. Eventually drugs were produced which were not only more active than the original, but in which many of the undesirable features had been modified or eliminated.

I think it is not too far-fetched to suggest that we have found in the field of antagonists of nucleic acid derivatives as related to cancer, substances of potential chemotherapeutic value - substances which stand to the chemotherapy of cancer about as sulfanilic acid might stand to the chemotherapy of bacterial diseases. A substance like 2,6-diaminopurine, for example, has only a small and rather questionable differential effect on sarcoma 180 in the whole mouse, but, as has been found by Dr. J. J. Biesele, it is very considerably more toxic to sarcoma 180 than to mouse embryonic tissue, when studied in tissue culture. The problem here may be essentially to learn how to deliver the purine to the cancer cell without allowing it to be dissipated in side reactions. The antifolics inhibit sarcoma 180 when tested in the whole mouse but have little or no effect on either the sarcoma or embryonic tissue in tissue culture. Does this mean that the antifolic itself is modified in the animal metabolism, or does it act by depriving the cell of some essential metabolite which is produced in a specific locus and then transported to the growing cell? Perhaps some other explanation will be found, but answers to such questions as these may lead to modified and better approaches to the problem of cancer chemotherapy. The activities of a number of the pyrimidine derivatives are not readily interpretable in terms of metabolite: anti-metabolite relationship but nevertheless point the way to work which should be done.

Undoubtedly many of the apparent openings to chemotherapeutic agents will be found, on exploration, to be blind alleys. However, the importance of the problem suggests that each should be explored with considerable diligence. The working out of these leads will be mainly if not completely empirical. Antimetabolite studies may produce leads but are no substitute for hard work - the kind of thoughtful work which has been termed "enlightened empiricism".

CARCINOGENESIS

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Since I have been called upon, I might make one brief comment. Dr. Berenblum referred to Dr. Haddow's discovery that many of the carcinogenic hydrocarbons inhibit tumor growth. In looking over the data which Dr. Haddow published in that regard, we were impressed with the few exceptions, i.e., that some non-carcinogenic compounds also inhibited tumor growth. He was interested in showing a correlation between the ability to inhibit tumor growth, and the property of carcinogenicity, and he showed a fairly interesting correlation in that regard; but there were a few exceptions which interested us. A few tumors were inhibited by compounds which were not carcinogenic, but resembled superficially the carcinogenic hydrocarbons in structure and did inhibit tumor growth.

It was at that point that we became interested and prepared a series of compounds similar to these compounds, to see if this property of inhibition of tumor growth was extensive. We prepared a number of azo dyes which simulated the ring structure of the hydrocarbons (J. Am. Chem. Soc., 71, 3010 (1949)). Some of these azo compounds showed inhibition of tumor growth (sarcoma 37 and Walker carcinoma 256, to be published in Cancer).

Later, he published some data in which he showed that the inhibition of tumor growth with the carcinogenic hydrocarbons was very much related to the protein content of the diet, and he pointed out that the observations he had made were that the carcinogenic hydrocarbons inhibited tumor growth when a fairly low protein diet was used, 5 per cent protein. When this was increased to 25 per cent, the effect was less apparent. Our own animals had received the higher protein diet.

It was at about this point, unfortunately, that we lost interest in the phenomenon, and I suspect it is at precisely that point that many of you would become very much interested in it.

CANCER CHEMOTHERAPY

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Dr. Shear has asked me to make some comments on the possible relationship of pyridoxine to the metabolism of neoplastic cells in man. We explored this possibility several years ago in clinical cancer chemotherapy and although the results were negative we learned several lessons.

The basis for the clinical experiment rested on a series of observations made by Dr. H. C. Stoerk and others. It had been noted that lymphoid tissue was dependent upon the vitamin, pyridoxine, for its maintenance and growth. In experimental animals placed on a pyridoxine deficient diet there was rapid involution of the thymus gland and other structures rich in lymphocytes. Stoerk further reported that in mice bearing a transplantable lymphosarcoma there was striking inhibition of the tumor growth when the animals were placed on a pyridoxine deficient diet and in addition were fed an analog of the vitamin, desoxypyridoxine.

Our clinical observations were carried out on a number of patients with disseminated lymphosarcoma and also patients with acute lymphatic leukemia. The patients were placed on a rigid pyridoxine deficient diet together with the oral administration of large doses of desoxypyridoxine. During a period of three weeks, no detectable changes in evident tumor masses or peripheral blood picture were noted and there was no evidence that the course of the disease had been modified. Biochemical studies in these patients failed to reveal an increased urinary xanthurenic acid excretion, a sign of pyridoxine deficiency in most lower species.

The lessons that we re-learned from this experience were three:

(1) The correlation between tumors in experimental animals and neoplastic disease in man is not known. Precise information on the biochemical similarities or dissimilarities between the animal and human disease would be of tremendous value for the evaluation of observations made in cancer chemotherapy studies in experimental animals.

(2) In many instances there are species differences in the metabolism of drugs and therefore the translation of animal observations to man can not be anticipated *a priori*.

(3) A very important consideration, it seems to me, is that if a chemical compound merely shows tumor inhibition in the experimental animal it is probably not wise to try this agent in man. This is so, I think, because a carcinostatic effect in man is too difficult to demonstrate due to the relative chronicity of malignant disease in man as compared with cancer in experimental animals. Pyridoxine deficiency inhibits the growth of lymphosarcoma in mice, urethane inhibits the Walter rat carcinoma, 8-azaguanine inhibits a number of experimental cancers but clinical observations have failed to duplicate the results. This does not mean that therapeutic regimens with these agents might not have a carcinostatic effect in man, it merely indicates that it is impractical to conduct an experiment on humans which will require many months or even years before a conclusion can be reached. For this reason I believe it is permissible to conclude that clinical trial should be reserved for chemical compounds which have been shown to be carcinolytic against experimental tumors.

PURINES AND PYRIMIDINES IN EXPERIMENTAL CANCER CHEMOTHERAPY

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The observation by Kidder in 1949 that a guanine analog, 2-amino-4-hydroxy-triazolopyrimidine, inhibits the growth of certain tumors has been confirmed and extended in our laboratory. In an attempt to gain some insight into structure-activity relationships of this carcinostatic chemical agent, a series of pyrimidines and purines have been synthesized by Dr. Morris Engelman at the Columbia University Cancer Research Institute and tested against a spectrum of neoplasms in experimental animals.

The technique of evaluation consists in transplanting the tumor fragments into the axillae of the appropriate hosts and instituting therapy 24 to 48 hours later. In every experiment a minimum of 10 animals was employed for the study of a compound, and untreated controls and 2-amino-4-hydroxy-triazolopyrimidine (azaguanine) treated series were always included. In previous experiments it had been determined that a daily dose of 50 mgm. per kilogram intraperitoneally of the azaguanine was uniformly effective and without apparent toxicity. The unknown chemical compounds were administered at doses equimolar to the azaguanine and also at maximally tolerated doses. The duration of an experiment was from 14 to 21 days. The single exception to the method just described was for the evaluation of chemotherapeutic effect against the Brown-Pearce tumor. In this case the tumor fragments were transplanted to the anterior chambers of rabbits' eyes, therapy was not initiated until 3 days after transplantation and the duration of drug administration was limited to 7 days. In all instances the animals were sacrificed at the termination of the experiment and the subcutaneous or intraocular tumors were dissected out and weighed.

Table 1 presents a key to the tumors used and their hosts. Tables 2 and 3 present the results of all of the compounds thus far studied.

TABLE 1

Tumor Designation*	Tumor Type	Host
755	Carcinoma of the breast	C57 black mice
RC	Carcinoma of the breast	dba mice
EO771	Carcinoma of the breast	C57 black mice
Brown-Pearce	Undifferentiated squamous cell carcinoma	All rabbits
Sarcoma 180	Undifferentiated sarcoma	Paris RIII and Longacre mice
$6C_3HED$	Lymphosarcoma	C3H mice
9417	Acute leukemia	AK mice
C1498	Acute myelocytic leukemia	C57 black mice

* All tumors are transplantable

TABLE 2

EXPERIMENTAL NEOPLASM

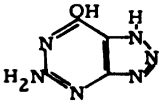
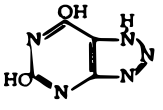
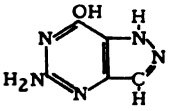
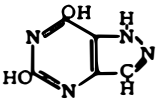
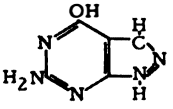
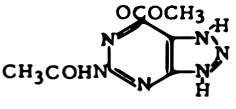
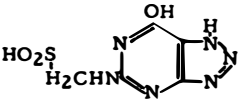
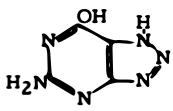
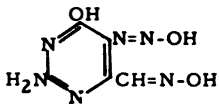
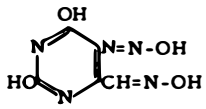
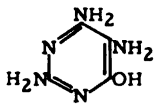
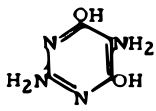
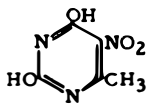
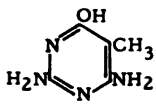
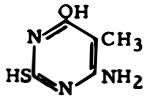
Compound	755	RC	EO771	Brown-Pearce	Sa. 180	$6C_3HED$	9417	C1498
	+	+	+	+	-	-	-	-
	-				-	-		-
	-				-	-		
	-							
	-				-			-
	+ -				-			-
	-				-			-

TABLE 3

EXPERIMENTAL NEOPLASM

Compound	755	RC	EO771	Brown-Pearce	Sa. 180	$6C_3HED$	9417	C1498
	+	+	+	+	-	-	-	-
	-				-			
	-							
	-							
	-							
	-				-			
	-							
	-							

As can be seen, no purine analog other than the 2-amino-4-hydroxy-triazolopyrimidine had any demonstrable effect against the tumors tested nor did any of the pyrimidines. Obviously at this time it is impossible to make any statements about structure-activity relationships in this series. This rather discouraging state of affairs is in part ameliorated by a series of interesting observations made by Dr. Jacob Kream and Dr. Ada Graff. Dr. Kream, who has been studying the deamination of guanine by liver guanase, demonstrated that both liver and tumor extracts are able to deaminate azaguanine. The product of such enzymatic action was found to be 2, 4-dihydroxytriazolopyrimidine. It is to be noted in Table 2 that this compound was tested and found to be inactive. In the hope of slowing the rate of inactivation, the diacetyl and 2-amino sulfoxylate derivatives of azaguanine were synthesized. These also proved inactive because, as Dr. Graff found, the acetyl and sulfoxylate radicles are not removed, at least in vitro, and the 2-amino group remains masked.

Although our results to date have been negative, the pursuit of structure-activity relationships in this series would appear to be potentially useful because (1) azaguanine probably interferes with nucleic acid metabolism and further understanding in this area could not fail to be of interest, (2) azaguanine appears to have a more selective action on neoplastic tissue than on normal tissue and, therefore, more potent carcinostatic or carcinoclastic agents might have clinical application and (3) azaguanine, in our experience, inhibits epithelial neoplasms but does not alter the growth of sarcoma or leukemia and, therefore, insight into its mechanism of action may lead to greater understanding of differences in metabolism in various types of malignant tumors.

CARCINOGENESIS

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It becomes increasingly apparent that the attempts at establishing consistent and generally applicable correlations between the structure and carcinogenicity of chemicals may suffer the same fate that has overtaken similar efforts aimed at correlating chemical structure and pharmacologic and toxic action of chemicals or the various types of microorganisms and their pathogenicity. There does not appear at the present time any possibility of linking in a common pattern the numerous aromatic carcinogens with the aliphatic ones or with the scarcely studied cancerigenic metals, such as arsenic, chromates, nickel, asbestos, selenium and beryllium, not to mention in this connection the non-ionizing and ionizing cancerigenic radiations.

Since the bulk of the available information on this subject is based on observations made with synthetic aromatic chemicals most of which have no practical importance to man, and because in most of the assay work only mice were used, it is impossible to state how much of the little we know of such correlations has any direct or indirect application to man. Doubtlessly, the terms "carcinogenicity and carcinogenic potency" are in need of reinterpretation. They should be defined in specific terms, such as species susceptibility, target organ, and route of contact, dose and vehicle used, before they acquire any real meaning.

The demonstration of correlations between the structure and cancerigenic potency of chemicals would be not only of distinct scientific importance, but also of eminent practical significance. The rapidly increasing number of environmental human cancerigens has created problems in connection with appropriate screening methods of chemicals that are of great urgency and complexity. Their solution would be simplified and hastened by the availability of information of such correlations, even if they should apply only to restricted groups of compounds.

However, the procurement of such data, obviously, depends not only on a much deeper insight into the carcinogen-host relation and their inter-reactions, but also on a widening of our present methods of bioassays, if we expect that this information may become of some value to the human cancer problem. For illustration, reference may be made to the extensive use of skin application of cancerigenic chemicals in experimental tests. Since about 75 to 95 per cent of human skin cancers can be cured with existing methods, any information as to correlation between structure and carcinogenicity of chemicals has only limited value. Likewise, the numerous studies on the hepatocarcinogenic action of certain azo compounds, while of definite value to the human problem, provide data restricted in their importance by the fact that primary liver cancer in man is uncommon. On the other hand, very little experimental work has been done to investigate the cancerigenic action of chemicals on the organs of the respiratory tract, although cancer of the lung has become a highly important tumor because of its rapidly increasing frequency and high rate of mortality.

It is apparent from these considerations that a great deal more information is needed before the human problem can benefit from the present studies on the correlation of structure and carcinogenic properties of chemicals.

CANCER CHEMOTHERAPY

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Drs. Stock and Shear have already discussed some phases of the usefulness of steroid hormones in the therapy of neoplastic disease in humans. Testosterone and the estrogens have been applied in cancer of the breast in females. Estrogens are also used in the therapy of cancer of the prostate, and recently the effects of large amounts of progesterone in cancer of the cervix have been reported. The influence of large amounts of adrenal cortical hormones (cortisone) and effects obtained after stimulation of the adrenal by ACTH on certain tumors of the lymphoid tissues are well recognized.

The correlation of chemical structure and antihormonogenic activity is still very little understood. If one considers that a slight change in the structure of a steroid hormone, often affords a striking change of biological action in most instances, a wide field for therapeutic trial is open as long as the chemist is willing to produce variations in the steroid structure and make compounds available in large enough amounts for testing. There seems to be now more hope that this will be possible than it was ten years ago. It seems that with many new steroids being made available, more thorough information will be obtained on the relation between structure, biological, and therapeutic activity on these compounds for the benefit of the suffering patients.

CANCER CHEMOTHERAPY

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Since I have a foot in both camps, I would like to put a plug in for the mouse. It has been somewhat maligned today.

I feel that clinical research on new compounds is a very expensive thing. Granted, it is the final proof of the pudding, but I think you have to have pretty good reason for going to the patient before you go there.

We have screened some three hundred compounds related to the folic acid antagonists against mouse leukemia. We must qualify this statement however. We have used a system in which the mice were on a normal diet, which I think arbitrarily rules out most of the weak antagonists. We have found that only the compounds which were full-dress folic acid antagonists - by that, I mean they contained the pteridine, the p-aminobenzoic acid, and the amino acid moieties and had an amino group in the 4-position of the pteridine ring, were active against this mouse leukemia. There was one other compound which was active, 2,6-diaminopurine, which is somewhat analogous to the 2,4-diaminopteridine. We thought, originally, that possibly the two worked the same way, but I think we have fairly definite evidence that 2,6-diaminopurine and A-methopterin (4-amino-N^{10}-methyl-pteroylglutamic acid) work in different ways on their effects on the mouse. We have a strain of mouse leukemia which has developed complete resistance to therapeutic doses of A-methopterin so that there is no prolongation of survival time. This same strain of resistant leukemia, however, is affected by 2,6-diaminopurine, and there is a significant prolongation of survival time.

One thing more I would like to mention; that is that when Dr. Shear introduced the folic acid antagonists, he called them the so-called folic acid antagonists. There is a great deal of controversy as to whether they are really folic acid antagonists, but we have definitely shown that at least the anti-leukemic effect of A-methopterin can be prevented by folic acid. It also might be appropriate to mention that this anti-leukemic effect can be prevented much more readily by the citrovorum factor. We feel that, on a dry weight basis, the citrovorum factor is probably twelve to twenty-five times as potent as folic acid in preventing the anti-leukemic effect. I hope Dr. Welch will tell us more about his very interesting studies on citrovorum factor shortly.

CANCER CHEMOTHERAPY

E. B. Schoenbach
Johns Hopkins University
Baltimore, Maryland

Dr. Shear, I do not think I understand your question too clearly. Therefore, I shall take the liberty of selecting the topic.

I think one could summarize a good deal of what has been said today, and our own attitude, in the words of Lewis Carroll that because we breathe when we sleep does not necessarily imply that we sleep when we breathe.

When we first found that the antifolic acid compound, 4-aminopteroylglutamic acid, exerted a definite inhibitory effect on the growth of sarcoma 180 and changed the cytologic appearance of the cells, we felt we should investigate to see whether these alterations were induced by the antifolic properties of this compound or whether the structural alteration in the configuration of the molecule had resulted in some new chemical compound which induced inhibition of tumor growth.

We, therefore, treated the animals with folic acid and the antimetabolite, and the tumor inhibition was reversed.

However, we could not demonstrate any change in the toxicity to the host of this antifolic acid compound in the presence of folic acid.

Time does not permit a detailed presentation of the various experiments which were performed, but one may summarize them by noting that, in acute toxicity, tests with a single injection, if the folic acid were given at least an hour before the antifolic acid compound, one could demonstrate protection to life and prevention of weight loss.

When less than an hour intervened, or the compound was given simultaneously, no protection whatsoever could be shown with the folic acid.

We also were able to show that, if a suitable interval intervened between the dosage of folic acid and the antifolic acid compound, suitable ratios of these two compounds could be used, and, still, protection would be observed; that is, we could use many times the lethal dose of antifolic acid compound and protect the mouse provided the ratio of folic acid to the antifolic acid was constant.

If one then gave repeated dosages of folic acid and antifolic acid, still trying to maintain the early injection of the folic acid, one could not protect the animal.

That was quite a stumbling block to us, and we have been unable to explain it. Dr. Welch's and Dr. Burchenal's observations, would indicate that the folic acid was converted to some other compound which protected the mouse. The intervening period of time between the injection of folic acid and the exhibition of the antagonist was probably the necessary interval for the *in vivo* conversion of the folic acid.

Our studies on mechanism of action have taken us far afield into nucleo-protein and steroid metabolism. We cannot discuss these at present, but the results are quite interesting and, I hope, will be available to everyone in published form soon.

CANCER CHEMOTHERAPY

A. D. Welch
Department of Pharmacology
School of Medicine
Western Reserve University
Cleveland, Ohio

That is quite a task you have set me. The subject of my remarks relates to a topic which the previous speakers have alluded to, namely, our interest in the citrovorum factor and the mechanism of action of aminopterin.

I cannot tell you about what we think may be the action of aminopterin without telling you a little bit about the citrovorum factor. Our interest in it developed as an outgrowth of our long concern with studies of the action of folic acid. It was reported in 1948, by Sauberlich and Baumann of Wisconsin, that a new factor was required by a microorganism called Leuconostoc citrovorum; this substance is referred to as the citrovorum factor. Later it was shown by Sauberlich that administration of folic acid to rats or human subjects increased the urinary output of the new factor.

I must skip over a great deal of evidence which has been offered by a number of contributors, particularly the ones mentioned, to indicate that this substance is a derivative of folic acid and not something which is synthesized as a result of a catalytic function of folic acid. It has prematurely been referred to as folinic acid, but, until the substance has been characterized, I think we will continue to refer to it as the citrovorum factor or factors.

When you study the urinary excretion of this material after giving folic acid, you find that there is a rather constant relationship between dosage and output of the citrovorum factor. We found, for example, in a study carried out in normal human subjects, that the output in units - and I must refer to it in units, because we have no absolutely pure material - following 5 mgm. oral doses of folic acid, is in the order of 50,000 units per day, instead of the two to four thousand units which normally are excreted daily. If ascorbic acid is given simultaneously with folic acid, there is approximately a three-fold additional increase in the output of the citrovorum factor. This intriguing observation I also cannot go into in great detail at this time.

It seemed logical, however, in trying to explain the urinary excretion of the new substance and the effect of ascorbic acid on it, to study the liver in vitro. My colleague, Dr. C. A. Nichol and I turned, therefore, to rat liver homogenates (which do not work) and to liver slices, and found that the latter are able to convert folic acid into the citrovorum factor or, at least, into materials which permit growth of the particular microorganism used in assaying for the new factor. Using a normal rat liver, a considerable formation of the citrovorum factor is observed during incubation of liver slices for two hours; the amount of activity can be doubled, approximately, by the addition, per gram of liver, of 100 micrograms of folic acid, for example, raising the amount from 1700 units to about 3400 units.

Use of liver obtained from rats deficient in folic acid gives even more striking results. The liver of the rat fed, during a period of several weeks, a purified diet free of folic acid and containing succinylsulfathiazole, contains negligible amounts of both folic acid and citrovorum factor - a further indication of the relation of the two substances. Incubation of liver slices from such an animal for a 2-hour period leads to the appearance of less than 100 units of activity, instead of nearly 2000 units, as is the case with the liver of normal rats. Addition of 100 micrograms of folic acid, under those conditions, leads to a striking increase in the amount of citrovorum factor. Thus, in one experiment, 60 units were found in the absence of folic acid, and 2500 units in its presence.

The addition of vitamin C will double the formation of citrovorum factor by liver slices from the normal rat; also, ascorbic acid will more than double the amount formed from added synthetic folic acid. However, ascorbate has little effect on the formation of the factor by the liver of the folic-deficient rat, unless folic acid is added. In other words, the 60 units which such a liver can form intrinsically, in vitro, is only increased by ascorbic acid to about 130 units. However, the addition of folic acid as well as ascorbic acid causes the formation of almost 13,000 units. There seems to be little doubt that this factor is produced from folic acid, and that the conversion is facilitated by ascorbic acid. It is suspected that the citrovorum factor is more closely related than is folic acid to a prosthetic group of which folic acid serves as a precursor.

It seemed to us possible, then, that aminopterin, the 4-amino analogue of folic acid, might block specifically this "activation" of folic acid. As Dr. Schoenbach has pointed out, many have wondered whether aminopterin functions exclusively by interfering with folic acid metabolism, or whether it has other important effects as well. If it affects only folic acid, and is truly an anti-metabolite of this vitamin, why, the question is asked, is its action in animals not effectively reversed by the metabolite it resembles?

Accordingly, aminopterin was tested in the liver slice system, and we were delighted to find that it blocks completely, or nearly completely, depending on the concentration, the formation of citrovorum factor from added folic acid. If the rat is treated with aminopterin before the liver is removed for slicing, the ability of the liver to form citrovorum factor from folic acid is found to be lacking.

Even more convincing proof that aminopterin prevents the conversion of folic acid to a physiologically utilizable substance has been obtained by the use of concentrates of this factor. Dr. Schoenbach and others have commented on the fact that the toxic effect of aminopterin cannot be reversed effectively by folic acid, if the latter is given simultaneously, and only slightly more efficiently if folic acid is given before the aminopterin. We had only a very small amount of a potent concentrate of the citrovorum factor which had been supplied to us by the Lederle Laboratories, through the courtesy of Dr. T. H. Jukes. Under conditions where daily injections of 5 mgm. of folic acid had absolutely no effect on the toxicity of 25 micrograms of aminopterin per day, injected simultaneously, and all animals died within a few days, administration of the concentrate in a dose of only 1.5 mgm. daily prevented completely the lethal effects of the analogue. This still crude material, containing approximately 250,000 units in the daily dose of 1.5 mg., not only prevented death, but, in the folic-deficient rats, growth, which had previously ceased, was initiated. Further rapid growth was maintained during the period of treatment despite daily dosage with the analogue.

I think it is safe to say that at least a major part of the action of aminopterin is due to a profound interference with the conversion of folic acid into a metabolically more active form of the vitamin. No doubt, in addition, the analogue interferes also with the utilization of the citrovorum factor. In other words, I think it may have a high affinity for at least one of the enzymes concerned with the conversion of folic acid to what we have termed citrovorum factor. In this manner, aminopterin prevents the effective utilization of folic acid, since its metabolic alteration appears to be essential to its biological efficacy as a vitamin. In addition, the utilization of the product of the conversion may be less efficient in the presence of aminopterin.

The role of ascorbic acid in the transformation is another story. However, it appears reasonable to suggest that it may play a role in one of at least two steps in the conversion of folic acid to citrovorum factor, perhaps in a reductive stage, since other reducing agents, such as gluco-ascorbic acid, also have the same effect as ascorbic acid.

The new factor may prove to be useful not only in the study of the enzyme systems involved in the metabolic alteration and utilization of folic acid for its hemopoietic and cell-growth function, but it may have a practical application in alleviating the manifestations of overdosage or of frank poisoning caused by aminopterin. However, it remains to be proved that the toxicity of the analogue, once it is fully developed, can be alleviated as effectively by the citrovorum factor, as the development of toxicity by aminopterin can be prevented by the simultaneous administration of a concentrate of this new derivative of folic acid.

How the abnormal cells of leukemic mice become resistant to aminopterin is most intriguing and deserves careful study. How the formation of the substance is disturbed, perhaps, in the megaloblastic anemias is the subject of investigative effort in progress in our laboratories,

in those of Dr. Burchenal, and in others. The next few months should add materially to our knowledge of the subject.

CANCER CHEMOTHERAPY

F. S. Philips
Sloan-Kettering Institute for Cancer Research
New York, New York

I do not think there is need to add further comment to the story of the antifolics. It seems clear that we are in position to understand their mechanism of action from Dr. Welch's lucid presentation. I believe that the mechanism by which antifolics act against some tumor cells is similar to the mechanism involved in their actions against bone marrow cells and the epithelium of the intestinal mucosa. Presumably, certain neoplastic and normal types of proliferating cells have more specific and possibly greater requirements for folic acid than other cells of the mammalian organism.

Unfortunately, such a mechanism is not useful in the ultimate therapeutic sense, since agents having a common effect in both normal and tumor cells have limited clinical value. A similar limitation of therapeutic usefulness is inherent in the application of "total body" irradiation and the nitrogen mustards to the treatment of neoplastic diseases. These agents have highly specific actions against all types of proliferating cells; and in some instances, they have proved temporarily, though significantly, effective in controlling neoplastic proliferations.

The nitrogen mustards are known to act through transformation in vivo into intermediates containing positively charged ethyleneimmonium rings. Sulfur mustards are also believed to act through in vivo transformation into ethylenesulfonium derivatives. The fact that nitrogen and sulfur mustards exhibit important biological properties in common is readily understood on the basis of their conversion in vivo into derivatives containing reactive ethylenonium moieties. This generalization was well established by investigations carried out during the recent war. More current research has provided additional information concerning the nature of the chemical configurations essential to mustard-like activity. Thus, compounds containing tertiary ethylenimine groups have been shown to inhibit and damage tumors and to elicit the profound pathological changes in normal hematopoietic tissues of mammals which characterize the actions of nitrogen and sulfur mustards. In addition, investigators in England have been finding that derivatives containing ethylenepoxy groups exert typical mustard-like activity against tumors. It would appear, then, that mustard-like properties are not only to be related to the ethylenonium configuration, but more generally to three-membered heterocyclic structures. Presumably, derivatives of three-membered heterocycles, which have as yet not been investigated, could also be expected to have similar biological properties.

The three-membered heterocycles mentioned above are characterized by high chemical reactivity and, when investigated, have been found to alkylate a large variety of important biochemical radicals. Alkylation takes place with groups in both simple organic molecules and in complex biochemical entities such as proteins or nucleic acids. The alkylating properties of the mustards have featured in explanations of their mechanism of action. In addition, certain hypotheses have attempted to account for the well-known fact that among nitrogen and sulfur mustard analogs only those containing at least two reactive groups per molecule elicit characteristic mustard-like actions. Accordingly, it has been suggested that mustard-like properties are to be related more to the capacity of single molecules to engage in multiple alkylations rather than to the possession of the three-membered heterocyclic configuration. Nevertheless, it remains unchallenged, at present, that only derivatives containing three-membered heterocycles have the capacity to elicit all of the characteristic actions of the mustards against normal and neoplastic proliferating cells both in vivo and in vitro. Furthermore, recent investigations with new compounds containing only one tertiary ethylenimine group reveal certain of these agents to inhibit Sarcoma 180 in vivo and to damage normal mammalian hematopoietic tissue in the characteristic manner of the mustards.

Dr. John Biesele in our laboratory has compared the cytological effects of mono-ethylenimines and mono-ethylenepoxy derivatives in dividing cells of the onion root tip with the effects of roentgen radiation, of a nitrogen mustard, and of a bis(ethylenimine) derivative. It is well-known that the characteristic chromosomal abnormalities caused by roentgen radiation in such cells can be duplicated by their exposure to HN2. Dr. Biesele has found the bis-(ethylenimine) to elici similar chromosomal changes. However, his most interesting findings concern the observation that ethylenimine and mono-ethylenimine derivatives, as well as a simple ethylenepoxy derivative such as glycidol (2,3-epoxy-propanol-1), cause chromosomal alterations which cannot be distinguished from those following treatment with roentgen radiation or HN2.

In view of the properties of certain mono-ethylenimine derivatives, it becomes difficult to accept the hypothesis that polyfunctional configurations are essential to mustard-like activity. Nevertheless, it remains true that compounds in which there are at least two ethylenimine moieties are 50- to 100-fold more toxic and more readily capable of producing damage to proliferating cells in vivo than are analogous mono-ethylenimine derivatives. For example, the LD_{50} of 2,4-bis-(ethylenimino)-6-amino-s-triazine in the rats is less than 1 mgm./kgm.; on the other hand, the LD_{50} of 2-ethylenimino-4,6-diamino-s-triazine is about 50 mgm./kgm. Moreover, in effective doses the mono-ethylenimines may cause lesions in tissues other than those containing proliferating cells. Thus, the LD_{50} of ethylenimine causes in the rat a severe necrotizing lesion of the renal papilla - an observation reported about 1890 by Ehrlich; when $4 \times LD_{50}$ doses of ethylenimine are administered typical mustard-like lesions are observed in bone marrow and lympoid organs. On the other hand, lesions caused by bis-(ethylenimines), like those caused by HN2, are restricted only to hematopoietic organs, intestinal epithelium and testis. It might be suggested that duplication of three-membered heterocyclic functions in molecules enhances the in vivo specificity of action against proliferating tissues.

DISCUSSION

DR. W.R. KIRNER (Director, Chemical-Biological Coordination Center): The compound CH_2-CH-CH_2OH (S) is fairly readily available if Dr. Philips is interested in testing it. It was obtained during the war when attempts were made to prepare BAL by a catalytic process. The method was not successful because dehydrogenation occurred producing the above compound. I believe a sample could be procured if Dr. Philips was interested in testing it in connection with his hypothesis.

CHAIRMAN SHEAR: I am sure that Dr. Philips, in his stimulating discussion, did not for a moment mean to imply that this postulated mode of action would be responsible for the modus operandi of other types of agents. As a matter of fact, we hope such will not prove to be the case, because the little progress that has already been made in the field of the chemical treatment of tumors has shown that even after a tumor responds, for a number of months, to a given agent, it may then, and usually does, become resistant.

If we have a variety of types of compounds which operate by means of different mechanisms, that would offer much more hope than if they all operated by the same mechanism.

Does that provoke comment from anyone?

DR. HOWARD E. SKIPPER (Southern Research Institute): Dr. Shear asked me to talk about carbamates which carried me away from things a little closer to my heart at the present moment. That is, some work on the mechanism of the anti-leukemic action of certain folic acid antagonists which fits in very well with Dr. Welch's observations; and secondly, some work on tracing 8-azoguanine (Kidder's guanazolo) which this group might find of interest.

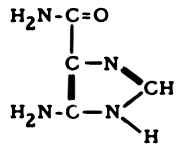
Taking up the work on labeled 8-azoguanine first, we have, working in cooperation with Dr. Stock and Dr. Sugiura of Sloan-Kettering Institute, observed the distribution of the active 2-carbon atom of this compound in C57 black mice with Eo771 tumors. The tumors did not selectively pick up the radioactive atom. Tumor and normal tissue throughout the mouse was found to contain active carbon from 8-azoguanine-2-C^{14} at 1, 6, and 24 hours after injection. Kidder had suggested that the tumor-inhibiting action of this compound might be due to differences in purine metabolism which might result in preferential 8-azoguanine incorporation in the nucleic acids of tumor tissue.

We have injected active 8-azoguanine into normal mice and after 6 and 24 hours isolated quite active nucleic acids from the viscera of the animals. The hydrolysis products of these viscera nucleic acids were then chromatographed (with a small amount of carrier 8-azoguanine) and we were pleased to find that much of the activity which resided in these nucleic acids was there as 8-azoguanine. Similar isolations with added "free" 8-azoguanine showed that the isolation procedure did not concentrate 8-azoguanine.

One cannot be too bold in interpretation of these results; however, we feel that the presence of this non-metabolite in nucleic acids might be expected to affect nucleic acid metabolism of the 8-azoguanine injected mice.

The second point I wanted to bring up has to do with some observations on the effect of aminopterin and A-methopterin on nucleic acid metabolism.

Perhaps most of you know of the interesting observation made by Dr. Woolley showing that E. coli inhibited by aminopterin build up 4-amino-5-carboxaminoimidazole.



4-amino-5-carboxamidoimidazole

You can see that this compound looks something like the purine skeleton minus the 2-carbon atom. It has been shown by Buchanan and his co-workers at the University of Pennsylvania that formate is the precursor of the 2-carbon in uric acid and presumably in nucleic acid purines. We have used the rate of incorporation of C^{14}-formate into nucleic acid purines as a means of estimating the rate of in vivo synthesis of nucleic acid purines and in turn, nucleic acids. Once one has established a base line for incorporation of formate, it is then possible to study the effects of various means of therapy on nucleic acid metabolism.

When mice were treated with A-methopterin or aminopterin, it is interesting to note that incorporation of formate into nucleic acid purines (over a six-hour period) is depressed to a small fraction of that seen in normal animals.

If, as has been suggested by Shive and co-workers, formyl folic acid (or more recently folinic acid or acids) is the prosthetic group of a co-enzyme system necessary for introduction of single carbon moieties into the purine skeleton, then these observations may help to explain the mechanism of the anti-leukemic action of folic acid antagonists.

CHAIRMAN SHEAR: Dr. Skipper, did you say that there was not a preferential concentration of the 8-azaguanine in the tumors? Did the tumors respond in any way which could be detected?

DR. HOWARD E. SKIPPER: These studies on distribution of labeled 8-azaguanine were only 6 or 24-hour experiments so no observations on tumor growth were possible. I think it is well established by the work of Dr. Gellhorn, Dr. Sugiura, and, of course, Dr. Kidder, that certain tumors (the Eo771 included) will respond to this compound.

With regard to the radioactive formate incorporation studies, we have observed that at 6 hours the specific activities of control viscera nucleic acid purines from mice were about seventeen times that of folic acid antagonist treated animals. These results have been confirmed by repeated experiments.

We feel that these results fit in very well with the observations of Woolley, who found that in the presence of aminopterin, E. coli apparently cannot incorporate the single carbon unit, the precursor of which is formate, into the 2-position of the purine skeleton.

DR. A. D. WELCH (Western Reserve University): I might add something to that.

The effect of aminopterin on the incorporation of formic acid indicates that the reaction may be catalyzed by folic acid or a derivative of it (perhaps the citrovorum factor) or a product of its metabolic activity, and it appears possible that a general function of folic acid is the transfer of a single carbon unit. That idea is not original with me, of course; a number of people familiar with the field are in this room and will be cognizant of speculation along these lines. Sakami and I reported in Atlantic City in April (1950) at the meeting of the Federated Societies that formic acid also is converted by the rat, both in vivo and in vitro, to the methyl group of methionine and we described findings indicating that folic acid, or a derivative of it, is involved in the synthesis of the methyl group, a synthesis hitherto considered not to be performed by mammalian species.

DR. HOWARD E. SKIPPER: One point I did not mention was that mice with leukemia incorporate formate into nucleic acids much more rapidly than do normal mice.

CHAIRMAN SHEAR: But, in your experiments with labeled formates, together with the experiments on the labeled 8-azaguanine, you have illuminated some questions of metabolism. So far as the treatment of tumors is concerned, just about a year ago there was tremendous publicity given to guanazolo as a possible chemotherapeutic agent. I wonder if you would care to comment, Dr. Gellhorn, on what results you got in patients with this substance.

DR. A. GELLHORN (Columbia University): I am enthusiastic about 8-azaguanine, but I have not considered its use in patients. The basis for this decision rests on the fact that guanazola, or 8-azaguanine, has had a purely carcinostatic action in all observations thus far recorded.

If a chemical compound inhibits the growth of an experimental tumor but fails to cause regression of the tumor it appears to me highly unlikely that it will show a qualitatively different effect on a malignant tumor in man and be carcinolytic. I do not wish to suggest that a carcinostatic agent for human neoplastic disease would not be a great step forward; however, the chronicity of cancer in man as compared with the mouse is such that the clinical trial of a purely growth inhibiting drug is not practical.

Although we have not given 8-azaguanine clinical trial, the drug has been used in other institutions. It is my understanding that no beneficial effects have been observed, but Karnofsky has recorded toxic manifestations in the form of skin rashes. The available clinical evidence is insufficient to determine whether the drug inhibits any human tumor.

I would like to pursue the discussion of 8-azaguanine started by Dr. Skipper. From the tracer studies with this chemical compound which he mentioned, I understand that there is no specific localization in the nucleic acids of tumor when compared to normal tissues. Does this imply that there is no selectivity in the action of 8-azaguanine on tumors? I do not believe that this necessarily follows. Barbiturates do not selectively localize in the central nervous system nor does digitalis concentrate in the myocardium; yet it cannot be denied that these drugs do have specificity of action. In our laboratory, Shapiro, Weiss and I have studied the specificity of 8-azaguanine by counting the mitoses in normal and neoplastic tissues of treated and control animals. We have found that the mitotic activity in tumors from treated animals is markedly reduced, whereas the rate of cell division in the intestinal crypts and in testicular epithelium is the same in treated and control animals. We feel that this indicates some specificity of action on tumor cells.

I would also like to ask Dr. Skipper a question. We have obtained evidence that the enzyme of normal tissues which deaminates guanine to xanthine is unable to distinguish 8-azaguanine and also deaminates this compound. The resulting xanthazola is completely inactive against tumors. In your isolation of the guanine analog from nucleic acids could you distinguish between 8-azaguanine and 8-oxaxanthine?

DR. HOWARD E. SKIPPER: I cannot answer that question positively, but I believe that our method of isolation (paper chromatography) would distinguish between guanazolo and deaminated guanazolo.

DR. A. D. WELCH: Did you find radioactivity in any of the other constituents of nucleic acids?

DR. HOWARD E. SKIPPER: Yes, we did, but I think we had something of a 10,000 or 20,000 safety factor in making this estimate, so there was not much doubt in our minds.

CHAIRMAN SHEAR: Among the novel ideas presented this afternoon was one which Dr. Gellhorn has mentioned. It has been an *a priori* assumption that one ought to look for a compound which collects preferentially in the tumor, and here was discussion of a compound for which the concentration in the tumor was lower than elsewhere.

There is another agent which is capable, at least on the first injection, of producing dramatic changes in some tumors, both in animals and in patients, viz. a polysaccharide fraction from certain bacteria. It had been expected, on the basis of the prevailing concept, that this material would be present in the tumor in much larger amounts than in the normal organs. However, when Seligman labeled it with radio-iodine, and tracer work was done it was found, somewhat to our astonishment, that the tumor contained much less of the labeled material than

did normal organs, such as liver and lung.

So, perhaps, we ought not to be unduly impressed with the requirement that a substance, to be of use in treatment of tumors, must necessarily collect preferentially in the tumor.

DR. W.R. KIRNER: In doing the analysis on nucleic acids, were they distinguished as to type?

DR. HOWARD E. SKIPPER: No. In this work we isolated combined nucleic acids and combined nucleic acid purines. Work is in progress in which desoxyribose nucleic acid and ribose nucleic acid as well as the individual purine and pyrimidine moieties of these polymers are being isolated for activity assays following injection of radioactive formate and known anti-cancer agents.

DR. W.R. KIRNER: Histologically and histochemically, there is evidence that, when aminopterin is given to animals bearing sarcoma 180, the nucleic acid continues to be formed in those cells. You might be able to show the differential incorporation of the formate carbon by separation of the two components.

DR. HOWARD E. SKIPPER: It is known that x-radiation will preferentially inhibit nucleic acid purine synthesis. Hevesy made such observations and we have confirmed them.

CHAIRMAN SHEAR: Your moderator no longer feels it his duty to needle those in attendance to contribute further to the discussion. I feel the moderator's duty has been accomplished, and his conscience is free; nevertheless, I do not want to give any implication of steamrollering or calling to an abrupt close this brief afternoon's discussion. Is there any final comment, question, or suggestion?

DR. C. CHESTER STOCK (Sloan-Kettering Institute for Cancer Research): I would like to talk briefly on a point Dr. Gellhorn raised. I believe he suggested that in tumor chemotherapy studies in which inhibition is observed as the criterion of value, the selected compounds might not have much value in the clinic. I think that is quite true for some tumors. The first slide I showed indicated that certain tumors are more sensitive to inhibitory actions than others, and that is particularly true with the rat tumors within the small group of effective compounds we have studied. One of the great misfortunes right now, I think, is that we are lacking in really adequate, effective compounds we can use as yardsticks to judge our chemotherapy screening programs.

However, just as an indication of tumor inhibition studies is the following. We have been through quite a large number of compounds; we have found nine which meet our maximum grade, positive grading. We do not mean that is the maximum we can get, but it is the maximum grading in the screening procedure. All nine of those have gone to clinical trial, I must say, not so much based on the sarcoma 180 results as the leukemia studies. All nine of those have shown some benefit clinically; seven of them are antifolics, and one is triethylenemelamine, which Dr. Gellhorn has had an opportunity to try. The ninth one is a bis-(β-chloroethyl)amine with a methoxyl pyridoxine as the third radical.

Certainly, all of those compounds are inadequate clinically, but they have shown some benefit. I am not just sure how much you want to have achieved before you consider they are worth while testing clinically.

Those have only shown inhibition in the growth of mouse tumors. I think their clinical limitations have been shown by the mouse tumors, in that the effects were temporary and there was not a large therapeutic index. Also, initiation of treatment could not be delayed long before activity against the tumor would be lost.

However, somewhat consistent with your idea of needing definite regressions, one of the antifolics and the other two compounds have caused regression in at least one rat tumor. That meets your criterion for selection of compounds, but still another tumor has shown a marked inhibition with these compounds but no regression. This is a rather small number of compounds to argue about or to base our arguments on, but I did want to bring out this point again of differences in the responses of different tumors. Thus with certain tumors you certainly might

want to take regression as the criterion, I think there might be certain tumors or certain test conditions in which you would not need to get regression before considering the compounds for clinical trials. If you want one which is really going to be a cure, then I would agree with you that remarkable anti-tumor effects must be observed in the experimental tumors.