Body Composition in Animals and Man: Proceedings of a Symposium Held May 4, 5, and 6, 1967, at the University of Missouri, Columbia (1968)

Pages
535

Size
5 x 9

ISBN
0309339936

Agricultural Board; Division of Biology and Agriculture; National Research Council

BODY COMPOSITION IN ANIMALS AND MAN

Proceedings of a Symposium
held
May 4, 5, and 6, 1967
at the
University of Missouri, Columbia

AGRICULTURAL BOARD
DIVISION OF BIOLOGY AND AGRICULTURE
NATIONAL RESEARCH COUNCIL

PUBLICATION 1598
NATIONAL ACADEMY OF SCIENCES
WASHINGTON, D.C. 1968

Available from

PRINTING AND PUBLISHING OFFICE
NATIONAL ACADEMY OF SCIENCES
2101 CONSTITUTION AVENUE
WASHINGTON, D. C. 20418

Library of Congress Catalog Card Number 68-62936

This symposium was organized by the National Academy of Sciences in cooperation with

- U.S. Department of Agriculture
  - Agricultural Research Service
  - Cooperative State Research Service
  - Economic Research Service
  - Consumer and Marketing Service
- U.S. Department of Health, Education, and Welfare
  - National Institutes of Health
- U.S. Atomic Energy Commission

The University of Missouri served as host, providing the services and facilities necessary for a successful conference.

PREFACE

The symposium on body composition in animals and man was conceived in recognition of the need for analyzing the status of information on the subject and methods of estimating body composition in the living animal, to determine the value of those methods as tools in genetic, nutritional, meat, and medical investigations. Knowing that a number of laboratories have recently planned, or are in the throes of planning, research concerned with the body composition of meat-producing animals, the Cooperative State Research Service of the U.S. Department of Agriculture suggested the probable need for such a symposium to the American Society of Animal Science. The Society brought the suggestion to the attention of the National Academy of Sciences, which in turn established a committee to determine the feasibility of, and eventually to plan, the symposium. This committee first selected the topics that should be included in the program, and then invited eminently qualified scientists to deal with these topics. The papers presented, together with open discussions of each session, constitute this volume of proceedings.

The major objectives of the symposium were to analyze and communicate certain aspects of the current knowledge of body composition and methods of estimating composition in the living animal. Further, it was hoped that such

aspects as the relative validity of various methods would be emphasized and that potentially fruitful lines of research might be explored.

A complete coverage of body-composition subject matter was not attempted. Rather, papers were selected to satisfy certain confining criteria. The most basic requirement of each paper was that it have as its foundation chemical composition determined by direct analysis of the whole body, or that it relate the results of indirect methods to directly determined body composition or, secondarily, to the results of another indirect method that has been validated by directly determined body composition. Thus, its use of directly determined body composition as the reference base line can be considered the "heart and mitochondria" of the symposium.

The symposium was planned and organized by the National Academy of Sciences–National Research Council with the support of the Agricultural Research Service, the Cooperative State Research Service, the Economic Research Service, and the Consumer and Marketing Service—all of the U.S. Department of Agriculture; the National Institutes of Health of the U.S. Department of Health, Education, and Welfare; and the U.S. Atomic Energy Commission.

The planning committee is indebted to many people. Advice and various services were provided at all stages of planning by C. F. Sierk of the Cooperative State Research Service, U.S. Department of Agriculture; G. P. Lynch of the Agricultural Research Service, U.S. Department of Agriculture; and H. B. Sprague, Executive Secretary of the Agricultural Board, National Research Council. Physical arrangements for the conference at the University of Missouri were very effectively made by a local committee: R. Dobbs, A. J. Dyer, G. B. Garner, Ruth N. Lutz, H. D. Naumann, and S. E. Zobrisky.

No institution in this country provides a more appropriate setting for a conference on body composition than the University of Missouri, for this is the land of Haigh, Moulton, and Trowbridge, who pioneered in the field of body composition almost 50 years ago. Thus, this symposium might well be considered a golden anniversary celebration of their pioneering studies of body composition in animals. This is also the place where Samuel Brody, a prodigious worker in related subjects, made novel contributions to biology.

COMMITTEE RESPONSIBLE FOR THE SYMPOSIUM

J. T. Reid, Chairman

B. C. Breidenstein
S. L. Hansard
H. H. Stonaker
S. E. Zobrisky

CONTENTS

INTRODUCTION

Josef Brožek
Lehigh University

INTERACTION OF HUMAN AND ANIMAL RESEARCH ON BODY COMPOSITION

The assigned title of this paper had a very cold, very academic ring to it. I was strongly tempted to add a more expressive subtitle. The phrase that came to my mind was "From Man to Beast and Back Again." This subtitle has merit, all right, but it has two disadvantages, one small and one large. First, I would have had to apologize to Drs. Allen and Krzywicki for borrowing their rhyme–they, not I, wrote a chapter entitled "From Body Water to Bone Mineral and Back Again."[1] Second, and more importantly, the sequence man–beast might be regarded as inappropriate, because most of the conference participants have been engaged in animal research. For them, this intellectual excursion should be entitled "From Beast to Man and Back Again."

Fortunately, this is not an occasion on which one must be concerned about protocol and rank. The title of the presentation indicates that the focal concept is the *interaction* between research on animal and human body composition. The interaction is viewed here, as it must be, as two-way traffic in methods, theories, and empirical data. The bridges lead clearly from man to beast as well as from beast to man. We have here a continuing dialogue or, if you prefer to think in terms of cybernetic models, a "reverberating circuit."

It is, then, the basic thesis of this paper that such dialogue and interaction are *essential* in research on body composition, as they are in all areas of biology and medicine. But when we look at the facts—at the history of research on body composition—we find that in the past the relationship between the two fields of endeavor has been *isolation* more frequently than *interaction*. We have not read each other's papers and journals as diligently as we should have. This has led to loss of forward motion and costly duplication of effort.

A BIT OF RETROSPECTIVE

My own research activities in the field of body composition have been limited to man. Consequently, I must disclaim at the very outset any expertness in the animal field. The Laboratory of Physiological Hygiene of the University of Minnesota, with which I have been associated for many years, has made only a brief excursion into the animal field. The study was concerned with the density of body fat extracted from the adipose tissues of man, some laboratory animals (dog, rat, rabbit, and guinea pig) and domestic animals (steer, pig, and lamb). In the domestic animals, but not in any of the other species, the density of internal fat was somewhat higher than the density of fat obtained from subcutaneous adipose tissue. The data were reported by Fidanza *et al.*[14]

My personal involvement in animal research on body composition has been indirect, organizational, and, if you wish, "inspirational." The interaction has involved several modes of contact.

Over the years, I have carried on extensive correspondence with students of animal body composition, in this country and abroad, encouraging them, clarifying details of methodology, pointing out issues of basic biological importance, and stressing the significance and implications of animal research for human biology. I have been very much pleased to see the growth of interest and research in this field—progress that is well documented by and at this symposium.

I kept in mind the interests and the contributions of the investigators of animal body composition when I organized the 1963 Conference on Body Composition, held under the sponsorship of the New York Academy of Sciences.[7] Animal research was well represented in the session on methodology.[2,19,33,38] Animal biologists also contributed to the sessions on potassium, the element considered to be an indicator of body composition,[20,23,27] and to the session on physiological considerations.[24,37]

In addition, an entire session was devoted to animal biology. The session was concerned, in part, with methodology.[4,15,25,30,31,35] Several participants explored changes in body composition during growth, with special

reference to nutrient intake.[16,26,40,42] Reports on feeding frequency and body composition[12] and on the influence of chronic acceleration[36] rounded out the session.

Even though the title of the 1963 London conference (and of the proceedings of the conference) was *Human Body Composition*,[8] two methodological papers concerned with animal body composition were included. They dealt, respectively, with measurement of the body volume of sheep[3] and with the interpretation of whole-body potassium measurements.[32]

In my editorial comment I emphasized that in experimental animal investigations one can tackle problems the investigations of which in human subjects are awkward, inefficient, imprecise, or outright impossible.[9]

Whenever the opportunity has presented itself, I have brought to the attention of human biologists the work and findings of their colleagues in the animal field, be it in the form of book reviews or of systematic reviews of the literature. Thus, in the journal *Human Biology* [*28* (2), 278-280, May 1956] I reviewed the volume entitled *Progress in the Physiology of Farm Animals*, edited by John Hammonds; in the *Quarterly Review of Biology* [*37* (1), 55, March 1962] I discussed Max Kleiber's *The Fire of Life: An Introduction to Animal Energetics*. In the version of that review prepared for *Human Biology* [*34* (4), 323–325, December 1962] I commented that neither the poetic title nor the subtitle of the book should discourage human biologists from reading it. I went on to say that the readers of *Human Biology* might be especially interested in the pages dealing with body composition, a *novum* in textbooks on animal nutrition, especially with regard to determining the composition of living animals.

In a systematic review[6] of recent literature on body composition that focused on human data, I made an extended comment concerning research on the body composition of animals. I made one general point and three specific ones: I noted that we can expect little help from animal studies regarding the specific quantitative parameters assumed in human body-composition models, such as the average mineral content of the body. Nevertheless, various methodological problems can be elucidated on the basis of animal data. But more than method is involved. In the past, important compositional concepts were defined, and valuable data were gathered by individuals concerned with the growth and development of farm animals. Thus, Moulton[29] formulated the concept of "chemical maturity," defined as a state in which the composition of the fat-free mass approximates constancy.

More specifically, while research with the traditional laboratory animals, from mice to dogs, cannot be neglected, farm animals, especially the pig, are of special interest in validating indirect methods in terms of criteria derived from anatomical and chemical analyses of carcasses.

Second, even though much of the work on animal body composition has

been done to improve "market quality," new data of fundamental importance to animal biology are gathered through the application of indirect methods in animal research. Thus, in studying the relationships between performance and body composition, Julian *et al.*[21] found that the "hot-blooded," lighter, and faster breeds of horses not only have a much greater volume of red cells (56.0 cm^3/kg) than the Percherons (37.8 cm^3/kg), but that they also have a higher water content (63.8% compared with 55.2%) and a substantially lower fat content (12.8% compared with 24.5%).

Finally, rearing animals on different "planes of nutrition" not only changes their body composition but importantly affects their longevity. Experiments carried on since 1917 on a variety of animal species indicate, with surprising consistency, that a high plane of nutrition during early life is not compatible with a long life-span.[34] Three groups of cows fed 88%, 100%, and 115%, respectively, of the Scandinavian standard allowances before the first calving and the normal (100 percent) allowances after the first calving, had average life-spans of 86.7, 80.1, and 67.2 months. Reid (reference 34, page 63) noted that a lower plane of nutrition and the resulting retardation of early growth are associated with a prolongation of the life-span in protozoa, water flies, fruit flies, silkworms, rats, mice, and cattle. In view of this overwhelming evidence, the pride of mothers, pediatricians, and baby-food manufacturers in babies' rapid growth and plumpness may be unfounded. In fact, there are few problems in human biology that call for attention more urgently than does the relation between early growth rate and adult morbidity and longevity.

SOME MISSING LINKS

As we have seen, studies of the body composition of animals and of man have vital points of contact, as well as concerns that are quite different.

In animal husbandry, economic considerations are primary, involving such criteria as rapid growth, efficiency of converting feed to meat, and—at least in some species—the desired body composition. In the proceedings of the recent Symposium on Swine in Biomedical Research, Cox[13] reminds those of us who may be too far removed from the realities of the marketplace that the value of the swine carcass depends on the relative proportions of fat and lean, with premiums paid for lean animals.

This kind of consideration is almost totally absent in human biology. Of course, twentieth century America also puts a premium on leanness in man (and woman!), but for other reasons. The internists and the insurance actuaries are distressed by the tendency of the American male to continue to grow around the middle long after he has stopped growing in height. Densitometry

as well as other techniques for the study of body composition indicate that what we put on, contrary to the wishful thinking of some, is *not* pure muscle.

Let us look at some data regarding age changes in body composition, obtained at the Laboratory of Physiological Hygiene of the University of Minnesota. Table 1 gives mean values of body density for men and women of specified age.

Data on body density and the estimated fat content [$F = (4.201/D) - 3.813$, reference 22] were obtained for five male groups. Each of the age groups represents a range of 2 to 6 years. The mean ages are 20, 25, 46, 50, and 55 years. In men, in the age range from 20 to 55 years, body density decreased from 1.072 to 1.041, with the estimated fat percentage rising from about 11% in the 20-year-old males to double this value (22%) at the age of 55.

The average weight of each of the five age groups coincided exactly with the standard weight (for age, sex, and height). The sample sizes ranged from 21 to 44 men per age group.

The data for women are more interesting, on several counts. The information is more satisfactory than for the males in that the whole age range has been covered, with no gaps. The subjects were grouped into three age categories (18–30, 31–45, and 46–67 years). The mean ages were 24, 39, and 56 years, respectively. The sample sizes ranged from 19 to 23 women per age group. Body density was strikingly lower for women, throughout.

Let us consider a sample of young men and women who are very close in age (25 years in men, 24 years in women). The average densities turn out to be 1.040 in women and 1.063 in men. This sex differential was maintained throughout the age range that we studied. In the three female groups, the body density decreased with increasing age, from 1.040 to 1.027 and 1.016, corresponding to the estimated fat content of 23, 28, and 32% of body weight.

The comparable data, then, are 14% for 25-year-old men versus 23% for women of similar age; in the middle-age range, the 55-year-old men averaged 22% fat, while their female counterparts held on to the 10% edge, with fat constituting some 32% of their body weight.

One additional comment, concerning the *relative* body weight (relative to the height–age standard, that is), may be useful. The female subjects in our sample tended to be somewhat below the standard weight; in other words, they were lighter than the "norm." Consequently, any sampling error in reference to relative weight is on the conservative side. Truly "standard" bodies would be still fatter.

In regard to sampling bias, then, we can be satisfied. Furthermore, data from other laboratories confirm both the age trends and the sex differences.

TABLE 1 Mean Values of Relative Body Weight, Density, and Estimated Fat Content as Percentage of Body Weight, by Sex and Age[a]

Items Compared	Men (Number in Group)					Women (Number in Group)		
	21	25	29	44	34	23	19	20
Age range (years)	18-22	23-29	45-47	48-52	53-57	18-30	31-45	46-67
Mean age (years)	20.3	25.2	46.0	50.0	54.6	24.2	39.1	56.0
Relative body weight (%)	100.0	99.9	100.2	99.9	100.2	95.3	97.3	95.3
Density (g/cc)	1.072	1.063	1.047	1.044	1.041	1.040	1.027	1.016
Fat (%)	11.0	14.0	20.0	21.0	22.0	23.0	28.0	32.0

[a]Minnesota data.

This information has been brought together, and I shall not tax your patience by a survey of the relevant literature.[10] The available evidence leaves no doubt about the trend in the adult American population toward lower density with increasing age. Second, there is a substantial sex difference in body density, upon which the age changes are superimposed.

We are on fairly safe ground when we interpret the significance of the decrease in density with age in both sexes principally in terms of body fat. We are safe, that is, when we limit ourselves to *qualitative statements.*

There are several reasons why we are less safe when we try to convert body-density values into total amount of fat in men and women differing in age. For one, we still do not know how accurately our model of the "reference man"[22] or the male "reference body"[11] –concepts basic to all attempts at converting body density into total body fat–applies to women.

We do know from the work of Trotter and her co-workers[5,39] that the density of female bones tends to be lower than the density of male bones. Consequently, the density of the fat-free mass in females is likely to be lower than that in males. This would automatically make women appear somewhat fatter than men when the same formula for converting density into fat content is used for both sexes.

Similar complications are present when we wish to compare adult individuals differing in age: the density of bones (of cervical vertebrae and of femurs, to be specific) decreases with age, both in men and women. To make things still worse, the age decrement is more pronounced in women than in men. Thus, other things remaining equal–which is hardly ever the case–the decrease in bone density would be reflected in a decrease in total body density. This would make densitometric estimates of the fat content of older individuals (especially of older women) somewhat higher than would correspond to reality. The precise amount of distortion is not yet known.

To some extent, human biologists can pull on their bootstraps and make progress in the elucidation of age changes in body composition. Clearly, here and elsewhere, a workable approach to a direct assessment of body fat, *in vivo*, is the answer–an important part of the answer, anyhow. But there is much to be said for a systematic study of the process of aging on a comparative basis, using appropriate animal species.

Please note that I am concerned about the *precision* with which we can convert body density into total body fat, not about the existence of age trends and sex differences. The presence of such trends and differences in body composition is attested to by several other approaches, including the "hydrometric" analysis of body composition, based on determination of the water content of the body.

For example, the data reported by Moore and his colleagues[28] indicate that the calculated body fat in the youngest group of their subjects (16–30

years of age) accounts for about 19% of body weight in the males and 30% in the females. In the older subjects, it rises to an average value of 29% in the males and 36% in the females.

Data on exchangeable potassium, also reported by Moore *et al.*,[28] indicate that ^{42}K, expressed as milliequivalents per kilogram of body weight, is lower in women than in men and decreases with age in both sexes. In males, the K_e value decreases from 48 to 45 to 37 mEq per kilogram of body weight; in females, the corresponding values are 38, 34, and 30 mEq. The data are interpreted by Moore and his co-workers as indicating that the "body cell mass"—their central compositional, somatolytic concept—decreases with age and is smaller in women. These trends are interpretable, at least in part, as the effect of increased body fat.

The part of the potassium decrease that is really interesting, and that so far has not been estimated with adequate precision, is the part that reflects an *absolute*, not a *relative*, decrease of "body cell mass"—a decrease beyond the effect of a larger fat content of the body. Moore views the "body cell mass," estimated from ^{42}K measurements, as the energy-exchanging body compartment. This makes intelligible the colorful phrase with which he describes the fundamental change in body composition with age: "It appears that during aging the 'engine shrinks within the chassis,' somewhat as it does in the wasting of a disease process."[28]

As I pointed out, it is the absolute decrease with age and the absolute sex difference, evaluated on a fat-free basis, that are of particular interest.

In terms of body composition, aging emerges as a dynamic process (or, more correctly, as a complex of processes). These changes may be viewed as a continuation of the changes taking place—at different rates, to be sure—from conception through childhood to adolescence.

There are important unsolved problems when we consider human *growth*, including the interpretation of the ^{40}K counts obtained in whole-body counters. In calculative fat-free weight, the critical item is the ratio of total K to fat-free weight. The calculation is based on the assumption that potassium, in milliequivalents, represents a constant fraction of the fat-free body weight:

$$\frac{\text{total K}}{\text{fat-free weight}} = c.$$

In the formula proposed by Forbes *et al.*,[18] the value of c is taken as 68.1.

The fat-free weight can then be calculated as

$$F\text{-}f\,(\text{kg}) = \frac{\text{measured total K (mEq)}}{68.1}.$$

Now, when we look at the data on the chemical composition of the human body reported by Widdowson and Dickerson,[41] we find that their value of K

is 69 mEq per kilogram of fat-free tissues. This value is for adult man and is encouragingly close to the value used by Forbes *et al.*[18]

So far, so good. However, when we look up Widdowson and Dickerson's figure for a full-term infant, we find that it contains not 69, as does the adult, but 53 mEq of potassium per kilogram of fat-free tissues.

We know little about the "chemical maturation" of the fat-free mass in man, considered with reference to this particular parameter of body composition. At present, and for a long time to come, we must look to the students of animal biology to provide information regarding the rate at which animal organisms increase the potassium content of their fat-free mass from birth to maturity.

Thus, one item on which additional information is needed—information comparative in character and including primates—concerns developmental changes in the potassium concentration of the fat-free mass.

ANIMAL VERSUS HUMAN RESEARCH

As compared with human studies, animal research on body composition has several important advantages, including rigorous, systematic experimentation and the possibility of checking the precision of indirect methods by direct chemical analysis. Although both points are important, I wish to stress the first one.

In this symposium, emphasis has been placed on the *validation* of indirect methods for the study of body composition. The statement introducing the program is quite specific:

> The major objectives of this Conference are to analyze and communicate certain aspects of the current knowledge of body composition, and methods of estimating composition *in the living animal*. Special emphasis will be given to the validation of indirect methods as based on direct chemical analysis of the whole body.

This continues to be a critical issue. Validation studies have been carried out in the past, of course, but there is still work to be done.

In addition to the feasibility of chemical whole-body analysis, in animal research there is also substantially greater freedom in the application of isotopes than in human studies (L. P. Novak, personal communication).

In animal biology the investigator may be concerned with a single species, such as the pig, or with a variety of species. The comparative point of view is likely to generate questions that are more general and theoretical in character, and ones that bring out more clearly the implications for man.

So far, a genuinely comparative viewpoint has not been strongly represented in research on body composition. Where research workers have been con-

cerned with more than one species, their interests have been limited, typically, to domesticated animals that are of prime economic importance—the pig, the sheep, the cow. This means that the more basic, theoretical considerations of body composition, approached on a broadly comparative basis, have not been tackled. The importance of a genuinely comparative point of view can hardly be overemphasized.

The relevance of the findings of animal research for man can be clarified by (a) the use of primates or other experimental animals that, in a particular respect, have proved serviceable as "models" for extrapolating the findings to man, or (b) the observations made in several species and examined with reference to the *consistency* of the observed phenomena. The neglect of research on the body composition of primates has been especially regrettable from the point of view of human biology.

Strictly parallel human and animal studies of the compositional changes during growth are needed. Such a comparative study of infant pigs and of human infants has been initiated by Filer and Fomon[17] at the University of Iowa. The same indirect methods are being applied to both species. In the pig, the indirect estimates of body composition are followed up by direct analyses. This is a move in the right direction.

SUMMARY AND CONCLUSIONS

As we have noted, the relations between animal and human research on body composition are complex. There is a total separation on some issues, parallelism in regard to other points, and areas of genuine interaction where the ideal of two-way traffic has been achieved.

In contrast to the primacy of economic considerations in animal husbandry, the primary criteria in applied human biology are health (including its psychological aspects), longevity, and performance capacity. These considerations and parameters could be built into animal research on body composition. In the past this has been done only to a very limited extent or not at all.

There is one last but important point: A great deal of animal research has been of the "applied" variety; yet applied research quickly gets out of breath. If it is to retain viability, it must incorporate more basic considerations. Fortunately, as Cox[13] has pointed out, the economic importance of carcass composition is providing incentive for investigation of the genetic and environmental factors involved in the differences in fat content, between breeds and within breeds. These are issues that have their analogues and close parallels in human biology.

REFERENCES

1. Allen, T. H., and H. J. Krzywicki. 1961. From body water to bone mineral and back again, p. 162–167. *In* J. Brožek and A. Henschel [ed.] Techniques for measuring body composition. Nat. Acad. Sci.–Nat. Res. Council, Washington, D.C.
2. Alsmeyer, R. H., R. L. Hiner, and J. W. Thornton. 1963. Ultrasonic measurements of fat and muscle thickness of cattle and swine. Ann. N. Y. Acad. Sci. 110:23–30.
3. Beeston, J. W. U. 1965. Determination of specific gravity of live sheep and its correlation with fat percentage, p. 49–50. *In* J. Brožek [ed.] Human body composition: approaches and applications. Pergamon Press, Oxford, England.
4. Bray, R. W. 1963. Biopsy and core techniques for estimating composition. Ann. N. Y. Acad. Sci. 110:302–306.
5. Broman, G. E., M. Trotter, and R. R. Peterson. 1958. The density of selected bones of the human skeleton. Amer. J. Phys. Anthropol. 16:197–212.
6. Brožek, J. 1961. Body composition. Science 134:920–930. 1961.
7. Brožek, J. [ed.]. 1963. Body composition. Parts 1 and 2. Ann. N. Y. Acad. Sci. 110:1–1018.
8. Brožek, J. [ed.]. 1965. Human body composition: approaches and applications. Pergamon Press, Oxford, England.
9. Brožek, J. 1965. Editor's addendum, p. 51–55. *In* J. Brožek [ed.] Human body composition: approaches and applications. Pergamon Press, Oxford, England. 1965.
10. Brožek, J. 1965. Age trends and adult sex differences in body composition, p. 23–35. *In* Homenaje a Juan Comas en su 65 anniversario, Vol. II, Antropología física. Editorial Libros de Mexico, Mexico, D.F.
11. Brožek, J., F. Grande, J. T. Anderson, and A. Keys. 1963. Densitometric analysis of body composition: revision of some quantitative assumptions. Ann. N. Y. Acad. Sci. 110:113–140.
12. Cohn, C. 1963. Feeding frequency and body composition. Ann. N. Y. Acad. Sci. 110:395–409.
13. Cox, D. F. 1966. Swine genetics and biomedical research, p. 1–12. *In* L. K. Bustad and R. O. McClellan [ed.] Swine in biomedical research. Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington.
14. Fidanza, F., A. Keys, and J. T. Anderson. 1953. Density of body fat in man and other mammals. J. Appl. Physiol. 6:252–256.
15. Feinstein, L., and R.L. Hiner. 1963. Anesthesia and its relationship to body composition. Ann. N. Y. Acad. Sci. 110:343–348.
16. Filer, L. J., Jr., and H. Churella. 1963. Relationship of body composition, chemical maturation, homeostasis, and diet in the newborn mammal. Ann. N. Y. Acad. Sci. 110:380–394.
17. Filer, L. J., Jr., G. M. Owen, and S. J. Fomon. 1966. Effect of age, sex and diet on carcass composition of infant pigs, p. 141–149. *In* L. K. Bustad and R. O. McClellan [ed.] Swine in biomedical research. Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington.
18. Forbes, G. B., J. Gallup, and J. B. Hursch. 1961. Estimation of total body fat from potassium-40 content. Science 133:101–102.
19. Gnaedinger, R. H., E. P. Reineke, A. M. Pearson, W. D. V. Huss, J. A. Wessel, and H. J. Montoye. 1963. Determination of body density by air displacement, helium dilution, and underwater weighing. Ann. N. Y. Acad. Sci. 110:96–108.

20. Hansard, S. L. 1963. Radiochemical procedures for estimating body composition in animals. Ann. N. Y. Acad. Sci. 110:229–245.
21. Julian, L. M., J. H. Lawrence, N. I. Berlin, and G. M. Hyde. 1956. Blood volume, body water and body fat of the horse. J. Appl. Physiol. 8:651–653.
22. Keys, A., and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:245–325.
23. Kirton, A. H., and A. M. Pearson. 1963. Relationships between potassium content and body composition. Ann. N. Y. Acad. Sci. 110:221-228.
24. Liebelt, R. A. 1963. Response of adipose tissue in experimental obesity as influenced by genetic, hormonal, and neurogenic factors. Ann. N. Y. Acad. Sci. 110:723–748.
25. Lynch, G. P., and G. H. Wellington. 1963. Predicting the whole body composition of living hogs from specific gravity determinations. Ann. N. Y. Acad. Sci. 110:318–326.
26. Martin, T. G., D. A. Garwood, W. V. Kessler, J. E. Christian, and F. N. Andrews. 1963. The influence of ration on body composition of growing farm animals. Ann. N. Y. Acad. Sci. 110:374–379.
27. Martin, T. G., W. V. Kessler, E. G. Stant, Jr., J. E. Christian, and F. N. Andrews. 1963. Body composition of calves and pigs measured by large volume liquid scintillation counting and conventional chemical analyses. Ann. N. Y. Acad. Sci. 110:213–220.
28. Moore, F. D., K. H. Olesen, J. D. McMurray, H. V. Parker, M. R. Ball, and C. M. Boyden. 1963. The body cell mass and its supporting environment: body composition in health and disease. W. B. Saunders, Philadelphia.
29. Moulton, C. R. 1923. Age and chemical development in mammals. J. Biol. Chem. 57:79–97.
30. Orme, L. E. 1963. Estimating composition from linear measurements, live probe, body weight. Ann. N. Y. Acad. Sci. 110:307–317.
31. Pearson, A. M. 1963. Implications of research on body composition for animal biology: an introductory statement. Ann. N. Y. Acad. Sci. 110:291–301.
32. Pfau, A. 1965. Interpretation of whole body potassium measurements, p. 57–60. *In* J. Brožek [ed.] Human body composition: approaches and applications. Pergamon Press, Oxford, England.
33. Pitts, G. C. 1963. Studies of gross body composition by direct dissection. Ann. N. Y. Acad. Sci. 110:11–22.
34. Reid, J. T. 1959. Plane of Nutrition and Livestock Performance, p. 56–64. *In* Proc. Cornell Nutrition Conference for Feed Manufacturers. Dept. of Poultry Husbandry, N.Y. State College of Agriculture, Cornell University, Ithaca, N.Y. 125 p.
35. Reid, J. T., A. Bensadoun, O. L. Paladines, and B. D. H. Van Niekerk. 1963. Body water estimations in relation to body composition and indirect calorimetry in ruminants. Ann. N. Y. Acad. Sci. 110:327–342.
36. Smith, A. H., and C. F. Kelly. 1963. Influence of chronic acceleration upon growth and body composition. Ann. N. Y. Acad. Sci. 110:410–424.
37. Steiner, G., and G. F. Cahill, Jr. 1963. Adipose tissue physiology. Ann. N. Y. Acad. Sci. 110:749–753.
38. Stouffer, F. R. 1963. Relationship of ultrasonic measurements and x-rays to body composition. Ann. N. Y. Acad. Sci. 110:31–39.
39. Trotter, M., G. E. Broman, and R. R. Peterson. 1959. Density of cervical vertebrae and comparison with densities of other bones. Amer. J. Phys. Anthropol. 17:19–25.

40. Weil, W. B., Jr., and W. M. Wallace. 1963. The effect of variable food intakes on growth and body composition. Ann. N. Y. Acad. Sci. 110:358–373.
41. Widdowson, E. M., and J. W. T. Dickerson. 1964. Chemical composition of the body, p. 2–247. *In* C. L. Comar and F. Bronnar [ed.] Mineral metabolism: an advanced treatise. Vol. 2, Part A. Academic Press, New York.
42. Wood, A. J., and T. D. D. Groves. 1963. Changes in body composition of the pig during early growth based on deuterium oxide dilution technique. Ann. N. Y. Acad. Sci. 110:349–357.

BIOLOGY

J. T. Reid, A. Bensadoun
L. S. Bull, J. H. Burton
P. A. Gleeson, I. K. Han
Y. D. Joo, D. E. Johnson
W. R. McManus, O. L. Paladines
J. W. Stroud, H. F. Tyrrell
B. D. H. Van Niekerk
and G. W. Wellington
Cornell University

SOME PECULIARITIES IN THE BODY COMPOSITION OF ANIMALS

Our initial interest in body composition arose mainly from its obvious value as a criterion of animal response to environmental treatments, especially those of a nutritional nature. In studies concerned with methods of estimating the gross chemical composition of the living animal and with the application of body-composition data, we have examined certain aspects of body composition in pigs, sheep, and cattle.

This report concerns the following subjects that reflect on the biological nature of body composition: (a) comparison of the compositional peculiarities of the porcine, ovine, and bovine species; (b) relationships between body weight and weight of chemical components; and (c) the mass-related homeostatic nature of the body composition of the ovine as influenced by frequency of meals, age, and dietary energy input, and by prolonged submaintenance energy intake followed by refeeding.

SPECIES PECULIARITIES

PROCEDURE

The relationships among the concentrations of the gross chemical components of the ingesta-free (empty) body; the fat-free, empty body; and the fat-free, dry body of pigs, sheep, and cattle were examined in correlation and regression analyses. For the same species, the heat-of-combustion values of protein and fat were derived by partitioning mathematically the total energy of the ash-free, dry body between protein and fat as described previously.[24]

The data studied came from a great variety of sources. The data for pigs, representing eight distinct breeds and one crossbred group and ranging in age from 1 to 923 days, were reported by many investigators (see references 2, 3, 10–19, 26, 29, 30, 31). Of the 714 pigs involved, sex was identified for only 248 (153 male castrates and 95 intact females).

The data for cattle represented at least seven pure breeds and five crossbred combinations. Of the 256 animals, 139 had beef conformation and 117 were of dairy-body types. The age range was from 1 to 4,860 days. Sex was designated for 249 animals (135 males and 114 females). The males were castrates, except for the 1-day-old males, which were intact. A more detailed description of the population and a summary of the original literature sources are given in the report of Reid *et al.*[25]

The data for sheep are based on body-composition measurements made at the Cornell University laboratory. Four distinct breeds and two crossbred populations representing a considerable range in body conformation, maturing rate, and mature size constituted the sheep studied. All animals were male castrates ranging from 90 to 895 days of age and from 12 to 67 kg of ingesta-free body weight.

The ranges in concentrations of the proximate chemical constituents of the three species studied are summarized in Table 1.

TABLE 1 Range in Chemical Composition of Bodies of Pigs, Sheep, and Cattle Studied

		Range in Composition of Empty Body (%)			
Species	Number in Group	Water	Fat	Protein	Ash
Pigs	714	30.7–80.8	1.1–61.5	8.3–19.6	1.3–5.6
Sheep	221	39.6–73.8	4.9–46.6	10.7–19.5	1.7–5.8
Cattle	256	39.8–77.6	1.8–44.6	12.4–20.6	3.0–6.1

RESULTS AND DISCUSSION

Relationship between the Concentrations of Water and Fat

Table 2 summarizes the correlation coefficients, prediction equations, and errors of estimate for the relationship between the concentrations of water and fat in pigs, sheep, and cattle. Although the correlation coefficients are speciously high, the range of data for each species is great (Table 1). Nevertheless, the sign of the coefficients shows that as the percentage of fat increases, the percentage of water decreases, and the squares of the coefficients indicate that 97.4 to 98.2% of the variation in fat concentration is associated with the variation in water concentration. As indicated by the regression coefficients, the rate of change in fat concentration per unit change in water percentage is greater in sheep (–1.3068) than in pigs (–1.1095) or cattle (–1.1182).

The relationships between the concentrations of water and fat were not different for females and castrate males (pigs and cattle) or for the various breeds within species (pigs, sheep, and cattle).

Composition of Fat-Free, Empty Body

As summarized in Table 3, the composition of the fat-free body varies both among and within species. For example, the concentration of protein was a little higher in the fat-free body of the ruminants than in that of the pig. Also, the coefficients of variation in the protein concentration of the fat-free body were 12.2% in pigs, 4.1% in sheep, and 7.1% in cattle.

Fat-free composition of the body was associated with body weight, age, and the weight of body fat, all of which were positively correlated with one another. With increasing body weight, age, and weight of fat, the percentages of water declined, and those of protein and mineral matter increased. However, the fat-free composition of castrate males was not different from that of intact females.

Composition of Fat-Free, Dry Body

Table 4 shows the mean composition of the fat-free, dry body. Reduction of the body to this two-component system minimized the coefficients of variation in the concentrations of protein and ash. For example, those for protein became 2.8% in pigs, 3.0% in sheep, and 2.1% in cattle, percentages that are considerably smaller than the corresponding coefficients of variation in protein concentration on the fat-free basis. Again, no differences associated with sex were observed.

TABLE 2 Relationships between the Concentrations of Water and Fat in Three Animal Species

Species	Number in Group	Correlation Coefficient	R^2	Prediction Equations[a]	$S_{y.x}$	Coefficient of Variation[b] (%)
Pigs	714	- 0.990	0.980	$Y = 89.11 - 1.1095\,X$	1.71	5.64
Sheep	221	- 0.991	0.982	$Y = 98.31 - 1.3068\,X$	1.03	4.90
Cattle	256	- 0.987	0.974	$Y = 84.29 - 1.1182\,X$	1.03	7.27

[a] Y = Fat in ingesta-free body (%); X = Water in ingesta-free body (%).
[b] $(S_{y.x} / \bar{Y}) \times 100$.

TABLE 3 Composition of the Fat-Free, Empty Body of Three Animal Species

Species	Number in Group	Water[a] (%)	Protein[a] (%)	Ash[a] (%)
Pigs	714	76.98 ± 2.69	19.18 ± 2.34	3.91 ± 0.79
Sheep[b]	221	74.89 ± 1.02	20.35 ± 0.84	4.76 ± 0.67
Cattle	256	72.91 ± 2.01	21.64 ± 1.53	5.34 ± 0.95

[a]Values are means ± standard deviations.
[b]Data for sheep on wool-free basis.

TABLE 4 Mean Composition of Fat-Free, Dry Body of Three Animal Species

Species	Number in Group	Protein[a] (%)	Ash[a] (%)
Pigs	714	83.42 ± 2.33	17.01 ± 1.69
Sheep[b]	221	81.09 ± 2.40	18.91 ± 2.39
Cattle	256	80.26 ± 1.69	19.74 ± 1.69

[a]Values are means ± standard deviations.
[b]Data for sheep on wool-free basis.

These observations, in combination with the respective species relationships between the concentrations of water and fat, make it possible to resolve body composition from either water or fat according to a schema proposed previously.[24,25]

Relationship of Sex and Dietary Treatments to Body Composition

In the considerations of the relationships between the percentages of chemical components, no evidence of differences attributable to sex or dietary treatment was detected. Also, by employing another approach we have failed to find unequivocal verification of the viewpoint often expressed that the female is fatter than the male. (It should be borne in mind that our males were castrates.) For example, in log weight of body fat–log of body weight transformations, female pigs contained more fat than male castrates of the same body weight from birth to the time they reached a body weight of 70 kg, at which

point pigs of the two sexes had the same weight of fat. Above a body weight of 70 kg, castrate males were fatter than females. Thus, the rate of increase in body fat was greater in the castrate male than in the female.

Despite a considerable range in dietary treatments imposed on the animals of all three species populations, the relationships between the concentrations of water and fat and the proportions of the fat-free, dry body consisting of protein and ash comprised one population within which the variation was relatively small for a given species. In sheep, the dietary treatments consisted of energy inputs ranging from starvation to *ad libitum* intakes, diets of various physical forms and chemical composition, and different meal frequencies. However, the interrelationships among the *concentrations of the gross chemical components* retained the same rigid patterns described above. (As will be discussed later, *the weight of body components* relative to body weight was markedly disturbed in sheep by submaintenance energy intakes followed by *ad libitum* feeding; whereas, even on the "weight" basis, the body composition of sheep maintained in continuous, positive energy balance was rigidly associated with body size.)

Energy Value of Body Protein and Fat in Three Animal Species

Since the German studies of the late nineteenth century, the calorific values of protein and fat most commonly employed have been 5.65, 5.70 and 9.5 kcal/g, respectively, for pigs, sheep, and cattle. For the most part, these values were based on the analysis of isolated muscle proteins of various states of purity and of extracted tissue lipids. Thus, the heat-of-combustion values have uncertain value for application to the conglomerate proteins and fats representing the total body.

In the present studies, the energy values were determined by partitioning the total energy between protein and fat in the ash-free, dry body, on the assumption that the amount of carbohydrate in the body is negligible. The values so derived are summarized in Table 5. These values for sheep and cattle agree well with those recently reported elsewhere.[8,20,24]

The heat of combustion of protein for all three species is markedly lower than the factor (5.65 kcal/g) commonly employed. Although the values for fat resemble the commonly used factor (9.5 kcal/g), those for the two ruminant species are somewhat lower than that for pigs. As the result of a detailed partitioning of the fatty acids of mixed lipids representing those of the total body,[4,5,6] we have observed that the average fatty-acid chain in body fat contains 17.5 C atoms in the pig and 17.2 C atoms in the sheep and that the fatty acids of the sheep with which we worked had only slightly more hydrogen than did the fatty acids of the pigs. Although other conditions, such as the

TABLE 5 Heat-of-Combustion Values of Protein and Fat in Bodies in Three Animal Species

Species	Number in Group	Protein[a] (kcal/g)	Fat[a] (kcal/g)
Pigs	172	5.348	9.608
Sheep[b]	221	5.411	9.414
Cattle	12	5.447	9.499

[a]Based on the partition of energy between protein and fat in the ash-free, dry body.
[b]Data for sheep on wool-free basis.

supporting structures for the fatty acids in the lipids, the geometry of molecules, and the lengths and angles of the chemical bonds, could be different and this could account for the difference in the heat-of-combustion value between the two species, the average difference of 0.3 C atom between the fatty acids of sheep and pigs could account for approximately 0.15 kcal/g of fat.

RELATIONSHIPS BETWEEN BODY WEIGHT AND THE WEIGHT OF CHEMICAL COMPONENTS

It is axiomatic that the weight of the chemical components of the body of the growing animal increases as the body weight increases. In previous studies [7,28] of body composition of the sheep, the weights of the chemical components were related to body weight in a predictable manner. As a consequence, it was proposed that a criterion of the adequacy of an indirect method for estimating body composition be whether the indirect method provides a more accurate estimate than does body weight alone.

During our previous studies with the sheep, it became clear that the weights of the proximal chemical components of the body increased rectilinearly with increasing body weight until the fat concentration of the body reached approximately 31%. Beginning with this concentration of fat, the weight of fat and the amount of body energy increase at an increasing rate as the body weight increases, and the weight of water and protein increase at a decreasing rate. Thus, the relationships between the chemical components and body weight of sheep are curvilinear at concentrations of body fat exceeding 31%. This observation is reminiscent of the findings of Pitts[23] that the proportion of lipocytes available to the fat-free body of guinea pigs in-

creases markedly, beginning at a level of approximately 25% of fat. He indicated that accretion of fat in the guinea pig is accomplished mainly by the saturation of existing adipose tissue but, in addition, by the engorgement of the new fat cells that appeared in very fat animals. The marked increase in fat per unit increase in body weight observed in the present studies suggests that sheep become compositionally mature when their body fat concentrations reach 31%, for at higher levels the chemical composition becomes less predictable from a common linear function.

The following section of this paper is concerned with relationships between body weight and the weights of chemical components and amount of energy in the bodies of sheep and with differences observed among certain breed populations. Also, the composition of pigs of one breed, but of three distinctly different body types, is examined.

PROCEDURE

The sheep employed in these studies consisted of 39 Rambouillet x Columbia crossbreds, 27 Hampshire x Suffolk x Shropshire crossbreds, 53 Suffolks, 54 Hampshires, 22 Corriedales, and 26 Shropshires. Within each breed population, body composition was determined over a wide range of body sizes. Various functional relationships were fitted between the weights of chemical components (as predictands) and the ingesta-free body weight (as the predictor). Among the models examined were $Y = a + bX$; $Y = aX^b$; and $Y = a + bX + c \log X$.

RESULTS AND DISCUSSION

Relationship between the Chemical Composition and Body Weight of Sheep

For the purposes of this study, the data were segregated into groups representing the individual breed populations; all animals, irrespective of fat concentration; and the animals containing less than 31% of fat. The data were fitted to the model, $Y = a + bX$; where Y = weight (kg) of chemical component or body energy (Mcal) and X = body weight (kg).

Table 6 shows certain statistical values concerned with the relationships between the chemical components and body weight in the total population (221) of sheep with no restriction on body-fat concentration. The same values derived from data representing 192 sheep containing less than 31% of fat are summarized in Table 7. A comparison of the data in the two tables reveals the decided influence of the 29 sheep that contained more than 31% of fat. The rate of increase in fat content and energy value per unit increase

TABLE 6 Relationship between Weights of Chemical Components and the Body Weight of All Sheep Studied, Irrespective of Fat Concentration or Breed

Component	Prediction Equation[a]	Correlation Coefficient	R^2	Coefficient of Variation[b] (%)
Water	$Y = 0.4067X + 5.313$	0.963	0.927	7.69
Fat	$Y = 0.4537X - 7.003$	0.945	0.893	25.34
Protein	$Y = 0.1190X + 1.199$	0.961	0.924	8.46
Energy	$Y = 4.9235X - 59.6920$	0.967	0.935	16.29

[a] Y = body component (kg) or energy (Mcal), and X = ingesta-free body weight (kg).
[b] Coefficient of variation (%) = $(S_{y \cdot x}/\bar{Y}) \times 100$.

TABLE 7 Relationship between Weight of Chemical Components and the Body Weight of Sheep Containing Less Than 31% of Fat

Component	Prediction Equation[a]	Correlation Coefficient	R^2	Coefficient of Variation[b] (%)
Water	$Y = 0.4776X + 3.511$	0.982	0.964	5.23
Fat	$Y = 0.3570X - 4.559$	0.943	0.889	21.31
Protein	$Y = 0.1386X + 0.705$	0.979	0.958	6.06
Energy	$Y = 4.1146X - 39.2214$	0.968	0.937	13.02

[a] Y = body component (kg) or energy (Mcal), and X = ingesta-free body weight (kg).
[b] Coefficient of variation (%) = $(S_{y \cdot x}/\bar{Y}) \times 100$.

in body weight was markedly greater for all animals (including the 29 very fat sheep) than for the restricted population containing less than 31% of fat. The rate of change in water and protein contents reflects that of fat. Despite the greater range of variables for the total population (221 sheep), the correlation coefficients between the chemical components and body weight are about the

same (fat and energy) or a little lower (water and protein) than those for the sheep containing less than 31% of fat.

The most marked difference between the data for the two populations was the size of the standard errors of estimate of body components. As a consequence, the coefficients of variation between the measured and predicted chemical components were 16 to 32% lower for the restricted population (i.e., less than 31% of fat) than for the total population. These data reflect the great contribution to variation provided by the data for the very fat (31 to 47%) sheep. Obviously, the relationship between accretion of the chemical components and body weight is of a different function above a concentration of 31% of fat than it is below. Thus, the data were examined by fitting curvilinear models (see Table 8).

TABLE 8 Relationship between Logarithms of Weights of Components and the Logarithm of Body Weight of All Sheep Studied, Irrespective of Fat Concentration or Breed

Component	Prediction Equation[a]	Correlation Coefficient	R^2	Coefficient of Variation[b] (%)
Water	$Y = 0.73976X + 0.15466$	0.975	0.951	2.22
Fat	$Y = 1.98776X - 2.18382$	0.936	0.876	16.14
Protein	$Y = 0.80148X - 0.50283$	0.974	0.949	4.55
Energy	$Y = 1.58987X - 0.43499$	0.970	0.941	3.45

[a] Y = logarithm of body components (kg) or energy (Mcal) and X = logarithm of ingesta-free body weight (kg).

[b] Coefficient of variation (%) = $(S_{y.x}/\bar{Y}) \times 100$.

In the treatment just described, breed of sheep was ignored, although distinct breed peculiarities were observed and are dealt with later in this report. However, the use of such prediction equations as those for sheep containing less than 31% of fat (Table 7) would refine many nutritional investigations in which growing sheep are employed. Usually sheep do not exceed 31% of fat until they have surpassed the conventional market size and condition.

Since in practice body weight is usually measured as either full-body weight or body weight measured after some degree of shrinkage, and since the prediction equations mentioned above employ the ingesta-free body weight, some means of transforming the measured body weight to the empty-body

weight is required. In the present studies, the following general equation was derived for estimating empty-body weight (EBW) from body weight measured after an 18- to 20-hr fast during which water was provided [this weight was designated shrunk-body weight (SBW)]: EBW (kg) = 0.9227 SBW_{kg} – 2.199. For this relationship, the R^2 value was 0.986, the $S_{y \cdot x}$ value was 1.53 kg, and the coefficient of variation between the estimated and measured EBW was only 4.7%. This general relationship ignores breeds and nutritional treatment. (Bensadoun *et al.* deal with some of the effects of dietary treatments on the amounts of gastrointestinal ingesta in a later paper in these proceedings; see page 452.)

Relationships between the Logarithms of the Chemical Components and the Logarithm of Body Weight

The data representing all animals (disregarding fat concentration) were fitted to the model, $Y = aX^b$; where Y = body component (kg) or body energy (Mcal) and X = empty-body weight (kg). Although the data were segregated by individual breeds also, only the data derived from the study of the total (221) population are summarized in Table 8. The functional relationships between the chemical components and the empty-body weight are described in Table 8 as $\log Y = \log a + b \log X$, with certain related statistical values.

As indicated by the R^2 values, from 88% (fat) to 94 or 95% (water, protein, and energy) of the variability in body components and energy is ascribable to variation in body weight (Table 8). However, for the total population of data, the coefficients of variation between the amounts of the measured body components and those predicted from the fitted function (i.e., from body weight) ranged from 21 to 64% of the size of the corresponding coefficients (Table 6) for the linear function (viz., $Y = a + bX$). In other words, the log–log transformations reduced the coefficients of variation of the corresponding linear functions by the following degrees: water, 71.1%; fat, 36.3%; protein, 46.2%; and energy, 78.8%.

Breed Peculiarities in the Body Composition of Sheep

On the basis of the log-component–log-body-weight equations derived from the individual breed populations, the weights of body components and the energy values of animals of five different body weights were computed. These data are shown in Table 9. Because of the undesirably large standard error of estimate of body fat, the amounts of body fat were computed from the water contents; prediction equations employed for the individual breeds corresponded with the prediction equation shown in Table 2 for all sheep.

TABLE 9 Among-Breed Peculiarities in Body Composition and Energy of Sheep (Wethers)

Breed	Body Components at Empty-Body Weight				
	20 kg	30 kg	40 kg	50 kg	60 kg
	Water (kg)				
Rambouillet x Columbia	14.2	18.3	22.0	25.3	28.4
Hampshire x Suffolk x Shropshire	12.9	17.7	22.1	26.2	30.2
Suffolk	13.5	18.4	23.0	27.4	31.5
Hampshire	13.4	18.0	22.1	26.0	29.6
Corriedale	12.4	16.6	20.4	24.0	27.4
Shropshire	12.4	16.7	20.6	24.2	27.7
	Fat (kg)				
Rambouillet x Columbia	0.9	5.4	10.4	15.9	21.8
Hampshire x Suffolk x Shropshire	2.9	6.7	10.8	15.2	19.9
Suffolk	2.0	5.3	9.1	13.2	17.7
Hampshire	2.1	5.9	10.3	15.1	20.2
Corriedale	3.4	8.0	13.1	18.4	24.0
Shropshire	3.5	7.8	12.5	17.7	23.1
	Protein (kg)				
Rambouillet x Columbia	3.8	5.0	6.1	7.2	8.1
Hampshire x Suffolk x Shropshire	3.3	4.6	5.9	7.1	8.2
Suffolk	3.6	5.1	6.4	7.7	8.9
Hampshire	3.5	4.9	6.1	7.3	8.5
Corriedale	3.5	4.6	5.4	6.2	7.0
Shropshire	3.3	4.5	5.6	6.8	7.8
	Energy (Mcal)				
Rambouillet x Columbia	39.7	78.2	126.4	183.5	248.9
Hampshire x Suffolk x Shropshire	44.7	84.0	131.3	185.7	246.5
Suffolk	38.7	74.5	118.5	169.9	228.0
Hampshire	39.6	78.4	127.2	185.1	251.6
Corriedale	51.2	96.5	151.3	214.3	285.0
Shropshire	48.9	93.6	148.5	212.2	284.2

Among the breeds studied, the Suffolks had the highest water and protein contents and the lowest fat contents, especially at body weights greater than 30 kg. On the other hand, Corriedales and Shropshires had considerably less water and protein and more fat and energy than the other breeds studied, and these differences became more pronounced as body weight increased.

Relationship between the Body Composition and Body Weight of Pigs

Although a detailed study of the relationship between body composition and body weight of pigs will be reported elsewhere, an aspect of the problem concerned with body type, back-fat thickness, and the location of body fat is considered here. The data reported by Mitchell and Hamilton[16] are employed for this purpose. These data are unique in that they represent Poland China pigs of three distinctly different body types, designated as chuffy, intermediate, and rangy. In 1929, Mitchell and Hamilton concluded:

> The carcass analyses revealed only inappreciable differences between types in spite of large differences in their market finish. The dressed carcasses of pigs of distinctly different type slaughtered at the same weight, although differing distinctly in market finish, analyzed very nearly the same. Apparently these carcasses differed in the distribution of fat but not in the content of fat.[16]

Although the data of Mitchell and Hamilton[16] represent pigs weighing from 40 to 272 lb (from 18 to 123 kg) on the empty-body basis and, therefore, pigs having a great range in composition, the present study of their data includes only the body composition of pigs ranging in empty-body weight from 90 to 100 kg. By means of covariance analysis, the weights of the chemical components in these pigs were adjusted to a common empty-body weight of 100 kg. The data are summarized in Table 10.

TABLE 10 Body Components of Poland China Pigs of Three Body Types[a]

Body Type	Number of Pigs	Water (kg)	Fat (kg)	Protein (kg)	Ash (kg)
Chuffy	15	45.2	40.9	12.9	2.3
Intermediate	26	44.8	41.5	12.4	2.2
Rangy	20	47.1	38.1	13.1	2.4

[a]Weight of body components adjusted by covariance analysis to an empty-body weight of 100 kg.

Differences in the weights of components among the three body types are very small. For example, as compared with the fat concentration of the rangy type (38.1% of empty body), the fat concentrations were only 2.8 and 3.4% greater in the chuffy and intermediate types, respectively. Because of the apparent difference between body types in the distribution of body fat, these data suggest that if the fat is not on the back or with the viscera, it must be

TABLE 11 Influence of Frequency of Meals on Body Weight Gain and Composition of Rats

Dietary Treatment	Feeding Period (days)	Total Gain		Composition of Empty Body			
		Body Weight (g)	Energy (kcal)	Water (%)	Fat (%)	Protein (%)	Ash (%)
Initial[a]	0	–	–	69.1	7.0	20.5	3.5
Fed *ad libitum*	14	85.8	235	65.4	9.9	21.0	3.7
Two meals per day	14	82.4	354	61.1	16.3	18.9	3.7

[a]Refers to body composition of 15 rats employed to establish composition at beginning of feeding period.

distributed in other parts such as the muscles, for the total amount of fat seems fairly rigidly associated with body size. This observation suggests the possibility that in breeding the "meat-type" hog, the back-fat thickness might have been reduced at the expense of increasing the intermuscular or intramuscular fat. Although it does appear that the size of the loin eye and other muscles have been improved by genetic methods, the practical situation might be that some of the fat located on the back or with the viscera in lard-type pigs may have become located in the improved pig in sites where the meat processor cannot readily cut it off. (On the other hand, at least the German Landrace breed of pigs appears to have less fat at 100 kg of body weight than did the Poland China pigs of any of the body types studied by Mitchell and Hamilton.)

MASS-RELATED HOMEOSTASIS OF BODY COMPOSITION IN SHEEP

During the past 9 years, we have investigated the effects of a variety of dietary treatments on the body composition and energetic efficiency of sheep. These treatments consisted of various energy and protein inputs, proportions of concentrates and forages in the diet, chemical compositions, physical forms, specific metabolites, and frequencies of meals. Despite marked differences in energetic efficiency between certain treatments, body composition and energy of sheep were found not to vary in a manner independent of the variability in body mass *when the animals have at least maintained body-energy equilibrium.*

In this report, the effects of frequency of meals, age-level of energy input, and prolonged ingestion of a submaintenance diet are considered in relation to the association of body composition with body weight.

Frequency of Meals

The response of rats was compared with that of sheep to diets provided in isocaloric amounts but at different meal frequencies. In rats, one member of a pair was fed *ad libitum*, and the other was provided the same amount of feed via stomach tube in two meals during the subsequent 24-hr period. The data obtained with three pairs of rats at the end of a 14-day feeding period (Table 11) are exemplary of those obtained in five other experiments involving 140 rats.

In an experiment with Suffolk sheep, two dietary treatments were imposed continuously during a 78- or 134-day period: eight meals per day and one meal per day. Both members of a pair ingested the same amount

TABLE 12 Influence of Frequency of Meals on Body Gain and Composition of Sheep[a]

Meals per Day	EBW Gain per Day[b] (g/MBS)	Composition of Empty Body[c]			
		Water (%)	Fat (%)	Protein (%)	Energy (kcal/g)
8	115 ± 20.4	59.3 ± 2.47	20.5 ± 3.40	16.4 ± 0.76	2.822 ± 0.267
1	92 ± 9.9	61.0 ± 2.82	19.5 ± 3.10	16.1 ± 0.51	2.692 ± 0.312

[a] Four sheep in each treatment group; feeding period, 78 or 134 days.
[b] Empty-body weight gain expressed as grams per kilogram of dry matter ingested per kilogram of metabolic weight (MBS).
[c] Values are means ± standard deviations.

of feed per unit of metabolic size per day. Four animals composed each treatment group; the feeding period was 78 days for two animals and 134 days for the other two animals. The gain in body weight and body composition are summarized in Table 12. (Similar data were obtained at the same time with a population of Corriedale sheep, but are not reported here.)

In the rat, meal frequency had a marked effect on body composition, but no effect on body-weight gain (Table 11). Also, rats ingesting the daily allowance in two meals utilized a much higher proportion of the dietary energy for fattening than did those allowed to nibble.

In sheep, body composition was not altered by meal frequency independently of the change in body weight. Frequent meals (eight meals per day) resulted in greater storage of energy and higher energetic efficiency of fattening than did one meal per day, but these effects were associated with an increase in body mass and its concomitant small increase in fat concentration. This relatively rigid association of body composition with body mass in sheep is distinctly different from the more plastic association that we and others[9] have observed in the rat.

Combined Effects of Age and Energy Input on the Relationship between Body Composition and Body Weight

An experiment was conducted with 26 sheep to partition the degree to which body composition is associated with age and body size. Seven of the animals were killed at the beginning of the experiment to establish the initial composition of the 19 sheep that were exposed to the experimental treatments. The sheep were divided into trios, each member of which was the same in initial body weight and age. The one odd sheep was exposed to one of the treatments (high energy input) and killed at a predetermined size. The beginning empty-body weights of the individual members of a trio ranged from 12 to 20 kg.

One member of each trio was provided a high level of feed (ca. 97 g of dry matter per kg of metabolic body weight per day) and was allowed to grow until he reached a predetermined body weight, at which time he was killed and analyzed. The two remaining members of the trio were fed a low level of feed (ca. 63 g of dry matter per kg of metabolic body weight per day). One member receiving the low level of feed was killed the same day (and hence at the same age) that his high-level mate was slaughtered. The other animal on the low level was continued until he reached the same body weight as that at which his high-level mate was slaughtered. Thus, relative to the age and size of the high-level member of a trio, one low-level animal was the same in age but considerably lower in body weight, and the other low-level animal was the same in body weight but was considerably older.

TABLE 13 Relative Dependence of Body Composition on Body Size and Age of Sheep as Modified by Level of Energy Input

Animal Number	Level of Intake per Day[a] (g/MBS)	Age (days)	EBW (kg)	Composition of Empty, Shorn Body				
				Water (%)	Fat (%)	Protein (%)	Ash (%)	Energy (kcal/g)
1	88	270	28.2	57.4	24.4	15.0	2.9	3.10
2	63	270	19.9	61.5	18.5	15.6	3.9	2.61
3	63	349	28.7	56.6	23.9	15.8	4.0	3.10
4	97	381	49.6	46.1	38.1	13.1	2.6	4.30
5	67	381	27.7	54.5	27.7	14.3	2.8	3.38
6	62	583	47.5	46.1	38.5	12.6	2.7	4.32
7	97	374	58.3	46.2	38.4	12.8	2.4	4.38
8	65	374	31.8	56.3	24.1	15.8	3.3	3.13
9	63	613	56.0	46.0	38.5	12.7	2.8	4.42

[a]Level of intake expressed as grams of dry matter ingested per kilogram of metabolic weight (MBS) per day, adjusted at weekly intervals; the mean energy gains (including wool) per kilogram of MBS per day were: low level, 31.3 kcal; high level, 58.3 kcal.

As an example of the data obtained, those for three trios are shown in Table 13. The body composition of animals of similar weight was approximately the same. As indicated by the data for the third trio (animals 7, 8, and 9), sheep as different in age as 374 and 613 days, but of about the same body weight, had very similar body composition.

A comparison was made of the standard partial regression coefficients for the individual chemical components (kilograms) on the empty-body weight (kilograms) with those computed for the regression of chemical components (kilograms) on age (days). Although this comparison revealed that the coefficients for empty-body weight were 7 to 10 times as large as those for age, normally, empty-body weight and age are correlated with each other and, in this study, the treatments that were imposed manipulated body size and age. As a consequence, R^2 values were computed and are summarized in Table 14. These values also indicate that empty-body weight is associated with a considerably greater proportion of the variability in body constituents than is age and that the addition of age to empty-body weight accounts for only 0.3 to 0.5% more of the variability than does body weight alone.

Observations in the present experiment agree with the conclusions of Tulloh,[27] who examined the body-composition data for pigs, cattle, and sheep that had been published by a number of investigators. Tulloh's study of the data of Pálsson and Vergés[21,22] revealed that the weights of muscle and fat dissected from various body parts of sheep were poorly related to age and nutritional history, but were strongly related to empty-body weight. (These data did not include sheep that had experienced a prolonged period of body-weight loss or that were older than 11 months.) In his study of data for sheep, cattle, and pigs, Tulloh[27] observed that the following ranges of the variance in the logarithms of the weights of bone (from 93.3 to 98.6%), muscle (from 98.0 to 98.7%), and fat (from 88.3 to 97.1%) were associated with the variability in the logarithm of the empty-body weight.

The Effect of Submaintenance Energy Intake on the Body Composition of Sheep

The effect of prolonged fasting followed by refeeding was investigated in an experiment consisting of two major treatments, designated as the control and partial fasting–refeeding. The control treatment consisted of feeding 12 sheep *ad libitum* (ca. 100 g of diet per kg of metabolic body weight per day). This level of dietary energy produced a continuously increasing, positive body-energy balance and resulted in an average body-weight gain of 0.36 lb per day during the first 28 days. At the end of the first 28-day period, five of the sheep were killed and analyzed. The remaining seven were continued for an

TABLE 14 Squares of Correlation Coefficients between Body Components and Age, Empty-Body Weight, and Age plus Empty-Body Weight

Body Components	Age	EBW	Age Plus EBW
Water	0.582	0.970	0.973
Fat	0.711	0.961	0.967
Protein	0.590	0.970	0.973
Energy	0.702	0.975	0.978

additional 30 days, during which the average body-weight gain was 0.34 lb per day. At the end of the 30-day period, these seven sheep were also killed and analyzed.

In the partial fasting–refeeding treatment, 12 similar sheep were fed a submaintenance diet for 26 days, during which the average body-weight loss was 0.44 lb per day. At the end of the 26-day period, five of the sheep were killed and analyzed. The remaining seven were then abruptly changed to an *ad libitum* feeding regime on which they remained for 52 days, during which the average rate of body-weight gain was 0.57 lb per day. (For the combined fasting–refeeding period of 78 days, the average body-weight gain was 0.23 lb per day.) At the end of the 52-day refeeding period, these seven sheep were also killed and analyzed.

The chemical components and energy value of the body were studied in relation to body weight. Partial fasting followed by refeeding resulted in a little more protein, considerably more water, but much less fat and energy at a given body weight than did an energy intake that produced a steady, positive body-energy gain. However, the relationships between the percentages of water and fat and the proportions of the fat-free, dry body consisting of protein were not different for the two populations of sheep.

As an example of the degree of difference between the two treatments, the relationship between body energy and empty-body weight is shown in Figure 1. The highest seven points for each treatment are directly comparable; these points represent the seven sheep that were kept in continuous, positive energy balance (*C*) for 58 days and the seven that were partially fasted for 26 days and then refed for 52 days (*F*). The other points associated with line *C* represent the five control animals killed after 28 days of feeding and the six animals killed at the beginning of the experiment to establish the initial composition of the animals exposed to the treatments. The five lowest points associated with line *F* represent the sheep killed after 26 days of partial fasting.

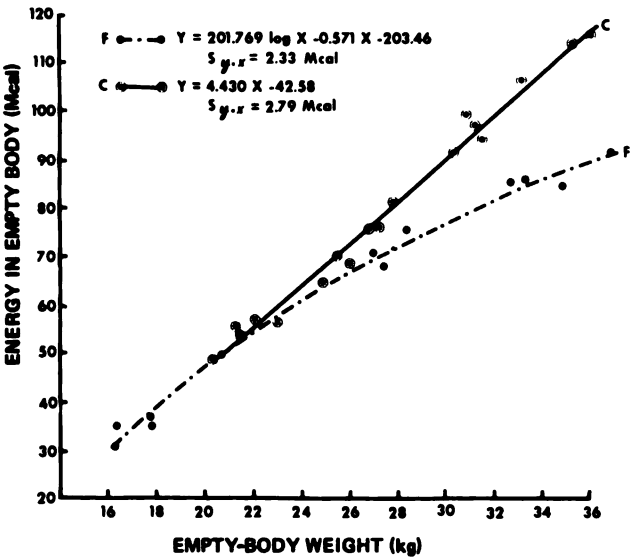


FIGURE 1 Relationships between body energy and empty-body weight of sheep maintained in positive energy balance (*C*) and of those fasted and refed (*F*).

The results of this experiment confirm one that is often reported: that the ingestion of a higher level of energy after a period of submaintenance feeding is accompanied by "compensatory" growth. During the 16-day refeeding period immediately following the partial-fasting period, the animals of the fasted–refed group gained 0.67 lb of body weight per day; for the last 14 days of the same period, the controls gained 0.35 lb per day, on the average. During the remainder of the experimental period, the fasted–refed animals (36 days) gained 0.53 lb of body weight per day, and the control animals (16 days) gained 0.33 lb per day.

Despite the high rate of body-weight gain during the last 36 days of the refeeding period, the body-fat gain by the fasted–refed sheep was only 3.56 g per kg of metabolic body weight per day, which is considerably lower than that (6.67 g) by the control animals during their last 16 days. These gains in body fat contrast with gains of 9.38 g for the fasted–refed animals during the first 16 days of the refeeding period and 5.89 g for the control animals during the last 14 days of the same period. On the other hand, the rate of protein accretion was a little greater for the fasted–refed group than for the control animals throughout the refeeding phase of the experiment.

The efficiency with which the metabolizable energy ingested above the maintenance requirement was utilized to form new body tissue was deter-

mined. (The maintenance requirement for metabolizable energy was determined in a separate experiment involving the measurement of energy storage in response to graduated inputs of the same diet.) For the 58-day period during which the control treatment was imposed, the mean efficiency was 46%. The efficiency with which the fasted–refed animals stored body energy was only 28% over that of the 78-day period. However, during the first 16 days of the refeeding period, the rate of energy conversion was 71%; during the last 14 days of the same period, the conversion rate for the control animals was 40%. During the last 36 days of the refeeding period, the efficiency of conversion was only 29% for the fasted–refed sheep, and during the last 16 days, the mean efficiency with which metabolizable energy was stored by control animals was 46%.

We do not yet have an explanation either for the exceedingly high energetic efficiency of body gain during the early part of the refeeding period or for the markedly diminished efficiency during the latter part, compared with the uniform, but intermediate, energetic efficiency of animals ingesting a level of energy that results in a continuous, increasingly positive body-energy balance. The recent studies of Anderson and Hollifield[1] suggest a possible explanation for the high energetic efficiency we observed during the early part of the refeeding period in animals previously ingesting the submaintenance diet. Those workers observed that the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase of rat adipose tissue decline during starvation and increase upon refeeding. Whether the reduced lipogenesis we observed during the latter part of the refeeding period is the result of atrophy of the hexosemonophosphate shunt enzymes is not known.

Significance of Prolonged Starvation to Relationship between Body Composition and Body Weight

Of a considerable number of dietary treatments we have imposed on sheep, the prolonged fasting–refeeding treatment was the only one found to disturb the mass-related homeostatic nature of body composition. All other treatments, irrespective of their drasticity, have maintained animals in at least a continuous, positive body-energy balance. Thus, equations for predicting the weight of chemical components from body weight derived from data representing sheep maintained in positive energy balance are not applicable to animals that have been fasted for an extended period and refed.

Despite the failure of such prediction equations when applied in the experiment cited above, body water in the same animals was estimated with a high degree of accuracy by means of the tritium-dilution method. The relationship between the total body water (water of body proper plus water of ingesta) determined by desiccation (Y) and the tritium space (X) was found

to be expressed by the equation: $Y = 0.955X + 0.415$. The coefficient of variation between the measured and estimated water contents was 2.1%, and the mean overestimation was equivalent to 2.6% of the measured water volume.

CONCLUSIONS

Certain interrelationships among the concentrations of the gross chemical constituents of the ingesta-free body of the porcine, ovine, and bovine have been quantified. The data examined within species represented a variety of breeds, body conformations, ages, sizes, nutritional histories, and sexes (though the animals were mainly intact females and male castrates). Despite the heterogeneous nature of the data, many of the relationships recorded have very small coefficients of variation. Thus, some of the relationships among the concentrations of body constituents are quite constant within species. As a consequence, the relationships derived have great utility in the resolution of the total composition and energy value of the body in experiments in which the concentration of either body water or fat can be estimated accurately.

Several differences in composition were detected among the three species. For example, the rate of change in fat concentration per unit change in the percentage of water is greater in sheep than in pigs or cattle. The fat-free body of pigs contains more water and less protein than does that of cattle; that of sheep contains intermediate concentrations. However, on the fat-free, dry basis the percentage of protein is highest in pigs, intermediate in sheep, and lowest in cattle.

The heat-of-combustion value of protein was lower and that of fat was higher in pigs than in the two ruminants. In animals of all three species, the heat-of-combustion values (5.35 to 5.45 kcal/g) of protein were considerably lower than the value (5.7 kcal/g) commonly applied.

In studies of 221 sheep representing six breed populations, the variation in the weights of the chemical components of the body was rigidly associated with the variation in the empty (and shrunk) body weight. The body components and energy increased rectilinearly with body weight until the concentration of fat in the body reached approximately 31%, irrespective of body size or age. At higher concentrations of body fat, the increases in the weights of chemical components and in the amount of energy were curvilinear functions of body weight. For the total population (221) of sheep representing a range in fat concentration of 4.9 to 46.6%, fitting the data to a double logarithmic model (viz., log weight of component = $a + b$ log body weight) revealed the following coefficients of variation between the measured and esti-

mated (from regression) *Y* values: water, 2.2%; protein, 4.5%; and energy, 3.4%. The higher coefficient of variation (16.1%) for fat is unexplained.

A variety of dietary treatments and differences in age failed to influence the body composition of the sheep independently of their effects upon body mass when a continuously positive body-energy balance was maintained. Of all the treatments we imposed, only prolonged submaintenance feeding followed by refeeding deranged the relationship between the weight of body components and body weight that we have observed in sheep kept in continuously positive energy balance. The rigidity of this relationship is distinctly greater in sheep than in rats. Although body weight was not affected, body composition of the rat was markedly pliant to meal frequency; similar treatments imposed on sheep did not affect body composition independently of the changes in body mass.

In general, the differences in body composition among breeds of sheep were small. However, a few are striking. Suffolks contained more water and protein and less fat and energy than any of the other breeds studied. On the other hand, Corriedales and Shropshires had less water and protein and more fat and energy than the other breeds.

The data obtained in the present studies, together with those of others, represent a considerable body of evidence that indicates that in the major domestic animals used for meat production, the weights of the chemical components of the body are strongly associated with body weight when healthy animals are kept in a continuously positive energy balance. For the most part, such animals are slaughtered prior to attaining mature size. However, the data recorded in this report for sheep include animals as old as 895 days (with empty-body weight as high as 150 lb) and, therefore, animals that were approaching maturity.

Although there is reluctance in some quarters to accept the viewpoint that a rigidly high degree of relationship exists between the weights of body components and body weight, the evidence is overwhelming when the conditions are as described here. Thus, it is suggested that body composition predicted from body weight can serve as a reference to which body composition estimated by indirect methods can be compared in order to determine the degree of adequacy of those methods. In addition, such relationships represent a refinement in certain kinds of experiments, particularly when growing animals are employed and a continuously positive body-energy balance is maintained.

Of a variety of indirect methods we have examined, only that based on tritium dilution has been consistently superior to body weight as a predictor. But, in ruminants, the use of the tritium-dilution method requires some means of correcting for the amount of water in the gastrointestinal tract with which tritium equilibrates. The coefficient of variation between the measured (by desiccation) total water content of the body and that estimated from tritium

dilution was only 2.1% in a population of sheep that included animals that were fasted and refed as well as those that were kept in a continuously positive energy balance.

These investigations were supported by Public Health Service Research Grant No. AM-02889 from the National Institute of Arthritis and Metabolic Diseases.

REFERENCES

1. Anderson, J., and G. Hollifield. 1966. The effects of starvation and refeeding on hexosemonophosphate shunt enzyme activity and DNA, RNA, and nitrogen content of rat adipose tissue. Metabolism 15:1098.
2. Armsby, H. P., and C. R. Moulton. 1925. The animal as a converter of matter and energy. The Chemical Catalog Co., Inc., New York.
3. Babatunde, G. M. 1965. Plane of nutrition of fattening pigs: its effect on the chemical composition of the empty body, edible carcass, and offal; and on carcass and performance characteristics of three weight groups of Yorkshire barrows and gilts. M.S. Thesis. Cornell University, Ithaca, N. Y.
4. Babatunde, G. M. 1967. Nutritional significance of essential fatty acids in swine diets. PhD Thesis. Cornell University, Ithaca, N. Y.
5. Babatunde, G. M., W. G. Pond, L. D. Van Vleck, G. H. Kroening, J. T. Reid, J. R. Stouffer, and G. H. Wellington. 1966. Relationships among some physical and chemical parameters of full- versus limited-fed Yorkshire pigs slaughtered at different live weights. J. Anim. Sci. 25:526.
6. Bensadoun, A., and J. T. Reid. 1965. Effect of physical form, composition and level of intake of diet on the fatty acid composition of the sheep carcass. J. Nutr. 87:239.
7. Bensadoun, A., B. D. H. Van Niekerk, O. L. Paladines, and J. T. Reid. 1963. Evaluation of antipyrine, N-acetyl-4-aminoantipyrine and shrunk body weight in predicting the chemical composition and energy value of the sheep body. J. Anim. Sci. 22:604.
8. Blaxter, K. L., and J. A. F. Rook. 1953. The heat of combustion of the tissues of cattle in relation to their chemical composition. Brit. J. Nutr. 7:83.
9. Cohn, C. 1963. Feeding frequency and body composition. Ann. N. Y. Acad. Sci. 110:395.
10. Ellis, N. R., and O. G. Hankins. 1925. Soft pork studies. I. Formation of fat in the pig on a ration moderately low in fat. J. Biol. Chem. 66:101.
11. Ellis, N. R., and Z. H. Zeller. 1934. Effect of quality and kind of feed on economy of gains and body composition of hogs. U.S. Dept. Agr. Tech. Bull. 412.
12. Emmett, A. D., H. S. Grindley, W. E. Joseph, and R. H. Williams. 1904. A study of the development of growing pigs with special reference to the influence of the quantity of protein consumed. Ill. Agr. Exp. Sta. Bull. 168.
13. Hogan, A. G., L. A. Weaver, A. T. Edinger, and E. A. Trowbridge. 1925. The relation of feed consumed to protein and energy retention. Mo. Agr. Exp. Sta. Bull. 73.
14. Lawes, J. B., and J. H. Gilbert. 1859. Experimental inquiry into the composition

of some of the animals fed and slaughtered as human food. Phil. Trans. Pt. II, 149:494.

15. Lowrey, R. S. 1961. Influence of energy level on protein utilization: description of a protein malnutrition condition in the young pig. PhD Thesis. Cornell University, Ithaca, N. Y.
16. Mitchell, H. H., and T. S. Hamilton. 1929. Swine type studies. III. The energy and protein requirements of growing swine and the utilization of feed energy in growth. Ill. Agr. Exp. Sta. Bull. 323.
17. Newlander, J. A., and C. H. Jones. 1935. Studies of the values of different grades of milk in infant feeding. Vt. Agr. Exp. Sta. Bull. 389.
18. Oslage, H. J. 1962. Untersuchungen uber die Korperzusammensetzung und der Stoffansatz wachsender Mastschweine und ihre Beeinflussung durch die Ernahrung. 2. Mitteilung. Korperzusammensetzung und Stoffansatz wachsender Mastschweine unter den Bedingungen normaler Ernahrung. Ztschr. Tierphysiol. Tierernahrung Futtermittelk. 17:357.
19. Oslage, H. J. 1963. Untersuchungen uber die Korperzusammensetzung und der Stoffansatz wachsender Mastschweine und ihre Beeinflussung durch die Ernahrung. 3. Mitteilung. Einfluss einer eingeschrankten Energiezufuhr im zweiten Teil der Mastperiode auf Korperzusammensetzung und Stoffansatz wachsender Mastschweine. Ztschr. Tierphysiol. Tierernahrung Futtermittelk. 18:14.
20. Paladines, O. L., J. T. Reid, A. Bensadoun, and B. D. H. Van Niekerk. 1964. Heat of combustion values of the protein and fat in the body and wool of sheep. J. Nutr. 82:145.
21. Pálsson, H., and J. B. Verges. 1952. Effects of plane of nutrition on growth and development of carcass quality in lambs. I. The effect of high and low planes of nutrition at different ages. J. Agr. Sci. 42:1.
22. Pálsson, H., and J. B. Verges. 1952. Effects of plane of nutrition on growth and development of carcass quality in lambs. II. Effects on lambs of 30 lb. carcass weight. J. Agr. Sci. 42:93.
23. Pitts, Grover C. 1963. Studies of gross body composition by direct dissection. Ann. N. Y. Acad. Sci. 110:11.
24. Reid, J. T., A. Bensadoun, O. L. Paladines, and B. D. H. Van Niekerk. 1963. Body water estimations in relation to body composition and indirect calorimetry in ruminants. Ann. N. Y. Acad. Sci. 110:327.
25. Reid, J. T., G. H. Wellington, and H. O. Dunn. 1955. Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. J. Dairy Sci. 38:1344.
26. Swanson, C. O. 1921. Effect of ration on development of pigs. J. Agr. Res. 27:279.
27. Tulloh, N. M. 1963. The carcase compositions of sheep, cattle, and pigs as functions of body weight. *In* Symposium on carcase composition and appraisal of meat animals. (CSIRO, Australia) 5:1.
28. Van Niekerk, B. D. H., J. T. Reid, A. Bensadoun, and O. L. Paladines. 1963. Urinary creatinine as an index of body composition. J. Nutr. 79:463.
29. Washburn, R. W., and C. H. Jones. 1916. Studies of the values of different grades of milk in infant feeding. Vt. Agr. Exp. Sta. Bull. 195.
30. Wood, A. J., and T. D. D. Groves. 1965. Body composition studies on the suckling pig. I. Moisture, chemical fat, total protein and total ash in relation to age and body weight. Can. J. Anim. Sci. 45:8.
31. Wood, T. B. 1926. Studies of the nutrition of young animals. I. Energy exchanges in the growing pig. J. Agr. Sci. 16:425.

Grover C. Pitts
and
T. Robert Bullard
University of Virginia

SOME INTERSPECIFIC ASPECTS OF BODY COMPOSITION IN MAMMALS

Studies of a number of laboratory and domesticated species of mammals have shown that fat is the most variable of gross body chemical components,[1,8,13] whereas in comparable age groups the composition of the fat-free body is relatively constant with respect to water,[8,10,13,15] nitrogen,[8,10,13] ash,[8,10] and anatomical components (G. C. Pitts, unpublished data). The few species show both similarities and differences. The present study is an attempt to determine what similarities and differences in body composition occur in a wide variety of mammalian species, both wild and domesticated. In addition, we will investigate whether data on body composition of one species have predictive value when applied to another and whether observed differences between species are related to body size. We shall deal primarily with fat, water, muscle, and bone in relation to the total fat-free body weight (FFBW).

MATERIALS AND METHODS

The 207 wild mammals used in this study were collected in the field in live traps, with few exceptions, and were killed immediately upon recovery. Some

of the smaller specimens were captured in snap traps. The bats were taken in nylon "mist" nets. Immature specimens, as judged by linear body measurements, were rejected. The Alaskan specimens were collected in the vicinity of Fairbanks, Paxon, Gulkana Glacier, Denali Highway near the McLaren River, and Steese Highway near Circle. The Brazilian specimens were collected in the vicinity of Salvador da Bahia, and those from Wisconsin, in the vicinity of Madison. The Virginia specimens were collected at many stations scattered throughout the state.

After total body weight was recorded, each animal was depilated with barium sulfide solution, a treatment that causes no statistically significant change in any of the parameters reported. Then the following components were separated by gross dissection: heart, liver, kidneys, spleen, empty gut, brain and spinal cord (central nervous system, or CNS), skeletal muscle, unscraped bone with marrow, skin with subcutaneous adipose tissue, internal adipose tissue, and a "remainder" category including shed blood. On each component we determined percentage of water, by freeze-drying to constant weight, and percentage of fat, by extraction with ethyl ether followed by petroleum ether. A more detailed description of the entire methodology has been presented elsewhere.[14,15]

Our sample of mammals was markedly expanded by calculations made on data in the literature on various species of large mammals. Wherever possible, these calculations were submitted for approval to the original authors, who are acknowledged in each case. However, it was not possible to calculate all parameters on all species. These calculations comprise data on 259 individuals, making a total sample of 466.

This sample is heterogeneous; it contains the following identifiable sources of variability: taxonomic, geographic, sexual, seasonal, and genetic. The complex taxonomic breakdown is evident in Tables 1 and 2, there being 11 orders, 23 families, 41 genera, and 49 species. Geographical distribution is as follows: Alaska 69, Virginia 65, Brazil 50, Wisconsin 23, and others 259; distribution by sex: 247 males, 171 females, and 48 castrates. Most of the Alaskan and Wisconsin specimens were collected in summer, and those from Virginia and Brazil in winter. Thus, it appears impossible to segregate all of these variables with this sample. The most readily demonstrable correlations are likely to be those strong enough to transcend the "noise" created by the unsegregated variables.

Corrected live weight (CLW) is defined as gross body weight, less the weight of gut content and fur. Total fat (or water) is obtained by summation of the fat (or water) from all components, and fatness is expressed as the ratio in percentage of total body fat to corrected live weight. Because variations in fat obscure the quantitative aspects of body composition, we have expressed

TABLE 1 Characteristics of the Experimental Animals

Family, Order, Common Name, and Species	Site	Number		CLW[a] (g ± SD)	Total Fat (g ± SD)	FFBW[b] (g ± SD)
		Male	Female			
Marsupialia						
Didelphiidae						
(Opossum)						
Didelphis marsupialis	Virginia	1	1	1,411	107	1,304
Didelphis sp.	Brazil	1	1	949.4	104.1	841.7
Insectivora						
Talpidae						
(Mole)						
Scalopus aquaticus	Virginia	1	–	44.64	1.23	43.41
Soricidae						
(Shrew)						
Sorex cinereus	Alaska	7	–	3.72 ± 1.10	0.16 ± 0.08	3.56 ± 1.01
Blarina brevicauda	Wisconsin	1	–	14.09	0.50	13.78
Chiroptera						
Vespertilionidae						
(Bats)						
Myotis lucifugus	Virginia	1	–	4.19	0.07	4.12
Eptesicus fuscus	Virginia	2	–	17.88	1.51	16.37
Nycteris borealis	Virginia	1	–	7.94	0.33	7.61
Molossidae						
Molossus major	Brazil	3	–	11.07	0.22	10.89
Phyllostomidae						
Artibeus jamaicensis	Brazil	5	9	40.47 ± 4.86	3.79 ± 2.55	36.18 ± 3.85
Artibeus lituratus	Brazil	1	4	63.65 ± 9.45	6.22 ± 2.37	57.19 ± 6.39

TABLE 1 Continued

Family, Order, Common Name, and Species	Site	Number Male	Number Female	CLW[a] (g ± SD)	Total Fat (g ± SD)	FFBW[b] (g ± SD)
Glossophaga soricina	Brazil	2	1	7.22	0.25	7.15
Phyllostomus discolor	Brazil	7	–	34.37 ± 3.82	2.38 ± 1.96	32.20 ± 2.25
Phyllostomus hastatus	Brazil	2	–	92.26	5.41	87.05
Sturnira lilium	Brazil	–	2	15.39	1.206	14.25
Vampyrops lineatus	Brazil	–	3	22.03	1.587	20.24
Primates						
Callitrichidae						
(Marmoset)						
Callithrix jacchus	Brazil	1	3	186.0	8.77	176.2
Edentata						
Dasypodidae						
(Armadillo)						
Euphractos sexcinctus	Brazil	2	–	2,459	252.2	2,123
Lagomorpha						
Ochotonidae						
(Pika)						
Ochotona collaris	Alaska	–	1	120.9	7.0	113.9
Rodentia						
Sciuridae						
(Marmots)						
Marmota monax	Virginia	1	–	1,638	88	1,550
Marmota monax	Alaska	–	1	2,750	985	1,765
Marmota caligata	Alaska	–	2	3,558	749	2,809

TABLE 1 Continued

Family, Order, Common Name, and Species	Site	Number Male	Number Female	CLW (g ± SD)	Total Fat (g ± SD)	FFBW (g ± SD)
(Chipmunk)						
Eutamias minimus	Wisconsin	16	6	60.70 ± 12.59	1.08 ± 0.63	59.08 ± 12.51
(Squirrels)						
Citellus undulatus	Alaska	3	1	479	21	458
Tamiasciurus hudsonicus	Virginia	–	1	166.5	3.6	162.9
Tamiasciurus hudsonicus	Alaska	9	2	192.8 ± 20.0	3.8 ± 2.4	189.0 ± 18.9
Sciurus carolinensis	Virginia	1	–	499	11	488
Castoridae						
(Beaver)						
Castor canadensis	Virginia	1	1	9,331	865	8,466
Castor canadensis	Alaska	1	–	17,640	2,013	15,620
Cricetidae						
(Mice)						
Peromyscus leucopus	Virginia	5	4	16.99 ± 2.09	0.59 ± 0.14	16.40 ± 2.03
Oryzomys palustris	Virginia	1	–	61.62	7.88	53.74
Lemmus trimucronatus	Alaska	3	2	41.62 ± 16.17	0.75 ± 0.38	40.87 ± 15.88
Clethrionomys gapperi	Virginia	1	–	18.34	0.14	18.20
Microtus pennsylvanicus	Virginia	2	5	31.38 ± 7.16	1.20 ± 0.47	30.18 ± 5.89
Clethrionomys rutilus	Alaska	10	10	25.27 ± 5.00	0.72 ± 0.32	24.55 ± 4.93
Microtus oeconomus	Alaska	1	8	24.83 ± 7.03	0.45 ± 0.01	24.38 ± 7.07
Microtus pinetorum	Virginia	1	6	19.41 ± 2.68	0.45 ± 0.22	18.96 ± 2.69
(Musk rat)						
Ondatra zibethica	Virginia	3	5	1,180 ± 117	86 ± 17	1,094 ± 114
Ondatra zibethica	Alaska	1	–	483	5	478

TABLE 1 Continued

Family, Order, Common Name, and Species	Site	Number Male	Number Female	CLW[a] (g ± SD)	Total Fat (g ± SD)	FFBW[b] (g ± SD)
Muridae						
(Mice)						
Mus musculus	Virginia	2	2	15.88	0.96	14.92
Albino mouse	Virginia	4	4	21.23 ± 2.45	1.95 ± 0.64	19.28 ± 2.12
Erethizontidae						
(Porcupine)						
Erethizon dorsatum	Alaska	1	1	5,339	674	4,725
Cuniculidae						
(Paca)						
Cuniculus paca	Brazil	1	–	1,565	196.5	1,368
Dasyproctidae						
(Agouti)						
Dasyprocta aguti	Brazil	1	1	2,097	263.4	1,833.8
Carnivora						
Procyonidae						
(Raccoon)						
Procyon lotor	Virginia	3	–	6,040	1,013	5,027

TABLE 1 Continued

Family, Order, Common Name, and Species	Site	Number Male	Number Female	CLW[a] (g ± SD)	Total Fat (g ± SD)	FFBW[b] (g ± SD)
Mustelidae						
(Ermine)						
Mustela erminea	Alaska	3	–	183.3	3.1	180.2
(Mink)						
Mustela vison	Virginia	2	–	1,032	66	966
(Wolverene)						
Gulo luscus	Alaska	–	1	9,362	562	8,800
Felidae						
(Lynx)						
Felis (Lynx) canadensis	Alaska	–	1	7,688	1,120	6,568
(Bobcat)						
Felis (Lynx) rufus	Virginia	1	2	6,152	738	5,414

[a]CLW (corrected live weight) = live weight, less weight of fur and gut content.

[b]FFBW = CLW, less total extractable fat.

TABLE 2 Characteristics of Species Extracted from the Literature[a]

Family, Order, Common Name, and Species	References	Number Male[b]	Number Female	Gross Body Weight (kg ± SD)	Total Fat (kg ± SD)	FFBW[c] (kg ± SD)
Lagomorpha						
Cavidae						
(Guinea pig)						
Cavia cobaya	14	26	20	0.684 ± 0.134	0.129 ± 0.066	0.555 ± 0.083
Artiodactyla						
Bovidae						
(Domestic cattle)	2	13	–	486 ± 68	165 ± 19	321 ± 34
	9	21	–	418 ± 195	113 ± 47	305 ± 99
	6,7	14	16	387 ± 70	100 ± 28	287 ± 41
Perissodactyla						
Equidae						
(Domestic horse)	4	2	8	611 ± 240	124 ± 58	487 ± 156
Cetacea						
Balaenopteridae						
(Fin Whale)						
Balaenoptera physalus	11	10	16	52,000 ± 8,400	17,800 ± 1,580	34,300 ± 5,760
(Blue Whale)						
Sibbaldus musculus	11	12	20	86,000 ± 18,000	32,100 ± 2,840	53,900 ± 11,190
Primates						
Hominidae						
(Man)	12	81	–	75.5 ± 11.5	11.8 ± 2.9	63.7 ± 7.3

[a] Calculated from published data, as indicated.
[b] All cattle listed as males are castrates.
[c] FFBW = corrected live weight (live weight, less weight of fur and gut content), less total extractable fat.

all other components (even adipose tissue) on the fat-free basis, and the fat-free condition will be understood without restatement in each case.

RESULTS AND DISCUSSION

Table 1 is an inventory by taxonomic group of some of our basic data; and Table 2 is a similar inventory of data recalculated from other investigators. Note that the number of individuals per species is statistically much more satisfactory in Table 2 than in Table 1.

Tables 3, 4, and 5 present percentage composition of the fat-free body of the Alaskan, Virginian, and Brazilian species, respectively. Tables 6, 7, and 8 present the percentage distribution of total fat among various organs and tissues of the Alaskan, Virginian, and Brazilian species, respectively. All statistical analyses on all species are not yet complete; hence, a few are missing from these tables and Table 9.

Comparative physiology and biochemistry are largely descriptive at this stage of their development, and Tables 1 through 8 reflect this fact. Below, we present several relationships with functional significance extracted from the total body of data, and we continue our search for others. Hopefully, others will be able to put these data to similar uses.

FATNESS VERSUS BODY SIZE

Our qualitative impression that larger mammalian species possess relatively more body fat than smaller ones is verified in Figure 1, where percentage of body fatness is plotted against fat-free body weight on log scales. Remembering that regression analysis requires randomly distributed Y values, we must acknowledge that percentage of body fatness may not comply. Since the significance of the relationship cannot be demonstrated otherwise, percentages are employed; and we have limited our objective to the demonstration of an interaction between the two parameters. The actual values of the constants in the regression equation may well be in doubt.

The relationship in Figure 1, which appears curvilinear on a semilog plot, may be represented by the equation

$$\text{Fatness} = 1.5\ (\text{FFBW})^{0.2}, \qquad (1)$$

where FFBW is fat-free body weight. Clearly, in this large, heterogeneous sample, body fatness is directly correlated with body size from less than 5% in mice to about 35% in blue whales.*

*Figure 1 spans the maximal range of body size from the shrew (3g) to the blue whale (86,000,000 g).

TABLE 3 Percentage Composition of the Fat-Free Body of Alaskan Mammals[a]

Number and Species	Heart	Liver	Kidneys	Spleen	Gut	CNS	Skin	Muscle	Bone	Bone Ash	Adipose	Remainder
1	Ochotona collaris											
	0.64	4.00	1.43	0.27	9.71	2.69	10.3	50.2	9.94	3.55	12.2	4.92
1	Marmota monax											
	0.55	7.48	0.83	0.21	5.22	0.67	14.7	46.4	8.86	3.25	16.9	2.06
2	Marmota caligata											
	0.59	5.14	0.67	0.235	5.26	0.725	11.6	59.5	9.15	3.18	7.40	2.31
4	Citellus undulatus											
	0.56	5.25	0.80	0.265	5.25	1.31	11.2	56.3	8.30	2.90	14.0	2.96
11	Tamiasciurus hudsonicus											
	0.89	3.03	0.90	0.16	3.86	2.91	11.5	60.4	9.61	3.30	13.5	3.96
	±0.08	±0.91	±0.12	±0.04	±0.71	±0.25	±0.9	±2.7	±0.34	±0.32	±6.8	±0.87
1	Castor canadensis											
	0.35	2.88	0.66	0.04	6.37	0.32	11.8	54.4	10.2	4.97	10.1	2.80
5	Lemmus trimucronatus											
	0.69	5.15	0.99	0.19	7.45	2.52	12.5	48.8	9.35	3.49	–	3.91
	±0.20	±1.01	±0.16	±0.05	±2.12	±1.49	±2.49	±3.8	±3.32	±1.27	–	±1.03

TABLE 3 Continued

Number and Species	Heart	Liver	Kidneys	Spleen	Gut	CNS	Skin	Muscle	Bone	Bone Ash	Adipose	Remainder
20	Clethrionomys rutilus											
	0.79	6.59	1.23	0.31	7.32	2.43	12.1	46.2	7.90	2.70	–	4.44
	±0.08	±1.60	±0.28	±0.16	±2.65	±0.61	±2.0	±4.6	±1.56	±0.36	–	±2.41
9	Microtus oeconomus											
	0.77	6.58	1.66	0.29	9.01	2.73	13.7	45.6	10.0	3.02	–	3.83
	±0.10	±0.91	±0.26	±0.15	±1.36	±0.82	±3.4	±5.8	±1.7	±0.29	–	±1.56
1	Ondatra zibethica											
	0.52	2.80	0.66	0.04	5.17	0.97	13.7	58.3	14.0	5.30	7.37	2.23
2	Erethizon dorsatum											
	0.51	5.40	1.08	0.10	7.72	0.80	15.5	46.5	12.2	3.83	18.1	3.38
3	Mustela erminea											
	1.04	3.56	1.06	0.255	3.73	3.71	11.3	58.1	12.2	3.63	26.8	3.55
1	Gulo luscus											
	0.92	4.15	0.71	0.23	3.51	0.97	9.30	59.9	9.99	4.23	12.8	3.58
1	Felis (Lynx) canadensis											
	0.42	3.86	0.54	0.17	2.45	1.60	8.79	66.1	9.61	4.27	6.40	3.00

[a]Expressed in terms of (fat-free component × 100) / FFBW.

TABLE 4 Percentage Composition of the Fat-Free Body of Virginian Mammals[a]

Number and Species	Heart	Liver	Kidneys	Spleen	Gut	CNS	Skin	Muscle	Bone	Bone Ash	Adipose	Remainder
2	Didelphis marsupialis											
	0.58	4.38	0.71	0.48	4.27	0.58	10.9	52.3	15.6	6.22	7.05	3.35
1	Scalopus aquaticus											
	0.78	5.03	1.29	0.47	5.65	2.32	13.9	50.4	12.2	4.11	–	1.56
1	Eptesicus fuscus											
	1.18	5.95	1.23	0.81	4.89	1.97	17.1	45.4	13.8	–	–	4.67
3	Procyon lotor											
	0.72	3.57	0.69	0.52	3.86	1.16	10.5	58.1	10.3	4.14	21.7	2.98
2	Mustela vison											
	0.79	3.93	0.87	0.50	3.64	1.87	11.7	60.2	8.31	3.02	10.8	3.32
3	Felis (Lynx) rufus											
	0.47	3.36	0.72	0.15	4.28	1.51	6.87	66.5	10.2	4.52	4.52	2.19
1	Marmota monax											
	0.42	4.11	–	0.08	5.90	0.88	14.6	52.6	9.20	2.91	15.2	3.70
1	Tamiasciurus hudsonicus											
	0.74	2.86	0.94	0.16	2.12	3.25	12.2	61.5	10.4	3.54	4.62	4.12
1	Sciurus carolinesis											
	0.58	2.71	0.62	0.36	1.62	1.82	12.7	62.8	10.6	4.43	5.10	5.34
2	Castor canadensis											
	0.33	3.34	0.86	0.05	6.25	0.63	14.0	54.6	10.6	3.32	17.0	2.87
9	Peromyscus leucopus											
	1.04	6.05	1.62	0.21	6.84	3.70	10.8	48.9	9.07	3.29	–	5.34
	±0.13	±1.02	±0.23	±0.04	±1.43	±0.45	±0.1	±2.1	±1.03	±0.49	–	±2.69

TABLE 4 Continued

Number and Species	Heart	Liver	Kidneys	Spleen	Gut	CNS	Skin	Muscle	Bone	Bone Ash	Adipose	Remainder
1	Oryzomys palustris											
	0.64	7.45	1.54	0.44	5.09	2.06	13.0	50.1	9.92	3.50	–	3.07
1	Clethrionomys gapperi											
	0.71	4.04	1.28	0.23	8.99	3.50	11.1	50.8	12.2	4.01	–	5.56
7	Microtus pennsylvanicus											
	0.86	7.41	1.78	0.26	8.82	2.52	10.7	47.9	8.57	3.92	–	5.00
	±0.11	±1.63	±0.27	±0.15	±1.47	±0.47	±1.9	±2.3	±1.45	±0.32	–	±1.60
7	Microtus pinetorum											
	0.79	7.40	1.34	0.70	7.71	2.98	9.80	49.9	9.82	3.89	–	2.92
	±0.06	±1.63	±0.48	±0.32	±1.82	±0.44	±0.90	±3.0	±1.06	±0.36	–	±0.67
8	Ondatra zibethica											
	0.32	2.95	0.48	0.05	3.59	0.65	12.9	62.1	10.6	4.81	7.38	2.00
	±0.04	±0.49	±0.05	±0.02	±0.45	±0.06	±1.3	±1.4	±1.1	±0.70	±2.07	±0.53
4	Mus musculus											
	1.03	6.79	1.92	0.49	6.06	3.22	12.4	47.4	8.16	2.99	–	4.24
8	Laboratory albino mice											
	0.70	8.09	2.11	1.16	9.20	2.35	12.6	42.3	6.92	2.75	–	5.62
	±0.07	±2.51	±0.43	±0.42	± 1.09	±0.22	±0.9	±2.7	++0.64	±0.20	–	±0.89

[a] Expressed in terms of (fat-free component × 100) / FFBW.

TABLE 5 Percentage Composition of the Fat-Free Body of Brazilian Mammals[a]

Number and Species	Heart	Liver	Kidneys	Spleen	Gut	CNS	Skin	Muscle	Bone	Bone Ash	Adipose	Remainder
2	Didelphis sp.											
	0.82	3.87	0.60	0.22	4.30	0.97	16.5	52.5	10.1	3.20	17.9	2.92
3	Molossus major											
	1.38	2.02	0.69	0.33	2.30	3.23	17.4	50.6	12.5	4.14	–	3.61
14	Artibeus jamaicensis											
	1.30	4.82	1.03	0.26	4.73	2.66	14.39	49.8	12.37	3.94	–	3.13
	± 0.23	±0.93	±0.10	±0.07	±0.78	±0.44	±1.14	±3.49	±1.37	±0.53	–	±0.82
5	Artibeus lituratus											
	1.29	4.44	1.11	0.42	2.54	2.12	15.70	50.8	14.15	4.96	–	3.03
	±0.31	±1.99	±0.25	±0.48	±0.53	±0.09	±2.98	±4.51	±3.21	±0.74	–	±0.52
3	Glossophaga soricina											
	1.37	2.81	0.82	0.18	2.56	5.18	16.38	54.0	9.64	1.62	–	4.21
7	Phyllostomus discolor											
	1.12	3.62	0.916	0.24	3.32	3.10	17.61	51.2	12.03	3.98	–	3.97
	±0.11	±0.83	±0.56	±0.21	±0.82	±1.07	±1.54	±2.15	±1.24	±0.70	–	±0.75

TABLE 5 Continued

Number and Species	Heart	Liver	Kidneys	Spleen	Gut	CNS	Skin	Muscle	Bone	Bone Ash	Adipose	Remainder
2	Phyllostomus hastatus											
	1.02	3.15	0.595	0.33	2.81	2.41	16.05	54.0	13.50	4.67	–	2.81
2	Sturnira lilium											
	1.12	5.71	1.52	0.25	4.22	4.36	15.35	44.4	14.30	3.58	–	3.17
3	Vampyrops lineatus											
	1.21	5.20	1.01	0.21	3.11	3.76	12.94	52.3	11.04	3.44	–	3.07
4	Callithrix jacchus											
	0.69	4.75	0.58	0.12	3.94	4.29	11.81	49.9	15.15	5.01	–	3.34
2	Euphractos sexcinctus											
	0.61	6.09	0.68	0.34	4.08	0.91	7.18	40.7	12.68	3.79	7.9	2.88
1	Cunniculus paca											
	0.57	5.10	1.01	0.24	5.40	2.12	13.42	53.9	10.30	2.39	–	1.73
2	Dasyprocta aguti											
	0.76	4.49	0.46	0.21	3.03	1.41	10.94	60.81	9.19	3.61	–	3.04

[a]Expressed in terms of (fat-free component x 100) / FFBW.

TABLE 6 Body Fat Distribution in Alaskan Mammals: Means and Standard Deviations

Number and Species	Total Fat as % of CLW	Distribution of Total Fat (%)						
		Heart	Liver	Kidney	Gut	Bone	Subcutaneous Depot	Internal Depots
1	Ochotona collaris							
	5.8	0.11	1.85	0.44	3.07	4.94	10.5	79.1
1	Marmota monax							
	35.8	0.05	0.50	0.07	0.40	1.22	19.9	77.8
2	Marmota caligata							
	17.5	0.12	2.75	0.27	1.52	5.40	8.44	81.5
4	Citellus undulatus							
	4.35	0.21	3.42	0.54	2.81	7.78	7.76	77.5
11	Tamiasciurus hudsonicus							
	1.97	0.61	4.61	1.02	3.40	11.4	14.2	64.7
	±1.13	±0.24	±1.36	±0.39	±1.86	±4.3	±4.8	±7.9
1	Castor canadensis							
	11.4	0.09	0.65	0.08	0.50	1.24	–	–
5	Lemmus trimucronatus							
	1.86	0.60	5.45	1.34	5.32	9.19	21.3	56.8
20	Clethrionomys rutilus							
	2.90	0.60	8.59	1.32	9.9	2.94	18.3	57.5
	±1.40	±0.60	±5.13	±0.56	±5.0	±1.35	±8.4	±7.7
9	Microtus oeconomus							
	2.08	0.96	9.97	2.62	9.89	3.52	24.6	48.4
	±0.89	±1.09	±5.34	±1.09	±5.98	±3.47	±11.4	±12.2
1	Ondatra zibethica							
	1.1	0.18	4.30	0.87	3.31	6.82	25.6	58.9
2	Erethizon dorsatum							
	11.0	0.10	2.18	0.33	1.73	6.25	30.3	59.1
3	Mustela erminea							
	1.53	0.89	12.2	1.75	5.12	10.8	17.4	51.9
1	Gulo luscus							
	6.0	0.35	2.85	0.25	8.67	12.7	10.1	65.0
1	Felis (Lynx) canadensis							
	14.6	0.26	0.57	0.31	0.81	9.48	5.65	82.9

A critical examination of some of the data in Figure 1 is essential. In the two series of whales, fat was extracted from all portions of the body except "meat" which was packed as food without extraction. Consequently, we estimated fatness from the equation

$$\text{Fatness} = \frac{(\text{oil yield} + \text{estimated oil content of meat}) \times 100}{\text{total body weight}}. \qquad (2)$$

TABLE 7 Body Fat Distribution in Virginian Mammals: Means and Standard Deviations

Number and Species	Total Fat as % of CLW	Distribution of Total Fat (%)						
		Heart	Liver	Kidney	Gut	Bone	Subcutaneous Depot	Internal Depots
2	Didelphis marsupialis							
	8.0	0.67	2.36	1.33	0.64	6.50	8.8	79.8
1	Scalopus aquaticus							
	2.8	0.41	4.48	0.81	3.58	6.51	11.6	72.6
1	Eptesicus fuscus							
	5.3	0.01	1.18	0.00	0.79	34.2	22.0	41.8
3	Procyon lotor							
	16.8	0.13	0.41	0.07	0.45	5.84	30.6	62.5
2	Mustela vison							
	6.1	0.09	6.39	0.28	1.08	2.10	38.9	51.3
3	Felis (Lynx) rufus							
	11.8	0.34	0.75	0.26	0.66	10.4	3.79	83.8
1	Marmota monax							
	5.4	0.18	5.07	0.09	2.27	6.04	22.0	64.4
1	Tamiasciurus hudsonicus							
	2.1	0.20	2.81	0.90	10.8	6.22	6.22	72.8
1	Sciurus carolinensis							
	2.2	0.47	2.87	0.68	1.62	10.1	11.3	73.8
2	Castor canadensis							
	11.0	0.08	0.35	0.23	0.39	1.09	–	–
9	Peromyscus leucopus							
	3.41	0.45	4.41	1.36	3.97	9.8	18.8	61.2
	±0.78	±0.30	±1.35	±0.42	±1.60	±2.2	±4.5	±4.4
1	Oryzomys palustris							
	12.8	0.06	1.93	0.30	1.02	3.36	43.0	50.4
1	Clethrionomys gapperi							
	0.8	0.71	7.86	3.57	10.7	1.43	28.6	47.1
7	Microtus pennsylvanicus							
	3.76	0.43	3.60	1.17	4.56	7.62	26.8	55.8
	±1.02	±0.22	±1.09	±0.27	±1.10	±3.88	±6.87	±4.1
7	Microtus pinetorum							
	2.36	0.26	7.45	1.24	4.50	9.0	23.9	53.7
	±1.21	±0.09	±3.54	±0.64	±2.37	±5.7	±6.5	±11.7
8	Ondatra zibethica							
	7.35	0.07	1.54	0.21	1.26	3.89	14.0	79.1
	±1.41	±0.05	±0.89	±0.08	±0.44	±1.08	±4.4	±5.0
4	Mus musculus							
	5.55	0.28	4.61	1.39	2.33	10.9	23.8	56.7
8	Laboratory albino mice							
	9.2	0.09	1.44	0.40	2.11	5.58	20.9	69.6
	±2.4	±0.05	±0.63	±0.05	±1.08	±2.72	±0.5	±5.5

TABLE 8 Body Fat Distribution in Brazilian Mammals: Means by Species

Number and Species	Total Fat as % of CLW	Distribution of Total Fat (%)				
		Heart	Liver	Kidney	Gut	Bone
2	Didelphis sp.					
	10.29	0.11	0.73	0.16	0.80	4.02
3	Molossus major					
	1.94	2.06	3.15	1.45	2.33	5.85
14	Artibeus jamaicensis					
	9.13	0.47	2.79	0.41	4.56	13.30
5	Artibeus lituratus					
	9.56	0.47	3.23	0.38	1.64	12.66
3	Glossophaga soricina					
	3.57	2.42	3.50	3.01	8.33	5.64
7	Phyllostomus discolor					
	6.60	0.66	2.02	0.57	3.21	15.41
2	Phyllostomus hastatus					
	5.80	0.64	2.25	0.25	2.29	18.29
2	Sturnira lilium					
	7.40	0.38	3.64	0.53	3.14	12.57
3	Vampyrops lineatus					
	7.00	0.55	1.60	0.34	2.94	12.47
4	Callithrix jacchus					
	4.40	0.42	6.14	0.47	7.83	16.65
2	Euphractos sexcinctus					
	9.66	0.18	3.40	0.30	1.13	1.41
1	Cuniculus paca					
	12.55	0.21	2.50	0.30	3.00	5.66
2	Dasyprocta aguti					
	12.32	0.28	2.51	0.23	1.88	7.31

Whale "meat" is assumed to be comparable with "flesh" of butchered cattle,[9] which we calculate to have approximately the same fatness as the total body. Since the fat associated with whale meat is a minor part of the whole, our assumption that it is one third extractable fat is not likely to introduce a large error into the calculation of whale body fatness.*

Caged mammals (mice, guinea pigs, and others) fall several standard errors of estimate above the curve in Figure 1, a finding in accord with Hayward's demonstration of increased fat accumulation in a wild species that has been caged under laboratory conditions.[3] Apparently, the relationship demonstrated in Figure 1 is applicable only to unrestrained animals in energy equi-

*Application of the constant factor for percentage of fat in meat to all individuals renders the standard deviation meaningless, and this was not calculated for the whales.

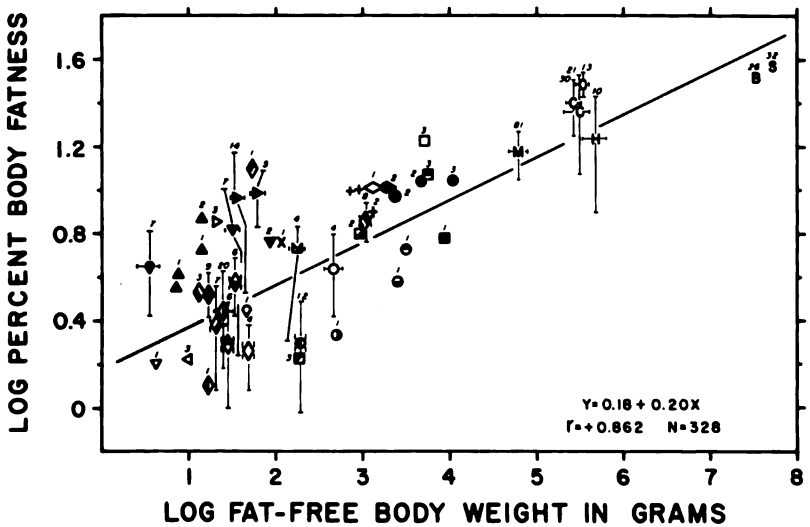


FIGURE 1 Relationship between log fatness (total extractable lipid × 100/CLW) and body size (log fat-free body weight). Although all individuals of a species are represented by a single symbol (identifiable in the key to symbols) located at the species mean, the statistics are based on the individual values. The line was fitted by the method of least squares. Number of individuals in each species is represented by the figures above the respective symbols. Vertical and horizontal lines passing through the symbols represent standard deviations.

KEY TO SYMBOLS

Didelphis	+	Euphractos	●	Ondatra	◆
Scalopus	○	Ochotona	X	Mus	◆
Sorex	◆	Marmota monax	⊖	Albino mouse	A
Blarina	◇	Marmota caligata	⊖	Erethizon	⊕
Myotis	▽	Eutamias	⊖	Cuniculus	◆
Eptesicus	△	Citellus	○	Dasyprocta	◆
Nycteris	▲	Tamiasciurus	◐	Procyon	□
Molossus	◁	Sciurus	◑	Mustela erminia	◪
Artibeus jamaicensis	▷	Castor	●	Mustela vison	◪
Artibeus lituratus	▷	Peromyscus	◆	Gulo	■
Glossophaga	▲	Oryzomys	◆	Felis	■
Phyllostomus discolor	▼	Lemmus	◇	Bos	C
Phyllostomus hastatus	▼	Clethrionomys rutilus	◆	Equus	H
Sturnira	▲	Clethrionomys gapperi	◆	Balaenoptera	B
Vampyrops	▷	Microtus pennsylvanicus	◆	Sibbaldus	S
Callithrix	M	Microtus oeconomus	◆	Homo (man)	M
Cavia (guinea pig)	G	Microtus pinetorum	◆		

librium with their natural environments. Does man meet these criteria? Certainly, older individuals and those in sedentary occupations do not. But the human series plotted in Figure 1 involved relatively active navy men with a mean age of 26.6 years.[12] Can the domesticated cattle and horses be justified in Figure 1? The selective processes of animal husbandry may produce changes in body composition that we are unable to evaluate at present. However, the energy equilibrium of a domesticated horse or cow on pasture is probably closely comparable with that of the feral animal on open range. In any event, restricting our consideration to those species smaller than man in Figure 1 reduces the correlation coefficient to +0.511, which is still significant at the 1% level.

We may speculate briefly on the biological significance of the relationship between fatness and body size. Probably predation is involved, at least at the small end of the body-size scale. Thus, fat mice with reduced mobility will be quickly gleaned from the population by predators, whereas individuals of larger species can accumulate body fat with impunity. An energetics analysis of the problem may be helpful. For example, in storing sufficient calories as adipose tissue to meet its energy needs for 2 days, a 30-g mouse will increase its body weight by more than 10%, whereas in accumulating a similar ration, a man will increase his body weight by less than 1%. This is attributable to the inverse relationship between metabolic rate and body size.[5] Considering the intense daily activity regimens of small mammals, can they "afford" to store and carry sufficient adipose tissue to be biologically significant as a source of energy?

BODY WATER VERSUS BODY SIZE

Evidence from guinea pigs[13,15] and cattle[2,6,9] reveal regulation of the water fraction of the fat-free body with a standard deviation of 1% or less. Figure 2 bears on the question, does constancy prevail on an interspecific basis? In this figure there are at least two subgroups; bats (triangular symbols) and terrestrial species. Clearly, in terrestrial species, log percent water in the fat-free body is negatively correlated with body size, ranging from about 78% in the smallest mammals to 71% in cattle. Many data in the literature do not conform with the relationship presented in Figure 2. Usually, this is because the values cited refer to something less than the total fat-free body, for example, fat-free eviscerated carcass, fat-free skinned eviscerated carcass, and others. We can only repeat the precaution expressed elsewhere,[15] that the expression for water fraction of the body must be standardized.

The biological significance of the negative correlation between water fraction of the fat-free body and body size (Figure 2, terrestrial species) may rest

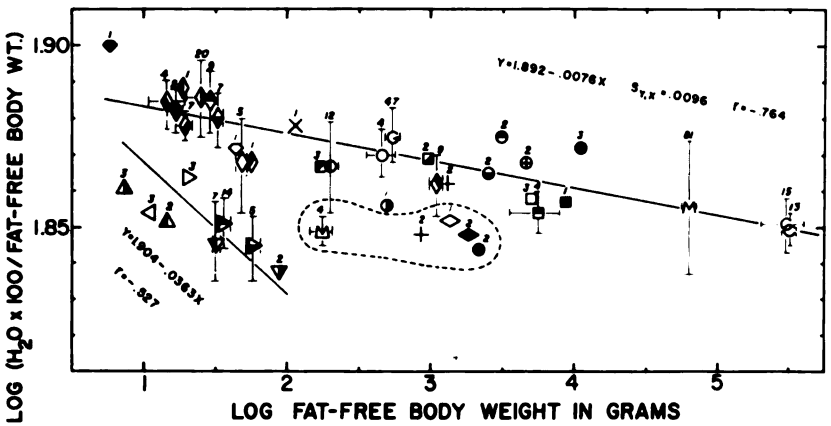


FIGURE 2 Relationship between log percent water in the fat-free body and log fat-free body weight. $S_{y \cdot x}$ is the standard error of estimate, that is, square root of the variance of estimate. See Figure 1 for further details.

in the following considerations. The impressive constancy of water fraction within a species and age group implies that:

1. The intracellular water fraction will be constant for each of the major cell types when freed of fatty inclusions, although the fraction may differ between cell types; for example, mouse liver will contain close to 74% of water, and gut will contain about 80% (G. C. Pitts, unpublished data).
2. The fractions of the various cells, tissues, and organs within the fat-free body will be constant; for example, the fat-free body of the mouse has 45% muscle and 6% gut (G. C. Pitts, unpublished data).
3. The volume of the extracellular fluid compartments will remain relatively constant in normal animals on an *ad libitum* regimen.

Dislocations in the above do occur, but the previously documented constancy in water fraction of the fat-free body speaks for itself. The decrease in water fraction observed in larger mammals (Figure 2) is most probably explained on the basis of (2) above. Physical and geometrical considerations dictate that weight will vary as the cube of a linear dimension and that structural strength will vary as the square of a linear dimension. For example, the weight of a long bone is a function of its volume, and the strength is a function of its cross-sectional area. Thus, larger mammals require a larger fraction of skeleton than do smaller ones. Since skeletal tissue is relatively "dry" (25 to 60% water), this would explain the relationship between water and size in Figure 2. These considerations are examined further in the next section.

The bats in Figure 2 (both Brazilian and Virginian) are statistically distinguishable as a group from terrestrial species. Analysis of covariance [17] reveals that the slopes of the two regression lines are not significantly different, but the two groups have different variances ($P < 0.01$). In other words, the "elevation" of the bat data is significantly below that of terrestrial species. The number and size range of the bat data must be expanded before more can be said about the nature of the regression or its biological significance.

The five species encircled with a broken line in Figure 2 are the terrestrial Brazilian species. In general, they appear drier than comparable temperate-zone species. This is understandable in the two armadillos (encircled asterisks) because of the presence of the body armor, which is of ectodermal origin and is low in water fraction. Of the remaining four species (nine individuals), two are more than 2 standard errors of estimate below the regression line. Thus, the probability is less than 0.05 that they belong to the population for which the regression line was calculated. These are all tropical rain-forest species, and we cannot explain why they should be drier than the temperate-zone species. Methodological error is a possible explanation. However, consistent technology was used throughout. Specimens from Alaska, Brazil, and Wisconsin were all packaged vaportight in aluminum foil and plastic, immediately deep-frozen at -20°F, and transferred to a shipping locker with dry ice; and all were flown to our Virginia laboratory with equal dispatch.

FRACTIONAL COMPOSITION OF THE FAT-FREE BODY VERSUS BODY SIZE

There are both theoretical and empirical reasons for expecting a difference in fractional composition between large and small mammalian species. Some of these have been mentioned. Statistical tests of these possibilities are presented in Table 9. The 116 specimens represented in this table include no bats.

The percentages of dry bone, bone ash, and muscle are the only components in Table 9 that are positively correlated with fat-free body weight; all the others have negative coefficients. Bone and muscle together constitute about 65% of the fat-free body. Consequently, with these massive components increasing in percentage, the several smaller components must, perforce, decrease. The disproportionate increase in mass of muscle and skeleton as one goes to larger mammals agrees with our prediction based on the relationship between change in mass and change in strength.

While dry bone and bone ash have statistically significant coefficients in Table 9, wet bone does not. Obviously, the water fraction of fat-free bone shows considerable variability. Most tissues and organs are relatively constant

TABLE 9 Correlation between Percentage Composition of the Fat-Free Body and Fat-Free Body Weight of Alaskan and Virginian Species[a]

Component	Number of Values	*r*	*P*
Bone	116	+0.129	>0.05
Bone (dry)	108	+0.339	<0.01
Bone ash	112	+0.266	<0.01
CNS	116	−0.500	<0.01
Gut	116	−0.211	<0.05
Heart	116	−0.441	<0.01
Kidney	116	−0.378	<0.01
Liver	114	−0.333	<0.01
Muscle	116	+0.321	<0.01
Skin	116	−0.104	>0.05
Spleen	112	−0.187	>0.05
Remainder	114	−0.233	<0.01
PERCENTAGE OF WATER IN MUSCLE	150	−0.353	<0.01

[a] About 18 individuals are omitted because of incomplete analyses. These are random, and we believe they cause no statistical bias.

in composition after correction is made for the storage fat present. By contrast, we believe that because bone water and bone fat compete for space in a marrow cavity of nearly constant volume, water of whole bone will show no greater constancy than fat. In other words, most organs accumulate fat by accretion, whereas the skeleton does so by displacement of soft tissues which are high in water content.

Constancy of water fraction in the fat-free body has the three implications listed under "Body Water versus Body Size" (page 64). Looking at these and at Table 9 again, we can assume that the disproportionate increase in dry bone and bone ash as body size increases is a change of the second type and might largely account for the decrease in fraction of water (Figure 2). Under these conditions, intracellular fraction of water need not change. Hoping that percentage of water in the largest soft-tissue component, muscle, might reflect intracellular water fraction, we calculated the correlation coefficient between its percentage of water and body size. The expectation that water fraction of muscle would be independent of body size is not borne out by the coefficient given in Table 9. Clearly, the larger a mammal, the smaller the fraction of water in its muscle. Using the lines of reasoning applied above to the body as a whole, we conclude that the decrease in muscle-water fraction with increasing body size may merely reflect an increase in the percentage of relatively dry sustentacular tissues associated with muscle, for example, tendons

and other connective tissues. This is homologous with our interpretation of the relationship between fractions of water and skeleton in the entire fat-free body. To generalize, larger species of mammals require a larger fraction of sustentacular tissues (both skeletal and nonskeletal) than smaller species do to support their bodies against the pull of gravity. This principle is difficult to demonstrate statistically except on samples with body size ranging through several orders of magnitude. However, simple hypertrophy of bone and muscle have been demonstrated within a species.[16]

SUMMARY

Body composition was studied in 466 adult mammals representing 11 orders, 23 families, 41 genera, and 49 species from Alaska, Virginia, Brazil, and Wisconsin. Descriptive data by species are presented on percentage composition of the fat-free body and percentage distribution of total fat among various organs and tissues. Regression of percentage of body fatness on fat-free body weight (an index of body size) is expressed by the equation:

$$\text{Fatness} = 1.5\ (\text{FFBW})^{0.2},$$

with fatness ranging from less than 5% in the smallest mammals to more than 35% in blue whales. Caged species are much fatter than predicted by this equation, which applies to unrestrained individuals in energy equilibrium with their natural environments. The percentage of muscle and of bone ash, dry bone, and other sustentacular tissues in the fat-free body increased with increasing body size. Because the skeleton and other sustentacular tissues are relatively dry, the increase of their fraction in larger mammals produces a decrease in the fraction of water in the fat-free body. Bats are drier than terrestrial species of equal size. The changes in composition of the fat-free body, which we found to be correlated with body size, are explicable, directly or indirectly, in terms of the proportionate amount of sustentacular tissue and muscle required to support and move the body against the pull of gravity.

This investigation was supported by a contract with the United States Air Force [AF 18(600)-855], a research grant [A-3354(A)] from the U.S. Public Health Service, and a National Aeronautics and Space Administration contract (NAS 2-1554).

We are deeply indebted to the following for invaluable assistance in arranging fieldwork and providing specimens: Dr. Peter R. Morrison, Institute of Arctic Biology, University of Alaska; Mr. I. T. Quinn, former executive

director, Virginia Commission of Game and Inland Fisheries, and numerous other persons in that commission; Professor Henry S. Mosby and Dr. M. A. Byrd, Virginia Cooperative Wildlife Research Unit, Blacksburg, Virginia; Numerous persons in the Arctic Aeromedical Laboratory, Alaskan Air Command, U.S. Air Force; The School of Medicine, University of Bahia, Brazil; and in particular, Professors Roberto Santos and José Simoes; Dean Paulo Sawaya, Faculty of Philosophy, Sciences and Letters, University of São Paulo, Brazil; Dr. Robert Rausch, Arctic Health Research Center, Anchorage, Alaska; Dr. Philip Hershkovitz, Chicago Natural History Museum.

Finally, we are indebted to J. J. Murray, Jr., and N. Sperelakis for reading and criticizing this manuscript.

REFERENCES

1. Behnke, A. R. 1941–1942. Physiologic studies pertaining to deep sea diving and aviation, especially in relation to the fat content and composition of the body. Harvey Lect., Ser. 37:198–226.
2. Haecker, T. L. 1920. Investigations in beef production. Minnesota Agr. Exp. Sta. Res. Bull. 193.
3. Hayward, J. S. 1965. The gross body composition of six geographic races of Peromyscus. Can. J. Zool. 43:297–308.
4. Julian, L. M., J. H. Lawrence, N. I. Berlin, and G. M. Hyde. 1956. Blood volume, body water and body fat of the horse. J. Appl. Physiol. 8:651–653.
5. Kleiber, M. 1947. Body size and metabolic rate. Physiol. Rev. 27:511–541.
6. Kraybill, H. F., H. L. Bitter, and O. G. Hankins. 1952. Body composition of cattle. II. Determination of fat and water content from measurement of body specific gravity. J. Appl. Physiol. 4:575–583.
7. Kraybill, H. F., O. G. Hankins, and H. L. Bitter. 1951. Body composition of cattle. I. Estimation of body fat from measurement *in vivo* of body water by use of antipyrine. J. Appl. Physiol. 3:681–689.
8. Moulton, C. R. 1923. Age and chemical development in mammals. J. Biol. Chem. 57:79–97.
9. Moulton, C. R., P. F. Trowbridge, and L. D. Haigh. 1922. Studies in animal nutrition. Mo. Agr. Exp. Sta. Res. Bull. 54.
10. Murray, J. A. 1922. The chemical composition of animal bodies. J. Agr. Sci. 12:103–110.
11. Nishiwaki, M. 1950. On the body weight of whales. Whales Res. Inst. (Tokyo) Sci. Rept. 4:184–209.
12. Osserman, E. F., G. C. Pitts, W. C. Welham, and A. R. Behnke. 1950. *In vivo* measurement of body fat and body water in a group of normal men. J. Appl. Physiol. 2:633–639.
13. Pace, N., and E. N. Rathbun. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158:685–692.

14. Pitts, G. C. 1956. Body fat accumulation in the guinea pig. Amer. J. Physiol. 185:41–48.
15. Pitts, G. C. 1962. Density and composition of the lean body compartment and its relationship to fatness. Amer. J. Physiol. 202:445–452.
16. Pitts, G. C. 1963. Studies of gross body composition by direct dissection. Ann. N. Y. Acad. Sci. 110:11–22.
17. Snedecor, G. W. 1956. Statistical methods, 5th ed. Iowa State University Press, Ames, Iowa.

E. M. Widdowson
University of Cambridge

BIOLOGICAL IMPLICATIONS OF BODY COMPOSITION

When Dr. Reid first invited me to speak at this conference and suggested as my subject "Biological Implications of Body Composition," I replied that I should be very pleased to give a paper under this title. But when I came to think about it in more detail and to start to prepare it, I realized that what I had rashly undertaken to do was to give a paper on the "why and wherefore of all things" concerning body composition—in other words, the biological implications of life itself. It was clearly quite beyond my powers to condense the whole body of information, so I have limited myself to saying something about those aspects of body composition that can be measured in the living body, in particular, body water and body potassium.

I need hardly mention the complicating effect of fat in considerations of total body water. It is well illustrated in Figure 1, which shows the amount of water in the developing fetus, both per 100 g of lean body tissue and per 100 g of whole body. The percentage of fat is also shown. The large fall in percentage of water in the whole body during the last part of gestation is due far more to the increase in fat than to any real fall in the percentage of water in the lean body mass.

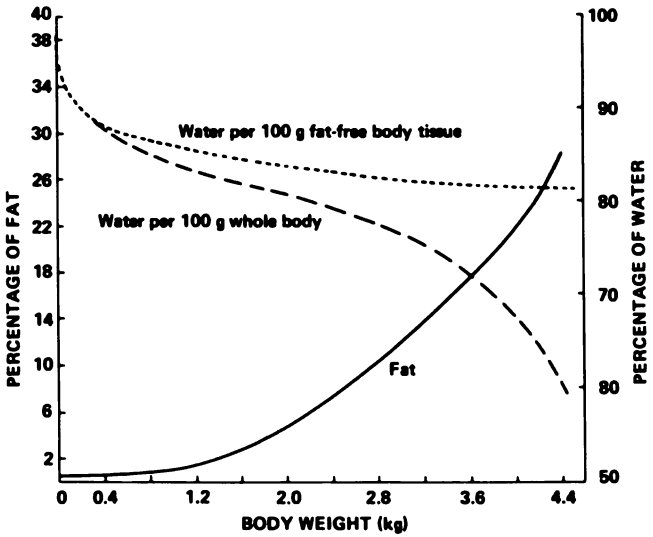


FIGURE 1 Fat and water in the body of the developing human fetus.

But when we eliminate the fat and consider the lean body mass, and the amount of total water and extracellular water in it, what do we really mean? The fraction that we call extracellular water forms a very tortuous continuum. It lies between cells and surrounds them; it is in the blood and lymphatic vessels and in the serous cavities. The intracellular fluids, on the other hand, do not form one continuous fluid mass but are many tiny droplets isolated by the restricted permeability of their cell membranes. All exchanges between one cell and another and between a cell and the external environment must take place through the extracellular fluids. The salts inside and outside the cells are characteristically different (Table 1). The chief cation of the extracellular fluid is sodium, but there are also important concentrations of calcium and potassium; and chloride and bicarbonate are the main anions. The chief cation in the cells is potassium, and the anions are mostly organic phosphates and proteins. Cells can and do differ from one another in their electrolyte makeup. The red blood cells of the cat and dog, for example, have sodium as their main cation, but all the other cells of their bodies have potassium. There are many intracellular fluids, but only one extracellular fluid, which varies only in a minor way from one part of the body to another—indeed, also from one species to another. I should perhaps qualify this by saying that there are certain specialized fluids outside the cellular mass that are really cellular secretions: intestinal fluids and the cerebrospinal, ocular, and aural fluids. Each of these has a different chemical makeup, as do all secretions, whether

TABLE 1 Electrolyte Structure of Extracellular and Intracellular Fluid

Structure	Extracellular Fluid (mEq/liter of water)	Intracellular Fluid (mEq/liter of water)
CATIONS		
Na	150	8
K	5.4	151
Ca	5.4	2
Mg	3.2	28
ANIONS		
Cl	110	–
HCO_3	29	10
PO_4	2.1	100
SO_4	1.1	10
Protein	17	65
Organic acids	6.5	4

they are internal or external. The ear fluids are particularly interesting because the perilymph has a composition similar to that of cerebrospinal fluid, while the vestibular endolymph contains 144 mEq/liter of potassium and only 16 mEq/liter of sodium.[10] This Na: K ratio makes it unique among extracellular fluids, as far as I know.

The cells in our bodies have been likened by Robinson to amoebas in a pond:

> Unlike most ponds, the size of the pond in the body is small compared with the volume of cells inhabiting it. The composition of the pond is altered by the metabolic products of the cells, but the cells are intolerant of changes in composition of their external environment, and an important part of physiology is concerned with the regulating mechanisms which have been developed to enable the over-crowded pond under our skins to maintain itself as though it had an unlimited volume.[9]

The cell membranes are generally permeable to water, and, in spite of their different electrolyte composition, the cells are in osmotic equilibrium with the extracellular fluid. The osmolar concentration of the extracellular fluid varies little during health; it is possibly the most constant of all the many biological constants, or steady states, as they are now often called. Two mechanisms bring this about; thirst and the antidiuretic hormone. Although the composition of the extracellular fluid is held so steady, as I have just said, its volume is much less rigidly guarded, and it can change quite extensively from day to day in health, and much more so with age, nutritional status, and disease.

What bearing has all this on the theory that there can be a lean body mass with a constant percentage of water in it? Great as this generalization is, it can

be only approximately true, for we know that the volume of the extracellular fluid is changing every minute as water is lost through the lungs, the skin, and the kidneys and is replaced by water absorbed from the gut. Any of you who have weighed yourselves repeatedly at frequent intervals will know how your weight may go up and down by a kilogram or more from day to day, and this must be due to retention and loss of extracellular fluid. The assumption that the lean body mass is constant in composition is clearly not strictly true even in a single individual from time to time. Still less is it true from one individual to another.

Most of the tissues contain more water per 100 g than the conventional 72% in the lean body mass, as shown here:

Skeletal muscle	79%
Heart	83
Skin (fat-free)	69
Liver	71
Brain	77
Kidney	81
Skeleton	36

The fat-free skin has only 69% of water, but it is the skeleton that is primarily responsible for bringing down the water from nearly 80% in the muscles and organs to 72% in the lean body mass. The whole skeleton of man contains about 36% of water, and it comprises 18% of the body weight. Table 2 shows the only values we have for the chemical analysis of "healthy" adult human bodies. Three of the five analyses were made in the United States and two in England, and it is immediately apparent that the water in the fat-free body tissue was not absolutely constant. In the four men, it varied from 69.4 to 73.0%. Because 99% of the calcium in the body is in the skeleton, we can take the amount of calcium per kilogram of fat-free body tissue as an index of the amount of bone; and it is satisfactory to see that the water is highest in the man with the least calcium, and lowest in the man with the most. The woman had high calcium and high water contents. The most likely explanation for this is to suppose that 100 g of her soft tissues, particularly her skeletal muscles, contained more water than those of a man. I would not be at all surprised if this turned out to be true.

Some time ago, workers pointed out that not only were the percentages of water and nitrogen in the lean body mass similar from one individual to another within a species, but they were also similar from one species to another. This is true, but largely for fortuitous reasons, for small four-legged mammals have small skeletons, as evidenced by the lower concentration of calcium in

TABLE 2 Composition of the Bodies of Five "Healthy" Human Adults

Composition of Fat-Free Body (g/100 g)		Age	Weight (kg)	Fat in Body (g/100 g)
MALE				
Water	69.4	46	53.8	19.4
Ca	2.40			
Water	70.4	60	73.5	27.0
Ca	2.40			
Water	73.0	48	62.0	4.3
Ca	2.07			
Water	72.5	25	71.8	14.9
Ca	2.13			
FEMALE				
Water	73.2	42	45.1	23.6
Ca	2.48			

their bodies (Table 3). Man has to have a large skeleton to support him in his upright position. Some other part of the body, possibly the hair in some animals, or in small animals, the skin with its proportionally greater area, must partly replace the action of the skeleton in larger animals in bringing down the percentage of water in the lean body tissue to about 72%.

Now I want to discuss the biological implication of the large volumes of extracellular fluid in the early stages of development. When the ovum is fertilized, the organism is entirely cellular, but there soon comes a stage when

TABLE 3 Composition of the Fat-Free Body Tissue of the Adults of Six Species

Species	Water (g/100 g)	Ca (g/100 g)
Man	72	2.2
Pig	75	1.2
Cat	74	1.3
Rabbit	73	1.3
Rat	72	1.2
Mouse	78	1.1

extracellular fluid forms an important part of it; in man this is when the blastocyst is formed 10 days after fertilization. The youngest fetuses that have been analyzed weighed less than a gram, and they contained from 93% to 95% of water,[4] so their bodies were more dilute than normal adult plasma. In the fetus of 20 weeks' gestation, the total water amounts to nearly 90% of the body weight, and nearly 70% of it is outside the cells. By term, the total water has fallen to about 69% in the whole body, or 82% in the lean body mass as shown in Figure 1. The intracellular water has risen, along with the increasing cell mass, and the extracellular fluid now exceeds the intracellular fluid by only a small amount. Six months after birth the position has been reversed, and in the adult the intracellular fraction exceeds the extracellular fraction by a factor of 2 or more.

There is good evidence that before birth the volume and composition of its fluids are maintained by the fetus itself. The concentration of some of the minerals, amino acids, and vitamins in the fetal plasma are higher than in the maternal plasma, which indicates that the placenta must be engaged in their active transport into the fetus; but the osmolar concentration of the fluid is about the same as that of the mother. Outside the body of the fetus, but within the fetal membranes, there is fluid in the amniotic sac, and in some species also in the allantoic sac. It seems that both the volume and composition of these fluids are also controlled by the fetus and are important to it.[7] The fetal kidney is active before birth and it secretes large volumes of hypotonic urine into the amniotic fluid. This urine is acid, and it contains very little sodium and practically no phosphate. We believe that the fetus drinks amniotic fluid and absorbs the water and salts from the gastrointestinal tract, thus completing the cycle and maintaining the "steady state" among its fluids necessary for its normal development. It has been estimated from studies in which methylene blue was injected into the amniotic cavity that the human fetus near term may drink about a liter of fluid a day. Thus, before birth the kidney is important in that it allows the fetus to maintain a desirable volume of amniotic fluid, but its excretory functions (in the postnatal sense) are nil. The placenta does it all.

After the baby is born, the urine it passes has much the same characteristics that it had before birth, and there is no evidence of any sudden change in renal function at the moment the baby is born. In other words, it does not at once start to excrete the sodium, chloride, and water from the extracellular fluid to bring the extracellular–intracellular proportions to the adult values. The volumes continue to change slowly and steadily, without any dramatic alterations at the time of birth. The essential thing that is going on in the fetus and newborn baby is, of course, the growth and multiplication of the cells. We found some evidence years ago that a large volume of extracellular fluid inhibited protein catabolism in the newborn pig.[8] I would like now to

put this in a more positive way and suggest that a large pond in relation to the volume of cells in it is essential in early life when the metabolically highly active cells are rapidly dividing and when the mechanisms for removing the products of metabolism and maintaining the constancy of the composition of the extracellular fluid are not as highly developed as they will be later in life. We know that fetal plasma has a higher concentration of potassium than adult plasma,[13] which looks as if the cell membrane has not yet acquired the efficiency in sodium and potassium transport it will have later.

The volume of extracellular fluids is often high in undernutrition. Here I think the reasons are different. Undernutrition, whether of a child or an adult, alters the relative proportions of the three great compartments of the body. The fat goes, and the cell mass shrinks, and with it the cell water. In sections of the skin or bone marrow of persons suffering from undernutrition, the fat cells are small and shrunken, and the spaces between them are taken by extracellular fluid. The perirenal tissues lose their fat and become gelatinous and watery. The muscle cells become smaller, and the spaces between them are filled with extracellular "gel." Many explanations have been brought forward to account for the increased extracellular fluid volume in undernutrition.[6] None of them explains all the facts; there are probably several causes. If and when the concentration of serum proteins falls, and consequently the colloidal osmotic pressure of the plasma falls, the edema may increase, according to Starling's principle, but the replacement of body space previously occupied by fat and cellular tissue is probably the most important cause of all.

Now a few words about the total body potassium. I am not so concerned with whether ^{40}K measures the total body potassium as with what it tells us when it does. We hope, of course, that it will give us a measure of the cell mass. This, however, depends on the relationships between the water, proteins, and potassium of the cell being relatively constant. Just as the body is a complex of organs and tissues, each with a different percentage of water, so the cells differ from one part of the body to another. Each organ and tissue has its characteristic cellular "makeup." We can get an idea of this from the ratio of potassium to intracellular protein within each organ, but even this is probably affected by age. This ratio in an adult man, expressed as milliequivalents of potassium per gram of intracellular protein nitrogen, ranges from 5.0 in the brain to 1.4 in the skeleton. The ratio in muscle and skin is about 3.4, and in liver it is about 2.7. The cells of the body are not homogeneous entities but are made up of cytoplasm and the cellular inclusions, of which the most important are the nucleus, mitochondria, and microsomes. These contain different concentrations of potassium. In calf thymus cells, for example, the concentration of potassium was about 4.5 times higher in the nucleus than in the cytoplasm.[5] In rat liver cells, the mitochondria contained 3 times as much potassium per 100 g as the microsomes.[2,11]

The amount of potassium in the whole body, therefore, depends not only on the extracellular and intracellular relationships within the body, but also on the contribution of the different organs, each with its own particular kind of cell; and the composition of the cells varies with their nutritional status and their age. Table 4 sets out some of these facts. The adult muscle cell, as we

TABLE 4 Ratio of Potassium (mEq) to Intracellular Protein Nitrogen (g) in Human Organs at Different Ages

Organ	Fetus at 20 Weeks Gestation	Full-Term Baby	Adult
Muscle	4.3	3.8	3.4
Skin	3.8	4.7	3.4
Heart	7.0	3.4	3.5
Liver	4.2	2.6	2.7
Brain	6.2	6.3	5.0

have seen, has about 3.4 mEq of potassium per g of intracellular protein nitrogen; but the small muscle cells of the fetus, with their large nuclei, have a higher ratio, which we found to be 4.7 at 14 weeks gestation, 4.3 at 20 weeks gestation, and 3.8 at birth. The contribution of the organs and tissues to the total body weight changes with age, and so does their contribution to the total body potassium. The muscle, for example, contains 33% of the body's potassium at birth and 62% in the adult. Values for skin are 16% at birth and 5% in the adult, and for brain, 19% at birth and 6% in the adult.

We do not know just how long the cells of the body maintain their integrity in severe undernutrition. They probably do so for a time, but there may come a point at which the metabolic activity of the cell wall fails, and sodium and chloride enter the cells and potassium leaves them. When this happens, the animal, or child, or adult, has probably reached a point of no return. We have seen this happen in the tissues of severely undernourished pigs that have died;[12] and Garrow *et al.*[1] observed it in Jamaica in children dying of severe protein and calorie deficiency. These workers have made the interesting observation, from both ^{40}K measurements of the head and chemical analysis of the brain, that the brains of these children are specifically deficient in potassium. If this is confirmed, it seems to be a very important observation. From the results of body-fluid volume measurements in adults who were protein deficient it has been suggested that the cells are overhydrated.[3] If this were so, one would expect a high concentration of potassium in the body, not a low one.

In this brief paper, I have been able to give only a few examples of the biological implications of body composition. I hope I have not presented

them to you as biological complications. I had a long list of many other things, and I can only hope that what I have presented will have demonstrated that the composition of the body is not a dull and static subject; it is dynamic, full of comings and goings and whys and wherefores. Our picture of it at one particular moment must always be thought of as one frame of a film. The picture we see depends on what has happened in the body in the past and what is happening at the moment. The picture may help us to predict what will happen in the future if we do not interfere. There is a reason for every change, whether physiological or pathological, and I believe that the fundamental reasons for all the changes in the composition of the body will be found in the cells themselves.

REFERENCES

1. Garrow, J. S., K. Fletcher, and D. Halliday. 1965. Body composition in severe infantile malnutrition. J. Clin. Invest. 44:417.
2. Griswold, R. L., and N. Pace. 1956. The intracellular distribution of metal ions in rat liver. Exp. Cell Res. 11:362.
3. Holmes, E. G., E. R. Jones, M. D. Lyle, and M. W. Stanier. 1956. Malnutrition in African adults. 3. Effect of diet on body composition. Brit. J. Nutr. 10:198.
4. Iob, V., and W. W. Swanson. 1934. Mineral growth of the human foetus. Amer. J. Diseases Children 47:302.
5. Itoh, S., and I. L. Schwartz. 1956. Sodium and potassium content of isolated nuclei. Nature 178:494.
6. McCance, R. A. 1951. The history, significance and aetiology of hunger oedema. *In* Studies of undernutrition, Wuppertal 1946-9. Med. Res. Council, Spec. Rept. Ser. 275:21.
7. McCance, R. A. 1964. Water and electrolyte metabolism of the foetus and newborn, p. 73. *In* J. H. P. Jonxis, H. K. A. Visser, and J. A. Troelstra [ed.] Nutricia symposium on "The adaptation of the newborn infant to extrauterine life." Leiden, Holland.
8. McCance, R. A., and E. M. Widdowson. 1957. Hypertonic expansion of the extracellular fluids. Acta Paediat. (Stockholm) 46:337.
9. Robinson, J. T. 1957. Functions of water in the body. Brit. Nutr. Soc. Proc. 16: 108.
10. Smith, C. A., O. H. Lowry, and M. L. Wu. 1954. Electrolytes of labyrinthine fluids. Laryngoscope (St. Louis) 64:141.
11. Thiers, R. E., and B. L. Vallee. 1957. Distribution of metals in subcellular fractions of rat liver. J. Biol. Chem. 226:911.
12. Widdowson, E. M. 1967. The place of experimental animals in the study of human malnutrition. *In* R. A. McCance and E. M. Widdowson [ed.] Calorie deficiencies and protein deficiencies. Churchill, London.
13. Widdowson, E. M., and R. A. McCance. 1956. The effect of development on the composition of the serum and extracellular fluids. Clin. Sci. 15:361.

Gilbert B. Forbes
University of Rochester

CHANGES IN BODY WATER AND ELECTROLYTE DURING GROWTH AND DEVELOPMENT

Growth means change, and not in size alone, for such aspects as relative organ size, body proportions, and organ function undergo distinct alterations. Adult tissues also differ from infant tissues in chemical composition, and it is the purpose of this brief report to discuss some of these. Most of the data deal with man. The compendium of Widdowson and Dickerson[11] should be consulted for greater detail.

Data on carcass analysis exist for the fetus and newborn in considerable numbers, and there are a few for adult man. Interest in the measurement of body composition in living subjects has stimulated the development of other methods, including the following:

1. Total body water, as determined by D_2O, THO, antipyrine, and urea dilution.
2. Extracellular fluid volume, as determined by inulin, thiocyanate, thiosulfate, stable Br, or ^{82}Br dilution; the apparent volumes of distribution gradually increase in this series. Stable Br and ^{82}Br can be used to estimate total exchangeable chloride.

3. Total exchangeable sodium and potassium, as determined by ^{24}Na and ^{42}K dilution, respectively.
4. Total body potassium, as determined by ^{40}K counting.

In the newborn infant, the correspondence between total body content determined by carcass analysis and by isotopic dilution is rather good. For the adult this is probably true for water, but ^{42}K and ^{82}Br measure only about 90 to 95% of body potassium and chloride, respectively, and the figure stands at 70% for ^{24}Na because of the large reservoir of nonexchangeable sodium in bone.[6] However, it is likely that the dilution methods do measure the metabolically active moiety in each case. In contrast to the situation in some animals, the problem of the water and electrolyte contained within the lumen of the gastrointestinal tract seems to be a minor one. The accuracy of the ^{40}K analyses depends on the calibration of the particular whole-body counter being used.

Attempts have been made to measure total body nitrogen and calcium by neutron activation, but the technique is an intricate one and involves deliberate exposure to neutrons.[1]

Table 1 presents data for the human fetus, newborn, and adult as determined by carcass analysis. Since body fat content varies considerably, and

TABLE 1 Body Composition (Fat-Free Basis) in Man at Different Stages of Development[a]

Component	Fetus at 500 g	Newborn	Adult
H_2O (%)	87	82	72
Na (mEq/kg)	95	90	86
Cl (mEq/kg)	70	57	50
K (mEq/kg)	40	49	68
N (%)	1.40	2.25	3.31
Ca (%)	0.45	0.89	2.15

[a] From Forbes (reference 6) and Widdowson and Dickerson (reference 11).

since neutral fat does not bind appreciable amounts of water or electrolyte, the data are expressed on the basis of fat-free wet weight.* For the body as a whole, the growth process involves a decline in H_2O, N, and Cl contents per unit weight, and an increase in the content of K, Na, and Ca.

Friis-Hansen[2] has partitioned body water into extracellular fluid (ECF) and intracellular fluid (ICF) compartments. At birth about one half of the total water can be accounted for as ECF; this value drops to 44% at 1 year

*The assumption that the fat-free tissues tend to exhibit a fairly constant composition in health provides the basis for an indirect method for estimating body fat.

and to 32% at adolescence. Thus growth is accompanied by a decreasing ratio of ECF to ICF.

These changes are in keeping with the hypothesis that postnatal growth takes place principally through an increase in cell size. Extracellular fluid is the only avenue connecting the cells with the outside world, and so it must be related in some way to the size of the cell surface. As the cell grows, surface area increases as the square of the linear dimension, and volume increases as the cube of the linear dimension, so that the surface:volume ratio declines. It is tempting to postulate that this geometric circumstance accounts for the decline in the ECF:ICF ratio during growth. If this is true, then geometric considerations place an upper limit on body size, for body cells must possess a certain mantle of ECF in order to function properly; and so the ultimate in cell size, and hence body size, will be reached at a point where this mantle is just sufficient to sustain metabolic equilibrium, at which point further growth is impossible without cell multiplication.

Figure 1 illustrates changes in human body composition. The fetal data represent carcass analysis, and the postnatal values indicate isotopic dilution.

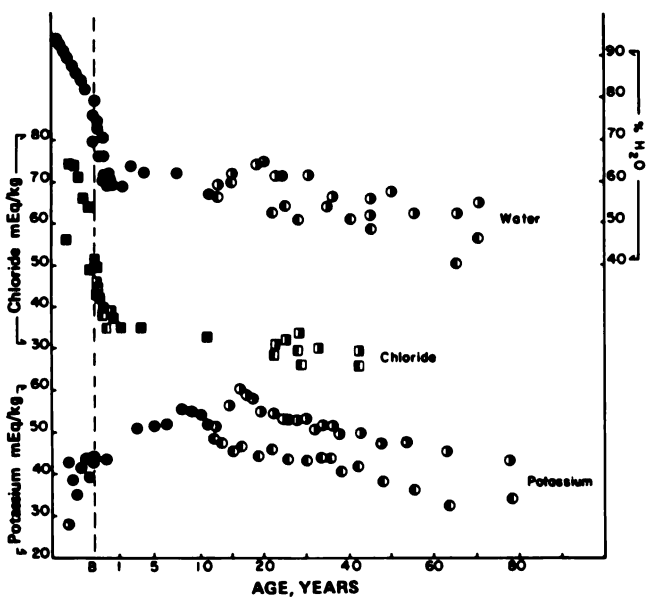


FIGURE 1 Total body content of H_2O, Cl, and K in relation to age. Each point is the average of several subjects. Symbols as follows: H_2O and K: Combined sexes ●, Males ◑, Females ◐; Cl: Combined sexes ■, Males ◨, Females ◧. From Forbes (reference 6), by permission of the publishers.

The data are referred to total weight, which includes a variable amount of fat. This circumstance accounts in part for the sex differences in adolescent and adult life, and for some of the decline in potassium and chloride content in the adult years. Women usually contain more fat than men, and the fat content rises during adult life in both sexes.

Figure 2 shows the changes in total body potassium, as estimated from ^{40}K measurements, during childhood and adolescence, compared with growth in

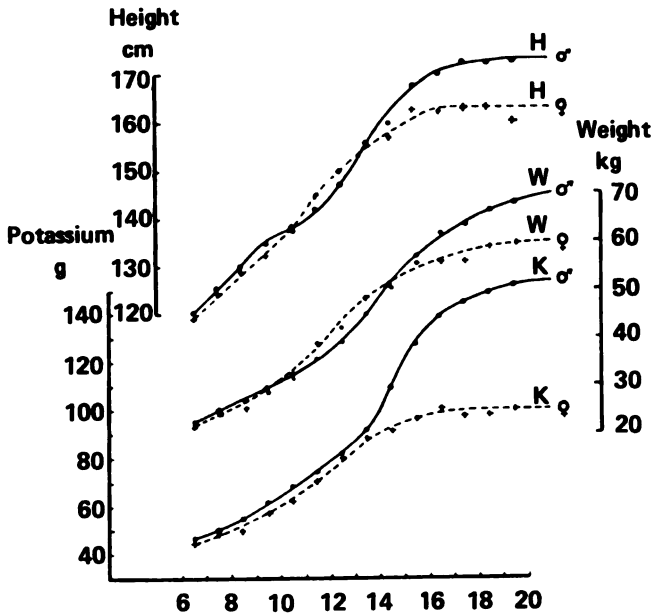


FIGURE 2 Median values for height, weight, and total body K (by ^{40}K) in a large series of West German children. From Oberhausen *et al.* (reference 10), by permission of authors and publisher.

weight and height. [10] The sex difference in total body K increments is proportionately greater than those for height and weight, which suggests that the male has a relatively larger cell mass. Figure 3 shows values obtained in my laboratory (Department of Pediatrics, University of Rochester) for lean body mass, calculated from ^{40}K content on the assumption that potassium forms a constant fraction of the lean body.[7] Fat is the difference between body weight and lean body mass. Sex differences in lean body mass and fat are readily apparent once the adolescent years are reached. The decline in body potassium during adult life is a reflection of the erosion of the cell mass which accompanies the aging process.

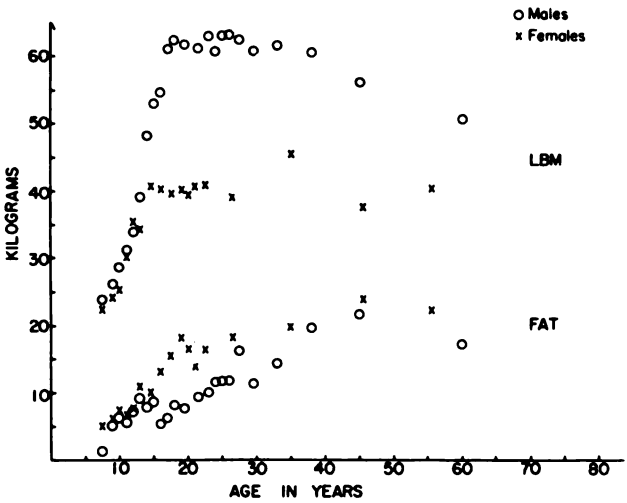


FIGURE 3 Average values for lean body mass (LBM) and fat, calculated from ^{40}K measurements, in a series of children and adults (Rochester counter).

In an attempt to systematize the changes that occur in body composition, some years ago we undertook to plot the data for total body water and electrolyte against body weight on double logarithmic coordinates. Plots were made for water, exchangeable sodium, chloride, and potassium, and in each instance they revealed a linear relationship between body content and body weight. Figure 4 shows such a plot for total body chloride, and those for other constituents are available in previous publications.[3,9] Similar plots, with rather similar coefficients, made for other mammalian species[4] indicate a good degree of interspecies correspondence.

It must be admitted that logarithmic plots tend to reduce the variability of data—hence the graphic beauty of the plot itself. Nonetheless, the mere existence of such a relationship, imperfect though it may be, suggests that there is a certain regularity in the change in body composition during growth. The differential form of the linear equation $\log y = k \log x + \log b$, where k and b are constants, is $dy/y = k \cdot dx/x$. This equation states simply that there is a constant proportionality between the specific rate of change of the constituent in question (y) and the specific growth rate of the body as a whole (x). When the growth rate is rapid, change in body composition is rapid, and vice versa; and as much change in body composition occurs during a weight gain from 1 to 10 kg as from 10 to 100 kg. Since the latter takes a much longer time, the change is less readily apparent in the child than in the infant.

Changes in composition of individual organs have also been studied.[11] Muscle, for instance, shows a decrease in water content and in ECF space during growth, while potassium and nitrogen increase. At the same time, the contribution of muscle to the total body weight increases.

Bone presents a somewhat different picture. The skeleton forms about the same percentage of total body weight in the newborn as in the adult, and the principal change involves maturation. As the skeleton grows, the proportion of cartilage, which in the human newborn comprises 30% of skeletal weight, falls almost to nothing in the adult, and it is this change that accounts in large part for the increasing calcium content of the body.[5] The remainder is accounted for by the increasing mineralization of bone cortex, a process that involves a reciprocal loss of water and little, if any, change in the content of organic matter.[8]

This all-too-brief résumé cannot do justice to the vast amount of data now available on the subject of body composition. Significant changes do occur during growth, and these changes may have an influence on a number of physiologic functions. The task for the future will be to elucidate the reasons for such changes and the mechanisms that control the rate of change, for in these may lie at least some of the mystery of the supervision of the growth process itself.

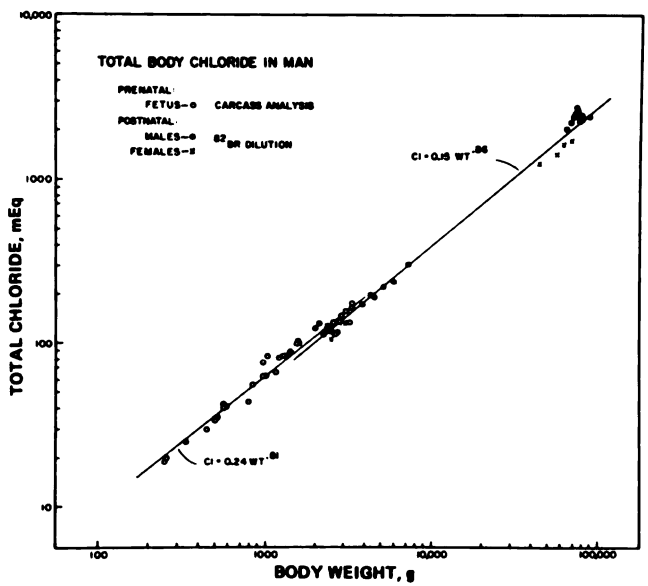


FIGURE 4 Log-log plot of total body Cl against body weight. From Forbes *et al.* (reference 9), by permission of the publishers.

This work was supported by grants from the U.S. Atomic Energy Commission and the National Institutes of Health.

REFERENCES

1. Anderson, J., S. B. Osborn, R. W. S. Tomlinson, D. Newton, L. Salmon, J. Rundo, and J. W. Smith. 1964. Neutron-activation analysis in man *in vivo.* Lancet 2:1201.
2. Friis-Hansen, B. 1958. Changes in body water compartments during growth. Acta Paediat. Supp. 110.
3. Forbes, G. B. 1952. Chemical growth in man. Pediatrics 9:58.
4. Forbes, G. B. 1955. Inorganic chemical heterogony in man and animals. Growth 19:75.
5. Forbes, G. B. 1960. Studies on sodium in bone. J. Pediatrics 56:180.
6. Forbes, G. B. 1962. Methods for determining composition of the human body. Pediatrics 29:477.
7. Forbes, G. B., and J. B. Hursh. 1963. Age and sex trends in lean body mass calculated from ^{40}K measurements: with a note on the theoretical basis for the procedure. Ann. N. Y. Acad. Sci. 110:255.
8. Forbes, G. B., and A. McCoord. 1963. Changes in bone sodium during growth in the rat. Growth 27:285.
9. Forbes, G. B., A. F. Reid, J. Bondurant, and J. Etheridge. 1956. Changes in total body chloride during growth. Pediatrics 17:334.
10. Oberhausen, E., W. Burmeister, and E. J. Huycke. 1965. Das Wachstum des Kalium-bestandes im Menschen gemessen mit dem Ganzkörperzahler. Ann. Paediat. 205:381.
11. Widdowson, E. M., and J. W. T. Dickerson. 1964. Chemical composition of the body. *In* C. L. Comar and F. Bronner [ed.] Mineral metabolism: An advanced treatise. Academic Press, New York.

Milton E. Bailey and
S. E. Zobrisky
University of Missouri

CHANGES IN PROTEINS DURING GROWTH AND DEVELOPMENT OF ANIMALS

Growth has been defined in many ways, and there is a diversity of opinion concerning what it should include. A definition of growth is operational and valuable when it is useful in solving a specific research problem, whether the problem is concerned with the crystal formation as an organic chemical or with the synthesis of fat or protein.

Among the physiological processes most intimately linked with the phenomenon of growth are those of nitrogen metabolism. This concerns not only the synthesis and anabolism of protein but also its interactions with other macromolecular constituents of animal bodies for the formation and maintenance of specific cells and tissues. Muscle growth, for instance, involves synthesis of specific protein monomers, precise alignment of these into structural elements peculiar to this tissue, and development of fibers according to muscle type and function. True muscle growth might be said to continue as long as its tissue protein increases in quantity.

The subject of this paper is very complex; it is concerned with many aspects of animal development and is influenced by many variables that are not discussed because they are beyond the scope of this paper.

Changes in muscle proteins are of major interest to researchers in animal

growth and development, and our subject matter will be imbalanced in our discussion of changes in these constituents.

BIOCHEMICAL CHANGES IN BODY PROTEINS

CHANGES IN DNA AND RNA

Intimately associated with protein synthesis in animal cells are the contents of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The relationship between these constituents and protein synthesis has been adequately reviewed during the last several years.[8,21,50,76] These and other references should be consulted for specific information concerning the relationship of nucleic acids to protein synthesis.

DNA Synthesis

One approach to study of early cellular change in DNA and RNA is through observations of compensatory growth. The mammalian liver constitutes an excellent system in which to analyze the biochemistry of growth, and some of the molecular aspects of compensatory growth of this and other tissues were recently outlined by Goss.[47]

The occurrence of weaning of the rat is an extremely important period of hepatic physiology. The rate of DNA synthesis climbs rapidly during this period,[85] and there is an abundance of polyploid and binucleated cells.[179] The adult liver, in contrast, has a very low mitotic rate and minimal DNA synthesis.[16,42] The DNA of liver is probably very stable under conditions of normal growth.

Many people have studied the synthesis of DNA during liver regeneration; but, in general, the rate of DNA synthesis reaches a maximum between 24 and 30 hours after partial hepatectomy and subsequently declines[10] to a minimal level characteristic of resting livers after 6 to 9 days.[62] In most of these studies, DNA synthesis was found to roughly parallel mitotic change of the organs and precede it by several hours, which indicates that DNA synthesis is prerequisite to mitosis.

Winick and Nobel[181] reported that early prenatal growth in the rat proceeds entirely by cell division. The rate of DNA synthesis, which presumably parallels cell division, decreases at different times for different organs. These investigators concluded that growth in the rat from 10 days after conception to maturity can be divided into three phases. Phase one consists of rapid cell division with cell size remaining constant; phase two consists of an increase in cell number and cell size as both DNA and protein

content rise, but as a result of decrease in the rate of DNA synthesis, protein increases out of proportion to DNA; and phase three consists of an increase in cell size only, as DNA synthesis stops and protein continues to accumulate. Growth finally ceases when protein synthesis and degradation equilibrate. These phases do not change abruptly, but merge gradually; this transition probably differs for the different organs and between the various animal species.

RNA Synthesis

According to Bell, [8] protein is very probably synthesized from the moment of fertilization. How the egg prepares itself for this event is not known, but extremely important is the time of protein synthesis relative to the supply of ribosomal, transfer, and messenger RNA. Apparently, sea-urchin eggs, and presumably others, are fully equipped at maturity with the three kinds of RNA required for protein synthesis. Addition of polyribonucleotides to cell-free extracts of unfertilized eggs promotes the incorporation of amino acids into proteins.[168,180] It appears, therefore, that ribosomal and transfer RNA are present in the unfertilized egg and are prepared to function when a template is provided.

Winick and Nobel[181] found that total organ RNA increased from 10 days after conception to maturity in the white rat. Except in liver, this increase is always proportional to increase in DNA and results in an RNA:DNA ratio that is constant for specific organs and does not vary with time.

Certain tissues such as the liver, heart, and skeletal muscle have high RNA:DNA ratios, whereas spleen, thymus, and other tissues have low ratios.[157] These data support the theory that tissues most actively engaged in protein synthesis are rich in RNA. High RNA:DNA ratios occur early in development, long before the observation of high protein:DNA ratios.

According to Goss,[47] in the partially hepatectomized animal, RNA synthesis in the liver is initiated very soon after the operation, even before DNA synthesis and mitosis begin. It reaches a maximum coincident with the maximum for DNA synthesis and mitosis and does not subside to normal levels of production until the liver is completely restored. Goss concluded that RNA synthesis is a good indication of the anabolic activity of the liver, and the same is probably true for other cells.

CHANGES IN BLOOD PROTEINS

A comprehensive review of the development of nonenzymatic proteins in relation to functional differentiation was published by Solomon.[159] An

important part of his paper includes discussion of change in serum proteins, changes in hemoglobin, and changes in proteins associated with immune reactions during development of animals. Many of the former group of proteins are synthesized by the liver, and their production can be used as an indication of liver development.

Serum Proteins

Solomon[159] cited several examples of proteins that are present in the serum only during embryonic development and that are characteristic of individual species of animals. The best example is calf fetuin, which was discovered by Pedersen.[135] There is a high concentration of fetuin in the sera of the newborn calf, but little in the sera of the adult.

The total serum proteins are present at a very low level in the newborn rat, and they increase rapidly to 78% of adult level within 3 weeks.[159] In this species, the albumin fraction increases rapidly after birth.[63]

Several types of serum proteins are associated with chick embryonic tissue. Between 10 and 20 days' incubation, α_2-globulin-E decreases in content. There is little or no consistent appearance of α_1-globulin until hatching, and then it remains at the adult level. Albumin does not appear as a distinct electrophoretic component until 9 to 11 days and may be derived from the α_2-globulin-E fraction.[148,175]

A fetuin-like fraction has also been found in pig blood; it migrated electrophoretically like α-globulin and decreased from 50 to 30% during fetal development.[116] The serum of the newborn pig is unusual in that it has a very low percentage of albumin (2%), which is one sixth that of the newborn calf, kid, and lamb. In the suckled pig, serum rises to a maximal value of 61% at 4 weeks of age. As soon as suckling begins, there is a tremendous increase in γ-globulin because of ingestion of clostral proteins via the gut, and this constituent was found to reach adult maximum at 2 to 6 months of age.[111]

The blood proteins of goats and sheep develop similarly. A fetuin-like component decreased from 13 to 6% during gestation. During this period, albumin increased from 20 to 40%, and the globulin fraction increased from 10 to 24% of this total protein.[5]

Serum proteins of the newborn calf contain only a small percentage of β-globulin (6%) but larger percentages of α-globulin (37%) and albumin (57%). As one would expect, after suckling there is a rapid increase in the γ-globulin level with compensating decreases in albumin and α-globulins.[78] γ-Globulin has been detected in the human fetus as early as 9 weeks of gestation, and it increases along with other globulin proteins during the last 6 months of gestation.[114] It has been reported to decrease to minimal values between birth and

3 to 4 months of age, then slowly increase to mean adult levels at 3 to 5 years.[128] The percentage of albumin in human blood was found to diminish until birth, then gradually increase.[138] However, the absolute quantities of albumin increased during these periods because of the net increase in total blood proteins.[114]

The synthesis of plasma proteins by the regenerating of liver is made possible by mobilization of RNA and amino acids, but the residual liver cell preferentially produces more cells, and only after a substantial amount of tissue has been replaced do they manufacture plasma proteins predominantly.[47]

Thus, after partial hepatectomy, only the concentration of plasma proteins decreases, and this is accompanied by a decrease in plasma as well as blood-cell volume.[101]

Hemoglobins

The ontogeny of three types of human hemoglobins consists of the appearance and early disappearance by 9 to 10 weeks of gestation of embryonic types of hemoglobins. Fetal hemoglobin in the young embryo is slowly replaced by adult hemoglobin. This replacement does not begin until around 13 weeks of gestation. After 8 months of gestation the adult hemoglobin represents only 10% of the total hemoglobin; but, subsequently, there is a rapid rise in adult hemoglobin which reaches 30% in the newborn infant. Fetal hemoglobin also disappears rather slowly after birth in goats, sheep, cattle, and monkeys. Conversely, the newborn rabbit and the hatching chick have no fetal hemoglobin. Fetal forms of hemoglobin have not been observed in cat, pig, or horse.[159]

Another important aspect of hemoglobin production is hemin synthesis, which has been studied extensively in recent years.[49] Data published by Moser and Flickinger[119] indicate that the addition of RNA to minced frog embryo tissue consistently stimulated the level of heme synthesis. Stimulation was also evoked by a mixture of the four ribonucleosides, and the mechanism probably involved production of enzymes responsible for iron porphyrin synthesis.

CHANGES IN MYOFIBRILLAR PROTEINS

Origin of Muscle Tissue

Information on the origin of muscle tissue and the manner in which it is assembled is difficult to find, but some pertinent information has been published. There are considerable differences of opinion concerning how the muscle cell is formed and how myofibrils accumulate. For several reasons,

described by Dreyfus *et al.*,[31] the protein biosynthesis of muscle is seldom studied. It is difficult to obtain pure nuclei from muscle because they sediment with the bulk of the myofibrils and because muscle is relatively poor in mitochondria and ribosomes and is difficult to homogenize without damaging the subcellular particles. The fact that muscle is poor in ribosomes and RNA partially accounts for the low synthesis of protein in muscle compared with other tissues.

The primitive embryonic cell that gives origin to muscle cells is the myoblast. Myoblasts that give rise to skeletal muscle fibers are apparently derived from mesenchyme and myotomes.[12,126] In striated-muscle fiber formation, the myoblasts begin to elongate and eventually produce cylinder-like structures. As the cell continues to grow, the nuclei increase in number and are centrally located. As the myofibrils increase in number, the nuclei move peripherally.[126]

Moscona,[118] from the study of myogenic cells of the chick embryo, cited indirect evidence that cytoplasmic granules may be the precursors of myofibrils. Characteristic granular inclusions were observed in the cytoplasm during early phases of cytodifferentiation.

The first fibrils formed are unstriated, and dots that delineate each sarcomere appear before the fibrils form tubes.[32] In mammals, the myofibrils just beneath the sarcolemma, that is, those forming the periphery of muscle fiber, are the first to become striated.

It is generally believed that after the second half of the intrauterine life, muscles increase in size by increasing the size of their fibers rather than the number; however, Goldspink[45] found that the number of muscle fibers in the bicep branchii of the albino mouse increased until they weighed 8 g. At this stage, differentiation of muscle tissue was complete. Subsequent muscle development with age or exercise was due to increase in the fiber diameter.

During embryonic development, fibrils grow in length from each end in complete sarcomere units.[74] After birth, the number of sarcomeres per fibril remains constant, and fiber length is increased by increasing the length of existing sarcomeres.[45]

Protein Synthesis and Turnover

McLean *et al.*[103] have shown that in muscle, mitochondria are as active as microsomes in the incorporation of isotopic amino acids into protein. Their results were further borne out by the ability of isolated muscle mitochondria to incorporate amino acids into proteins.[104] Amino acid uptake by isolated mitochondria was found to be dependent upon the supply of adenosine triphosphate which could be generated by oxidative phosphorylation or by

added adenosine triphosphate generating systems. Incorporation was also aided by the presence of magnesium ion and was stimulated by pancreatic ribonuclease. This evidence has been supplemented by data that indicate that mitochondria of rat liver incorporate amino acids into proteins to a small extent.[145,166]

It is possible that myofibrils can themselves synthesize proteins. In this regard, Perry and Zydowo[137] isolated a ribonucleoprotein from myofibrils of chicken and rabbit skeletal muscle. The presence of ribonucleoprotein is particularly marked in tropomyosin which comes from a variety of species and muscle types as a crystallizable complex, nucleotropomyosin containing up to 20% of nucleic acid.[59] Smaller amounts of RNA have been reported in purified myosin preparations.[110]

A number of workers, including Caspersson and Thorell,[20] have produced cytochemical evidence that nucleic acid is localized in the myofibrils. The evidence cited diminishes the possibility that nuclear contamination contributes more than a small fraction of the total myofibrillar RNA, but is inconclusive as to whether the RNA is part of the filamentous structure of the myofibril or is localized in some other cytoplasmic element such as the sarcoplasmic reticulum.

Studies by Winnick and Winnick,[182] who used labeled adenine and ^{14}C-labeled amino acids to determine the rate of formation of skeletal-muscle protein, support the view that considerable amounts of the myofibrillar proteins were derived from mitochondria and microsomes. However, the possibility that myofibrils themselves participate in protein synthesis was not excluded. A nucleoprotein fraction separated from myofibrillar protein was found to concentrate certain isotopic amino acids.

Regardless of the exact mechanism of protein synthesis in muscle, the consensus is that it is in a state of dynamic equilibrium with other body protein synthesis. Shemin and Rittenberg[154] showed that muscle proteins can be divided into two groups: the rapidly metabolized and the slowly metabolized. A third group, the inert proteins, was added by Dreyfus *et al.*[31] The first group includes aldolases and other sarcoplasmic proteins, and the second group includes proteins of the myofibrils. The third group, of course, includes collagen, elastin, and reticulin.

The half-life of mouse muscle aldolase was found by Schapira *et al.*,[147] using ^{14}C-tagging, to be 20 days. This enzyme undergoes molecular turnover according to the law of dynamic equilibrium.[31] There are changes between free and bound amino acids, which may or may not involve complete hydrolysis and synthesis of the protein molecules. Other water-soluble proteins exhibited an exponential-like decrease in radioactivity for about 80 days.

In animals fed a diet containing 24% of protein, the incorporation of ^{14}C-glycine into myosin was maximal by the 36th hour; the radioactivity of

myosin remained constant until about the 30th day and then decreased to a lower plateau. Similar results were obtained with ^{14}C-valine. Since the radioactivity of myosin is very similar to that of the total myofibrillar protein, the curve for the radioactivity of myosin apparently reflects the life-span of the myofibrils.

Dreyfus *et al.*[31] concluded that the myofibrils display a well-defined life-span of about 30 days (the life-span for mice myofibrils is 20 days). The authors concluded that myosin is relatively inert and its metabolic behavior can be compared with hemoglobin. If the radioactive-free glycine was diluted by the use of high-protein diet, the myosin molecule had a slow but definite molecular turnover.

Simpson and Velick[156] and Heimberg and Velick,[64] using ^{14}C-amino acids, found that certain sarcoplasmic proteins were synthesized from the same amino acid pool although the turnover rates differed. All of the peptide bonds in labeled enzyme molecules were formed within a relatively short time, although the rate at which the total pool of a given enzyme in muscle was replaced was slow. This work was extended to include myofibrillar proteins,[169] and it was concluded that H-meromyosin, actin, and the glycolytic enzymes were synthesized from the same amino acid precursor pools. Further, it was suggested that subunits of myosin (H- and L-meromyosins) were synthesized independently and that they turn over metabolically at different rates.

During the study of myosin from rats, Kruh *et al.*[97] found very little over-all decay of radioactivity after injection of ^{14}C-amino acids, but they observed turnover of amino acids. Evidence favored the view that glycine and phenylalanine were transformed into serine and tyrosine after having been incorporated into myosin. A three-step mechanism for the metabolism of radioactive amino acids in this protein was described. After incorporation into protein, the amino acids were liberated and passed through a pool, not mixed with the general pool, and were later reincorporated into the protein chain. The turnover was presumed to be purely internal (i.e., within the myofibrils) and was not involved in the general metabolic turnover of the rest of the body because there was no over-all decay in the radioactivity of myosin.

Effect of Hormones

Growth hormones Protein metabolism is acutely affected by hormones. The action of growth hormone on protein synthesis has been adequately reviewed by Korner,[93] who concluded that the hormone controls the ability of liver microsomes to assemble amino acids into peptide chains. The changes found in the microsomes as a result of hormone treatment of the rat could be accounted for entirely by changes in the ribosomal part of the microsomes.

There is also evidence[93] that growth hormone exerts its protein anabolic

effect by stimulating the secretion of insulin from the β-cells of the islets of Langerhans. Insulin has also been found to stimulate incorporation of precursors into RNA[184] and amino acids into liver protein.[136]

Evidence has been supplied [33,79] that urinary hydroxyproline is increased by growth hormone. This increase is probably related to formation of new collagenous tissue. Collagen content in muscle of rats has also been shown to depend upon the presence of growth hormone. Myosin, on the other hand, requires the presence of growth hormone and thyroxin for its synthesis. Thyroxin alone increases myosin deposition in muscle but has no effect on collagen in muscle, bone, or skin of thyroidectomized-hypophysectomized rats.[151]

Androgens The administration of testosterone and several other steroid hormones markedly decreases nitrogen excretion and increases protein deposition in kidney, liver, muscle, and accessory sex tissue.[88] The precise mechanism of this protein-anabolic action has not been fully explained, but Wilson[178] demonstrated that the primary effect was on the conversion of soluble RNA-amino complexes to microsomal ribonucleoprotein. The effect apparently was localized in the microsome, although the ultimate mechanism by which this physiological action is measured awaits explanation. A similar study by Kassenaar *et al.*[82] is compatible with the concept that androgens stimulate release of messenger RNA and act at the ribosomal level.

Kochakian[90] recently described studies concerning regulation of growth by androgens. Growth was qualified in terms of changes in nitrogen balance, changes in body weight, and changes in weight of skeletal muscle. A positive nitrogen balance was produced within 24 hours in the dog by the administration of an extract of male urine, and the administration of testosterone propionate to the castrate rat diminished urinary nitrogen excretion within 24 hours. The normal rat was less responsive and required a higher dose of androgen.[89]

The administration of growth hormone to the nonhypophysectomized castrate rat at maximal dose level produced a response in nitrogen excretion and change in body weight practically identical with that of the androgen. The simultaneous administration of growth hormone and testosterone propionate to the castrate rat resulted in summation of their effects on nitrogen balance and increased body weight. The induced protein synthesis occurred primarily in muscle, but the liver, kidney, seminal vesicles, and prostate were also affected. Apparently the two hormones have separate and nonconflicting roles in regulating protein metabolism.

Kochakian *et al.*[92] found that the decrease in rate of growth of muscles in the rat after castration was proportionate to the decrease in rate of animal growth. Administration of testosterone propionate reversed the effects of

castration. Korner and Young[94] found that methyl androstenediol increased the body weights of rats with a parallel increase in the weights of several skeletal muscles. The protein content changed in proportion to weight.

Certain skeletal muscles of the guinea pig were found markedly dependent upon androgens for full development.[133] These results were confirmed and extended to heart muscle of the guinea pig.[91] The weight changes after castration and treatment with androgen were accompanied by proportionate changes in protein.

The myosins, collagens, and sarcoplasmic proteins of the guinea pig temporal muscle decreased after castration and could be restored by testosterone,[152] but these effects were not demonstrable with the thigh muscles of the male rat.[153] Likewise, the myoglobin content of some of these muscles was unchanged by the above treatments.

The amino acid activating step in protein synthesis apparently is important in the protein anabolic action of androgens. The specific activities of the amino acid activating enzymes of the temporal muscle of guinea pigs changed because of the absence or presence of androgen. The effect appeared to be relatively the same for each enzyme studied, and in each case it paralleled changes in the weight of this muscle. Incorporation of ^{14}C-leucine and ^{14}C-phenylalanine into protein by a microsomal preparation was decreased by castration and increased to above normal by testosterone administration.[90] These results were confirmed by Breuer and Florini,[13] who demonstrated that androgen administration restored protein synthesis. Cell-free preparations of ribosomes were affected by androgen administration in conversion of amino acids to protein. Androgen administration restored protein synthesis to normal.

Estrogens In contrast to the response of skeletal muscle to androgens, estrogens appear to influence smooth musculature of the reproductive organs. Muscular hypertrophy of the uterine wall due to estrogenic stimulation has been discussed in detail,[24] and similar results have been observed in many cases in the oviduct as well as in the muscular parts of the male reproductive organs.[164]

The influence of orally administered stilbestrol on connective tissues of lamb skeletal muscle has been studied by McIntosh *et al.*,[102] who observed that the hormone increased the connective tissue content in direct proportion to its concentration. There was also evidence that stilbestrol increased the collagen content in skin. The latter results are similar to those of Anastassiadis *et al.*,[1] who found that estrogen increased the collagen content of the skin of immature pullets.

During studies of regulation of protein synthesis by androgens and estrogen, Wilson[178] concluded that the acceleration of protein synthesis in accessory sex tissue of the rat by these hormones is due to enhancement of peptide bonding of soluble RNA-amino acids to form microsomal ribonucleoprotein. Since this mechanism of stimulation is closely associated with that described for growth hormone, it is possible that the mechanism of protein synthesis stimulation by sex hormones is also involved directly with the activity of growth hormones. Studies cited above for androgen, however, would indicate that this is not necessarily the case.

CHANGES IN ENZYMES

Excellent reviews of enzyme development in relation to functional differentiation have been published by Moog[113] and by Beckett and Bourne.[7] These reviews should be referred to for specific information on this subject.

Organ Tissue

The activities of several enzymes increase extensively in chick embryonic tissues. Peptidase increases sixteenfold during the first 5 to 7 days and thereafter only slightly.[159] Cytochrome oxidase and succinic dehydrogenase activity rise steadily from 7 to 20 days.[27] Malic, glutamic, and lactic acid dehydrogenases rise twofold to threefold between 7 and 15 days, then decline sharply.[158]

An important aspect of enzyme development in animals is concerned with the glycogen-storage function of the liver. It was found several years ago[26] that glycogen appears in chick liver during the first week when previously high mitotic activity falls to a low level. In the fetal guinea pig liver, glycogen is not detectable until 56 days.[61] It then is produced very rapidly to term and decreases almost to zero again. Thereafter, it begins to accumulate. Uridylic diphosphoglucose (UDP)-glucosyl-transferase also increases from about 58 days to term when it plateaus at its adult value. UDP-glucose-pyrophosphorylase also rises at about the same time, and phosphorylase increases about fivefold from 64 days after conception to 2 days after birth, then declines.[95]

The human infant can oxidize phenylalanine and tyrosine at birth, but the premature infant is unable to do so.[96] The fetal rat, rabbit, pig,[86] and guinea pig[48] are likewise unable to catalyze oxidation of these amino acids. The necessary enzymes are usually produced in these animals shortly after birth.

Although there are numerous enzymes in the kidney at birth in most species of animals, they are low in functional activity. The over-all picture is one of immaturity, but in many animals the kidney is not completely de-

veloped at birth. Although enzymes are in short supply during the period of dependent infancy, their activities are accelerated tremendously upon requirement when the animal is capable of independent maintenance.[113]

The small intestine, like other parts of the gastrointestinal tract, begins full-scale enzyme activity immediately after birth or hatching. Thereafter, the enzyme systems develop coincidentally with requirements for converting foods to digestible materials. These enzymes, like many others in animal tissues, begin to accumulate in anticipation of their biochemical functions.[113]

Muscle Cells

Myosin is a rather unusual protein in that it serves as muscle structural material, part of the contractile protein, and as an enzyme. A major question would be concerned with the point in the development of the animals at which these activities begin to function.

Ebert[34] used an immunochemical procedure to determine when cardiac myosin first appeared in the chick embryo. He demonstrated that a cross-reactive substance is distributed in the epiblast at stage three (early primitive streak) but is limited to the heart-forming areas by the time the head process appears. Actin is not found before the head-process stage, when it, too, is localized in the heart-forming areas.[35] These results did not indicate whether myosin on first appearance was enzymatically competent or whether the enzymatic site differentiates independently of the antigenic site. However, Klein[87] was able to detect myosin-associated adenosinetriphosphatase activity 2½ days after the heart had begun to pulsate, but more sensitive measures are needed to determine the early development of these proteins.

Skeletal-muscle myosin seems to appear later than the cardiac variety. Holtzer *et al.*[74] demonstrated the presence of myosin in brachial somes of the latter after about 52 hours. The antibody was clearly localized on myofibrils that were shown to be present earlier than can be seen with iron-hematoxylin staining. Contraction or addition of adenosine triphosphate (ATP) to glycerinated myoblasts was not observed until stage 21.[74]

A material reacting with antiserum to adult myosin or actomyosin was demonstrated in tailbud stages of both *Rana pipiens* and *Triturus* shortly before the beginning of movement.[129] Adenosinetriphosphatase activity apparently begins at about the same stage in *Rana pipiens*.[6]

Even though the antigenic properties of larval and adult actomyosin of *Rana pipiens* were found to be similar,[124] the adenosinetriphosphatase of proteins from the two sources was strikingly different. The adenosinetriphosphatase activity of the larval enzyme was activated by both Mg^{2+} and Ca^{2+} at both high and low KCl concentrations; whereas adult actomyosin was

affected only at low salt concentrations in the presence of Ca^{2+}, a pH optimum between 7 and 8 was found for the larval enzyme, and one between 9 and 10 was found for the adult enzyme. The larval enzyme had adenylpyrophosphate activity, opposed to strict adenosinetriphosphatase activity of the adult form. At the free-swimming tadpole stage, the specific activity was almost three times that of adult actomyosin. The shift to adult characteristics occurred just before metamorphosis.

Actomyosin accumulated rapidly in the chick embryo, rising from about 10 mg per g of leg muscle at 10 days to about 25 mg per g at hatching; the adult maximum of 60 mg per g is attained gradually.[25,67] The myofibrillar fraction of breast muscle increases about the same magnitude during the first month after hatching.[143] The Ca^{2+} adenosinetriphosphatase activity of the myofibrillar proteins almost doubled between the thirteenth day and hatching.[144] The adenosinetriphosphatase activity per gram of total muscle protein increases gradually after hatching.

Herrman *et al.*[69] reported the occurrence of myosin in the rat with weak adenosinetriphosphatase activity as early as the fourteenth day. Although de Villafranca[28] was unable to find myosin at 15 days, he could demonstrate contraction at 16 days; at 19 days, myosin was easily detected. The specific activity of adenosinetriphosphatase increased rapidly from day 19 *in utero* to 20 days after birth and continued to increase at a slower rate. Myosin continued to accumulate relative to whole-muscle growth until adulthood.[68]

These studies indicate that there is clearly a differentiation of myosin during growth with respect to both level and characteristics of adenosinetriphosphatase activity. Both of these obviously could be influenced by changes in the H-meromyosin fraction of myosin. Robinson[144] reported that water extracts of chick leg muscle in the late embryonic stages have adenosinetriphosphatase with chemical characteristics like those associated with myofibrillar adenosinetriphosphatase. At the cytochemical level, material reacting with fluorescent antimyosin is at first diffused throughout the myofibril but later assumes a banded pattern in which only the A-bands react.[39,74]

THE MORPHOGENESIS OF FIBROUS PROTEINS

The cellular biosynthesis of animal proteins has been extensively studied during the past several years, and a discussion of this subject is beyond the scope of this paper. Considerably less is known about the biosynthesis of protein fibers, but this, too, has received much recent attention. It has been well established that the biogenesis of certain types of protein fibers *in vitro* is the result of polymerization or aggregation of precursor macromolecules.[109]

Aggregation of rather large units has been suggested for F-actin[163] and for

F-insulin.[134] Other proteins believed to be formed by aggregation include collagen,[52] keratin,[41] feather keratin,[3] fibroin,[108] trypsinogen,[51] paramyosin,[72] and meromyosin.[139]

The polymerization mechanism leading to fibrillar protein structure has been studied in several model systems.[140] The general morphology of the process of aggregation of globular proteins was discussed by Pauling,[134] who described the aggregation of protein monomers as follows: The compact particle formed of a single folded polypeptide chain is asymmetrical and, therefore, slight change in structure or environment, or both, may lead to aggregation of the monomer. The structural changes necessary to bring about the change may be slight (oxidation, reduction, or removal of side chains) or extensive (unfolding of the monomeric polypeptide for example). During some stage of the process of structural modification, monomeric molecules develop complementary regions with sufficient energy (20 kcal/mole) of interaction to form a bond. A single bond such as a –S–S bond would serve to hold the two protein molecules together, but other forms, such as van der Waals forces, hydrogen bonds, and electrostatic attraction, may also be involved.

From the study of a variety of proteins, it is evident that polymerization may be due to various and different mechanisms.[140] Tropomyosin, a protein complex with pentosenucleic acid,[155] has globulin-like properties suggesting an asymmetrically distributed electrical charge. In solution free from salt it undergoes end-to-end aggregation with a resultant large rise in viscosity. Hexamers are formed, which result in a length-to-width ratio of about 20:1 for the individual particle.[83] The bond is purely electrostatic, as indicated by the immediate fall in relative viscosity when salt is added to a salt-free sol. In this particular protein, the absence of terminal amino groups suggests that the monomer is cyclic polypeptide.

The polymerization of actin, on the other hand, depends on an entirely different mechanism. The monomers appear to be linked through the nucleotide prosthetic group of the molecules by a divalent metallic ion. In the presence of strong chelating agents, such as ethylenediamine-tetraacetic acid which removes all traces of metallic ions from the protein, the monomer alone is present.[167] Dissociation also occurs at high pH, probably as a result of electrostatic repulsion between monomers when a high proportion of the acidic groups are ionized. The ultrastructures of these fibers have been thoroughly investigated by Hanson and Lowy.[60]

In the case of myosin, the monomer is extremely complex, and the unit appears to contain both cyclic and open chains. The myosin monomer is an elongated structure with a length of about 1,600 Å and a diameter of 20 to 40 Å.[186] The molecule consists of a rod-shaped, 2- or 3-stranded[100] helical

coil 15 Å in diameter and 1,300 Å in length and a globular head 40 Å in diameter and 350 Å in length.[142] The head is envisioned as consisting of three polypeptide chains folded in tertiary structure characteristic of an elongated globular protein.[185] Lateral as well as end-to-end aggregations occur in solution, and possibly disulfide bonds between cysteine residues participate in polymerization. It can be assumed that on either side of the center of the filament, myosin molecules are laid down with opposite polarity, the head end always being oriented away from the center.[75]

CHANGES IN COLLAGEN

The best example for the formation of morphologically specific structure from precursors produced at the macromolecular level has been offered in the physical-chemical studies of collagen. Recent studies have been revealing in terms of the structure of collagen and have added much to the clarification of the mechanism of *in vitro* fibrogenesis, which possibly relates to the *in vivo* physiological process. Observations on the production of different morphological forms of collagen by controlling conditions of pH and ionic strength and by the addition of different noncollagenous substances may be extremely important to problems of morphogenesis of organisms.[56,70,150]

The most common characteristic of collagen fibrils is an axial periodicity of about 640 Å with detailed intraperiod fine structure, which can be observed with the electron microscope and detected by x-ray diffraction in collagen fibers of most phyla, ranging from sponges to mammals.[56,107] Electron-microscopic analysis of several structural forms of collagen reconstituted from solution led Gross *et al.*[55] to characterize the unit building block or molecule as tropocollagen. This molecule was considered to be approximately 3,000 Å long and about 50 Å wide. Physical-chemical studies by Boedtker and Doty[9] confirmed the existence of this particle in solution. It has been suggested that the manner in which these highly asymmetric particles lined up gave rise to different forms of collagen.[54]

Collagens from various sources can be dissolved at acid, neutral, and moderately alkaline pH without apparent degradation of the molecule.[53,57,71,130] These collagens can then be precipitated from solution by various procedures such as dialysis, slight heating, neutralization, or addition of salt. Important factors determining the kind of structure obtained are collagen concentration, the amount and kind of salt added, pH, and temperature.[54]

Three natural forms of collagen fibers have been reconstituted: those having a normal axial period of 640 Å, those having an axial period of 220 Å, and those having no periodicity. These are also interconvertible under appropriate conditions. Two other unusual forms have been observed by Highberger

et al.[71] The "fibrous long spacing" (FLS) collagen has an axial period of about 2,400 Å and can be produced by the addition of α-acid glycoprotein of blood serum to a solution of acid-extracted collagen followed by dialysis against water.[71] It is convertible to collagen having the 640-Å period by dissolving in acid and dialyzing against saline. The use of less glycoprotein results in formation of a mixture of FLS and 640-Å-type fibrils. Lower concentration resulted in formation of the usual 640-Å-type fibrils only. Morphogenetically, these results are important because two radically different structural forms could be produced by interaction of two molecular species simply by altering the concentration of one of them.

Another unnatural structural type of collagen was discovered in precipitation of a neutral phosphate extract of fresh connective tissue.[150] This type took the form of short segments of crystallites of varying width having average lengths of 240 Å. They were called "segment long spacing" (SLS), and they contain ATP.

Many different agents can cause the formation of FLS collagen. Among these agents are thrombin, mushroom tyrosinase, *Clostridium* collagenase, chondroitin sulfate, sulfated dextrin, and gum arabic. The common factor responsible for FLS-inducing properties is still unknown.[54]

The collagen fibers of newborn animals usually are small and are surrounded by viscous mucopolysaccharide ground substance. The growth of these fibers can possibly proceed in two phases. The tropocollagen monomers aggregate in the axial direction, then grow in thickness by apposition.[170] The growth of collagen fiber has been described in microscopic and electron-microscopic studies of Linke.[99]

An actual scheme of fibrogenesis was proposed by Jackson.[77] The fibroblast apparently synthesizes the fiber precursor tropocollagen described above. This is secreted by the fibroblast into the extracellular space. These submicroscopic fibrils can accumulate monomers by aggregation in the axial direction and can grow in thickness by apposition to form visible histocollagen, which is citrate-soluble and contains fairly strong cross lengths. Further, particles of neutral salt-soluble collagen are added on, making the fibers thicker and longer. The cross-length core of these fibers may be strong and may include mucoprotein, now extractable only with alkali.[11] Outer fibers most recently laid down may still have very weak cross-links and are still soluble in citrate buffer. Finally, the whole fiber matures with age and the cross-linkage increases in strength; hence, the proportion soluble in citrate buffer decreases.

At present, the best founded theory for the aging of collagen seems to be that in the stable and nonmetabolizing mature collagen macromolecules the number of hydrogen bonds increases, possibly through physical factors associated with Brownian molecular movement. In addition, substances may exist that speed up the process of aging by increasing the cross-linking, perhaps by

the formation of ester cross bonds. These might be either intrinsic or extrinsic factors.[170]

Despite the fact that aged collagen is considered essentially inert metabolically, there apparently is some turnover of this protein in certain tissues even in the adult animal. Carniero,[17] using ^{3}H-proline and radioautographic procedures, concluded that collagen in the periodonate membrane (the ligament attaching teeth to the alveolus) turns over rapidly compared with other tissues such as bones, cementum, and dentine. Of several tissues studied by Kao *et al.*,[81] including aorta, skin, tendon, and uterus, only the latter showed appreciable synthesis and turnover of collagen.

Collagen is a major organic component of bone and cartilage and of dentine of teeth. Bone develops by transformation of connective tissue into calcified connective tissue and can be considered as crystalline collagen, which is intimately associated with hydroxyapatite and calcium phosphate.[125] Undoubtedly, the growth of collagen in bone is similar to that proposed above for the development of fibrils; but in bone, collagen has the ability to react with minerals to form organic–inorganic matrices. Despite extensive investigation, the arrangement of the collagen fibrillar bundles in the lamellar system has not yet been completely elucidated.[43,44]

Osteoblasts are connective-tissue cells that produce bone. Examination of sections of bones from growing animals sacrificed 30 minutes after injection of labeled ^{3}H-glycine reveals the label principally in osteoblasts. Four hours later the labeled product is located principally in bone adjacent to osteoblasts. Seven to 45 days later, the labeled product is farther from the osteoblasts because of progressive cellular activity.[18] ^{45}Ca and ^{32}P injected into growing animals resulted in concentration of mineral near osteoblasts, but labeled minerals were not confined to this region. These and similar results indicate that continuous exchange of minerals occurs normally in bone.

Tonna[165] recently described a series of radioautographic studies of mice 5 to 52 weeks of age in which tritiated amino acids were used. A distinctive pattern of protein synthesis was observed among the periosteal and endosteal surface of bone, which accounted for the familiar growth and remodeling dynamics of the skeleton. Collagen synthesis diminished significantly after bone formation and growth ceased. The activity diminished further as aging progressed; and active protein synthesis by old periosteal cells, even when traumatized, was not observed. Following trauma, however, activation of periosteal-cell proliferation resulted from an increased population of both bone and cartilage cells capable of intense protein synthesis at the site of trauma and along the entire shaft.

Several investigators have concluded that collagen turnover in tissues is low, but it is somewhat greater in bone than in other tissues. Neuberger,[127] using ^{14}C-glycine as a tracer, found that collagen turnover was almost 3 times

as great in bone of old rats as in tendon from the same animals. In young animals, the turnover of collagen in bone was about 6 times as great as that in tendon and about twice as great as that in skin. Over all, it was found that collagen turnover was quite low compared with that of other proteins.

QUANTITATIVE CHANGES IN BODY PROTEINS

CHANGES IN INDIVIDUAL MUSCLE PROTEINS

Data in the literature indicate that there is an increase in intracellular protein during growth. The relative changes in muscle proteins with age are somewhat consistent in various mammals as reported by different investigators and summarized in Table 1.[30,46,65] Robinson[143] found that total protein nitrogen

TABLE 1 Growth Changes in Concentration of Structural Proteins in Mammalian Skeletal Muscle (g/100 g tissue)[a]

	Immature		Adult	
Species and Reference	SP[b]	MP[c]	SP[b]	MP[c]
Human[30]	3.2	11.0	4.3	12.8
Pig[30]	3.5	10.6	5.2	12.8
Cattle[65]	5.0	8.4	5.3	13.0
Rat[65]	–	–	4.5	11.5
Rat[46]	4.1	10.0	5.2	11.2

[a]From Gordon *et al.*[46]
[b]Sarcoplasmic protein.
[c]Myofibrillar protein.

per 100 g of muscle in chicken increased progressively from birth and leveled off at 100 g of weight. Myofibrillar protein represented an increasingly larger proportion of the total protein, whereas percentage of sarcoplasmic protein decreased during growth. The rapid increase in actomyosin during the first month of growth in chicks was confirmed by Herrmann.[67]

This type of study was extended to human and porcine muscle by Dickerson and Widdowson,[30] who quantitated changes in all nitrogen constituents of "thigh" muscle during growth and development. Nonprotein nitrogen attained adult levels by the age of 4 to 7 months in man and by the age of 4 to 6 weeks

in porcine (3.2 and 4.1 g/kg of fresh tissue, respectively). The concentration of total protein nitrogen increased to the same minimal level (28 g/kg of fresh tissue) during development of both species, even though the newborn human had a higher initial concentration than swine. During fetal life, the concentration of sarcoplasmic proteins changed very little in human muscle but apparently decreased in porcine muscle. Postnatally, the concentration of sarcoplasmic protein nitrogen increased in both species. Concentration (grams per kilogram) of fibrillar protein nitrogen increased with age and was greater than that of sarcoplasmic protein during most stages of development. Most changes in quantity of fibrillar proteins occurred within 4 to 6 weeks in the swine, but in man increases were evident after 7 months. Changes in the protein components of the muscle cell continued to occur in both species until maturity. The myofibrillar proteins were more concentrated than the sarcoplasmic-protein portion at most stages of development in the human and porcine muscle.

Maximal values of extracellular protein nitrogen in muscles were 4.6 and 3.2 g/kg of fresh muscle tissue for humans and swine, respectively, during the suckling period. These values declined to about 1.1 g/kg of fresh tissue in adult muscle and were very similar in the two species. The change in collagen nitrogen paralleled changes in extracellular nitrogen. These changes in the proportions of the protein fractions are basically the same as those reported in developing chick muscle by Robinson[143] and Dickerson.[29] In the human, the swine, and the chick, nitrogen contributed by the extracellular fraction increased to a maximum and subsequently decreased to a lower level in the adult. It has been theorized[30] that extracellular protein increases while cells are increasing in number and decreases while cells are increasing in size. The extracellular proteins are distributed in skeletal muscle as endomysium, perimysium, and epimysium around the individual fibers, fasciculi, and muscle bundles, respectively.

Nucleoprotein comprises a large percentage of protein during early development of animals. The larger nuclei in prenatal muscle cells represent a higher nuclear-to-cytoplasmic ratio than in more mature cells. It is logical to assume that the nucleus has a greater influence on the composition of the cell during early gestation than in early postnatal or adult cells. Robinson[144] found that in 10-g chick embryos as much as 66% of the nitrogen of the muscle fibrillar fraction came from nucleoproteins.

Data concerning changes in muscle proteins during growth of cattle were discussed in detail by Helander.[65] Later, Helander[66] reviewed changes in proteins of several species of animals, but was particularly concerned with the effects of activity on muscle development. Helander pointed out the difficulty associated with ascribing age periods of animals when studying muscle develop-

ment, because exercise and certain pathological conditions are also important. It was concluded that exercise was accompanied by muscle hypertrophy, and that inactivity resulted in hypotrophy. The amount of myofibrillar proteins increased with activity and diminished with restricted activity. These results would be expected, but some previous work indicated that hypertrophy due to exercise was caused by increased quantities of sarcoplasmic protein and not the contractible proteins.[117]

GROSS CHANGES IN PROTEIN

Changes in Whole Bodies

The literature abounds with studies of the influence of species, age, and nutritional intake on the changes of total muscle and body proteins in animals. Von Bezold[171] was among the first to illustrate that the composition of mammals, birds, and amphibia was characteristic of the species as well as their stages of development. Later, it was reported[98] that cattle had a higher percentage of protein than sheep or swine. These results have been confirmed,[120] and further comparison has been made of nitrogen distribution within mammalian species, particularly between swine and humans.

Further study[120,122,123] resulted in the conclusion that the chemical composition of mammalian bodies of the same species could be predicted if the fat content were known, because the nonfat portion was of the same approximate percentage composition during growth, regardless of fatness. It was generalized[120] that mammals attain "chemical maturity" in regard to water, protein, and mineral on a fat-free basis at an age equal to 4.0 or 4.5% of their total life expectancy. The proportions of these substances are not constant since percentage of water decreases and percentage of protein[2] and ash[141] increase as animals become older.

Ellis and Hankins[36] and, more recently, Manners and McCrea,[106] Dickerson and Widdowson,[30] and Elson *et al.*[37] also observed increases in protein content of swine as age progressed. The percentage of protein appeared to reach a maximum at about 150 to 200 days of age[36] and subsequently decreased.

The age of maximum protein level in swine was found to coincide with the age at which muscle fibers of the *semitendinosus* (175 days) and *longissimus* (150 days) muscles of swine attain 95 to 99% of their maximum diameter.[23,38] This is shown in Figure 1, which is a semilogarithmic plot of muscle-fiber diameter versus age of swine. The initial rate of increase was very rapid: the muscle-fiber diameter increased 100% during the first 25 days after birth. The rate of increase in muscle-fiber diameter decreased between 100 and 125 days, and was essentially zero after 150 days.

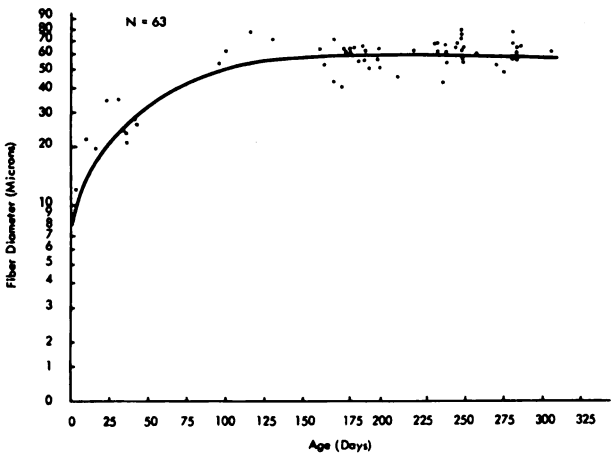


FIGURE 1 Change in *longissimus*-muscle-fiber diameter with increase in age of swine. Data after Chrystall, reference 23.

Muscle-fiber diameter and body weight were significantly correlated: 0.68 and 0.86 in swine,[23,161] and 0.76 in sheep.[40] Similarly, muscle-fiber diameter and age were significantly correlated: 0.84 and 0.81 in swine,[19,23] and 0.74 in sheep.[80] The diameters of *longissimus* muscle fibers have also been shown to be closely associated (T = 0.81) with the nitrogen content of the empty bodies of swine.[23]

A semilogarithmic plot of total protein and empty-body weights of swine (Figure 2) is quite similar to the curve (Figure 1) concerned with changes in muscle-fiber diameter during growth. Some of the data in Figure 2 from the study by Swanson [164] are lower than the average values because many animals in this study were on protein-deficient diets, which apparently affected body-protein concentrations. These data also exemplify the effects of undernutrition on the muscle mass of animals at specific weights. Some animals of the same body weight in this study differed as much as sevenfold in fat content.

For empty-body weights below 15 and 20 kg, there was very little variation about the line representing protein content. One may question whether this uniform protein increase during early development of the pig was due to some innate growth mechanism or to a response to the quality of the diet provided by the lactating sow. Both factors probably have an influence, as pointed out as a result of similar work with rats.[146]

Assuming that the swine included in these studies were weaned at 8 weeks (15 to 18 kg live weight), one might further associate the larger deviations in protein content beyond weaning with the adequacy of growing and fattening rations to meet the animal requirements for maximum protein retention.

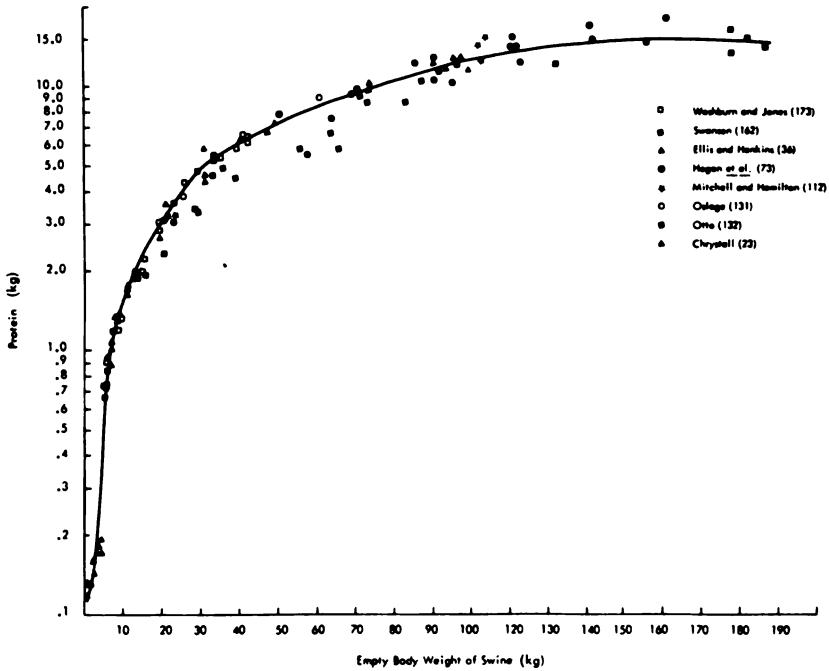


FIGURE 2 Change in kilograms of protein with increase in empty-body weight of swine.

These deviations obviously represent the accumulated effects of the total environment and genetic potential of these animals. Other investigations concerned with the increase in protein as related to age or body weight, or both, include Bailey *et al.*,[4] Brody,[14] Moulton,[120] and Zobrisky.[187] The investigation of Bailey *et al.*[4] is concerned with protein-to-water ratios as indices of physiological maturity. Generally, moisture content decreases as protein content increases[4] and, similarly, the nitrogen–potassium ratio increases in humans and swine during maturation.[138,177] It should be recognized, however, that some organs and tissues mature faster than others.[3] The heart, liver, and kidney at birth more nearly approximate their adult compositions than the skin and skeletal muscle.

Proteins of Various Body Constituents

The distribution of protein of various body components as a percentage of the total protein in the empty body of growing fattening swine is presented in Figure 3. The proportion of the total protein found in the flesh or soft

tissue increased from about 65% in the 25-kg swine to 76% in the 160-kg swine.[131] The percentage of protein in the soft tissue compared with whole-body weight decreased from 15.0 to 13.0%. The amount of protein in the soft tissue increased relative to the total protein in the body with each successive gain in weight because of the greater increase in weight of soft tissue compared with the other body components. Data in Figure 4 were available to separate the protein of the soft tissues into that proportion found in the muscle, fat, and skin from 110- and 160-kg swine; they indicate that approximately 52% of the protein in the empty body of swine was in the muscle, whereas the values were 10.1 and 12.6% in fat, and 10.5 and 11.3% in skin from the 110- and 160-kg animals, respectively.

Figure 3 further illustrates that 8.9 to 12.1% of the total body protein was found in the skeleton. These values are considerably lower than the value of 18.7% reported for porcine by Kemme,[84] 18% for humans reported by Widdowson and Dickerson,[177] and 16 to 26% reported here for cattle (Table 2). The discrepancy is possibly due to the amount of tissue left on the bone, that is, definitiveness in separation and nutritional state of the animal at death.

The proportion of the total protein in the internal organs and offal[131] declined steadily from about 16 to 7% during the 135-kg increase in live weight.

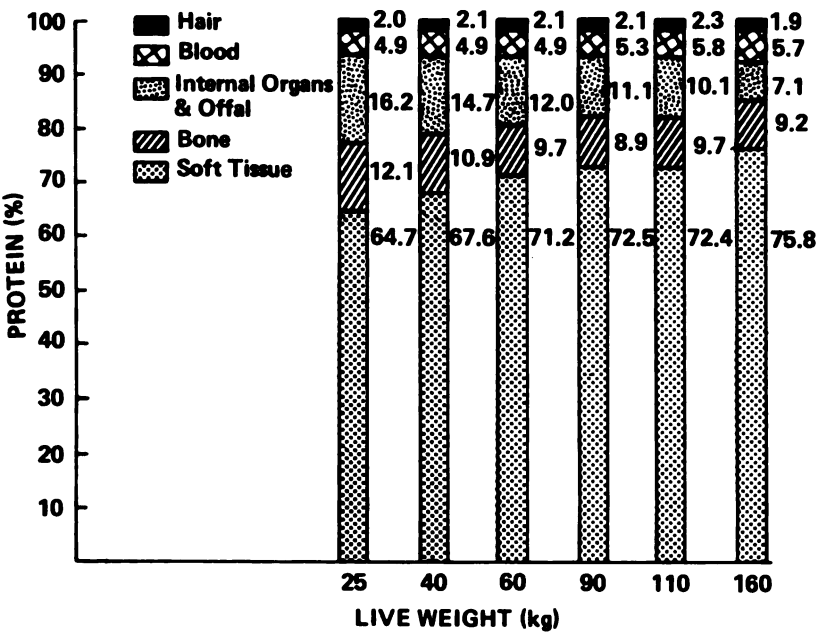


FIGURE 3 The distribution of protein in the growing fattening swine (fresh-tissue basis). Reworked from data of Oslage.[131]

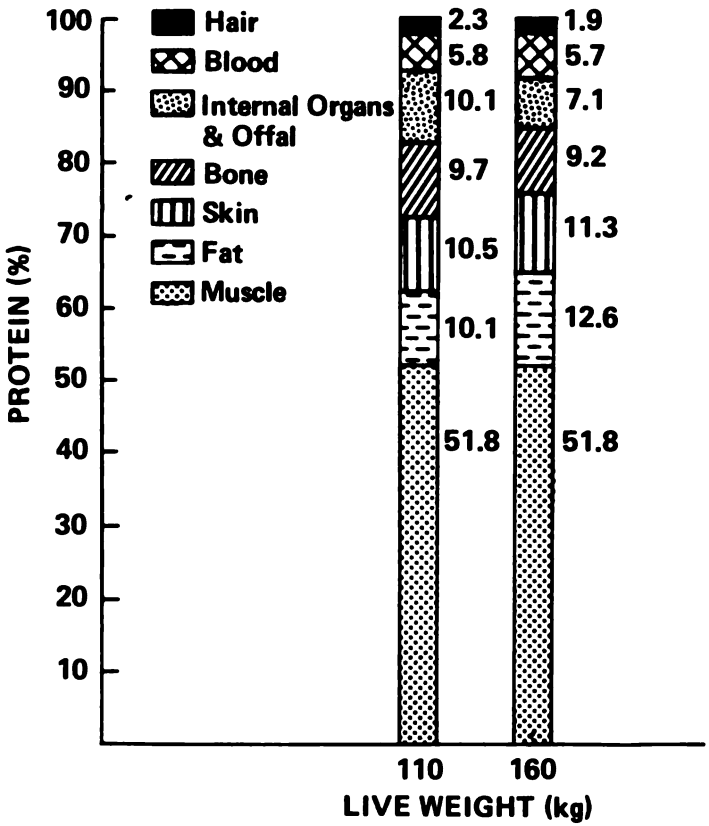


FIGURE 4 The distribution of protein in 110- and 160-kg swine (fresh-tissue basis). Reworked from data of Oslage.[131]

These results support those of McMeekan,[105] who reported that the internal organs comprised a larger portion of body weight in young swine.

About 5 to 6% of the total protein of the empty swine bodies was in the blood. This portion of the protein increased slightly as weight increased. Similarly, the percentage of protein in the blood (fresh basis) increased from 16.5% at 25 kg to 19.8% at 110 kg of empty-body weight. Although the protein content of the hair ranged from 82 to 86%, this constituted approximately 2% of the total protein of the body.[131]

CHANGES IN PROTEINS OF CATTLE

The relationship between the protein (nitrogen x 6.25) content and the empty-body weight of cattle is presented in Figure 5. The three groups of

TABLE 2 Distribution of Protein in the Empty Body of Cattle (Fresh Tissues)[a]

Group and Age[b] (months)	Empty-Body Weight (kg)	Total Protein (kg)	Total Protein (%)	Distribution of Protein: Blood (%)	Organs (%)	Hair and Hide (%)	Skeleton (%)	Fat and Lean (%)
GROUP 1								
3.0	98.1	19.37	19.74	5.47	8.72	17.09	23.23	45.48
5.5	172.8	29.75	17.22	5.88	10.52	14.72	20.76	48.08
8.5	171.5	31.38	18.30	4.97	8.95	15.55	18.73	51.77
11.0	281.3	48.74	17.32	4.55	8.15	17.39	16.56	53.30
18.0	459.0	76.06	16.57	4.54	6.73	14.42	17.18	57.77
21.0	475.9	76.64	16.10	5.71	7.67	18.50	16.47	51.59
34.0	671.9	90.93	13.53	6.11	6.25	16.62	18.47	58.58
39.5	786.0	98.42	12.52	5.65	6.29	15.62	16.32	47.44
44.5	771.0	98.76	12.81	5.57	7.08	14.80	18.22	54.27
48.0	814.9	99.94	12.26	5.88	6.62	17.19	19.75	50.50
GROUP 2								
3.0	78.1	15.18	19.44	4.94	7.77	15.22	25.95	41.11
5.5	99.4	19.31	19.43	5.18	8.08	16.52	23.61	46.60
8.5	121.0	22.13	18.29	5.65	9.31	15.81	21.19	48.03
11.0	159.0	29.32	18.44	3.85	8.53	18.11	19.40	50.09
26.0	337.8	62.69	18.56	4.29	6.87	18.44	18.64	51.74
34.0	418.9	75.19	17.95	5.73	6.39	15.46	20.11	52.24
40.0	428.0	79.20	18.50	4.58	6.23	16.72	19.17	53.24
44.5	444.0	88.92	20.03	4.84	6.46	18.26	18.92	51.34
48.0	493.9	88.70	17.96	5.28	7.13	19.24	20.52	48.95
GROUP 3								
3.0	71.0	14.19	19.99	6.13	9.23	15.01	24.66	44.96
5.5	86.0	17.43	20.27	4.99	8.95	16.87	23.29	45.90
8.5	90.0	17.31	19.23	4.33	9.36	15.54	24.55	46.22
11.0	137.7	24.99	18.15	4.72	8.24	16.76	20.25	50.01
18.5	192.0	36.19	18.85	4.67	7.60	16.58	19.34	51.80
26.0	265.5	51.18	19.28	4.88	6.58	19.71	19.53	49.55
40.5	332.0	65.56	19.75	4.48	7.05	18.11	21.06	49.27
45.0	391.5	77.94	19.91	5.45	6.91	20.40	20.05	54.97
48.0	407.8	81.44	19.97	5.21	6.75	17.34	18.86	51.77

[a] Original data from Moulton *et al.*[120]
[b] Group 1 fed *ad libitum*; group 2 fed for growth, little fattening; group 3 fed for "restricted" growth.

cattle from which these data were obtained ranged in age from 1 day to 48 months. The cattle in group 1 were fed *ad libitum*; those in group 2 were fed for growth with little fat development, and they gained about 0.45 kg per

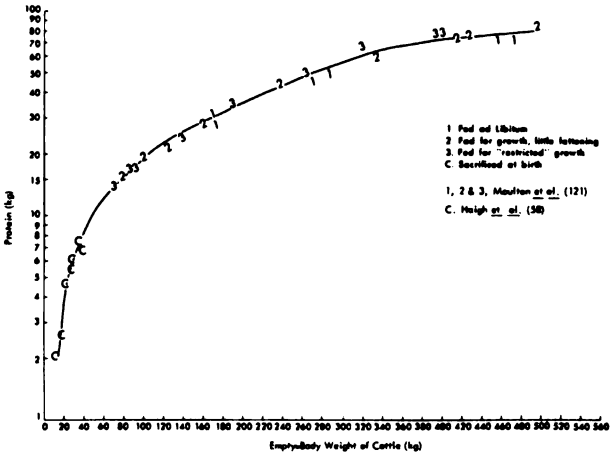


FIGURE 5 Change in protein with increase in empty-body weight of cattle.

day, whereas those in group 3 were fed for "restricted" growth and gained about 0.31 kg per day.[121] The calves (c) were from group 1 and were sacrificed at birth.[58] The proximity of each datum point to the curve established by all the data suggests that the absolute increase in protein content conformed rather closely to the average for all empty-body weights, irrespective of nutritional treatment. The shape of this curve is very similar to the curves illustrating the increase in nitrogen in cattle, sheep, swine, humans, and other mammals.[14,120] The protein–empty-body curve shown in Figure 5 for cattle increased rapidly initially, then at a progressively slower rate relative to increments in body weight up to about 160 kg. At approximately 160 kg, protein deposition again increased more rapidly and subsequently diminished.

The distribution of protein of various body components as a percentage of the total protein in the empty body of some of the cattle in the earlier Missouri investigations[121] is presented in Table 2.

Weight of empty body and percentage of protein (nitrogen x 6.25) increased with age. Within an age classification, percentage of protein was inversely associated with body weight. Similar results were obtained for swine by Wood and Groves[183] and Spray and Widdowson.[160] Total protein as a percentage of the empty-body weight declined most rapidly (7.5%) in the cattle that were fed the most liberally (group 1). In group 2 (the cattle fed to attain moderate body-weight gain), the percentage of protein declined about 1.5% during the 45-month period, whereas the percentage of protein of the cattle fed to maintain restricted growth (group 3) remained about constant.

The blood protein contributed from 5.0 to 5.5% of the total protein in the body. The variation noted in protein of the blood in Table 2 may be attributed to the difficulty in actually collecting the blood at the time of sacrifice.

The portion of protein in the internal organs was also very similar for all cattle, regardless of weight or nutritional treatment. In other words, about 7.7% of all the protein in the empty body was in the organs. The variation about this average was approximately the same within each treatment.

Cattle that were fed more liberally (group 1) had a smaller portion of their total protein in the hair plus hide and also in the skeleton. The average amount of protein in the hair plus hide for the cattle within each successive nutritional regime was: group 1, 16.3%; group 2, 17.1%; group 3, 17.4%. The range was approximately ±2% in each group.

The skeletons from the cattle fed more liberally, moderately, and for "restricted" growth contained an average of 18.3%, 20.8%, and 21.3% of the total body protein and ranged as follows: group 1, 16.3 to 23.2%; group 2, 18.6 to 25.9%; and group 3, 18.9 to 24.7%. The general trend was for the older, heavier cattle in each group to contain a slightly smaller proportion of the total protein in the skeleton.

The soft tissue, that is, all the separable skeletal muscle and fat of the cattle fed *ad libitum*, contained an average of 3% more of the total protein of empty body than the animals fed to make moderate and restricted gains. There was a tendency for the heavier cattle within each treatment to have a greater portion of their total protein in the composite separable lean and fat tissue. This tendency was similar but lower than that observed for fattening swine (compare Figure 3 with Table 2).

These data indicate that the percentage distribution of protein in various parts relative to the total in the empty body was stable; the values changed little during the 4-year period. However, on the lower levels of nutrition, the total protein present actually decreased, whereas the concentration or percentage of protein of empty-body weight increased. These results support the idea advanced by Wallace[172] and Waterlow *et al.*[174] concerning the homeostaticability of animals to maintain a high degree of constancy in essential body composition even though absolute lean and fat weight is altered nutritionally. Dickerson and Widdowson[30] observed that the proportion of the total nitrogen in humans contributed by various amino acids during growth and maturation was essentially constant. Similarly, Chinn[22] found little change in the muscle-to-nonmuscle protein ratio of rats ranging from 81 to 188 days of age.

Data were not available to determine the portion of the total protein of the empty body associated with separable fat. However, the percentages of protein in the separable fat of the round, loin, and kidney knob for the Missouri cattle on the three nutritional treatments and several ages are presented in Table 3.

These results indicate that the lower the level of nutritional intake, the higher the percentage of protein in the separable fat of the round, loin, and

TABLE 3 Effects of Age, Weight, and Nutritional Level on the Percentage of Protein in Cattle Fat[a]

Age (months)	Weight (kg)	Ration[b]	Protein in Separable Fat		
			Round (%)	Loin (%)	Kidney Knob (%)
3	98.1	1	13.8	8.8	3.7
3	78.1	2	16.7	8.7	4.4
3	71.0	3	16.0	13.7	7.7
8.5	171.5	1	8.4	6.2	1.9
8.5	121.0	2	9.0	5.4	2.8
8.5	90.0	3	14.5	9.6	5.9
21-26	475.9	1	5.6	3.3	1.3
21-26	337.8	2	9.9	5.4	2.9
21-26	265.5	3	10.0	6.9	2.9
40	786.0	1	4.7	2.2	1.2
40	428.0	2	10.4	5.3	2.1
40	332.0	3	14.0	7.7	2.8
48	814.9	1	4.2	2.4	1.2
48	493.9	2	4.8	4.1	1.1
48	407.8	3	9.1	3.8	2.6

[a] Reworked from Moulton *et al.*[120]
[b] 1 = liberal; 2 = moderate; 3 = restricted.

kidney knobs regardless of the age of the cattle. Also, within each nutritional treatment, as the cattle became more advanced in age, the percentage of protein in the fatty tissue decreased. The kidney-knob fat contained the lowest and the fat of the round the greatest percentage of protein.

CHANGES IN PROTEIN OF HUMANS

The limitations in determining the composition of human bodies are very ably discussed elsewhere.[15,115,176] It is sufficient to say, comparatively, that less is known about the composition of whole human bodies than of other animals. Even though many sophisticated research techniques are available, as recently as 1965, Widdowson stated, "They can never replace straightforward chemical analysis for arriving at the total amount of any substance within the body." Nevertheless, data published by Dickerson and Widdowson[30] are presented to show the distribution of most of the protein in the human body (Table 4).

These data indicate that the skin, skeleton, and skeletal muscle contain over 70% of the total protein in the body, whereas the skin and skeleton alone

TABLE 4 Protein of the Skin, Skeleton, and Skeletal Muscle Relative to the Total Protein in the Fat-Free Adult Human Body[a]

Constituent	Total Protein (kg)	Distribution of Total Protein (%)	Distribution of Collagen Protein (%)
Total body	11.50	–	–
Skin	1.10	9.5	8.5
Skeleton	2.07	18.1	14.8
Muscle	5.35	46.3	1.5

[a]From Widdowson and Dickerson.[177]

contain more than 27%. The collagen of the skin and skeleton accounted for about 25% of the total body protein.

If these values are representative of the human population and are further compared with results previously presented for cattle and swine, it can be generalized that the distribution of protein in the components studied is similar in the bodies of humans, cattle, swine, and possibly all mammals.

SUMMARY ANALYSIS

The protein content of animals is dynamic during growth and development, although the greatest changes occur during the prenatal period. The proteins formed during this period are believed to be precursors of adult proteins, but they have not been completely characterized. In most species there is a gradual increase in total proteins during early development, but the relative levels of the different proteins do not change proportionally and are dependent upon the state of cellular differentiation.

Growth can be divided into three phases involving DNA, RNA, and protein synthesis. Phase 1 consists of rapid DNA and RNA synthesis during rapid cell division; phase 2 involves rapid DNA, RNA, and protein synthesis concerned with cell multiplication and hypertrophy; and phase 3 entails only cellular hypertrophy and rapid protein synthesis. The synthesis of RNA generally parallels that of DNA, although the ratio of RNA and DNA may differ in the various organs and other tissues. High RNA-to-DNA ratios occur during early development.

Embryonic animals produce blood-serum proteins characteristic of individual species and different from those of mature animals. An outstanding example is calf fetuin. Characteristic types of immature hemoglobins are also present during prenatal development.

Various hormones acutely affect protein metabolism, and the activities of growth and the sex hormones are involved in protein synthesis at the microsomal level.

Collagen and muscle contractile proteins are ideal for model studies of fibrous-protein morphogenesis, and considerable knowledge has been gained concerning fibrogenesis of these proteins.

Enzyme development and activities innately respond to specific anticipated biochemical functions. Their activity levels are usually maintained as required by the normal metabolism of the biological system. This is possibly best exemplified by the kidney enzymes. In many animals the functional activity of kidney enzymes is immature at birth, but it accelerates tremendously when the animals are capable of independence.

Muscle fibers apparently grow in complete sarcomere units during embryonic development, and subsequently they grow by elongation of these units as well. Growth during the second half of intrauterine life is believed to be due to hypertrophy of fibers and not to an increase in their numbers. The turnover of muscle and related proteins is dependent upon their function.

At equivalent age levels, the relative changes in muscle proteins are constant in many animal species. The major quantitative changes occur in early life, whereas those occurring in the adult animal are concerned with an attempt to maintain anabolic and catabolic equilibrium.

The concentration of myofibrillar proteins is greater than that of the sarcoplasmic proteins during most stages of development, and apparently, hypertrophy due to exercise increases the proportion of myofibrillar proteins. Conversely, the percentage of extracellular protein nitrogen (mostly collagen) decreases during growth but may increase somewhat in the mature animal.

The percentage distribution of protein in various parts of the body relative to total empty-body protein is stable during postnatal growth. The animal body is able to maintain to high degree the essential protein composition regardless of differences in age, weight, or nutrient intake. During extreme undernutrition, the percentage of protein on an empty-body basis increases, although the absolute quantity of protein decreases. The percentage of protein of fatty tissue decreases as age advances, but it increases as nutrient intake is lowered.

Generally, muscle comprises approximately 50% of the total body protein, whereas bone contains about 20%. Blood contains around 5% of the total body protein, whereas the remainder is approximately equally divided between skin, organs, and fatty tissues.

This paper is a contribution from the Missouri Agriculture Experiment Station. Journal Series Number 5228.

REFERENCES

1. Anastassiadis, P. A., W. A. Maw, and R. H. Common. 1955. Studies on the glycoproteins of the domestic fowl. II. The hexosamine content of certain tissues of the sexually immature pullet and some effects thereon of the gonadal hormones. Can. J. Biochem. Physiol. 33:627.
2. Armsby, H. P. 1908. Feeding for meat production. U.S. Dept. Agr. Bur. Anim. Ind. Bull. 108.
3. Astbury, W. T., and T. C. Marwick. 1932. X-ray interpretation of the molecular structure of feather keratin. Nature 130:309.
4. Bailey, C. B., W. D. Kitts, and A. J. Wood. 1960. Changes in the gross chemical composition of the mouse during growth in relation to the assessment of physiological age. Can. J. Anim. Sci. 40:143.
5. Barboriak, J. J., G. Meschia, D. H. Barron, and G. R. Cowgill. 1958. Blood plasma proteins in fetal goats and sheep. Proc. Soc. Exp. Biol. Med. 98:635.
6. Barth, L. G., and L. J. Barth. 1951. The relation of adenosine triphosphate to yolk utilization in the frog's egg. J. Exp. Zool. 116:99.
7. Beckett, E. B., and G. H. Bourne. 1960. Histochemistry of developing skeletal and cardiac muscle. Vol. 1, p. 87. *In* G. H. Bourne [ed.] The structure and function of muscle. Academic Press, New York.
8. Bell, E. 1965. Molecular and cellular aspects of development. Harper & Row, New York.
9. Boedtker, H., and P. Doty. 1956. The native and denatured states of soluble collagen. J. Am. Chem. Soc. 78:4267.
10. Bollum, F. J., and V. R. Potter. 1959. Nucleic acid metabolism in regenerating rat liver. VI. Soluble enzymes which convert thymidine to thymidine phosphates and DNA. Cancer Res. 19:561.
11. Bowes, J. H., R. G. Elliott, and J. A. Moss. 1955. The composition of collagen and acid soluble collagen of bovine skin. Biochem. J. 61:143.
12. Boyd, J. D. 1960. Development of striated muscle. Vol. 1, p. 64. *In* G. H. Bourne [ed.] The structure and function of muscle. Academic Press, New York.
13. Breuer, C. D., and J. R. Florini. 1965. Amino acid incorporation into protein by cell-free systems from rat skeletal muscle. IV. Effects of animal age, androgens and anabolic agents on activity of muscles ribosomes. Biochem. 4:1544.
14. Brody, S. 1945. Bioenergetics and growth. Reinhold Publishing Corp., New York.
15. Brožek, J. 1965. Methods for the study of body composition: some recent advances and developments. Symposia of the Society for the Study of Human Biology. 7:1. *In* J. Brožek [ed.] Human body composition: approaches and applications. Pergamon Press, Oxford, England.
16. Brues, A. M., M. M. Tracy, and W. E. Conn. 1944. Nucleic acids of rat liver and hepatoma: their metabolic turnover in relation to growth. J. Biol. Chem. 155:619.
17. Carniero, J. 1965. Synthesis and turnover of collagen in periodontal tissue, 14:247. *In* C. P. Leblon, and K. B. Warren [ed.] The use of radio-autography in investigating protein synthesis. Academic Press, New York.
18. Carniero, J., and C. P. Leblond. 1959. Role of osteoblasts and odontoblasts in secreting the collagen of bone and dentin, as shown by radio-autography in mice given triton-labeled glycine. Exp. Cell Res. 18:291.

19. Carpenter, Z. L., R. G. Kauffman, R. W. Bray, E. J. Briskey, and K. G. Weckel. 1963. Factors influencing quality in pork. A histological observation. J. Food Sci. 28:467.
20. Caspersson, T., and B. Thorell. 1942. The localization of the adenylic acids in striated muscle fibers. Acta Physiol. Scand. 4:97.
21. Chantreene, H. 1961. The biosynthesis of proteins. Pergamon Press, Oxford, England, p. 57.
22. Chinn, K. S. K. 1966. Potassium and creatinine as indexes of muscle and non-muscle protein in rats. J. Nutr. 90:323.
23. Chrystall, B. B. 1967. Some chemical and physical parameters of swine during growth. M.S. Thesis, Univ. Missouri.
24. Csapo, A. 1959. Function and regulation of myometrium. Ann. N. Y. Acad. Sci. 75:790.
25. Csapo, A., and H. Herrmann. 1951. Quantitative changes in contractible proteins of chick skeletal muscle during and after embyronic development. Amer. J. Physiol. 165:701.
26. Dalton, A. J. 1937. The functional differentiation of hepatic cells of the chick embryo. Anat. Rec. 68:393.
27. Davidson, J. 1957. Activity of certain metabolic enzymes during development of the chick embryo. Growth 21:287.
28. de Villafranca, G. W. 1954. Adenosine-triphosphatase activity in developing rat muscle. J. Exp. Zool. 127:367.
29. Dickerson, J. W. T. 1960. The effect of growth on the composition of avian muscle. Biochem. J. 75:33.
30. Dickerson, J. W. T., and E. M. Widdowson. 1960. Chemical changes in skeletal muscle during development. Biochem. J. 74:247.
31. Dreyfus, J. C., J. Kruh, and G. Schapira. 1962. Muscle protein metabolism in normal and diseased states, p. 327. *In* G. F. Grass [ed.] Protein metabolism. Springer Verlag, Berlin.
32. Duesberg, J. 1960. J. Arch. Zellfosch 4:602. 1909. Cited by J. D. Boyd, 1:63. *In* G. H. Bourne [ed.] Structure and function of muscle. Academic Press, New York.
33. Dull, T. A., L. Causing, and R. H. Henneman. 1962. Urinary total hydroxyproline as an index of connective tissues turnover in bone. J. Clin. Invest. 41:1355.
34. Ebert, J. D. 1953. An analysis of the synthesis and distribution of contractible proteins myosin in the development of the heart. Proc. Nat. Acad. Sci. U. S. 39:333.
35. Ebert, J. D., R. A. Tolman, A. M. Mun, and J. F. Albright. 1955. The molecular basis of the first heart beats. Ann. N. Y. Acad. Sci. 60:968.
36. Ellis, N. R., and O. G. Hankins. 1925. Soft pork studies. I. Formation of fat in the pig on a ration moderately low in fat. J. Biol. Chem. 66:102.
37. Elson, C. E., W. A. Fuller, E. A. Kline, and L. N. Hazel. 1963. Effect of age on growth of porcine muscle. J. Anim. Sci. 22:946. (Abstr.)
38. Elson, C. E., E. A. Kline, W. A. Fuller, and L. N. Hazel. 1961. Effect of age on growth of porcine muscle. J. Anim. Sci. 20:916. (Abstr.)
39. Engel, W. K., and B. Horvath. 1960. Myofibril formation in cultured skeletal muscle cells studied with antimyosin fluorescent antibody. J. Exp. Zool. 144:209.
40. Everitt, G. C. 1964. Component analysis of meat production using biopsy techniques. Carcass composition and appraisal of meat animals. CSIRO, Australia.
41. Farrant, J. L., A. L. G. Reese, and E. H. Mercer. 1947. Structure of fibrous protein. Nature 159:535.

42. Furst, S. S., P. M. Roll, and G. W. Brown. 1950. On the renewal of the purines of the desoxypentose and pentose nucleic acids. J. Biol. Chem. 183:251.
43. Glimcher, M. J. 1959. The macromolecular aggregation state of collagen and biological specificity, p. 97. *In* I. H. Page [ed.] Calcification in connective tissue, thrombosis and atherosclerosis. Academic Press, New York.
44. Glimcher, M. J. 1961. The role of the macromolecular aggregation state and reactivity of collagen in calcification, p. 53. *In* M. V. Edds, Jr. [ed.] Macromolecular complexes. The Ronald Press Co., New York.
45. Goldspink, G. 1962. Studies on post embryonic growth and development of skeletal muscle. Proc. Roy. Irish Acad. 62b:135.
46. Gordon, E. E., K. Kowalski, and M. Fritts. 1966. Muscle proteins and DNA in rat quadriceps during growth. Am. J. Physiol. 210:1033.
47. Goss, R. J. 1964. Adaptive growth. Logos Press, London.
48. Goswami, M. N. O., and W. E. Knox. 1961. Developmental changes of p-hydroxylpyruvate-oxidase activity in mammalian liver. Biochem. Biophys. Acta 50:35.
49. Granick, S., and D. Mauzerall. 1961. The metabolism of heme and chlorophyll, 2:525. *In* D. M. Greenberg [ed.] Metabolic pathways. Academic Press, New York.
50. Grant, P. 1965. Informational molecules and embryonic development, 1:482. *In* R. Weber [ed.] The biochemistry of animal development. Academic Press, New York.
51. Gross, J. 1951. Fiber formation in trypsinogen solutions: an electron optical study. Proc. Soc. Exp. Biol. Med. 78:241.
52. Gross, J. 1956. The behavior of collagen units as a model in morphogenesis. J. Biophys. Biochem. Cytol. 2:261.
53. Gross, J. 1958. Studies on the formation of collagen. I. Properties and fractionation of neutral salt extracts of normal guinea pig connective tissue. J. Exp. Med. 107:247.
54. Gross, J. 1965. The behavior of collagen units as a model in morphogenesis, p. 416. *In* E. Bull [ed.] Molecular and cellular aspects of development. Harper & Row, New York.
55. Gross, J., J. H. Highberger, and F. O. Schmitt. 1954. Collagen structure considered as states of aggregation of a kinetic unit. The tropo-collagen particle. Proc. Nat. Acad. Sci. U.S. 40:679.
56. Gross, J., F. O. Schmitt, and J. H. Highberger. 1952. Josiah Macy, Jr., Conf. Metabol. Interrelations 4:32.
57. Gross, J., Z. Sokal, and M. Rouguie. 1956. Structural and chemical studies on the connective tissue of marine sponges. J. Histochem. Cytochem. 4:227.
58. Haigh, L. D., C. R. Moulton, and P. F. Trowbridge. 1920. Composition of the bovine at birth. Missouri Agr. Exp. Sta. Res. Bull. 38.
59. Hamoir, G. 1951. Further investigations on fish tropomyosin and fish nucleotropomyosin. Biochem. J. 50:140.
60. Hanson, J., and J. Lowy. 1963. The structure of F-actin and of actin filaments isolated from muscle. J. Mol. Biol. 6:46.
61. Hard, W. L., O. Renolds, and M. Winbury. 1944. Carbohydrates, fat and moisture relationships in the pregnant, fetal and newborn guinea pig. J. Exp. Zool. 96:189.
62. Hecht, L. I., and V. R. Potter. 1958. Nucleic acid metabolism in regenerating rat liver. Cancer Res. 18:186.
63. Heim, W. G. 1961. The serum proteins of the rat during development. J. Embryol. Exp. Morphol. 9:53.

64. Heimberg, M., and S. P. Velick. 1954. The synthesis of aldolase and phosphorylase in rabbits. J. Biol. Chem. 208:725.
65. Helander, E. 1957. On quantitative muscle protein determination. Acta Physiol. Scand. 41: Suppl., 141.
66. Helander, E. 1966. General consideration of muscle development, p. 19. *In* E. J. Briskey, R. G. Cassens, and J. C. Trautman [ed.] The physiology and biochemistry of muscle as a food. Univ. Wisconsin Press.
67. Herrmann, H. 1963. Quantitative studies of protein synthesis in some embryonic tissues, p. 85. *In* M. Locke [ed.] Cytodifferentiation and macromolecular synthesis. Academic Press, New York.
68. Herrmann, H., and J. S. Nicholas. 1948. Quantitative changes in muscle protein fractions during rat development. J. Exp. Zool. 107:165.
69. Herrmann, H., J. S. Nicholas, and M. E. Vosgian. 1949. Liberation of inorganic phosphate from adenosinetriphosphate by fractions derived from developing rat muscle. Proc. Soc. Exp. Biol. Med. 72:455.
70. Highberger, J. H., J. Gross, and F. O. Schmitt. 1950. Electron microscope observations of certain fibrous structures obtained from connective tissue extracts. J. Am. Chem. Soc. 72:3321.
71. Highberger, J. H., J. Gross, and F. O. Schmitt. 1951. The interaction of mucoprotein with soluble collagen. An electron microscope study. Proc. Nat. Acad. Sci. U.S. 37:286.
72. Hodge, A. J. 1952. A new type of periodic structure obtained by reconstitution of paramyosin from acid solutions. Proc. Nat. Acad. Sci. U.S. 38:850.
73. Hogan, A. G., L. A. Weaver, A. T. Edinger, and E. A. Trowbridge. 1925. The relation of food consumed to protein and energy retention in swine. Missouri Agr. Exp. Sta. Res. Bull. 73.
74. Holtzer, H., J. M. Marshall, and H. Finck. 1957. An analysis of myogenesis by the use of flourescent antimyosin. J. Biophys. Biochem. Cytol. 3:705.
75. Huxley, H. E. 1963. Electron microscope studies of the structure of natural and synthetic protein filaments from striated muscle. J. Mol. Biol. 7:281.
76. Ingram, V. M. 1965. The biosynthesis of macromolecules. W. A. Benjamin, Inc., New York.
77. Jackson, D. S. 1957. Connective tissue growth stimulated by carrageenin. I. The formation and removal of collagen. Biochem. J. 65:277.
78. Jameson, E., C. Alvarez-Tostado, and H. H. Sortor. 1942. Electrophometic studies on new-born calf serum. Proc. Soc. Exp. Biol. Med. 51:163.
79. Jasin, H. E., C. Fink, D. Smiley, and M. Ziff. 1962. Influence of growth on total urinary hydroxyproline. J. Clin. Invest. 41:1368.
80. Joubert, D. M. 1956. Analysis of factors influencing post-natal growth and development of muscle fiber. J. Agr. Sci. 47:59.
81. Kao, K. Y. T., D. M. Hilker, and T. H. McGavack. 1961. Connective tissue. V. Comparison of synthesis and turnover of collagen and elastin in tissues of rat at several ages. Proc. Soc. Exp. Biol. Med. 106:335.
82. Kassenaar, A. A. H., A. Querido, and A. Haak. 1962. Effects of anabolic steroids on nucleic acid and protein metabolism, p. 222. *In* F. Gross [ed.] Protein metabolism. Springer Verlag, Berlin.
83. Kay, C. M., and K. Bailey. 1960. Light scattering in solutions of native and guanidinated rabbit tropomyosin. Biochem. Biophys. Acta 40:149.

84. Kemme, J. 1962. PhD Dissertation. Univ. Göttingen. [Cited by H. J. Oslage. The distribution of nitrogen protein and fat in the body of growing swine. Zuchtungskunde 37:338. 1965.]
85. Kennedy, G. C., W. M. Pearce, and D. M. V. Parriot. 1958. Liver growth in the lactating rat. J. Endocrinol. 17:158.
86. Kenney, F. T., and N. Kretchmer. 1959. Hepatic metabolism of phenylalanine during development. J. Clin. Invest. 38:2189.
87. Klein, R. L. 1961. A possible correlation between ATPase activity and Na content of embryonic chick heart. Am. J. Physiol. 201:858.
88. Kochakian, C. D. 1950. The mechanism of the protein anabolic action of testosterone propionate, p. 113. *In* E. S. Gordon [ed.] Symposium on steroid hormones. Univ. Wisconsin Press, Madison.
89. Kochakian, C. D. 1964. Protein anabolic properties of androgens. Alabama J. Med. Sci. 1:24.
90. Kochakian, C. D. 1966. Regulation of muscle growth by androgens, p. 81. *In* E. J. Briskey, R. G. Cassens, and J. C. Trautman [ed.] The physiology and biochemistry of muscle as a food. Univ. Wisconsin Press, Madison.
91. Kochakian, C. D., J. H. Humm, and M. N. Bartlett. 1948. Effect of steroids on the body weight, temporal muscle and organs of the guinea pig. Am. J. Physiol. 155:242.
92. Kochakian, C. D., C. Tillotson, and G. L. Endahl. 1956. Castration and the growth of muscles in the rat. Endocrinology 58:225.
93. Korner, A. 1962. The effect of growth hormone on protein synthesis, p. 8. *In* F. Gross [ed.] Protein metabolism. Springer Verlag, Berlin.
94. Korner, A., and F. G. Young. 1955. The influence of methyl androsterediol on the body weight and carcass composition of the rat. J. Endocrinol. 13:78.
95. Kornfeld, R., and D. H. Brown. 1963. The activity of some enzymes of glycogen metabolism in fetal and neonatal guinea pig liver. J. Biol. Chem. 238:1604.
96. Kretchmer, N. 1959. Enzymatic patterns during development. Pediatrics 23:606.
97. Kruh, J., J. C. Dreyfus, G. Schapira, and P. Padieu. 1957. Nonuniform incorporation of glycine-2-C^{14} into rabbit hemoglobin *in vivo* and *in vitro*. J. Biol. Chem. 228:113.
98. Lawes, J. B., and J. H. Gilbert. 1861. On the composition of oxen, sheep, and pigs and of their increase whilst fattening. J. Roy. Agr. Soc. Eng. 21:1.
99. Linke, K. W. 1955. Elektronenmkroskopische untersuchung uber die differenziekung de interzellulansubstanz der menschlichen lederhaut. Z. Zellforsch. Mikroskop. Anat. 42:331.
100. Lowey, A., and C. Cohen. 1962. Studies on the structure of myosin. J. Mol. Biol. 4:293.
101. Lowtance, P., and A. Chanutin. 1942. The effect of partial hepatectomy on blood volume in the white rat. Am. J. Physiol. 135:606.
102. McIntosh, E. N., D. C. Acker, and E. A. Kline. 1961. Influence of orally administered stilbestrol on connective tissue of skeletal muscle of lambs fed varying levels of protein. J. Agr. Food Chem. 9:418.
103. McLean, J. R., G. L. Cohn, I. K. Brandt, and M. U. Simpson. 1958. Incorporation of labeled amino acids into the protein of muscle and liver mitochondria. J. Biol. Chem. 233:657.
104. McLean, J. R., G. L. Cohn, and M. U. Simpson. 1961. Amino acid incorporation into isolated mitochondria. Federation Proc. 15:312.

105. McMeekan, C. P. 1941. Growth and development in the pig, with special reference to carcass quality characters. II. The influence of the plane of nutrition on growth and development. J. Agr. Sci. 30:387.
106. Manners, M. J., and M. R. McCrea. 1963. Changes in the chemical composition of sow reared piglets during first month of life. Brit. J. Nutr. 17:495.
107. Marks, M. H., R. S. Bear, and C. H. Blake. 1949. X-ray diffraction evidence of collagen-type protein fibers in the *echinolermata, collenterata* and *parifera*. J. Exp. Zool. 111:55.
108. Mercer, E. H. 1951. Formation of silk fibre by the silkworm. Nature 168:792.
109. Mercer, E. H. 1958. Electron microscopy and the biosynthesis of fibres, p. 113. *In* W. Montagna, and R. A. Ellis [ed.] The biology of hair growth. Academic Press, Inc., New York.
110. Mihalyi, E., E. Laki, and M. I. Knoller. 1957. Nucleic acid and nucleotide content of myosin preparations. Arch. Biochem. Biophys. 68:130.
111. Miller, E. R., D. E. Ullrey, I. Ackerman, D. A. Schmidt, J. A. Hoefer, and R. W. Luecke. 1961. Swine hematology from birth to maturity. I. Serum proteins. J. Anim. Sci. 20:31.
112. Mitchell, H. H., and T. S. Hamilton. 1929. Swine type studies. III. The energy and protein requirements of growing swine and the utilization of feed energy in growth. Illinois Agr. Exp. Sta. Res. Bull. 323.
113. Moog, F. 1965. Enzyme development in relation to functional differentiation, 1:307. *In* R. Weber [ed.] The biochemistry of animal development. Academic Press, New York.
114. Moore, D. H., R. M. DuPan, and C. L. Buxton. 1949. An electrophoretic study of maternal fetal and infant sera. Am. J. Obstet. Gynecol. 57:312.
115. Moore, F. D., K. D. Olsen, J. D. McMurrey, H. V. Parker, M. B. Ball, and C. M. Bayden. 1963. The body cell mass and its supporting environment. Saunders, Philadelphia and London.
116. Moore, D. H., S. C. Shen, and C. S. Alexander. 1945. The plasma of developing chick and pig embryos. Proc. Soc. Exp. Biol. Med. 58:307.
117. Morpurgo, B. 1897. Uber die postembryonal enwickeling der overgestreifter von wessen ratten. Ana. Anz. Bd. 15:200. [Cited by H. H. Donaldson. The rat. 1924.]
118. Moscona, A. 1955. Cytoplasmic granules in myogenic cells. Exp. Cell Res. 9:377.
119. Moser, C. R., and R. A. Flickinger. 1965. Hemin synthesis in developing frog embryo and its stimulation by frog liver RNA. Develop. Biol. 12:117.
120. Moulton, C. R. 1923. Age and chemical development in mammals. J. Biol. Chem. 57:79.
121. Moulton, C. R., P. F. Trowbridge, and L. D. Haigh. 1922. Studies in animal nutrition. III. Changes in chemical composition on different planes of nutrition. Missouri Agr. Exp. Sta. Res. Bull. 55.
122. Murray, J. A. 1919. Meat production. J. Agr. Sci. 9:174.
123. Murray, J. A. 1922. The chemical composition of animal bodies. J. Agr. Sci. 12:103.
124. Nass, M. K. K. 1962. Developmental changes in frog actomyosin characteristics. Develop. Biol. 4:289.
125. Needham, A. E. 1964. The growth process of animals. D. Van Nostrand Co., Inc., New York.
126. Nelson, O. E. 1953. Comparative embryology of the vertebrates. The Blakiston Co., Inc., New York.

127. Neuberger, A. 1953. The metabolism of collagen from liver, bone, skin and tendon in the normal rat. Biochem. J. 53:47.
128. Oberman, J. W., K. O. Gregory, F. G. Burke, S. Ross, and E. C. Rice. 1956. Electrophoretic analysis of serum proteins in infants and children. I. Normal values from birth to adolescence. New Engl. J. Med. 255:743.
129. Ogawa, Y. 1962. Biochemical relation between actin and myosin synthesis during the differentiation of skeletal muscle tissues of *triturus*. Exp. Cell Res. 26:526.
130. Orekovitch, V. H., and V. O. Shpikiter. 1958. Procollagens. Science 127:1371.
131. Oslage, H. J. 1965. The distribution of nitrogen, protein, and fat in the body of growing swine. Zuchtungskunde 37:338.
132. Otto, E. 1966. Der Fleisch-und fettansatz von schweinen verschiedenen gewichtes. Die Fleisch Wirtschaft 46:1242.
133. Papanicolaou, G. V., and E. A. Falk. 1938. General muscular hypertrophy induced by androgenic hormone. Science 87:238.
134. Pauling, L. 1953. Aggregation of globular proteins. Discussions Faraday Soc. 13:170.
135. Pedersen, K. O. 1945. Fetuin, a new globulin isolated from serum. Nature 154:575.
136. Penhos, J. C., and M. E. Krahl. 1962. Insulin stimulus of leucine incorporation in rat liver protein. Am. J. Physiol. 202:349.
137. Perry, S. V., and M. A. Zydowo. 1959. A ribonucleoprotein of skeletal muscle and its relation to the myofibril. Biochem. J. 72:682.
138. Pfau, P. 1954. Die serumproteine von feten, neugeborenen und ubertrogenen saulingen. Arch. Gynakol. 185:208.
139. Philpott, D. E., and A. G. Szent-Gyorgyi. 1954. The structure of light-meromyosin: an electron microscopic study. Biochem. Biophys. Acta 15:165.
140. Picken, L. 1960. The organization of cells and other organisms. The Clarendon Press, Oxford, England.
141. Reid, J. T., G. H. Wellington, and H. O. Dunn. 1955. Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. J. Dairy Sci. 38:1344.
142. Rice, R. V. 1964. Electron microscopy of macromolecules from myosin solutions, p. 41. *In* J. Gergely [ed.] Biochemistry of muscle contraction. Little, Brown and Company, Boston.
143. Robinson, D. S. 1952. Changes in the protein composition of chick muscle during development. Biochem. J. 52:621.
144. Robinson, D. S. 1952. Changes in the nucleoprotein content of chick muscle during development. Biochem. J. 52:628.
145. Roodyn, D. B., P. J. Reis, and T. S. Work. 1961. Protein synthesis in mitochondria. Requirements for the incorporation of radioactive amino acids into mitochondrial proteins. Biochem. J. 80:9.
146. Rosenthal, J., and F. Doljanski. 1961. Biochemical growth patterns of normal and radio thyroidectomized rats. Growth 25:347.
147. Schapira, G., J. Kruh, J. C. Dreyfus, and F. Schapira. 1960. The molecular turnover of muscle aldolase. J. Biol. Chem. 235:1738.
148. Schjeide, O. A., and N. Ragan. 1957. Studies on the New Hampshire chicken embryo. VIII. Glycoprotein of serum. J. Biol. Chem. 227:1035.
149. Schmitt, K. O. 1956. Macromolecular interaction pattern in biological systems. Proc. Am. Phil. Soc. 100:476.

150. Schmitt, K. O., J. Gross, and J. H. Highberger. 1953. A new particle type in certain connective tissue extracts. Proc. Nat. Acad. Sci. U.S. 39:459.
151. Scow, R. O. 1952. Effect of testosterone on muscle and other tissue and on carcass composition in hypophysectomized thyroidectomized and gonadectomized male rats. Endocrinology 51:42.
152. Scow, R. O., and S. N. Hagan. 1955. Effect of testosterone propionate on myosin, collagen and other protein fractions of striated muscles of gonadectomized male guinea pig. Am. J. Physiol. 180:31.
153. Scow, R. O., and S. N. Hagan. 1957. Effect of testosterone propionate on myosin, collagen and other protein fractions in striated muscle of gonadectomized rats. Endocrinology 60:273.
154. Shemin, D., and D. Rittenberg. 1944. Some interrelationships in general nitrogen metabolism. J. Biol. Chem. 153:401.
155. Sheng, P. K., and T. C. Tsao. 1955. A comparative study of nucleotropomyosin from different sources. Sci. Sinica. (Peking) 4:157.
156. Simpson, M. U., and S. P. Velick. 1954. The synthesis of aldolase and glyceraldehyde 3-phosphate dehydrogenase in the rabbit. J. Biol. Chem. 208:61.
157. Smellie, R. M. S. 1955. The metabolism of nucleic acids, 2:9. *In* E. Chargaff and J. N. Davidson [ed.] The nucleic acids. Academic Press, New York.
158. Solomon, J. B. 1959. Changes in the distribution of glutamic, lactic and malic dehydrogenases in liver cell fractions during development of the chick embryo. Develop. Biol. 1:182.
159. Solomon, J. B. 1965. Development of monezymatic proteins in relation to functional differentiation, 1:367. *In* R. Weber [ed.] The biochemistry of animal development. Academic Press, New York.
160. Spray, C. M., and E. M. Widdowson. 1950. The effects of growth and development on composition of mammals. Brit. J. Nutr. 4:332.
161. Staun, H. 1963. Various factors affecting number and size of muscle fibers in the pig. Acta. Agr. Scand. 13:292.
162. Swanson, C. O. 1921. Effect of rations on the development of pigs. J. Agr. Res. 21:279.
163. Szent-Gyorgyi, A. 1947. Chemistry of muscular contraction. Academic Press, New York.
164. Szirmai, J. A. 1962. Histological aspects of the action of androgens and oestrogens, p. 45. *In* F. Gross [ed.] Protein metabolism. Springer Verlag, Berlin.
165. Tonna, E. A. 1956. Protein synthesis and cells of the skeletal system. *In* The use of radioautography in investigating protein synthesis. Int. Soc. Cell Biol. Symp. 4:247.
166. Truman, D. E. S., and A. Korner. 1962. Incorporation of amino acids into the protein of isolated mitochondria. Biochem. J. 83:588.
167. Tsao, T. C., and K. Bailey. 1953. Aspects of polymerization in proteins of the muscle fibril. Discussions Farady Soc. 13:145.
168. Tyler, A. 1963. The manipulations of macromolecular substances during fertilization and early development of animal eggs. Am. Zool. 3:109.
169. Velick, S. P. 1956. The metabolism of myosin, the micromyosins, actin and tropomyosin in the rabbit. Biochem. Biophys. Acta 20:228.
170. Verzar, F. 1964. Aging of the collagen fiber, 2:243. *In* D. A. Hall [ed.] International review of connective tissue research. Academic Press, New York.
171. Von Bezold, A. 1857. Untersuchungen uber die vertheiling wasser, organischer materia und anorganischen verbindungen im thierreiche. Z. Wiss. Zool. 8:487.

172. Wallace, W. M. 1959. Nitrogen content of the body and its relation to retention and loss of nitrogen. Federation Proc. 18:1125.
173. Washburn, R. M., and C. H. Jones. 1916. Studies of the value of different grades of milk in infant feeding. Vermont Agr. Exp. Sta. Bull. 195.
174. Waterlow, J. C., J. Cravioto, and J. M. L. Stephen. 1960. Protein malnutrition in man, 15:139. *In* C. B. Anfinsen, Jr., M. L. Anson, K. Bailey, and J. T. Edsall [ed.] Advances in protein chemistry. Academic Press, New York.
175. Weller, E. M., and A. T. Schechtman. 1962. Ontogeny of serum proteins in the chicken. 1. Paper electrophoretic studies. Develop. Biol. 4:517.
176. Widdowson, E. M. 1965. Chemical Analyses of the body, p. 31. *In* J. Brožek [ed.] Human body composition: approaches and applications. Pergamon Press, Oxford, England.
177. Widdowson, E. M., and J. W. T. Dickerson. 1960. The effect of growth and function on the chemical composition of soft tissues. Biochem. J. 77:30.
178. Wilson, J. D. 1962. Regulation of protein synthesis by androgens and estrogens, p. 26. *In* F. Gross [ed.] Protein metabolism. Springer Verlag, Berlin.
179. Wilson, J. W., and E. H. Leduc. 1948. The occurrence and formation of binucleate and multinucleate cells and polyploid nuclei in the mouse liver. Am. J. Anat. 82:353.
180. Wilt, F. H., and T. Hultin. 1962. Stimulation of phenylalanine incorporation by polyuridylic acid in homogenales of sea urchin eggs. Biochem. Biophys. Res. Comm. 9:313.
181. Winick, M., and A. Nobel. 1965. Quantitative changes in DNA, RNA, and protein during prenatal and postnatal growth in the rat. Develop. Biol. 12:451.
182. Winnick, R. E., and T. Winnick. 1960. Protein synthesis in skeletal muscles with emphasis on myofibrils. J. Biol. Chem. 235:2657.
183. Wood, A. J., and T. D. D. Groves. 1965. Body composition studies on the suckling pig. I. Moisture, chemical fat, protein and ash in relation to age and body weight. Can. J. Anim. Sci. 45:8.
184. Wool, I. G. 1963. Effect of insulin on nucleic acid synthesis in isolated rat diaphragm. Biochem. Biophys. Acta. 68:28.
185. Young, D. M., S. Himmelfarb, and W. F. Harrington. 1965. The structure assembly of the polypeptide chains of heavy meromyosin. J. Biol. Chem. 240:2428.
186. Zobel, C. R., and F. D. Carlson. 1963. An electronic microscopic investigation of myosin and some of its aggregates. J. Mol. Biol. 7:78.
187. Zobrisky, S. E. 1957. Physical composition of swine during growth and fattening. PhD Dissertation. Univ. Missouri.

J. T. Reid, Presiding

GENERAL DISCUSSION

M. A. FLYNN In the cells of the newborn there is a large amount of potassium. Can we further say, on the basis of amount of potassium per liter of intracellular water, that the quantity of potassium would be greater than the amount that we usually accept, 150 mEq per liter of intracellular water?

E. M. WIDDOWSON This is a very good question, and as far as I know there is no answer to it because, of course, we have no direct way of getting at the composition of intracellular water. We have to get at it indirectly, and I think that what you say may well be true. If you take the cell as a whole, I think this is perfectly true, and there may well be a difference in the composition of the intracellular water between the fetus and the adult. But, of course, I think we must emphasize that if we are thinking of the tissue as a whole or of the body as a whole, quantitatively, the changing proportions of cells to extracellular fluid is much greater than any change within the cell. Perhaps I did not make that quite clear, but that is the big change, and these other changes are more minor ones.

B. A. PANARETTO Dr. Widdowson, I rise to make a comment rather than to ask a question. I think you said during your talk that you saw a breakdown

of cells in the release of intracellular potassium only in swine that had been starved almost to the point of death. We have been thinking along similar lines in Australia for the last 2 to 3 years, and we have been able to produce this effect very nicely by stressing undernourished animals. We have used cold as the stress, although we could have used other stressing agents. The premortal picture is one of cellular breakdown: the release of cellular contents into the circulation and into the urine. This occurs quickly although some animals do recover, so death is not invariable. Also, the adrenal cortex seems to be involved because one can prolong, or at least ameliorate, the effects of the stress by using adrenal-cortico steroids prior to applying the stress. The last point I want to make is that histologically, the adrenal glands are quite abnormal.

M. MARESH I would like to ask Dr. Forbes if he has any way of speculating about when, during infancy or childhood, the potassium concentration changes from the newborn level to the figure that we use in the later childhood years, that is, the adult figure.

G. B. FORBES This is the difficult question. The point is that there are no data on composition of children obtained by proper analysis. We have good data on the fetus and the newborn, and a few data on adults, not as much as we should have, but very, very little, if any, data on people of intermediate age ranges. So the matter is that of speculating about the concentration of material in the fat-free body. We know it is different in the infant than it is in the adult; the question is when is the change complete. All I can say is that I would like to speculate that the change is most rapid in very early life. I do this on the basis of my double logarithmic plots, which, if they are correct, indicate that in the first year of life a larger degree of change should occur than in later years. I think, however, that the answer is that we really do not know. In our own ^{40}K work, we assume, without any valid reason for doing so, that the concentration of potassium in the fat-free body in the 7-year-old is about the same as it is in the adult. Now, if it is a little different in the adult, it would not hurt us very much, but this is all we have been able to do.

R. M. MALINA I would like to direct my question to Dr. Forbes. I was very much interested in your concept of the changing composition of the skeleton relative to skeletal maturation. Have you compared any of your data, in terms of maturation of the skeleton, with perhaps the hand-wrist atlases, for example, the Tanner-Whitehouse British standards?

G. B. FORBES I am not sure what that would tell us. The data I presented were obtained by others and represent a very extensive and laborious dissection of the skeleton, and I merely presented the relative weight of the cartilage

versus the noncartilage components of the skeleton. Now, whether this could be produced by x-ray or not, I am not so sure. One would have to have a way of estimating the volume of the skeleton from x-rays and also of estimating the volume of the cartilage, and I am not sure this can be done as accurately as one would want to do it. Did I understand your question correctly? Obviously, an x-ray of the wrist of the newborn baby shows there is very little calcium, but an x-ray of the wrist of the adult shows that there is a lot of calcium there, so there has been a change, but I do not know how well it can be quantitated. Perhaps Dr. Maresh has a better answer than I do.

J. T. REID Dr. Maresh, would you like to comment on that?

M. MARESH I think there are all kinds of good theories on the application of x-rays to such problems. However, I do not think there is an easy solution to this particular problem.

G. LESSER I would like to expand slightly on Dr. Forbes' comments on potassium changes with aging. We have no information on growth, because we have studied only adults in aging. Dr. Forbes made a comment that most of the fall in potassium per body weight reflected increased fattening. I am sure that this is true for the largest part, but I think there is appreciable evidence by Maresh to show that there is probably an absolute decrease in potassium relative to the fat-free body as well, which accentuates this change with adults. For example, there was work done about 10 years ago in which it was found that potassium in young and old adult populations showed a great deal of change even when potassium was expressed per unit of body height, which would not be expected to change too much with aging. In addition to this, our indirect fat studies on total populations represented at least good estimates for the fat-free body in these people. If we take those we have in a population that sticks very much to American standards, just by plotting the data for the fat-free body of the large groups that Anderson and Langham and others have studied and their body potassiums corrected for body fat, it appears very definitely that potassium per unit of fat-free body tends to fall. I think it would be odd to observe also that total potassium per liter of body water fell. Body water seems to be a constant portion of the lean (or the fat-free body) in aging also. I think that by now, while there is not absolute evidence, it does appear that in senescence there is a fall in the total potassium and probably also in the cell content. I wonder if you would comment briefly.

G. B. FORBES I do not think I should now, Dr. Lesser. The most important work that has been done in this field has been done by you, and I think your comments are most worthwhile. Certainly you have shown that the ratio

of extracellular water to total body water does, as you determine it, increase slightly with senescence, and one would predict, therefore, that potassium would reflect this as well.

A. R. BEHNKE I have been admiring Dr. Forbes' footwork here, but I do not want Dr. Bailey to go home and tell his wife that no one asked him a question. My question is this: In athletes it is possible to increase the muscle mass by as much as 40% by isometric contractions. It is possible to put on 30, 40, or even 50 pounds of muscle mass. It should be possible also in cattle to induce this marked increase in muscle mass. What is the nature of this hypertrophy?

M. E. BAILEY This is not a new question, of course, and all of us are familiar with the phenomenon. Some of the older literature would indicate that hypertrophy due to increased activity is due to increase in sarcoplasmic protein. This does not seem reasonable to me. Helander, a Swedish worker, recently published a review including his own data which involved many years of work on this problem. According to this worker, muscle hypertrophy resulting from exercise is due to an increase in quantity of myofibrillar protein. This increase could occur in several ways, and I would guess that hypertrophy in this particular instance is due to an increase in the number of myofilaments of the muscle fibers and not to increases in their individual sizes.

G. C. PITTS I would like to make two comments, if I may. While admiring the data that Dr. Widdowson reported, I was a little bit disappointed to find that her fractions of water in the fat-free body of various species did not fall in line with the relationship that I just reported, namely, that the muscle water was inversely related to the size of the animal's body. I would have said that the values on cattle were too high, and that the values on rats were too low. These comments may or may not apply to her data, but as a precaution, I would like to point out that when I first began to search the literature for comparative data, I found that I had to be very careful. First of all, you have to control your age group very carefully. This has been made clear in the reports of several people. Second, when an author says he has determined the total water of a carcass, he might mean, as I did, that he has corrected the carcass for the weight of fur and gut contents. Or, he might have ground up the fur and gut contents with everything else, which makes a big difference; or, when he says the total water of the body, he might be talking about the eviscerated carcass. In some cases he might be talking about the eviscerated skinned carcass. All of these things occur, and we have to be very careful that we are comparing similar situations here. Now, in my own report, I presented values of 70 to 71% on cattle and up to 78 to 79% on mice and shrews, and I think I was able to demonstrate a regression line that has some

significance, only because the whole series was done by a careful, standardized technique.

May I make one more comment? Dr. Widdowson tended to emphasize variations in the various fractions or in the fat-free body, whereas I emphasized the constancy in these fractions. I think that this stems from the fact that she is extrapolating from work on human beings, and I, on small mammals. If you look at a great deal of the data derived by direct determination of a variety of mammals, you see work on cattle, guinea pigs, rats, and other animals, and in almost every case, the standard deviation is plus or minus above 1 percent of the mean. This includes methodological error. I believe biological error will have a standard deviation smaller than this. Now this caused me some concern, as it did Dr. Widdowson, when I thought of human beings who pick up a kilogram of weight one day and a pound the next day. I would point out that if a fellow goes out drinking beer with the boys, he might come home weighing 2 kilograms more than when he left. But by the time you get him on the scales, he has already had half or more of this ingested volume in his blood, and this contributes to the gross weight of the human being. But, with my small mammals and direct determinations, I correct for water in the bladder and gut content and for the weight of the hair and what-all. Also, I would persist in feeling that the fraction of water in the fat-free body is regulated with the precision that compares well with the regulation of, say, pH in plasma, or pCO_2 in arterial blood.

S. L. HANSARD We say a lot about the effect of different treatments on animals as they increase in weight. I wonder if Dr. Bailey has made any attempt, for instance, on the massive data that he reported to separate the effects of age and weight, that is, the effects or the changes with age if we maintain a constant weight. Now, this is not too important in mature animals, but when we speak of weight changes with age from the fetus to mature animals, this is a big factor—one I think we are going to have to consider.

M. E. BAILEY This would involve a somewhat different analysis from that discussed. However, the data studied indicate that for animals of the same age in which nutrient intake was restricted, the protein gain relative to body weight was similar or somewhat greater than for animals fed higher levels. When animals of approximately equal weights that differ considerably in age are compared under the conditions of restricted and *ad libitum* feeding, the restricted-fed animals contained a greater proportion of protein to empty-body weight.

G. M. WARD I was interested in Dr. Forbes' postulate (if I got it correctly) that growth may be limited by the maximum of the extracellular water mass. If this is a possibility, then as senescence occurs in animals, shouldn't the extracellular volume be decreasing rather than increasing?

G. B. FORBES I offer this as pure speculation, Dr. Ward, but I think there is an explanation from the relative increase in extracellular water. The fact that connective tissue may form a larger component of the muscle mass in the aged than in the young and that connective tissue has a higher extracellular fluid component relative to its total water than does muscle *per se* indicates that if one had a change with aging in the relative contents of connective tissue and muscle fibers *per se*, one would expect the ECF to increase relatively. I cannot speculate any more than that.

A. M. PEARSON We have been interested in the variation that occurs from muscle to muscle within the same animal. In looking at it both from the standpoint of potassium and from the standpoint of water, we find that there is variation from muscle to muscle on the fat-free basis. We have assumed that one of the reasons for this, as Dr. Forbes mentioned, is the different type of protein that is present. We have not looked at this yet, but perhaps we are thinking that in the case of some muscles we may have a high connective-tissue content and in others, a high content of contractile proteins, and that these may account for the variations we find in potassium, for example. We were wondering if anyone had any similar experience?

E. M. WIDDOWSON I have not had any experience myself, but Dr. Flear in England has made measurements of chloride in different muscles.

A. M. PEARSON Chloride or potassium?

E. M. WIDDOWSON Yes, he did potassium as well, but he has seen the same thing, big variations from one muscle to another.

K. HANSEN I would like to ask Dr. Bailey the converse of the question asked by Dr. Behnke: What happens to protein in muscles during atrophy, particularly during denervation of either lower motor neurons or upper motor neurons?

M. E. BAILEY I'm sorry I have no direct answer for that. We assume that in the case of hypertrophy there is an increase in myofibrillar proteins, which is reciprocally related to your question. In a diminution in size of a muscle, you might possibly find a decreased content of this particular type of protein and perhaps slightly more sarcoplasm and fat associated with the muscle cell than are normally found. There are numerous disease syndromes associated with loss of muscle mass during disuse atrophy. Much recent work in this regard has been done on the hydrolytic enzymes, particularly cathepsin localized in the so-called lyzosomes of muscle and other cells. Work at California under the direction of Dr. Tappel in regard to protein catabolism in muscular dystrophy shows that there is actually a decrease in contractile protein, and that there are increases in peptides and free amino acids.

E. C. ANDERSON In view of Dr. Behnke's remarks about the spectacular effect of isometric exercises on athletes, I would like to ask the animal husbandry people in general if they have tried improving the diameter of sirloin steaks by isometric exercising of a cow.

R. T. PRESTON Can you tell me how to teach these animals to do isometric contractions? I think there is someone, maybe Dr. Garrett would comment on this, who did some work with swine standing on their hind legs while feeding.

W. N. GARRETT I am looking around for Dr. Heitman, who actually did the work. I saw him earlier today but I do not see him volunteering. I really do not have any detailed comments to make on this except that perhaps they were able to change the muscle of the ham somewhat by having the animal stand on its hind legs to eat, and in some experiments they also ran a treadmill at the same time. There were complicating factors, I believe, in that the animal in this uncomfortable position would not eat as much. So then, the complicating factor of slower growth was confounded with the difference in muscle size. If Dr. Heitman comes in later, he can comment more fully on this experiment.

A. R. BEHNKE I think I can add something to that. Put straps on the animals' hind legs, have the animals pull against a spring, and stimulate them by mild electrical stimulation applied to the caudal extremity or area. Then, the animals will pull against resistance. All that is necessary to cause muscle to hypertrophy is resistance to exercise. This would not be practical were it not for the recent finding that either a period of 10 minutes or intermittent periods lasting 2 or 3 minutes several times a day during which the resistance exercise approaches the maximal will produce sufficient muscle fatigue to cause the muscular hypertrophy that I spoke of.

Now I want to make one remark about the hibernating of quiescent muscle. It is quite amazing that no one has studied either muscular hypertrophy or the hibernating muscles of an animal before and after hibernation. Even people like my good friend Manuel Moralis, who is an authority in the field, cannot give me an answer. I did see some work at one time to show that the amount of adenosine triphosphate in the muscles of the hibernating animal was about one half that in the muscles of the active animals.

H. H. STONAKER I would like to direct two questions to Dr. Pitts. First, has the muscle–bone ratio, independent of fat deposition (or on the fat constant basis), been observed through this range of species as a function of the body size? Second, has the body composition of these various species been determined under quite different climatological conditions, such as tropical

versus arctic? Have these kinds of association been drawn from your data as yet?

G. C. PITTS With respect to the first question, I must admit that these data are quite old, but I am still in the process of working them out, and I do not have the answer to your question on muscle–bone ratio.

The other question: yes, I have looked into this, and I could find only one particularly significant difference between arctic- and tropic-zone mammals. That was a significantly greater percentage of the total fat present in the subcutaneous depot of arctic mammals than in temperate-zone mammals. One can reason from this and say, "Well, if it (fat) can serve as an insulator, it will do an arctic mammal the most good," and this is the only significant difference between most of the groups that I have been able to demonstrate so far. If it is possible to sort out some of those other interfering variables, I hope to find other things.

QUESTIONER I understand that a hippopotamus is a very lean animal. Is this correct?

G. C. PITTS I don't know one. I would love to work with a hippopotamus, if I could find a patient technician who would dissect him for me.

J. T. REID I think we might have one more question. Dr. Martin.

T. G. MARTIN I would like to ask Dr. Widdowson if analyses of whole-body potassium and sodium content would be likely to give us any concrete leads concerning the relative volumes of intracellular and intercellular fluids?

E. M. WIDDOWSON I think whole-body potassium would certainly give us a good measure of intracellular fluid, but not of sodium. Of course, as Dr. Forbes said, a considerable amount of sodium in the body is in the skeleton. Only a fraction of the body sodium is associated with the extracellular fluid. Chloride would be a much better bet than sodium.

STATISTICS

H. W. Norton
University of Illinois

OPPORTUNITIES AND PITFALLS IN THE MATHEMATICS OF BODY-COMPOSITION STUDIES

It is appropriate to commence by delimiting the topic. The literature indicates plainly that most of the mathematics used in the study of body composition is statistics, and this appears likely to continue for some time.

Statistics is only making sense out of data, and I have not detected opportunities that seem more specific. This brings me to pitfalls and how to recognize them. A description of statistical mistakes could be based on general experience, but then it might happen that none of you would hear anything of interest. Hence "pitfall" will mean here an error into which someone has fallen in body-composition studies. My source is *Body Composition*, the report of the 1963 conference.[1] Little will be said about specific measurement techniques or about specific problems of body-composition research, such as compartment analysis. Attention will be focused mainly on problems of statistical analysis of body-composition data, with interpretation, and with actual errors.

In dealing with errors, there will be no effort to disguise sources: surely we are all concerned chiefly with the advance of science and the exposure of error. I make more than my share of errors, and still manage with an effort to welcome correction. The prevalence of statistical errors in the report of

the 1963 conference leads me to think that most of you will be struck at least a glancing blow: I hope you will feel benefited.

There will be little or nothing new in my remarks. In particular, many of these matters were dealt with by Harrington in his contribution to the 1963 conference, though it is possible to disagree with him on some points.

To begin, let us dispose of some myths. A simple one, which is fairly common, is the notion that the scale unit, or least count, of a measuring device limits the accuracy of a mean value of a sample of measurements and that the mean should not be reported beyond the units of measurement. If tenths of a scale division are estimated, it may be supposed that the mean is worth reporting to tenths. In fact, the scale unit is ordinarily of little importance to the accuracy of the mean. A rule for rounding a mean value, or any other statistic, should be such that the error introduced by rounding is itself of little importance. An effect of rounding is to increase the variance. Just as a mean value can be improved by increasing the sample size, an increase in variance can be regarded as a reduction in sample size, that is, as discarding a portion of the data. If we evaluate the standard error of the mean (or other statistic), divide it by three, and take the position of the first significant digit to be the position of the last digit to be retained in the mean, we have a rule that conserves at least 99% of the data. An old British sample of 8,585 heights of men to the nearest inch yields a mean of 67.4583503 in. with a standard error of 0.0277638 in. One third of the standard error is 0.0093, so that we must retain the third decimal in rounding the mean if we are to conserve at least 99% of the data, though obviously two decimals will almost satisfy the rule. Hence, we report a mean of 67.458 ± 0.02776 in., the same rule being applied to the standard error.

Now consider what has been lost by measurement to the nearest whole inch. The sample variance is increased by what can be called the recording variance, in this case the variance of errors about equally distributed from -½ to +½ in., having a variance of 0.0833. Hence, the variance, 6.62 in.^{2}, was inflated by 0.0833, or about 1.28%, and more precise measurement would have led to an equally accurate mean height from a sample of only 8,477. This illustrates the rounding rule again. The standard deviation was 2.572 in., so height should have been measured to tenths of inches, according to the rule, to conserve at least 99% of the information in each datum; but measurement to the nearest whole inch nearly satisfies the rule.

Does it make sense to report the mean height to the third decimal in inches? Probably not, even for such a large sample. Remember that the discussion has concerned a purely statistical consideration, the precision of measurement. Accuracy is another matter. The obvious importance of posture, and the known effect of time of day (or perhaps time since last

sleep) are big enough to destroy our interest in the third decimal, and probably in the second, unless the procedure is very carefully standardized and described. In fact, it may mean little to report an average value beyond the precision of single measurements, but if so, it is because the accuracy of single measurements is limiting, not the precision.

Another myth is that, in approaching an experimental study, the experimenter should have no desire concerning the outcome, and even no preconception. That is nonsense, if only because it is impossible. What is necessary is an appropriate statistical design, with a suitable element of randomness, which will permit unbiased estimation of treatment effects and of the experimental error variance, and an objective evaluation procedure, or, if not objective, at least not influenced by the experimenter's prejudices or hopes. As is widely recognized, clinical evaluation of medical treatments requires both the patient and the evaluating clinician to be ignorant of the treatment received by the patient being evaluated. This may be difficult because side effects, such as jaundice, may enable the clinician, and perhaps even the patient, to recognize at a glance which treatment the patient has received.

Another myth is that ratios of ratios, or ratios with a common factor, should not be used. Remember that the object is to make sense out of the data. That license is broad enough to include ratios of ratios. It may be hard to think straight about them, and likewise about ratios with a common factor, or so-called spurious correlations, or correlation of a part with the whole, but none is proscribed: use them with discretion, like any other statistic or method, but do not be discouraged from using what you need.

Enough of myths.

Most statistical analyses of body-composition data use correlation or regression or covariance analysis. To begin, the correlation coefficient is a comparatively meaningless and useless statistic.[2] It tells only two things. Its sign indicates whether the dependent variable increases or decreases as the independent variable increases. Its square is the fraction of the variability of the dependent variable which is associated with, or ascribable to, or explainable on the basis of, variation in the independent variable. Both are readily obtained without calculating the correlation coefficient itself. As is now generally realized, correlation does not demonstrate a cause-and-effect relation, no matter how close the correlation is.

Estimation of the correlation coefficient requires a random sample from a bivariate population, an uncommon thing at best. Unless the data constitute such a random sample, the sample correlation will not be an unbiased estimate of the population correlation, and there will be no possibility of sound statistical comparison with another value of the correlation coefficient except zero. In fact, the regression coefficient is nearly always to be preferred.

An unbiased estimate of the regression coefficient requires that values of the dependent variable be unselected. Values of the independent variable may be chosen at random, or equally spaced, to include unusually large and unusually small values, or in any other desired way independent of the value of the dependent variable. As long as dependent-variable values are ignored in sampling, the regression coefficient and the regression line will be unbiased, but the correlation will be biased if the standard deviation of the independent variable is biased. A deliberate effort to include a wide range of values of the independent variable may often be good strategy, both by increasing the sensitivity of the test of deviation from linear regression (and of any test of homogeneity of deviations from regression) and by increasing the precision of the sample regression coefficient. Nor is anything lost from evaluation of the closeness of relationship, which should be based on the deviation mean square, not on the correlation coefficient.

Testing the significance of a regression coefficient is precisely equivalent to testing the significance of the corresponding correlation coefficient. If two samples are to be compared, comparison of the regression coefficients will be straightforward if sampling is random with respect to the dependent variable. The regression coefficient is more informative than the correlation coefficient because it has dimensions, being expressed in pounds per inch, grams per liter, milliosmols per minute, and so on.

In testing the significance of the difference between two correlation coefficients, Fisher's z-transformation is sometimes mentioned as if it solves all problems. It does not. If the two correlations involve a common dependent variable, a test is needed that takes into account the fact that the sample comes from a population with an unknown correlation between the two independent variables. This unknown value is sometimes called a nuisance parameter. An exact test of significance is available, and it may give a much different result from routine application of the z-transformation. To illustrate, suppose we have a sample of 1,000, in which the correlations between a dependent variable and two independent variables are 0.1 and 0.2. By the z-transformation we would arrive at 2.286 for t, significant at the 5% level. The exact test involves the sample correlation between the two independent variables. If this correlation is –0.7, 0.0, or +0.7, the respective values of t are 1.91, 2.35, and 4.27.

Reference to randomness of sampling of the dependent variable raises the question of what variable to label dependent. A purely semantic difficulty is removed by thinking of predictor variables and a predictand. The predictors will not usually be independent of each other or of the predictand, so "dependent" and "independent" are somewhat unsuitable terms. As the predictand is the variable whose errors are minimized in the least squares arithmetic, it is important that the variable treated as dependent be exposed

to sampling error. For example, if response is to be used to estimate the effective dose of a physiologically active material, and the basic relation is established by testing several doses of the pure substance, it will be meaningless to calculate the regression of dose on response, though that might seem to be appropriate if the relation is to be used to estimate dose from response. Because response, not dose, is exposed to sampling error, the regression of response on dose should be used to infer the unknown dose that elicited an observed response.

Sometimes one encounters statements such as "Assignment of the x-axis to fat values . . . expresses our belief that fat is the independent variable." Enough has been said to indicate that a choice between dependent and independent, based on some concept of the underlying physiological relationship, may be statistically inappropriate. However, this example, in which the other variable is fat-free body weight, is clearly a case in which both variables result from a complex of causes, including diet and exercise, in particular, and neither can be regarded as independent in the sense of causality.

Another point concerns manipulations of the dependent variable. It is common in nutrition experiments to analyze weight gains. Also, covariance adjustment of weight gains to equal initial weight is usually worthwhile. It is not always realized that analyzing gains is precisely equivalent to analyzing final weights if covariance is used to adjust both to equal initial weight. The sole difference is that the gains regression coefficient will be less by unity, the result of subtracting initial weight from both sides of the final-weight observation equations. When the significance tests give identical results, it is because they are mathematically equivalent, not "for lack of statistically significant differences in initial weights."

The choice of functional relationship is important. There are two distinct common objectives of curve fitting. The simpler one is to provide a formula usable for estimating the value of the dependent variable. For this use, simplicity of the selected function, especially simplicity of calculation, is the aim, and a polynomial is often used. The more complex and more important objective is to approximate the natural relationship. To do this, the selected function should satisfy all the obvious requirements. For example, to be considered for representing body weight during growth to maturity, a function should approach asymptotically an upper value which, for this example, is the mature weight. In using cyclopropane to measure body fat, it has been proposed that the body must be considered to have a minimum of three compartments, and the function fitted to the data therefore includes three distinct exponential terms.

Data on fatness in guinea pigs exercised from weaning at 21 days of age to "retirement" at 240 days of age were given in a graph similar to Figure 1, with a footnote that "The fatness curve was fitted visually." The point at 240

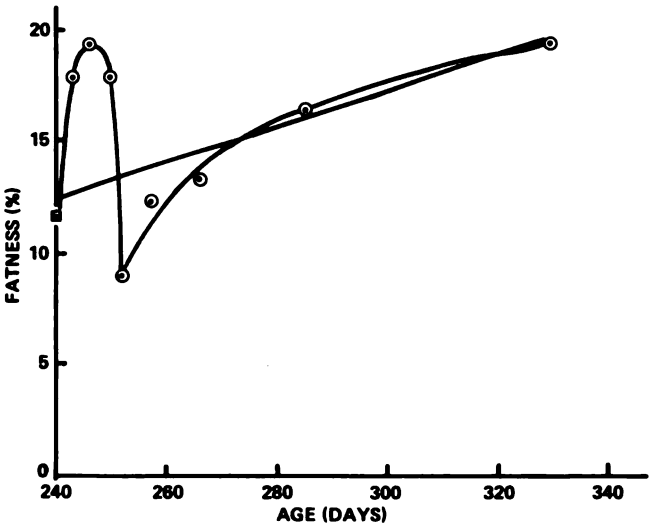


FIGURE 1 Effect of retirement on fatness.

days is the mean of 12 animals, with a standard deviation of 3.7 units of percentage of fatness, and the other eight points represent single individuals. What can be learned from these data? I have found only one comparison significant at the 5% level. The datum after 6 days of retirement was pointed to as being more than two standard deviations above the 12-datum mean at 240 days, but the *t*-test does not reach the 5% level. The linear regression line fits the nine points adequately: the mean square deviation being about 18, with 8 degrees of freedom; the error mean square being about 14, with 11 degrees of freedom. However, the 1 degree of freedom for linear regression achieves a mean square of only about 58, appreciably short of the 5% level. In fact, despite whatever plausibility the visually fitted curve may have, the data do not give significant indication of any effect except that average fatness was higher for retired animals than for those terminated at 240 days of age.

In further discussion of the same experiment, it is said to be "invalid" to compare the exercised group to the sedentary group as a whole (that is, I suppose, to compare their mean values), and that the role of exercise can be properly assessed only if the influence of fat is held constant. First it should be noted that any comparison should have a definite objective. There is no invalidity to a direct comparison, say, a *t*-test, of whether the exercised and sedentary animals were of equal average fat-free body weight. The answer was unexpected, the exercised animals being the lower, contrary to the known

tendency of exercise to cause muscle hypertrophy. Hence it was suggested, in effect, that the regression of fat-free body weight on total body fat for sedentary animals should be used to adjust for the "effect" of total body fat; and it was concluded that the exercised group was "obviously heavier, 7 of the 12 values falling 2½ or more standard errors of estimate above the regression line." This procedure was unconventional, and covariance analysis should have been used. The result is, indeed, that the exercised group has the higher adjusted mean, but not enough higher to reach the 5% significance level.

Does the covariance adjustment yield the answer to an interesting question? Of course, it answers the usual formal question: whether sedentary animals and exercised animals of equal total body fat are equal in fat-free body weight. What remains obscure in this example is the role of fat-free adipose tissue: whether it should be included in fat-free body weight, and whether adjustment to equal total body fat is the adjustment that is really wanted. These are not statistical questions. Regression of fat-free body weight on total body weight is significant beyond reasonable doubt, indicating clearly that each increment of fat is accompanied by an approximately equal increment of fat-free body weight, but the data do not indicate the composition of the increment of fat-free body weight.

A question about correlation concerns the possible values of the third correlation among three variables when two correlations are known. If the correlation of A with B is r, and of B with C is s, what is the correlation of A with C? Obviously there is some reason to expect a value rs. In fact, it must lie in the range $rs \pm t$, where t is the square root of $(1 - r^2)(1 - s^2)$. This may be much narrower than the possible range (–1 to +1), but will usually be so wide as to seem of little use. For example, if $r = 0.6$ and $s = 0.8$, the third correlation must lie in the range from 0 to 0.96. These limits apply not to the population value but to the value calculable from the given sample of values of A and C. Hence, even these wide limits may fail to include the population correlation.

Harrington, in *Body Composition,* quoted a correlation of 0.96 between red-cell volume and total excess protein, but pointed out that the correlation of protein with body weight was 0.99, which suggested to him that red-cell volume would be of little use for differentiating these animals on the protein scale at the same body weight. This raises the question of the partial correlation between red-cell volume and body weight. We can calculate that the total correlation must lie between 0.9109 and 0.9899, the third and fourth decimals being given for clarity. This range corresponds directly to the possible range, –1 to +1, for the partial correlation. If the total correlation is 0.92, the partial correlation is –0.77. Can we say anything useful about the partial correlation when the total correlation is unknown? I think not. In

passing, you may like to note that the quoted correlations are based on 40 pairs of data. On the basis of the rounding rule stated earlier, these correlations should be reported to three decimals. Also, it is worthwhile to point out the bias in these correlations: The 40 animals were in two groups receiving different amounts of feed, leading to a gross difference in body weight, and lower correlations should be anticipated in any data that have not had the range of the independent variable artificially expanded. Specifically, the correlation for the low-intake animals was probably about 0.71.

Supposing no real understanding of the natural relations involved, what is the general approach to statistical analysis of data that should be employed to arrive at an empirical formula for estimating one variable from the values of several others? This is the typical multiple-regression problem, and several points require attention. First, multiple-regression analysis is least squares analysis. As mentioned elsewhere, that means that the variable taken to be dependent must sample an error variability to be minimized by the least squares process. Further, this error variability should be homogeneous. This is familiar in the use of a pooled error term. Pooling is appropriate when the quantities pooled are samples of the same error variance. In biological data, the error variance frequently increases as the value of the dependent variable increases. For example, if final weight is observed after various feeding periods, it is common to find that the variability of final weight is greater for those fed longer. If a regression of final weight on feeding period is calculated in the ordinary way, the error sum of squares will be a mixture of samples of unequal population variances, and tests of significance of goodness of fit or of the regression coefficient may be seriously misleading. The error variance indicates how near to the subgroup mean the regression need pass for a satisfactory fit. If the errors are treated as if all have the same variance, the line may deviate significantly for the earlier points from the location that would be found by a correct analysis, that is, taking account of heterogeneity of error variance.

Transformation of the dependent variable, perhaps to logarithms, may make the error variances homogeneous. An attendant effect may be to change a linear relation to curvilinear, but this can sometimes be overcome by transforming the independent variable(s) also. The alternative is to utilize statistical weights in the multiple-regression arithmetic. If theoretical weights are available, this is straightforward and will result in a sum of squares of deviations from regression which is distributed in the chi-square distribution if the regression function is suitable. If statistical weights have to be estimated from the data, the deviation mean square can sometimes be referred to the *F*-distribution.

Second, curvilinearity should be sought. How far one should go in this direction is not easy to say. Of course, if a relation is understood well enough

to suggest that it is probably not linear, several powers of the independent variable may be needed for an adequate fit and should be included so they can be tested. The results should be interpreted with common sense. For example, if 10 points that come from a linear relationship are available for analysis, and a ninth-degree polynomial is fitted so as to make a thorough test, there will be eight regression coefficients for powers above the first. By hypothesis, they should be nonsignificant, but, at the 5% level, the chance that at least one of eight will be judged significant is 0.34. An apparently significant coefficient should probably be disregarded if the coefficient of the next lower power is not significant, and almost surely if the next two lower coefficients are not significant. It is worth noting that the sum of squares accounted for cannot decrease when a term is added to the model. Hence, the statement "We . . . found the r for the third-order equation to be approximately the same as for the parabolic form, but . . . markedly diminished for the fourth order" indicates a mistake in arithmetic.

In seeking curvilinearity, products of independent variables should not be overlooked, xy being no less appropriate than x^2 and y^2, and it might be the only second-degree term needed. If the regression is $z = a + bx + cy + dxy$, the regression of z on x is linear for fixed y, with regression coefficient $b + dy$, and similarly for regression on y. This is a real enough possibility, and can be recognized only by including xy among the independent variables tested.

What are we to think of the case in which the quadratic regression coefficient is significant beyond reasonable doubt, but the linear coefficient is nonsignificant? Some would discard the linear term. This is done automatically by some so-called step-wise multiple regression computer programs, which Yates[3] has said will be rejected by experienced statisticians. Of course, there are natural phenomena that require a term of second degree, an example being the distance moved, as a function of time, by an object under the influence of gravity. Data for objects initially at rest require only t^2 as an independent variable, the physical relation being $d = at^2$. A little grasp of this relation might lead to a choice of data starting at rest, but with a more complex situation, the best choice might not appear. Suppose, instead, that we chose, or could acquire, data for some fixed initial speed, s. The physical relation would be $d = st + at^2$, and we might find s "nonsignificant" if we made appreciable errors of observation. In fact, when a relation is quadratic, the value of the partial linear regression coefficient is affected by the choice of origin of the independent variable. For one origin its value is zero, and for nearby origins it will be judged nonsignificant. In my view, such a nonsignificant term should usually be retained in the model, on the ground that it would be too great a coincidence to have selected that origin for which the linear coefficient was exactly zero. Hence, the best available value should be

retained, and should have its influence not only on the error mean square and upon values estimated from the regression equation, but especially upon the error variance of estimated values.

After fitting any model, the fit should be tested. One way is to compare the deviation mean square to the error mean square. The deviation mean square will ordinarily have several degrees of freedom. A more sensitive test, with only one degree of freedom, is sometimes possible. For example, it is a better test of the adequacy of a straight line to test the quadratic term for significance. If the fit is satisfactory, individual partial regression coefficients can be tested for significance, or groups can be tested jointly by fitting a reduced model and testing the change in the deviation mean square. If the fit is not satisfactory, some change in the model is indicated. Tests of partial regression coefficients may nevertheless be useful, but they should use the deviation mean square, not the error mean square.

The ease of multiple-regression arithmetic, especially when computerized, may lead some to fit polynomials of high degree, involving many partial regression coefficients, and to overlook the great gain in simplicity and understanding that can result from a more thoughtful choice of function. An exponential function approaching a horizontal asymptote from below, with only three adjustable constants, may fit growth data far better than a second-degree polynomial, also with three adjustable constants, and may give a more reasonable extrapolated value for ages above those observed.

Given a statistically adequate fit, the next question concerns the practical utility of the model. This is primarily a question of the deviation mean square, and it (or its square root, the standard error of estimate) should always be reported. It tells how near to the several datum points the fitted function passes. The correlation coefficient can be manipulated within wide limits by selection of values of the independent variable(s), but such selection has no effect on the deviation mean square.

A secondary practical consideration concerns convenience of use, whether a simpler function would fit well enough for a specified practical use, even though statistically inadequate, or whether another function entirely might be more practical. These questions have to be considered in the individual case.

The use of regression equations can raise puzzling questions. In one case it was said that

> Since there is a random counting error . . . , it is not surprising that at times potassium values predict lean body weights somewhat in excess of total body weight. We do not feel justified in discarding such cases but it would obviously be absurd to make computations based on lean body weights in excess of body weights . . . where this occurred the lean body weight was taken to be the same as total body weight and body fat was taken to be zero.

Perhaps no one will disagree with this procedure if the sole purpose is to get, say, an estimate of fatness for individual clinical use, and it is understandable that a negative fatness does not appeal. However, it is when computations are to be made that these absurd values are to be used unaltered. As a simple example, suppose the average fatness of a group is the desired value. One usual desideratum underlying the use of a statistical relationship is that it give unbiased estimates of individual values. If a statistical formula is unbiased, and if there are individual values near some boundary (such as weight zero), absurd values will indeed sometimes result from sampling errors. If such absurd values are "corrected" by reduction to the permissible limit, some of the sampling errors are being reduced, but there is no way to make a compensating reduction in sampling errors that happen to be in the opposite direction. Hence, the average value will be biased. This can be appreciated in another way, for the simple case of linear relations, because the average estimated y can be obtained not only by averaging the estimated individual y's but also by inserting the average value of x into the equation. Even the average x might lead to an absurd estimate of y, but that is surely better than to adopt a nonabsurd value reached by biasing some of the data, if only because it gives a warning regarding the estimated value.

REFERENCES

1. Brožek, J. [ed.]. 1963. Body Composition. Ann. N. Y. Acad. Sci. 110:1–1018.
2. Tukey, J. W. 1954. Causation, regression and path analysis. *In* O. Kempthorne, T. A. Bancroft, J. W. Gowen, and J. L. Lush [ed.] Statistics and mathematics in biology.
3. Yates, F. 1966. Computers: the second revolution in statistics. Biometrics 22:233–251.

L. N. Hazel
Iowa State University

INVITED DISCUSSION

Dr. Norton has done an excellent job of summarizing the useful statistical tools available for use in biological investigations and illustrating some of the common pitfalls that are encountered frequently in their use or, perhaps, misuse. Having accepted the task of acting as discussant for this section, I must find a few points to emphasize differently, or to re-emphasize, or to disagree with to some extent. But my remarks are made in the hope of ensuring clarity and do not indicate that any broad area of disagreement exists with the statements and principles he has outlined.

While statistics will be most useful in comparing methods and techniques involved in measuring body composition and in estimating parameters related to composition, these statistics scarcely can be a goal in themselves. Mathematics will increase in usefulness as accurate estimates of statistical parameters become available. Then these can be utilized in the necessarily complex mathematical equations to describe the obviously complex growth rates of organs and tissues and of the interactions among them. We need more emphasis on rates of cell multiplication and loss in individual organs and of the changes in their chemical content. Then mathematics can become fully useful

for describing biological phenomena, as has been the case for describing the physical world.

Dr. Norton's comment concerning the unimportance of preconception concerning the outcome of an experiment deserves re-emphasis. The important points are in developing the hypothesis to be the subject for experimentation and in choosing a statistical design that will give an unbiased answer. The researcher's own knowledge and experience frequently will and should suggest the probable nature or direction of the outcome.

Dr. Norton's warning concerning that "comparatively meaningless and useless statistic," the correlation coefficient, probably was occasioned because he has seen us use it wrongly so often, rather than because he wanted us to accept those critical adjectives literally. His later comments seem to bear out this interpretation, because they make a good handbook of how to use correlation coefficients correctly, or at least of how to avoid the errors frequently made in their use.

The most distasteful fact about the regression coefficient is that one must choose a dependent variable and one or more independent ones. Frequently the choice may be self-evident; frequently it must be made more or less arbitrarily; at other times the choice must have a purely statistical basis, because only one of the variables has the statistical properties required of the dependent variable. Because the words "independent" and "dependent" imply cause-and-effect relationship, Dr. Norton's suggestion to substitute the terms "predictor" and "predictand" has considerable merit where no biological basis exists for identifying the variables that represent each of the effects.

Dr. Norton has pointed out that the dependent variable must be unselected to obtain unbiased estimates of the regression coefficient, but that selection of the independent variable does not cause bias as it does with the correlation coefficient. He has also suggested that a deliberate effort to include a wide range of values of the independent variable may be good strategy. This practice, if used consistently, is likely to have more disadvantage than otherwise. First, if nonlinearity is not evident in the bivariate distribution of variates that are usually encountered in our biological problems, we are not likely to make serious errors of interpretation by ignoring its existence. Second, deliberately choosing values outside our usual experience ensures that they are uncommon, if not actually unusual! The extra variation introduced thereby may obscure or minimize an important relationship that exists in more homogeneous data.

Third, error variances (deviation mean squares) are likely to be more heterogeneous when the independent variable is made to vary widely. For example, the ^{40}K count of 3-year-old children is considerably less variable than that of 30-year-old adults. Fourth, this practice is likely to open the door to the very

statistical misuses that we propose to warn you about. You may be tempted to compute a correlation coefficient from such data or to infer that the variance in count accounted for by age means a great deal more than the obvious fact that body weight in the two age groups is vastly different.

Dr. Norton has passed over without comment a questionable practice that is becoming increasingly common in these days of easy computer analysis. When computation was more difficult, relationships usually were not studied unless they were thought to have biological meaning. Now that it costs so little to "look and see," many nonsense regressions are calculated. Taken individually, 5 out of 100 of these will prove to be statistically significant when, in fact, the true relation is zero. This practice of examining the statistical significance of many relationships and retaining only those that are larger distorts the probability distributions of the retained variables in an extremely complex manner. I don't say not to engage in the practice but to only say "be careful" may be minimizing the danger. If the conclusions to be drawn after such treatment are very important, try conducting another experiment. You should be surprised if the retained variables are not less important in the second experiment than in the first.

NOTES ADDED AFTER ATTENDING THE CONFERENCE

As presented and misinterpreted so frequently in this conference, Dr. Norton's criticism of "that comparatively meaningless and useless statistic," the correlation coefficient, was unduly mild!

Chemical determinations are not without error; in fact, I thought there was evidence that some contained relatively greater error than ^{40}K counts. Repeated independent sampling may indicate the magnitude of the error, but does not necessarily do so if a bias peculiar to each individual item in the sample persists over the entire sampling period. If we wish to measure a variable t, whose real variance is σ_t^2, we may use a chemical determination, X_1, which involves some error such that $X_1 = t + e_1$ with variance $\sigma_t^2 + \sigma_{e_1}^2$. A predictor variable $X_2 = bt + e_2$ with variance $b^2\ \sigma_t^2 + \sigma_{e_2}^2$ may be more closely associated with t than is X_1, but we fail to recognize it because we can only observe X_1 and X_2. Some clue may be obtained as to which of two predictor variables contains the greater error by examining their relationships with other variables expected to be closely related to t.

DENSITOMETRIC METHODS

A. M. Pearson
R. W. Purchas
and E. P. Reineke
Michigan State University

THEORY AND POTENTIAL USEFULNESS OF BODY DENSITY AS A PREDICTOR OF BODY COMPOSITION

Discovery of the principle of density is credited to the Greek scientist Archimedes in about 200 B.C. Presumably while he was taking a bath, he pondered the problem of how to measure the purity of the Emperor's crown and observed that a body displaces a volume equal to its own. Although Archimedes reportedly made his original observations on the human body, subsequent applications were primarily on metallic substances until Robertson[63] attempted to estimate the density of 10 "middling-sized" men, who "were bribed" to submerge themselves in a tank filled with water. Results were unsatisfactory, so the author complained, with great scientific fervor, that the men "were more interested in the bribe than in the experiment."

RATIONALE FOR DENSITY

The Archimedean principle, as already stated, is based upon the fact that a body displaces a volume equal to its own. Thus, substances can be compared on a basis of their weight per unit of volume. Density is expressed in relation to the density of a reference standard—usually water—at 20° C. In the case of gases, however, the reference standard is generally air.

The elements in the periodic table vary widely in density, with hydrogen and uranium being the lowest- and highest-density naturally occurring elements, with values of 9×10^{-5} and 19.05 g/ml, respectively.[41] Since hydrogen is a gas and uranium is a solid, a more meaningful comparison is obtained by comparing lithium (the least dense solid), at a density of 0.534, with uranium.[41]

To illustrate a further point, rather wide differences occur in the density of adjacent elements in the periodic table. For example, lithium, at 0.534 g/ml can be compared with beryllium (the next least dense solid), which has a density of 1.816.[41] This can be contrasted to the values of 1.10 for lean and 0.90 for fat that are commonly given.[6]

The rationale for estimating fatness or leanness, or both, from density is based upon the assumption that the body can be considered a two-component system, with the components being of different but constant densities.[33] If this is the case and the densities of the components are known, the proportions of the two components can be estimated from the density of the whole body.[5,33]

With animals other than humans where empirical relationships can be established quite easily, the two components do not have to be specified but are usually considered to be the fatty tissues and the fat-free body. There is considerable evidence that the fat-free body is fairly constant in composition in mature animals.[17,31,46–49] However, the water content of the fat-free body is not constant in young, growing animals, which indicates that density measurements are not likely to be as accurate for predicting the composition of the immature.[24,48,50] Variation in the density-body-composition relationship may also result from density variation within a tissue.[19]

In work with humans, where empirical relationships cannot be established so easily, relationships between density and body composition have been derived by taking the densities of the two components being considered and incorporating them in the theoretical equation of Keys and Brožek.[33] Originally, the two constant density components considered were the fat and the fat-free body. Behnke *et al.*[7] introduced the concept of "lean body mass," which includes the essential body lipids in addition to the fat-free body. More recently, Minnesota workers[12,33] have utilized a two-component system consisting of a "reference body" (which contains 15.3% of fat) and "obesity tissues." The latter system has been complicated by the fact that "obesity tissues" differ in composition depending upon whether the subject is gaining or losing weight.[12] Pitts[57–59] has shown this concept to be further complicated with the discovery that there is an increase in the nonfatty supporting tissues as an animal increases in fatness.

The relative density of extracted fat at 37° C for seven species was found to range from 0.9000 for man to 0.9212 for the internal fat from the lamb,

according to Fidanza *et al.*[19] The data summarized in Table 1 verify the relatively small amount of variation in the density of fat. This is true not only between species (Table 1) but also within the same species. Morales *et al.*[47] reported that the differences in density of the perirenal, intramuscular, and subcutaneous fat samples of the guinea pig were not statistically significant.

TABLE 1 Density Values for Fat and Lean Tissues

Tissue and Species	Reference	Density
Fat, cattle	Bieber *et al.*[8]	0.912
Fat, cattle	Kraybill *et al.*[39]	0.891
Fat, guinea pig	Morales *et al.*[47]	0.912
Fat, human	Hodgman[29]	0.918
Fat, pig	Kraybill *et al.*[40]	0.914
Fat-free body, man and guinea pig	Morales *et al.*[47]	1.100
Protein, lean beef	Bieber *et al.*[8]	1.312
Protein, theoretical	Schmidt[64]	1.250

The single body component other than fat that would be expected to influence density the most is bone. Values for bone density reported by Lange[41] vary from 1.7 to 2.0, although the state of the bone (i.e., wet or dry) is not given. These values are higher than a density of 1.43 for raw bone given by Morales *et al.*[47] Even though variation in bone density might be expected to be a major source of variation in estimating body composition from body density, such does not appear to be the case,[2,47] presumably because bone comprises a relatively small proportion of the body.

METHODS OF MEASURING DENSITY

Since density is the weight per unit of volume, the major problem in determining density resolves itself into measurement of volume. The volume measurement can be accomplished by determining the volume of either the gas or the water that is displaced. Although measurement of volume would appear to be a simple procedure, such is not the case, especially in live animals. Even water displacement, which is the simplest method, has numerous pitfalls, and air-displacement procedures for measuring volume are even more complicated (see references 1, 7, 13–15, 18, 20, 23, 26, 28, 30, 32, 36, 38, 42, 43, 45, 51, 52, 55, 56, 69).

Measurement of the volume of water displaced is relatively simple for eviscerated carcasses or cuts of meat, where the major problem seems to be one

of exercising care to prevent erroneous values due to the trapping of air. However, measurement of volume with the intact animal is complicated by the sizable volume of air trapped in the respiratory passages and lungs. Failure to correct for the volume of air in the lungs appeared to be the major reason that the early studies of Robertson[63] and others as reviewed by Boyd[10] were doomed to failure. The first successful attempts to measure body volume of intact animals by water displacement were achieved by Behnke *et al.*,[7] who made corrections for lung volume. Nevertheless, correction for lung volume is still one of the major difficulties in using the water-displacement procedure.

The air-displacement procedure of measuring volume has the advantage of requiring no corrections for lung volume because there is a free exchange between the gaseous atmosphere surrounding the body and the air in the respiratory tract. The advantages of this system are obvious, but measurement of volume by this procedure requires a closed system because the gases displaced are the constituents of the surrounding atmosphere. Measurement is therefore difficult and involves corrections for temperature and humidity and for changes in the concentration of gases as a result of respiratory exchange.[23,28,43] A gas-tight chamber of known volume is utilized. The person, animal, or object to be measured can be enclosed in this type of chamber. The volume can then be determined either by the dilution principle with an inert gas, such as helium, or by pressure changes utilizing the basic formula:

$$VP = V'P'.$$

The changes in gas pressure can be either positive,[55,56] where a known amount of gas is added, or negative,[43,55] where a vacuum is drawn and the change in pressure is used to calculate volume.

WATER DISPLACEMENT

Following the early work of Robertson,[63] Spivak[69] also attempted to determine the density of human subjects by underwater weighing, but similarly failed to recognize the importance of correcting for lung volume. Boyd[10] reviewed the earlier work on density and obesity by concluding that obesity should decrease density. The first successful attempts to measure body volume by underwater weighing were achieved by Behnke[5] after correcting for the pneumatic volume at the time of weighing. Later, Behnke *et al.*[7] demonstrated that underwater weighing gave logical estimates for body composition on correcting for lung volume, which was achieved by weighing at the completion of a maximal inspiration and at the end of maximal expiration. Subsequent determination of residual air in the lungs was determined by dilution and was utilized in correcting the body volume. In spite of the elegant work of Behnke and others,[5–7,21,25,74] underwater weighing is not

a simple technique and its use requires special training. Furthermore, the method cannot be easily adapted to the infirm or the very young, and it is not practical for use with intact living animals. Modifications of the method, whereby the subject has been immersed until only the nose and upper portion of the head are above water have been utilized.[76,77] In this case, corrections for the lung spaces and the unemerged portion of the head have been obtained by helium dilution.

Application of underwater weighing to measurement of the density of eviscerated carcasses from both large and small animals has been used extensively for estimating relative fatness. The classic study by Morales *et al.*[47] provided basic information that demonstrated the validity of the procedure and gave rise to the fundamental equations for estimating fatness of the guinea pig and man from specific-gravity values. Density obtained by underwater weighing has been shown to be a useful technique for measuring the composition of carcasses and cuts from farm animals (see references 3, 11, 16, 22, 30, 35, 54, 57, 60, 61, 70, 75) and has also been widely used for laboratory animals.[15,53,57-59,62] In spite of its usefulness, underwater weighing cannot be considered a panacea by those interested in composition. Although it can provide useful information to the researcher, it cannot be readily applied where body-composition information would be of the most value, that is, for determining the composition of the very young and infirm or in selecting farm animals for breeding purposes.

AIR DISPLACEMENT

According to Spivak,[69] the first attempts to determine body density by air displacement were made by Jaeger more than 80 years ago. He used the Kopp volumeter, which was fairly accurate for inert objects, but gave poor results with living subjects because of pressure changes resulting from gaseous exchange, vaporization of water, and increases in air temperatures. Pfaundler[55] constructed a chamber for measuring the density of cadavers from young children, but obtained poor correlations between density values obtained by underwater weighing. The poor relationship was probably due to his failure to correct for air in the respiratory system of the cadavers on determining density by underwater weighing, since the air-displacement technique appeared to be repeatable on subsequent readings for the same cadaver. In order to obtain good repeatability, it was necessary to allow the temperature of the cadavers to fall to that of the room before making the measurements. Pfaundler's procedure[55] made use of negative pressures or vacuum.

Pfleiderer[56] added positive pressures by using compressed gas and reported a mean error of only 1 to 2% in body volume. Kohlrausch[38] used the air-displacement technique to study the effects of exercise on the body density

of dogs. He noted that one dog subjected to heavy muscular exercise declined in weight from 10,805 to 9,060 g, while specific gravity increased from 1.054 to 1.074. Using the changes in density and weight, the author calculated that fat content declined from 1,217 to 609 g, while active muscle mass calculated from the basal metabolic rate increased from 1,676 to 1,750 g.

Böhnenkamp and Schmäh[9] measured the volume of human subjects by adding known amounts of oxygen. They attempted to minimize the effects of variations caused by the humidity by completely saturating the air with water vapor. Corrections were also made for temperature changes. Estimated density values were 1.095 for men and 1.070 for women. Noyons and Jongbloed[51] criticized the method of Böhnenkamp and Schmäh[9] and modified the procedure in calculating the body volume of cats; they weighed at different pressures and used both positive and negative pressures. Measurement of density in human subjects by Jongbloed and Noyons[32] resulted in an average of 1.080. Positive pressures were reported to be more comfortable than negative ones.[56]

A sine wave of changing volume was used by Wedgewood and Newman[73] to correct for the gradual changes in volume associated with the increased heat, vapor, and air exchange. Liuzzo *et al.*[43] obtained promising results on estimating the density of guinea pigs by air displacement. More recently, Hix *et al.*[27] obtained good agreement between both body volumes and densities determined by air displacement and helium dilution, but they failed to obtain such promising estimates on a modified procedure for measuring the density of live pigs.[28] Falkner has also used the air-displacement method for estimating the composition of babies.[18]

Although the air-displacement procedure for measuring body volume is based upon a simple, straightforward principle, its success has been rather limited. Corrections for temperature and humidity are essential for accuracy.

HELIUM DILUTION

The helium-dilution procedure for measuring body volume was developed by Walser and Stein,[71] who applied it in determining the density of live cats and compared results with underwater weighing of the eviscerated carcasses. In the helium-dilution procedure, the animal is placed in a closed chamber of known volume, and an exactly measured volume of helium gas is injected into the chamber. After thorough mixing, a sample is removed and analyzed. The gas is diluted in proportion to the amount of space in the chamber, which is determined by the volume of the animal.

The most extensive series of studies on the helium-dilution procedure of measuring body volume have been carried out by Siri.[65–68] The greatest im-

provements in his method were a more accurate procedure for measuring helium and incorporation of corrections for relative humidity and temperature into his formula. Siri[66] later estimated the magnitude of the errors that could be tolerated in each detail of design to reduce the standard deviation for measuring volume to ±0.1 liter. Modifications of the helium-dilution method have also been utilized by Foman *et al.*[20] for determining the volume of infants, by Hix *et al.*[27] for men and women, and by Gnaedinger *et al.*[23] and Hix *et al.*[28] for live pigs. Results have been variable except for the study on humans by Hix *et al.*,[27] where correlations of 0.96 and 0.91 were obtained for density values determined by helium dilution and air displacement for men and women, respectively.

Gnaedinger *et al.*[23] have pointed out some of the major problems in the helium-dilution procedure. Using pigs weighing from 181 to 220 lb with a range of 27.4 to 41.1% in fat content, they calculated that for each change of 0.001 unit in density there would be a change of 0.5% in fat content. To obtain the density of a 95.49-kg pig accurately to the third decimal, assuming there were no errors in weighing, would require that the volume be measured to an accuracy of 0.1 liter or 1 part in 1,000. These authors pointed out the problem of correcting for relative humidity and temperature, but stated that the activity of the animals in the chamber was probably the greatest source of error.

Although the helium-dilution procedure for determining density has not to date proven accurate enough for precise estimations of density, the method has some inherent advantages. By more precise control of the variables and incorporation of corrections, it should be possible to measure volume with enough accuracy to predict density within ±0.001 unit.

EQUATIONS FOR ESTIMATING BODY COMPOSITION

A great many equations have been calculated for estimating body composition from density. The data in Table 2 summarize the equations from a number of studies and provide information on several species. As can be seen, there is a wide divergence in the equations for predicting composition from specific gravity or density, as the case may be. The divergence in the equations is true not only between species but also within the same species. This suggests that it is difficult to take the equation derived from one population and apply it to any other population. On the other hand, the similarity between equations indicates that comparative values derived from using a single equation may be meaningful, except in cases of abnormally large weight changes or in abnormal hydration.[6]

TABLE 2 Formulas for Calculating Percentage of Fat in Body from Density or Specific Gravity

Species and Condition	Reference	Number of Animals	Density or Specific Gravity		Fat Percentage		"r" Value Density versus % Fat	Formula for Calculating Percentage of Fat from Specific Gravity or Density
			Range	Mean	Range	Mean		
Cattle, whole animal minus lungs	Kraybill *et al.*[39]	30	1.017–1.070	1.045	13.6–39.5	25.1	–.96	$\%\ \text{Fat} = 100\left(\frac{4.476}{\text{sp. gr.}} - 4.034\right)$
Guinea pig, eviscerated and shaved	Rathbun and Pace[62]	50	1.021–1.096	–	1.5–35.8	–	–.97	$\%\ \text{Fat} = 100\left(\frac{5.132}{\text{sp. gr.}} - 4.694\right)$
Guinea pig, theoretical value for eviscerated-calculated	Rathbun and Pace[62]	50	–	–	–	–	–	$\%\ \text{Fat} = 100\left(\frac{5.362}{\text{sp. gr.}} - 4.880\right)$
Guinea pig, whole animal	Rathbun and Pace[62]	50	–	–	–	–	–	$\%\ \text{Fat} = 100\left(\frac{5.501}{\text{sp. gr.}} - 5.031\right)$
Guinea pig, eviscerated and shaved	Pitts[57]	72	–	1.052	–	18.3	–.95	$\%\ \text{Fat} = 100\left(\frac{4.183}{\text{sp. gr.}} - 3.790\right)$
Man, calculated	Rathbun and Pace[62]	–	1.002–1.100	–	0.0–49.3	–	–	$\%\ \text{Fat} = 100\left(\frac{5.548}{\text{sp. gr.}} - 5.044\right)$
Man, calculated	Keys and Brožek[33]	–	–	–	–	–	–	$\%\ \text{Fat} = 100\left(\frac{4.201}{\text{density}} - 3.813\right)$
Man, calculated	Siri[65]	–	–	–	–	–	–	$\%\ \text{Fat} = 100\left(\frac{4.950}{\text{density}} - 4.500\right)$
Man, calculated	Behnke[6]	–	–	–	–	–	–	$\%\ \text{Fat} = 100\left(\frac{5.053}{\text{density}} - 4.614\right)$
Pig, carcass	Kraybill *et al.*[40]	40	0.990–1.057	1.016	17.8–53.1	38.4	–.93	$\%\ \text{Fat} = 100\left(\frac{5.405}{\text{sp. gr.}} - 4.914\right)$
Pig, whole–theoretical	Gnaedinger *et al.*[23]	24	1.015–1.042	1.031	31.9–46.8	37.4	–.94	[a]
Pig, whole–theoretical	Hix *et al.*[28]	24	1.016–1.051	1.032	28.2–41.3	35.3	–.73	[a]
Rat, eviscerated and shaved	Da Costa and Clayton[15]	99	–	1.053	–	35.1	–.73	[b]
Sheep, carcass	Barton and Kirton[3]	15	1.009–1.049	–	26.1–45.4	–	–.85	$\%\ \text{Fat} = 100\left(\frac{5.680}{\text{sp. gr.}} - 5.138\right)$
Sheep, carcass	Kirton and Barton[35]	58	1.009–1.054	1.029	24.9–54.3	40.4	–.88	% Fat = 554.1 – 499.2 × sp. gr
Sheep, carcass	Garrett *et al.*[22]	20	1.036–1.075	1.055	14.3–37.5	23.5	–.90	% Fat = 556.6 – 505.0 × sp. gr.
Sheep, carcass	Khandekar *et al.*[34]	24	1.029–1.084	1.054	–	–	–.98	% Fat = 590.76 – 535.06 × sp. gr.
Sheep, carcass	Timon and Bichard[70]	83	–	1.047	–	–	–.93	% Fat = 603.7 – 550.1 × sp. gr.

[a] Specific-gravity values were obtained by air displacement and helium dilution and were not sufficiently accurate to use to predict fat content.

[b] Values were not calculated.

SOME THEORETICAL ASPECTS OF GAS-DISPLACEMENT TECHNIQUES

The involvement of a number of variables in estimating body volume by gaseous displacement means that there is an infinite number of possible systems. An attempt is made below to classify some of the forms of the two-chamber system, which is exemplified by the work of Liuzzo *et al.*,[43] and to assess ways in which its effectiveness can be or has been improved.

Increased effectiveness may be considered to be an increase in the accuracy with which the unknown volume can be measured, and it depends upon the following factors:

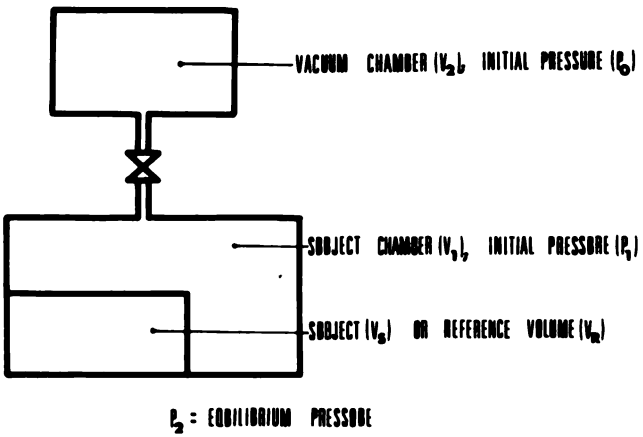
1. The accuracy of measuring the variable upon which the subject's volume is dependent. Pressure will be the only such variable considered herein.
2. The change in pressure per unit change in subject volume.
3. The accuracy with which variables influencing the pressure–volume relationship can be measured and utilized in corrections.

The accuracy of measuring pressure may be increased by improved instrumentation, but may also be influenced by the type of system. Although modifications are possible, the systems basically are either a simple two-chamber type or a single-chamber type.

Figure 1 shows a simplified diagram of the basic two-chamber system. In addition, two forms of the basic equation are presented. Equation 1 enables one to calculate subject volume from the equilibrium pressure and other known values. Equation 2 enables one to estimate the equilibrium pressure if the subject volume is known. This arrangement is considered both as a simple system (utilized directly by Liuzzo *et al.*,[43] Gnaedinger *et al.*,[23] and Kodama and Pace[37]) and as a modification by the suppressed zero system of Loh[44] and Hix *et al.*[28]

In the simple system (Figure 2), the subject volume (V_s) is estimated directly from the equilibrium pressure (P_2) by using Equation 1. The main disadvantage is that wide variations occur in P_2 if the subject volumes differ widely. Such variations could be reduced considerably by adding known volumes to the subject volume so that the total volume in the subject chamber varies as little as possible. However, an absolute pressure of at least half an atmosphere must be measured, and this is more difficult to accomplish than measurement of low differential pressures varying to the same extent.

With the suppressed zero system (Figure 2), the equilibrium pressure is estimated from a known reference volume (V_r) by using Equation 2. Then, ΔP (a low differential pressure) is measured directly, P_2 is estimated by dif-



BASIC EQUATIONS:

$$V_s = V_1 - V_2 \frac{P_2 - P_0}{P_1 - P_2} \quad \text{----------(1)}$$

$$P_2 = \frac{P_1|V_1 - V_s| + V_2 P_0}{V_2 + V_1 - V_s} \quad \text{----------(2)}$$

FIGURE 1 A diagram of the two-chamber system showing the symbols used and the basic equations enabling calculation of subject volume (1) or equilibrium pressure (2).

ference ($P_{2'} - \Delta P$), and V_s is estimated from P_2 by using Equation 1. By judicious selection of V_r, the difference in pressure (ΔP) can be kept small so that a more accurate and sensitive pressure-measuring device may be used than in the previous system. Loh[44] developed the concept of the suppressed zero system, which can be readily adapted to either the air-displacement or the helium-dilution method. Several variations are possible. For example, the mirror-image systems may be connected by a U-tube manometer so that $P_{2'}$ and P_2 can be produced and compared simultaneously; or a single system may be used in which the two pressures may be produced at different times. With the latter system, the pressure created ($P_{2'}$) on using the reference volume may be maintained on one side of the manometer, while the subject is used to create P_2.

The following generalizations may be made in regard to maximizing the pressure change per unit change in subject volume:

1. The response improves as the volume of the subject increases in proportion to the volume of the subject chamber.

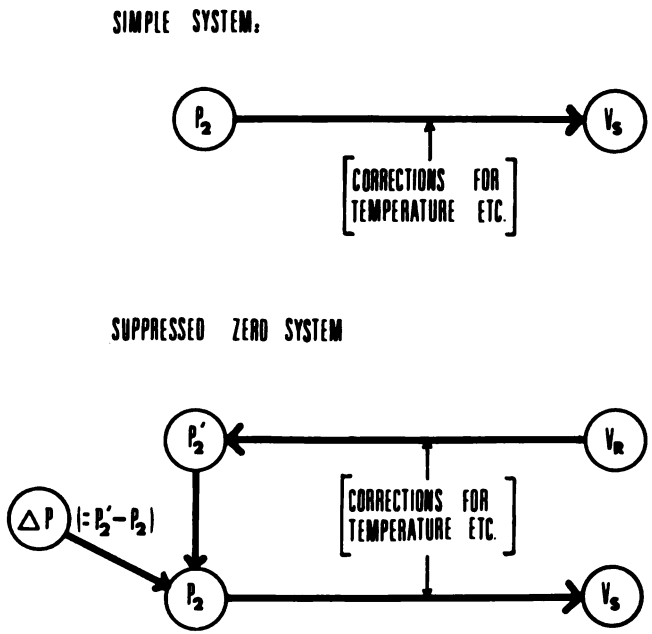


FIGURE 2 Flow diagrams showing the sequence of steps taken in calculating the subject volume by using a simple system and a suppressed zero system.

2. The response increases as the initial pressure in the vacuum chamber decreases. This means that the greatest possible vacuum should be drawn on the evacuation chamber. Low pressures also have an advantage in that they can be accurately measured more easily with instruments such as the McLeod gauge.

3. The response is affected by the relative volumes of the vacuum chamber and the subject chamber volume minus the subject volume. The best response is achieved when the two volumes are equal.[37]

4. The response increases as the final equilibrium pressure decreases.

From a practical viewpoint, however, the conditions employed are limited by the fact that the subject can withstand only a certain vacuum and can occupy easily only a limited proportion of the subject chamber. By giving these limits definite values and using the appropriate equation, optimum conditions can be estimated for particular cases.

The third and final influence on over-all accuracy that is discussed is the accuracy with which variables affecting the pressure–volume relationship can be measured or corrected, or both. A number of workers have analyzed the

importance of such variables.[23,44,65] Siri[65] also assessed the relative importance of biological and technological errors in the estimation of body composition from body density. His conclusions, based on a number of assumptions, included the claim that there is little to be gained from measuring density to an accuracy of more than 0.005 g/ml, when it is the only measurement being made. This gives an indication of the accuracy that is required. The two main variables affecting the pressure–volume relationship appear to be temperature and relative humidity. Liuzzo *et al.*[43] incorporated corrections for temperature in their equation but concluded that errors in measurement of temperature and relative humidity were the major sources of over-all error. After correcting for temperature and relative humidity, Gnaedinger *et al.*[23] still found only low relationships between body fat and estimated body density. They suggested that the movement of the animals in the chamber and the lack of accuracy in measuring temperature and humidity were the main sources of error. Hix *et al.*[28] also considered relative-humidity measurements to be a major source of error. Falkner[18] and Kodama and Pace[37] used extrapolation procedures to correct for changes in conditions that take place after the two chambers have been opened to each other. Such extrapolation procedures are commonly used with gas-dilution techniques as well.[65]

Although only a two-chamber system has been considered above, a single chamber may also be used quite effectively, as indicated by Beeston.[4] The basis of any gas-displacement technique is that the gas remaining in the sealed subject chamber when it is occupied by the subject can be treated in certain ways so that the effect of the treatment will depend directly on the volume occupied by this gas, and indirectly on the subject volume. With the two-chamber system, the treatments have generally involved opening the chamber to a second chamber that differs in pressure or gas concentration from the subject chamber. The measured effects are then the pressure change or the gas-concentration change, respectively.

With a single-chamber system, the treatment may involve a change in the volume of the chamber after it is sealed, as described by Beeston,[4] or it may involve the addition of gas to the chamber, as outlined by Foman *et al.*[20] In the former case, the effect measured is the pressure change, whereas in the latter, it may be the change in gas concentration or the change in pressure.

With the single-chamber system, Beeston[4] changed the volume of the sealed chamber containing the subject by pumping water into it. He then measured the difference in the volume of water required to reach a particular pressure with and without the subject present. Using this approach, he has reported a correlation of 0.97 between the specific gravity and total body fat of 14 mature sheep ranging in live weight from 14 to 55 kg.

Results such as these suggest that gas-displacement techniques are at least potentially useful, but there has been no definitive work done that enables identification of the most useful system. It seems, however, from the work reviewed here that it is possible to devise systems and to correct for important influencing factors such as temperature and relative humidity, so that the errors associated with density measurements are small compared with errors associated with the relationship between density and body composition.

SUMMARY

The rationale for estimating body composition from density has been reviewed along with the major procedures for estimating density. Although there are a great many pitfalls in measurement of density, it appears that one should be able to measure density accurately enough to make a reasonable estimate of composition. The relatively small differences in density between different body tissues and the variation within a single tissue would appear to be the limiting factors in accurately estimating composition from density.

REFERENCES

1. Babineau, L.-M., and E. Pagé. 1955. On body fat and water in rats. Can. J. Biochem. Physiol. 33:970.
2. Baker, P. T. 1966. Human bone mineral variability and body composition estimates, p. 69. *In* J. Brožek [ed.] Techniques for measuring body composition. Conf. Quartermaster Res. and Eng. Center, Natick, Mass., Jan. 22–23, 1959. National Academy of Sciences–National Research Council, Washington, D.C.
3. Barton, R. A., and A. H. Kirton. 1956. Determination of fat in mutton carcasses by measurement of specific gravity. Nature 178:920.
4. Beeston, J. W. U. 1965. Determination of specific gravity of live sheep and its correlation with fat percentage, p. 49. *In* J. Brožek [ed.] Human body composition; approaches and applications. Pergamon Press, New York.
5. Behnke, A. R. 1941–1942. Physiologic studies pertaining to deep sea diving and aviation, especially in relation to the fat content and composition of the body. Harvey Lect., Ser. 37:198.
6. Behnke, A. R. 1961. Comment on determination of whole body density and a resume of body composition data, p. 118. *In* J. Brožek [ed.] Techniques for measuring body composition. Conf. Quartermaster Res. and Eng. Center, Natick, Mass., Jan. 22–23, 1959. National Academy of Sciences–National Research Council, Washington, D.C.
7. Behnke, A. R., B. G. Feen, and W. C. Welham. 1942. Specific gravity of healthy men. J. Amer. Med. Ass. 118:495.

8. Bieber, D. D., R. L. Saffle, and L. D. Kamstra. 1961. Calculation of fat and protein content of beef from specific gravity and moisture. J. Anim. Sci. 20:239.
9. Böhnenkamp, H., and J. Schmäh. 1931. Untersuchungen zu den Grundlagen des Energie und Stoffwechsels. IV. Mitteilung das reinvolunmen Sowie die spezifirche Dichte des Menschen und die Bestimmungsweise dieser Grossen. Pfluger's Arch. 228:100.
10. Boyd, E. 1933. The specific gravity of the human body. Hum. Biol. 5:646.
11. Brown, C. J., J. C. Hillier, and J. A. Whatley. 1951. Specific gravity as a measure of the fat content of the pork carcass. J. Anim. Sci. 10:97.
12. Brožek, J., F. Grande, J. T. Anderson, and A. Keys. 1963. Densitometric analysis of body composition: Revision of some quantitative assumptions. Ann. N. Y. Acad. Sci. 110:113.
13. Brožek, J., and A. Keys. 1950–1951. Evaluation of leanness-fatness in man: a survey of methods. Nutr. Abst. and Rev. 20:247.
14. Buskirk, E. R. 1961. Underwater weighing and body density: A review of procedures, p. 90. *In* J. Brožek [ed.] Techniques for measuring body composition. Conf. Quartermaster Res. and Eng. Center, Natick, Mass. Jan. 22–23, 1959. National Academy of Sciences–National Research Council, Washington, D.C.
15. Da Costa, E., and R. Clayton. 1950. Studies of dietary restriction and rehabilitation. 2. Interrelationships among the fat and water content and specific gravity of the total carcass of the albino rat. J. Nutr. 41:597.
16. Doornenbal, H., G. H. Wellington, and J. R. Stouffer. 1962. Comparison of methods used for carcass evaluation in swine. J. Anim. Sci. 21:464.
17. Elsley, F. W. H., I. McDonald, and V. R. Fowler. 1964. The effect of plane of nutrition on the carcasses of pigs and lambs when variations in fat content are excluded. Anim. Prod. 6:141.
18. Falkner, F. 1963. An air displacement procedure for measuring body volume in babies. A preliminary communication. Ann. N. Y. Acad. Sci. 110:75.
19. Fidanza, F., A. Keys, and J. T. Anderson. 1953. Density of body fat in man and other mammals. J. Appl. Physiol. 6:252.
20. Foman, S. J., R. L. Jensen, and G. M. Owen. 1963. Determination of body volume of infants by a method of helium displacement. Ann. N. Y. Acad. Sci. 110:80.
21. Garn, S. M., and P. Nolan, Jr. 1963. A tank to measure body volume by water displacement (BOVOTA). Ann. N. Y. Acad. Sci. 110:91.
22. Garrett, W. N., J. H. Meyer, and G. P. Lofgreen. 1959. The comparative energy requirements of sheep and cattle for maintenance and gain. J. Anim. Sci. 18:528.
23. Gnaedinger, R. H., E. P. Reineke, A. M. Pearson, W. D. Van Huss, Janet A. Wessel, H. J. Montoye. 1963. Determination of body density by air displacement, helium dilution and underwater weighing. Ann. N. Y. Acad. Sci. 110:96.
24. Gnaedinger, R. H., A. M. Pearson, E. P. Reineke, and V. M. Hix. 1963. Body composition of market weight pigs. J. Anim. Sci. 22:495.
25. Goldman, R. F., and E. R. Buskirk. 1961. Body volume measurement by underwater weighing: Description of a method, p. 78.. *In* J. Brožek [ed.] Techniques for measuring body composition. Conf. Quartermaster Res. and Eng. Center, Natick, Mass., Jan. 22–23, 1959. National Academy of Sciences–National Research Council, Washington, D.C.
26. Goldman, R. F., B. Bullen, and C. Seltzer. 1963. Changes in specific gravity and body fat in overweight female adolescents as a result of weight reduction. Ann. N. Y. Acad. Sci. 110:913.

27. Hix, V. M., A. M. Pearson, and E. P. Reineke. 1964. Specific gravity of human subjects by air displacement and helium dilution. J. Appl. Physiol. 19:955.
28. Hix, V. M., A. M. Pearson, E. P. Reineke, T. A. Gillett, and L. J. Giacoletto. 1967. Determination of specific gravity of live hogs by suppressed zero techniques. J. Anim. Sci. 26:50.
29. Hodgman, C. D. 1961. Handbook of chemistry and physics. 43rd ed. Chemical Rubber Publishing Co., Cleveland, Ohio.
30. Holme, D. W., W. E. Coey, and K. L. Robinson. 1963. The prediction of pig carcass composition from measurements of carcass density. J. Agr. Sci. 61:9.
31. Huff, R. L., and D. D. Feller. 1956. Relation of circulating red cell volume to body density and obesity. J. Clin. Invest. 35:1.
32. Jongbloed, J., and A. K. M. Noyons. 1938. Die Bestimmung des wehren Volumens und des spezifischen Gewichtes von Menschen mittels Luftdruckveraanderung. Pfluger's Arch. 240:197.
33. Keys, A., and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:245.
34. Khandekar, V. N., W. R. McManus, and C. L. Goldstone. 1965. Some indices of the carcass composition of Dorset Horn top-cross lambs. II. Specific gravity as an index of the fat content of the carcass and of various joints. J. Agr. Sci. 65:155.
35. Kirton, A. H., and R. A. Barton. 1958. Specific gravity as an index of the fat content of mutton carcasses and joints. N. Z. J. Agr. Res. 1:633.
36. Kline, E. A., G. C. Ashton, and J. Kastelic. 1955. The effect of chilling time on the specific gravity of hog carcasses and upon the correlation between specific gravity and measures of fatness. J. Anim. Sci. 14:1231.
37. Kodama, A. M., and N. Pace. 1963. A simple decompression method for *in vivo* body fat estimation in small animals. J. Appl. Physiol. 18:1272.
38. Kohlrausch, W. 1929. Methodik zur quantitativen Bestimmung der Korperstoffe *in vivo*. Arbietsphysiol. 2:23.
39. Kraybill, H. F., O. G. Hankins, and H. L. Bitter. 1952. Body composition of cattle. II. Determination of fat and water content from measurement of specific gravity. J. Appl. Physiol. 4:575.
40. Kraybill, H. F., E. R. Goode, R. S. B. Robertson, and H. S. Sloane. 1953. *In vivo* measurement of body fat and body water in swine. J. Appl. Physiol. 6:27.
41. Lange, N. A. 1946. Handbook of chemistry. 6th ed. Handbook Publishers, Inc. Sandusky, Ohio.
42. Lim, P. T. K. 1963. Critical evaluation of the pneumatic method for determining body volume: Its history and technique. Ann. N. Y. Acad. Sci. 110:72.
43. Liuzzo, J. A., E. P. Reineke, and A. M. Pearson. 1958. An air displacement method for determining specific gravity. J. Anim. Sci. 17:513.
44. Loh, Y. C. 1956. Measurement of human body density. Sci. Lab. Rept. 4–28. Ford Motor Company, Dearborn, Mich.
45. Lynch, G. P., and G. H. Wellington. 1963. Predicting the whole body composition of living hogs from specific gravity determinations. Ann. N. Y. Acad. Sci. 110:318.
46. Messinger, W. J., and J. M. Steele. 1949. Relationship of body specific gravity to body fat and water content. Proc. Soc. Exp. Biol. Med. 70:316.
47. Morales, M. F., E. N. Rathbun, R. E. Smith, and N. Pace. 1945. Studies on body composition. II. Theoretical considerations regarding the major body tissue components, with suggestions for application to men. J. Biol. Chem. 158:677.
48. Moulton, C. R. 1923. Age and chemical development in mammals. J. Biol. Chem. 57:79.

49. Murray, J. A. 1922. The chemical composition of animal bodies. J. Agr. Sci. 12:103.
50. Myhre, L. G., and W. V. Kessler. 1966. Body density and potassium 40 measurements of body composition as related to age. J. Appl. Physiol. 21:1251.
51. Noyons, A. K. M., and J. Jongbloed. 1935. Uber die Bestimmung des wahren Volumens und des spezifischen Gewichtes von Mensch und Tier mit Hilfe von Luftdruckveraan derung. Pfluger's Arch. 235:588.
52. Osserman, E. F., G. C. Pitts, W. C. Welham, and A. R. Behnke. 1950. *In vivo* measurement of body fat and body water in a group of normal men. J. Appl. Physiol. 2:633.
53. Pace, N., and E. N. Rathbun. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158:685.
54. Pearson, A. M., L. J. Bratzler, R. J. Deans, J. F. Price, J. A. Hoefer, E. P. Reineke, and R. W. Luecke. 1956. The use of specific gravity of certain untrimmed pork cuts as a measure of carcass value. J. Anim. Sci. 15:86.
55. Pfaundler, M. 1916. Korpermass–Studien an Kinder. IV. Vom Korpervolumen und der Korperdichte. Z. Kinderheilk. 14:123.
56. Pfleiderer, H. 1929. Methodik des Bestimmung des spezifischen Gewichts am Lebenden (Antropopyknometrie). Klin. Wschr. 47:2191.
57. Pitts, G. C. 1956. Body fat accumulation in the guinea pig. Amer. J. Physiol. 185:41.
58. Pitts, G. C. 1962. Density and composition of the lean body compartment and its relationship to fatness. Amer. J. Physiol. 202:445.
59. Pitts, G. C. 1963. Studies of body composition by direct dissection. Ann. N. Y. Acad. Sci. 110:11.
60. Pradham, S. L., W. R. McManus, C. L. Goldstone, R. F. Hart, and V. N. Khandekar. 1966. Indices of carcass composition of Dorset Horn top-cross lambs. III. Relationships between chemical composition, specific gravity and weight of carcasses and joints. J. Agr. Sci. 66:41.
61. Price, J. F., A. M. Pearson, and E. J. Benne. 1957. Specific gravity and chemical composition of the untrimmed ham as related to leanness of pork carcasses. J. Anim. Sci. 16:85.
62. Rathbun, E. N., N. Pace. 1945. Studies on body composition. I. The determination of total body fat by means of body specific gravity. J. Biol. Chem. 158:667.
63. Robertson, J. 1757. An essay towards ascertaining the specific gravity of living man. Phil. Trans. Roy. Soc. (London) 50:30.
64. Schmidt, C. L. A. 1944. The chemistry of the amino acids and proteins. 2nd ed. Charles C Thomas, Springfield, Ill.
65. Siri, W. E. 1955. An apparatus for measuring human body volume. Univ. California Radiation Laboratory 3228, Berkeley.
66. Siri, W. E. 1956. Apparatus for measuring human body volume. Rev. Sci. Instr. 27:729.
67. Siri, W. E. 1956. Body composition from fluid spaces and density: analysis of methods. Univ. California Radiation Laboratory 3349, Berkeley.
68. Siri, W. E. 1961. Body volume measurement by gas dilution, p. 108. *In* J. Brožek [ed.] Techniques for measuring body composition. Conf. Quartermaster Res. and Eng. Center, Natick, Mass., Jan. 22–23, 1959. National Academy of Sciences–National Research Council, Washington, D.C.

69. Spivak, O. D. 1915. The specific gravity of the human body. Arch. Intern. Med. 15:628.
70. Timon, V. M., and M. Bichard. 1965. Quantitative estimates of lamb carcass composition. 2. Specific gravity determination. Anim. Prod. 7:183.
71. Walser, M., and S. N. Stein. 1953. Determination of specific gravity of intact animals by helium displacement. Comparison with water displacement. Proc. Soc. Exp. Biol. Med. 82:774.
72. Wedgewood, R. J., J. R. Breckenridge, and R. W. Newman. 1953. Measurement of body volume by air displacement. Federation Proc. 12:151.
73. Wedgewood, R. J., and R. W. Newman. 1953. Measurement of body fat by air displacement. Amer. J. Physiol. Anthropol. 11:260.
74. Welham, W. C., and A. R. Behnke. 1942. The specific gravity of healthy men. J. Amer. Med. Ass. 118:498.
75. Whiteman, J. V., J. A. Whatley, and J. C. Hillier. 1953. A further investigation of specific gravity as a measure of pork carcass value. J. Anim. Sci. 12:859.
76. Young, C. M., B. A. Gehring, S. H. Merrill, and M. E. Kerr. 1960. Metabolic responses of young women while reducing: Body fatness and nitrogen metabolism. J. Amer. Diet. Ass. 36:447.
77. Young, C. M., J. Blondin, R. Tensuan, and J. H. Fryer. 1963. Body composition studies of older women, thirty to seventy years of age. Ann. N. Y. Acad. Sci. 110:589.

W. N. Garrett
University of California, Davis

EXPERIENCES IN THE USE OF BODY DENSITY AS AN ESTIMATOR OF BODY COMPOSITION OF ANIMALS

Early experiments[41] indicated a similar chemical composition for morphologically similar animals. Other experiments in the nineteenth century observed the reduction in water content (percentage) that accompanied the fattening process in farm animals.[14,36] The classic work of Lawes and Gilbert[23,24] led Murray[31] to conclude that the chemical composition becomes obvious once the fat content is known. Later, Murray[32] and Moulton[30] confirmed the conclusion that the relative fatness of animals of the same species does not influence the composition of the fat-free body. These workers also found that the water content of the fat-free body decreased slightly with age. Since this early work, considerable evidence has accumulated to support the generalization that the percentages of water, protein, and ash are a remarkably constant proportion of the fat-free body of animals of the same age and species. Keys and Brožek[19] critically reviewed the information available in 1953.

From these generalizations it is apparent that one can consider that an animal consists of two compartments: fat and a fat-free portion. Accordingly, it is possible to derive an estimating equation based on body density if the density of fat and the fat-free portion is known. This is the approach used by

Rathbun and Pace[37] and Morales *et al.*[29] However, a statistical procedure relating body density or specific gravity to one of the two compartments (usually some measure of body fat) has been more common (see references 4–6, 11, 19, 22, 35, 40). The statistical procedure eliminates the need for any assumptions or knowledge regarding the density of fat or the fat-free material. However, the relationships between the various body components still must be known or estimated in order to resolve the gross body composition.

The purpose of this paper is to examine some of the relationships that have been determined between body specific gravity or density and the chemically determined components of the animal body. In the majority of cases, the relationship will, in fact, be between carcass specific gravity or density and either carcass composition or the composition of whole empty body. A very high relationship between the composition of the carcass and the composition of the empty body is to be expected because the carcass is a large portion of the total empty body. Results with sheep,[11] with swine,[12] and with cattle (reported later in this paper) indicate correlation coefficients on the order of 0.99 between percentage of carcass fat and percentage of empty-body fat.

SHEEP

The information in Table 1 and Figure 1 indicates some of the published relationships between carcass specific gravity and percentage of fat in the ovine. The correlation coefficients are all negative and statistically significant, but range from –0.49 to –0.98.

The data indicate a standard error of estimate of around 3% and a coefficient of variation of about 10% in predicting carcass fat from carcass specific gravity. By means of this estimate and a table of the minimum number of replicates necessary to demonstrate significant treatment differences (10% of the mean) at the 5% level of significance given in Paterson,[34] it is evident that about 9 replicates (animals) would be necessary to establish a significant difference of 10% of the mean in percentage of carcass fat from two experimental groups.

The experiments in which low correlation coefficients were obtained[9,20] resulted in regression equations that are considerably different from the bulk of the data (see Figure 1). Further analysis reveals that the mean specific gravity of fat and the fat-free body calculated from the seven other regression equations is 0.912 ±0.022 and 1.100 ±0.010, respectively (the value following the mean is the standard deviation). These values agree well with the density of lamb fat given by Fidanza *et al.*[8] and the density of fat-free tissue suggested by Behnke.[3] Similar values calculated from the two most

TABLE 1 Linear Estimates of the Relationship between Chemically Determined Carcass Fat (Y)[a] and the Carcass Specific Gravity (X) of the Ovine

Number and Description of Animals	Range in Chemically Determined Fat (%)	Temperature °C		Estimating Equation	Correlation Coefficient	Standard Error of Estimate (%)	Reference
		Water	Carcass				
53 ewes	24.9–61.7	17–20	11–12[b]	$Y = 554.1 - 499.2X$	−0.91	3.2	Kirton and Barton[19]
20 whiteface wether lambs	14.3–37.5	24–25	3–4[c]	$Y = 556.6 - 505.0X$	−0.90	2.6	Garrett *et al.*[11]
29 lambs	13.2–34.0	24–25	3–4[c]	$Y = 463.7 - 418.4X$	−0.84	2.6	Meyer[28]
20 crossbred wether lambs	25.2–40.6	15	[d]	$Y = 295.2 - 255.8X$	−0.56	3.3	Kirton and Barton[20]
30 crossbred lambs	13.6–37.9	24–25	3–4[c]	$Y = 770.69 - 707.05X$	−0.89	3.6	Spurlock and Bradford[39]
65 crossbred lambs[a]	34.1 ± 4.0[e,f]		[f]	$Y = 269.79 - 228.4X$	−0.49	3.5	Field *et al.*[9]
83 wether lambs[a]	27.9 ± 3.9[e]	11–20	11–20	$Y = 603.7 - 550.1X$	−0.93	3.0	Timon and Bichard[40]
66 lambs[a]	10.1–40.0	3–4	3–4	$Y = 590.76 - 535.06X$	−0.98	1.3	Khandekar *et al.*[18]
35 sheep[g]	14.2–48.6	24–25	3–4[c]	$Y = 632.8 - 578.7X$	−0.95	3.3	Garrett (unpublished, 1966)

[a] Physically separated fat.
[b] Surface temperature.
[c] Deep body temperature.
[d] Not recorded, but chilled overnight.
[e] Mean and standard deviation.
[f] Water and carcasses in 3 to 4°C cooler for 48 hours before specific gravity was measured.
[g] Density rather than specific gravity for this group of 15 lambs and 20 yearlings.

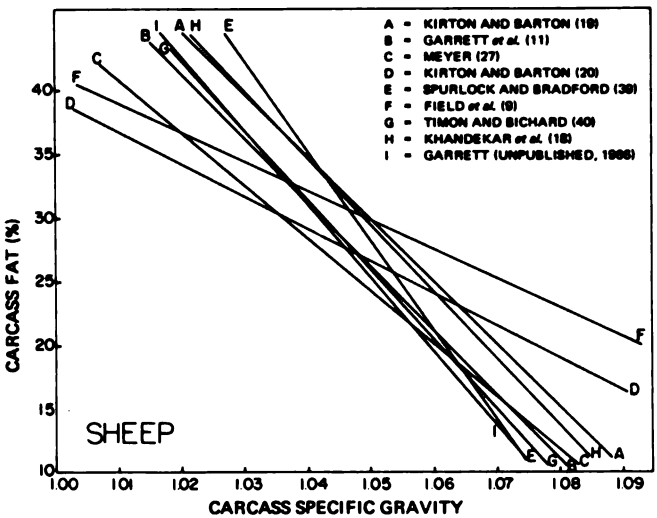


FIGURE 1 Linear estimates of the relationship between specific gravity and percentage of fat of the sheep carcass.

discordant regression lines average 0.75 as the specific gravity of fat and over 1.16 as the specific gravity of the fat-free body.

The theoretical regression line calculated by assuming the specific gravity of sheep fat to be 0.912 and the specific gravity of the fat-free body to be 1.100 is: percentage of carcass fat = 585.10 – 531.91 (carcass specific gravity). This regression line is shown in Figure 4 (page 181).

Meyer[27] used a multiple regression equation that included carcass weight as well as carcass specific gravity to estimate percentage of carcass fat. The multiple correlation coefficient was 0.96 with a standard error of estimate of 1.40. For this particular sample, the inclusion of carcass weight in the predicting equation reduced the coefficient of variation by almost 50%. However, this method does not always result in a significant reduction in the standard error of estimate. The data from 35 sheep (last line, Table 1) and the data of Spurlock and Bradford (reference 39; personal communication) were analyzed by the procedure used by Meyer.[27] There was no significant improvement in the correlation coefficients and only a 10% decrease in the standard error of estimate for these two samples.

In some experiments the energy per unit weight of carcass may be of more interest than percentage of carcass fat. The data from 35 sheep (last group, Table 1) indicate a correlation coefficient of –0.94 between carcass density and kilocalories per gram of carcass. The standard error of estimate was 0.29.

This results in a coefficient of variation of 7.6% and a reduction in the number of replicates necessary to demonstrate significant treatment differences of 10% of the mean to six. The multiple regression of carcass weight and carcass density against kilocalories per gram of carcass increased the correlation to 0.95 and reduced the standard error of estimate to 0.27. It is of interest that carcass weight by itself was correlated (0.79) with kilocalories per gram of carcass with a coefficient of variation of 14%. Carcass density was responsible in this study for reducing the coefficient of variation by 46% over the use of carcass weight alone to predict the kilocalories per gram of carcass. For comparison, Paladines *et al.*[33] reported coefficients of variation of 12% and 14% for two groups of sheep when total kilocalories of ingesta-free body was the dependent variable and weight of the ingesta-free body was the independent variable. Gardner *et al.*[10] report a correlation coefficient of 0.96 for a similar regression.

From these results it seems apparent that carcass density can be a useful predictor of carcass composition in the ovine. It is also clear that some investigators have found rather weak relationships between carcass specific gravity and percentage of carcass fat. One reason for this may be the range in fat content of the sample group. Some other reasons for this variability, as discussed by Meyer[27] revolve around the difficulty of removing the air sometimes found trapped beneath the fascia of a sheep carcass, especially carcasses from thin animals. Also, the temperature of the water and the carcass can affect the numerical value of carcass specific gravity.[17,21] No attempt has been made to correct all published data to an equivalent water and carcass temperature. The temperature differences shown in Table 1 could result in a slight shift in the regression line, but should not have a major influence on the correlation coefficient or the standard error of estimate.

CATTLE

Published information concerning the relationships between empty (ingesta-free) body or carcass composition and the specific gravity or density of the carcass or the empty body is limited. Kraybill *et al.*[22] determined the specific gravity of the carcasses and the empty bodies (less the blood, hide, and lungs) of 30 beef animals. The correlation coefficient between carcass specific gravity and empty-body specific gravity was 0.989. Empty-body specific gravity was correlated (–0.956) with percentage of physically separated fat. The standard error of estimate was not given, but it appears to be on the order of 2.0%. These relationships obtained by Kraybill *et al.*[22] and those of Reid *et al.*[38] have been used extensively by workers in California to estimate body

composition of beef cattle from carcass specific gravity.[11,25,28] Recent reports by Zinn *et al.*[42] and Albin *et al.*[2] indicate that the crude protein content of beef cattle estimated from carcass specific gravity by using the relationships established by Kraybill *et al.*[22] and Reid *et al.*[38] (as used by the California group) are higher than those determined by laboratory analysis. (The results of the study reported later in this paper support this observation.)

Guenther *et al.*[13] presented an equation to estimate fat content of a beef carcass based on the specific gravity of the carcass and the specific gravity of the fat and the lean body. A correlation coefficient of 0.928 was found between chemically determined fat and estimated fat by using the data from the 51 animals involved in determining the working equation. A correlation of 0.995 was obtained when the same equation was used to estimate the fat content of 18 other beef carcasses.

Preliminary results are presented from a study involving the chemical analysis of 48 beef steers approximately 15 ± 3 months of age. The relationship of the density of the right half of the carcass to right-half-carcass composition (chemically determined) and empty-body composition was studied. Table 2 shows the range in body composition of the animals. Some simple

TABLE 2 Range and Mean Composition of the Carcass and the Empty Body of 48 Beef Steers

Component	Carcass		Empty Body[a]		Correlation Coefficient[c]
	Range	Mean[b]	Range	Mean[b]	
Weight (kg)	146 - 322	216.5 ± 41.6	228 - 463	325.1 ± 57.0	0.99
Water (%)	44.4 - 63.0	52.3 ± 4.5	46.7 - 64.0	54.6 ± 4.4	0.99
Fat (%)	15.1 - 38.8	27.9 ± 6.0	14.6 - 35.7	25.1 ± 5.7	0.99
Nitrogen (%)	2.11 - 2.98	2.54 ± 0.24	2.25 - 3.01	2.69 ± 0.19	0.96
Ash (%)	3.08 - 5.16	4.23 ± 0.43	2.90 - 4.32	3.76 ± 0.31	0.96
Density (g/cc)	1.033 - 1.074	1.056 ± 0.011			
Energy (kcal/g)	2.43 - 4.43	3.48 ± 0.51	2.37 - 4.18	3.27 ± 0.48	0.99

[a] Entire animal without the contents of the gastrointestinal tract.
[b] Mean and standard deviation.
[c] Between the carcass and the empty body.

linear relationships between the chemically determined components of the beef carcass and the empty body with carcass density are shown in Table 3. All correlation coefficients between the major components of the carcass and similar components of the empty body were either 0.99 or 0.98.

The information in Table 3 indicates reasonably good correlations between chemically determined fat (ether extract) and carcass density. The correlation

TABLE 3 Some Simple Linear Relationships between Chemically Determined Components of the Beef Carcass and the Empty Body with Carcass Density (*X*)

Item	Correlation Coefficient	Standard Error of Estimate	Coefficient of Variation	Replicates to Measure Differences[a] 5%	10%	Estimating Equation
CARCASS PARAMETERS *(Y)*						
Ether extract (%)	−0.95	1.94	7.0	17	6	$Y = 587.86 - 530.45X$
Water (%)	0.91	1.89	3.6	7	3	$Y = 375.20X - 343.80$
Nitrogen (%)	0.93	0.09	3.5	6	3	$Y = 20.00X - 18.57$
Energy (kcal/g)	−0.94	0.18	5.1	9	4	$Y = 49.54 - 43.63X$
EMPTY-BODY PARAMETERS *(Y)*						
Ether extract (%)	−0.96	1.59	6.4	13	5	$Y = 551.38 - 498.50X$
Water (%)	0.93	1.60	2.9	5	3	$Y = 378.74X - 345.18$
Nitrogen (%)	0.92	0.08	2.9	5	3	$Y = 15.97X - 14.17$
Energy (kcal/g)	−0.95	0.15	4.5	8	4	$Y = 47.58 - 41.97X$

[a] As percentage of the mean at the 5% level of significance assuming a minimum of 30 degrees of freedom for the error variance.

coefficient (0.96) between carcass density and percentage of empty-body fat is almost identical with that (0.956) reported by Kraybill *et al.*[22] It is interesting, however, that carcass density is more highly correlated with empty-body parameters than with carcass parameters. No explanation is readily apparent for this finding, but it is consistent for each item.

Figure 2 compares the several equations given by Kraybill *et al.*[22] for predicting whole-body fat from a specific-gravity measurement and the regression equation given in Table 3.

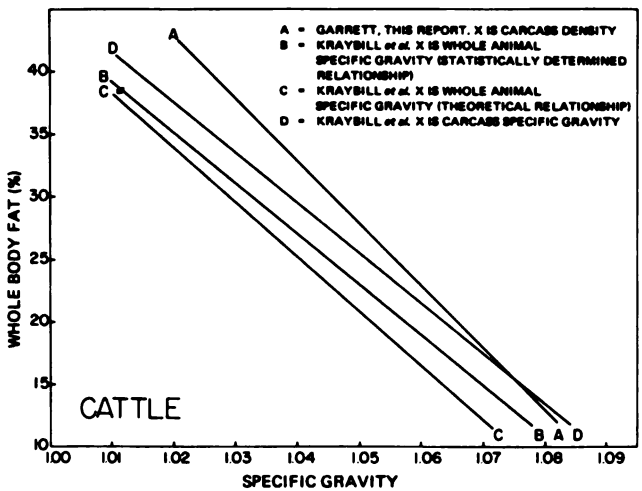


FIGURE 2 Comparison of some regression equations to predict the fat content of the beef animal.

Table 4 gives the results of a multiple regression analysis with carcass density and carcass weight as the independent variables. The addition of carcass weight was of no value in improving the precision of predicting either fat or energy content of the carcass or the empty body. Weight of carcass and the empty body were significantly correlated (all at about 0.7) to percentage of ether extract and kilocalories per gram of carcass or empty body, but the coefficients of variation were generally 50% higher than when carcass density was the only predictor.

It is possible that some slight but significant improvements can be made in predicting the various components of the body or the carcass from carcass density by the use of more complex estimating equations. Information on this point is unavailable at this writing.

TABLE 4 The Relationship between Carcass Weight (X_1),[a] Carcass Density (X_2), and Some Chemically Determined Parameters (Y) of the Carcass and Empty Body of Beef Cattle

Item	Multiple Correlation Coefficient	Standard Regression Coefficients X_1	X_2	Standard Error of Estimate	Coefficient of Variation	Estimating Equation
Carcass parameters *(Y)*						
Ether extract (%)	0.95	0.023	−0.93	1.95	7.0	$Y = 576.99 + 0.335X_1 - 520.84X_2$
Energy (kcal/g)	0.94	0.022	−0.92	0.18	5.2	$Y = 48.65 + 0.0275X_1 - 42.84X_2$
Empty body parameters *(Y)*						
Ether extract (%)	0.96	0.003	−0.94	1.60	6.4	$Y = 536.96 + 0.444X_1 - 485.75X_2$
Energy (kcal/g)	0.95	0.044	−0.92	0.15	4.6	$Y = 45.92 + 0.051X_1 - 40.51X_2$

[a]The units of carcass weight are hundreds of kilograms.

In general, comparable coefficients of variation resulting from the relationships between carcass density and various parameters of carcass composition were lower for cattle than for sheep.

SWINE

Several reports in the literature concern the relationship between carcass specific gravity and the chemical composition of the swine carcass. Some correlation coefficients of interest are shown in Table 5. Considerably more literature reports the use of specific gravity to measure the fat content of certain cuts or parts of the swine carcass. The comprehensive review by Hornicke[15] is an excellent source of information regarding many aspects of body composition in swine and other species.

The data in Table 5 indicate a similar trend in the correlation coefficients between specific gravity or density and the fat content of the swine carcass to those found with sheep and cattle.

The regression equations relating percentage of carcass fat to carcass specific gravity are shown in Figure 3. Line *F* in Figure 3 was determined with live hogs and was included for comparison but can be ignored in this discussion of the relationship between carcass fat and carcass specific gravity. In general, the regression equations are somewhat variable, similar to the situation with lambs discussed earlier (page 171). If the most discordant regression line (line *C*, Figure 3) is eliminated from consideration, a composite or theoretical line based on the specific-gravity value for swine fat (0.943 ± 0.014, mean and standard deviation) and the fat-free body (1.106 ± 0.010), as determined from the other regression equations in Table 5, can be calculated. The composite regression equation by this procedure is: percentage of carcass fat = 678.53 - 613.50 (carcass specific gravity). This line is shown in Figure 4.

Based on the data in Table 5, the standard error of estimate for predicting percentage of carcass fat in the swine carcass from carcass specific gravity is about 1.7%. With a mean fat content of 40%, the coefficient of variation is a comparatively low 4.3%. Thus, to demonstrate a 10% difference between means at the 5% level would require four replications.

DISCUSSION

On the basis of this review, a very high relationship is indicated between carcass specific gravity and the carcass fat content of our three major meat animal species. The standard errors of estimate attached to the regression

TABLE 5 Linear Estimates of the Relationship between Chemically or Physically Determined Fat Content *(Y)* and Specific Gravity of the Swine Carcass *(X)*

Number and Description of Animals	Range in Fat Content (%)	Temperature (°C)		Estimating Equation	Correlation Coefficient	Standard Error of Estimate (%)	Reference
		Water	Carcass				
66 Duroc hogs	54.6 ± 4.1[a]	–	–	–	−0.75	–	Brown *et al.*[4]
10 Berkshire hogs	35.8–46.9	–	4	–	−0.95	–	Doornenbal *et al.*[7]
20 hogs[b]	22.7–41.3	–	–	$Y = 536.3 - 493.8X$	−0.71	–	Lynch and Wellington[26]
42 large white	43.5 ± 4.9[a]	3–10	*c*	$Y = 68.3 - 0.062X^d$	−0.95	1.53	Joblin[6]
64 mixed	35.0 ± 4.4[a]	*c*	*c*	$Y = 60.2 - 0.058X^d$	−0.92	1.75	Joblin[6]
24 castrates	48.4 ± 4.7[a]	4–7	3–4	$Y = 296.7 - 237.4X$	−0.96	0.89	Adam and Smith[1]
52 hogs	36.9 ± 5.8[a]	4–7	4–7	$Y = 641.0 - 577.4X$	−0.95	1.72	Adam and Smith[1]
17 Duroc barrows	33.2–49.7	24	3–4	$Y = 694.96 - 630.00X$	−0.95	1.64	Hintz (unpublished, 1967)
41 crossbred	30.6–47.9	–	–	$Y = 100\ (\frac{7.244}{X} - 6.561)$	−0.92	2.0	Hornicke[15]

[a] Mean and standard deviation.
[b] Live hog density and whole-body percentage of fat.
[c] Probably 3 to 10°C. Corrections were made for variations in water temperature.
[d] X = [(specific gravity) − 1.000] × 10^4.

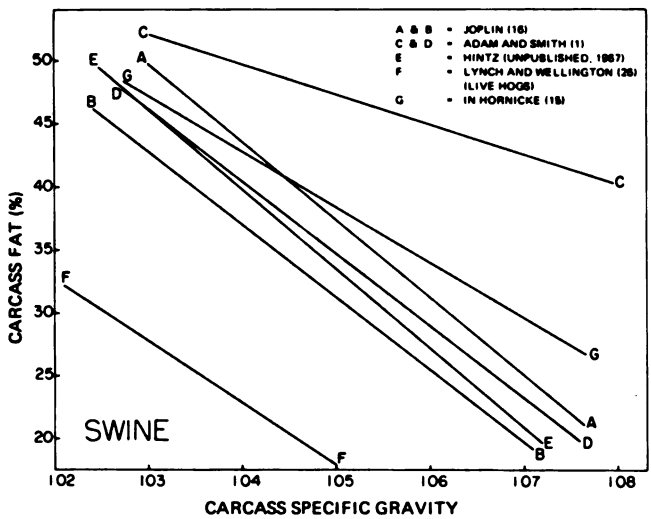


FIGURE 3 Linear estimates of the relationship between carcass fat and carcass specific gravity of swine.

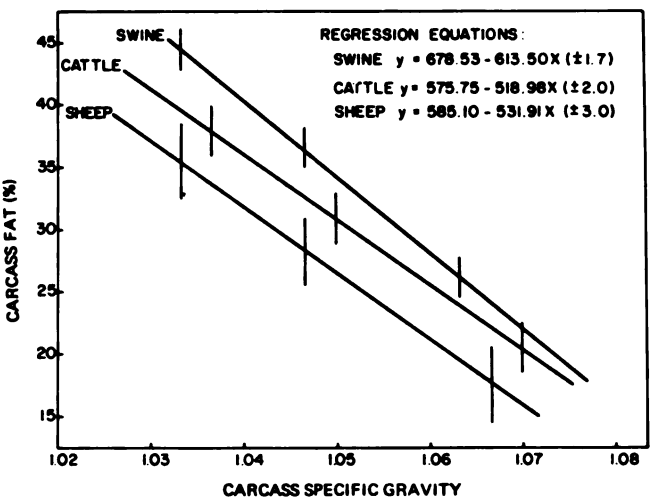


FIGURE 4 Comparison of regression equations to predict carcass fat from carcass specific gravity in three species.

equation are too high to be very precise in predicting the composition of an individual animal. However, in experiments where replication is possible, the use of specific gravity to demonstrate differences in body composition between groups of animals would be a valuable research tool.

Figure 4 shows a comparison of the "composite" regression equations between carcass specific gravity and percentage of carcass fat as determined in the three species. The standard errors shown indicate a slight increase in the precision of predicting carcass fat in swine compared with sheep and, to a lesser extent, cattle. Whether the differences between species are of physiological or technical origin cannot be determined with certainty from the data. Probably both physiology and method play a role.

Specific gravity is easily determined on the commercially dressed carcass. There is no monetary loss because the carcass remains intact and unharmed by the water. The measurement can be made at a cooperating slaughter plant using portable equipment just as easily as it can be done under laboratory conditions. The information obtained is useful in many experimental situations and does lead to more precise measures of animal response to experimental treatments.[11,25,27]

That different investigators (or the same investigator at different times) obtain slightly variable to markedly variable regression equations to predict body composition from carcass specific gravity of the same species must be recognized. Why this occurs is not apparent. It is unlikely that the major cause is physiological (although the influence of age on the composition of the fat-free body cannot be overlooked), but this has yet to be proved. Slight differences in water temperature cannot account for all the variation. Variable carcass temperatures, especially for fat carcasses, would have more influence on the relationship because the modulus of expansion of fat is on the order of 7 times that of water (calculated from the data of Fidanza *et al.*[8] and information in Hornicke[15]). Trapped air could be a major source of variation, but this could not account, for example, for the shift of line *C*, in Figure 3, which is in the opposite direction.

The following procedure is recommended with a view toward standardizing the density determination of animal carcasses:

1. Have the carcass deep tissue temperature at 3 to 5° C. In most coolers, this will occur after chilling for 36 to 48 hours.

2. If the water temperature is very different from the carcass temperature, make appropriate corrections in the calculated density.

$$\text{Carcass density} = \frac{\text{weight in air}}{\text{weight in air} - \text{weight in water}} \times \text{density of the water.}$$

3. Be very careful that trapped air is removed or released. This seems to be more of a problem in carcasses from which the hide has been pulled rather than cut.
4. Make the underwater weighing as rapidly as possible.

SUMMARY

Most of the information available regarding the use of carcass density or specific gravity to estimate carcass or empty-body composition of cattle, sheep, and swine indicates a moderate to high degree of usefulness, depending upon the parameter estimated and the species. The variation associated with predicting body or carcass composition from specific gravity or density measurements is too great to be very precise in determining the composition of a single individual. However, the technique is useful to detect reasonable differences (above 5% of the mean) in body composition between small groups of animals. The results to date indicate that the precision of the technique may be greater for swine and cattle than for sheep, but this cannot be considered a statement of fact. There has been considerable unexplained variation in the results from different investigators indicating a need, perhaps, for standardization of the technique.

REFERENCES

1. Adam, J. L., and W. C. Smith. 1964. The use of specific gravity and its reciprocal in predicting the carcass composition of pigs slaughtered at three weights. Anim. Prod. 6:97.
2. Albin, R. C., D. W. Zinn, S. E. Curl, and G. H. Tatsch. 1967. Growth and fattening of the bovine. III. Effect of energy intake upon carcass composition. J. Anim. Sci. 26:209.
3. Behnke, A. R. 1945. Absorption and elimination of gases from a body in relation to its fat content. Medicine 24:359.
4. Brown, C. J., J. C. Hillier, and J. A. Whatley. 1951. Specific gravity as a measure of the fat content of the pork carcass. J. Anim. Sci. 10:97.
5. Clawson, A. J., B. E. Sheffy, and J. T. Reid. 1955. Some effects of feeding chlortetracycline upon the carcass characteristics and the body composition of swine and a scheme for the resolution of the body composition. J. Anim. Sci. 14:1122.
6. Da Costa, E., and R. Clayton. 1950. Studies of dietary restriction and rehabilitation. II. Interrelationships among fat, water content and specific gravity of the total carcass of the albino rat. J. Nutr. 41:597.
7. Doornenbal, H., G. H. Wellington, and J. R. Stouffer. 1962. Comparison of the methods used for carcass evaluation in swine. J. Anim. Sci. 21:464.

8. Fidanza, F., A. Keys, and J. T. Anderson. 1953–1954. Density of fat in man and other mammals. J. Appl. Physiol. 6:252.
9. Field, R. A., J. D. Kemp, and W. Y. Varney. 1963. Indices for lamb carcass composition. J. Anim. Sci. 22:218.
10. Gardner, R. W., D. E. Hogue, and A. Bensadoun. 1964. Body composition and efficiency of growth of suckling lambs as affected by level of feed intake. J. Anim. Sci. 23:943.
11. Garrett, W. N., J. H. Meyer, and G. P. Lofgreen. 1959. The comparative energy requirements of sheep and cattle for maintenance and gain. J. Anim. Sci. 18:528.
12. Gnaedinger, R. H., A. M. Pearson, E. P. Reineke, and V. M. Hix. 1962. Contribution of body compartments to the composition of pigs. J. Anim. Sci. 21:981.
13. Guenther, J. J., J. A. Stuedemann, S. A. Ewing, and R. D. Morrison. 1967. Determination of beef carcass fat content from carcass specific gravity measurements. J. Anim. Sci. 26:210.
14. Henneberg, W. 1881. Uber Fleisch und Fettproduction in verschied-enem Alter und bei verschied-enem. Ernaehr. Z. Biol. 17:295.
15. Hornicke, H. 1961. Methoden zur Bestimmung der Korperzusammensetzung lebender Schweind. Z. Tierphysiol., Tierernaehr. Futtermittelk. 16:237; 267; 331; 344. *ibid.*, 17:28. 1962.
16. Joblin, A. D. H. 1966. The estimation of carcass composition in bacon-weight pigs. II. The use of specific gravity. N. Z. J. Agr. Res. 9:227.
17. Keys, A., and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:235.
18. Khandekar, V. N., W. R. McManus, and C. L. Goldstone. 1965. Some indices of carcass composition of Dorset Horn top-cross lambs. II. Specific gravity as an index of the fat content of the carcass and various joints. J. Agr. Sci. 65:155.
19. Kirton, A. H., and R. A. Barton. 1958. Specific gravity as an index of the fat content of mutton carcasses and various joints. N. Z. J. Agr. Res. 1:633.
20. Kirton, A. H., and R. A. Barton. 1962. Study of some indices of the chemical composition of lamb carcasses. J. Anim. Sci. 21:553.
21. Kline, E. A., G. C. Ashton, and J. Kasterlic. 1955. The effect of chilling time on the specific gravity of hog carcasses and upon the correlation between the specific gravity and measures of fatness. J. Anim. Sci. 14:1230.
22. Kraybill, H. F., H. L. Bitter, and O. G. Hankins. 1951–1952. Body composition of cattle. II. Determination of fat and water content from measurement of body specific gravity. J. Appl. Physiol. 4:575.
23. Lawes, J. B., and J. H. Gilbert. 1859. Experimental inquiry into the composition of some of the animals fed and slaughtered as human food. Phil. Trans., Pt. II:494.
24. Lawes, J. B., and J. H. Gilbert. 1861. On the composition of oxen, sheep and pigs and of their increase whilst fattening. J. Roy. Agr. Soc. Engl. 21:1.
25. Lofgreen, G. P. 1965. A comparative slaughter technique for determining net energy values with beef cattle. *In* K. L. Blaxter [ed.] Third symposium on energy metabolism. Academic Press, Inc., London.
26. Lynch, G. P., and G. H. Wellington. 1963. Predicting the whole body composition of living hogs from specific gravity determinations. Ann. N. Y. Acad. Sci. 110:318.
27. Meyer, J. H. 1962. Removing sources of error in lamb feeding experiments. J. Anim. Sci. 21:127.
28. Meyer, J. H., G. P. Lofgreen, and W. N. Garrett. 1960. A proposed method for removing sources of error in beef cattle feeding experiments. J. Anim. Sci. 19:1123.

29. Morales, M. F., E. N. Rathbun, R. E. Smith, and N. Pace. 1945. Studies on body composition. II. Theoretical considerations regarding the major body tissue components with suggestions for application to man. J. Biol. Chem. 158:677.
30. Moulton, C. R. 1923. Age and chemical development in mammals. J. Biol. Chem. 57:79.
31. Murray, J. A. 1919. Meat production. J. Agr. Sci. 9:174.
32. Murray, J. A. 1922. The chemical composition of animal bodies. J. Agr. Sci. 12:103.
33. Paladines, O. L., J. T. Reid, B. D. H. Van Niekerk, and A. Bensadoun. 1964. Energy utilization by sheep as influenced by the physical form, composition and level of intake of diet. J. Nutr. 83:49.
34. Paterson, D. D. 1939. Statistical technique in agricultural research. McGraw-Hill Book Co., Inc., New York.
35. Pace, N., and E. N. Rathbun. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158:685.
36. Pfeiffer, L. 1887. Uber den Fettgehalt des Korpers und verschiedener Theile desselben bei mageren und fetten Thieren. Z. Biol. 23:340.
37. Rathbun, E. N., and N. Pace. 1945. Studies on body composition. I. Determination of body fat by means of the body specific gravity. J. Biol. Chem. 158:667.
38. Reid, J. T., G. H. Wellington, and H. O. Dunn. 1955. Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. J. Dairy Sci. 38:1344.
39. Spurlock, G. M., and G. E. Bradford. 1965. Comparison of systems of lamb carcass evaluation. J. Anim. Sci. 24:1086.
40. Timon, V. M., and M. Bichard. 1965. Quantitative estimates of lamb carcass composition. II. Specific gravity determination. Anim. Prod. 7:183.
41. Von Bezold, A. 1857. Untersuchungen uber die Vertheilung von Wasser, organischer Materia und anorganischen Verbindungen im Tierreiche. Z. Wiss. Zoöl. 8:487.
42. Zinn, D. W., R. C. Albin, S. E. Curl, and C. T. Gaskins. 1966. Growth and fattening of the bovine. II. Post weaning protein and gross energy composition. Proc. West. Sec. Amer. Soc. Anim. Sci. 17:151.

Albert R. Behnke
University of California

INVITED DISCUSSION

The data and discerning analyses by Dr. Pearson and co-workers and by Dr. Garrett stimulate the following comments pertaining to the relationship of densitometry and body composition.

It is a simple procedure to determine by the Archimedean principle the volume of the human body and the volumes of carcasses of animals. There are few, if any, pitfalls in this straightforward technique. The difficulty is the translation of values for density into estimates of body fat. It is unfortunate that the scholarly criticism that has colored the literature in this field seems to have inhibited wide application, at least in man, of a basic corporeal parameter in somolytic analysis. Except for the systematic investigations of Grover Pitts and the limited, high quality analyses of Fidanza, Allen, and Krzywicki, and of Widdowson, Forbes, and Mitchell on cadavers, there are virtually no data relating tissue densities to chemical analysis.

METHODOLOGY

The progress from hydrostatic weighing to the helium-dilution technique as developed for man by Siri has been noteworthy, and so have been the more

difficult approaches to measuring the net volume of live animals. From the papers presented, it is certain that gas dilution, displacement, and pressurization techniques require scrupulous control of temperature and humidity. These logical and inviting avenues have not yet led to the goal of certain reliability in animal husbandry. A noteworthy advance by Beeston is the employment of water in a single chamber to reduce volume and subsequently, to determine residual volume by the hydrostatic-induced elevation of pressure on an adiabatic basis. The technique applied to anesthetized sheep appears to be highly satisfactory.

INTERPRETATION OF DENSITOMETRIC DATA

The accurate estimate of body fat from a single number substituted in an equation depends in large measure upon the degree of constancy of the density of the dichotomous fractions, fat and lean tissue. Lipid extracted from adipose tissue by solvents at specified temperature appears to be remarkably constant both in composition and in density for man and animals not fed unsaturated oils. The density of the lean mass is influenced by hydration and the ratio of muscle to skeletal mass. In this symposium, the matter of variation in water content of the lean mass has been brought up repeatedly. A clarifying distinction may be the difference between inbred strains of small animals carefully regulated as to diet and its mineral content, and the larger animal and man that have not been systematically controlled with respect to these variables. In the immature animal, in different species, and certainly in patients, there may be large differences in the percentage of body water referred to lean mass. It would be naive not to take into account factors that earlier could not be incorporated into a single equation, namely, hydration of the newborn, age and species, state of health, and environmental temperature. An important contribution by Siri was his estimate of body fat in man from a combined determination of total body water and of density.

It is instructive to calculate the error in fat estimates if the density of the lean mass is altered either by increase in skeletal mass or hydration. If mean *d lean* is taken as 1.100, and mean lean body weight (LBW) as 62 kg, skeletal weight including marrow will be approximately 10 kg (16.1%). An increase of bone weight to 12 kg (+ 20%) will raise *d lean* to 1.108. On the other hand, if body hydration were increased by 4 liters (about 10%), *d lean* would be reduced to 1.093. If *d fat* is constant (0.90), then from Siri's equation, percentage of fat = (495.0/*d body*) – 4.500, under conditions where *d lean* is 1.100. If the density of an individual is 1.050, then from the equation percentage of fat = 21.4; if *d lean* were 1.108 (increased skeletal mass), and the equation

adjusted to accommodate this constant, then percentage of fat = 23.9; if *d lean* were 1.093 (overhydration) and if the equation were altered, then percentage of fat = 19.1.

EQUATIONS

In the hyperbolic equation as initially formulated by Morales *et al.*, $F/W = (a/d\ body) - b$, one can derive by extrapolation toward each asymptote the respective densities of the two basic components, fat and lean, from the constants a and b. In the linear regression equations frequently formulated, the values of the prime constants are "concealed" and cannot be derived from extrapolation to 0% fat on the one extreme and 100% fat on the other hypothetical limit.

The equation of Keys and Brožek formulated in 1953 and widely used for the calculation of human fat [$F/W = (4.201/d\ body) - 3.813$] yields, on extrapolation, a value of 0.873 for *d fat*, which is far below the contribution from their laboratory of 0.901, determined chemically for human fat. This equation subsequently was corrected, and it is essentially Siri's formulation for the computation of fat from density alone. One may derive nearly identical percentages of fat by substitution of specific gravity (d + 0.005 or 0.006, water at skin temperature, approximately 31 to 34° C) in the Rathbun-Pace formula. Essentially, without benefit of formula, one may employ our first approximation, namely, that 0.002 specific gravity (sg) units are equivalent to 1% of fat.

Nevertheless, one is overwhelmed by the number of equations cited in the papers presented for the simple case of carcass fat from one species. One may take five equations listed in Table 2, page 175, as an example of the calculation of fat in the sheep's carcass. In the range of fat between 20 and 40% of body weight, the relationship to density is closely linear so that no significant error is introduced by a shift from the hyperbolic to the linear regression expression. The following percentages of fat were derived from each of the five equations for densities of 1.060, 1.040, and 1.020:

DENSITY	EQUATION				
	1	2	3	4	5
1.060	22.1	23.9	21.3	24.8	20.6
1.040	32.8	34.9	31.4	36.4	31.6
1.020	43.1	44.9	41.5	47.1	42.6

ACCURACY OF ESTIMATE OF FAT FROM DENSITOMETRY

If, by inspection, one is able to judge the percentage of fat in the animal carcass (range 20 to 50%) with an error of perhaps no more than 5%, then the range of variation by use of the different formulas approaches the 5% level. What is not known, except for derivation from hyperbolic equation 1, are the density values for extracted lipid and, more important, for the lean mass. From equation 1, the extrapolations are: *d fat*, 0.905 and *d lean*, 1.106. Part of the variation in the equations may be accounted for by temperature levels and differences between water and the carcass. Encouraging are the standard errors of estimate of 2.6 to 3.6% for the ovine, in Garrett's Table 1 (page 172) for carcass fat determined by hydrostatic weighing and by chemical analysis.

CONCLUDING REMARKS

The volume of the carcass can be measured with an accuracy of 1 part in 1,000 (± 0.001) and the percentage of fat to ± 0.5%, if *d fat* and *d lean* are constant. To attain this degree of accuracy it is necessary to compile data for *d fat* and *d lean* in animals classified according to species, age, conditions of hydration and diet, and temperatures of water and carcass at the time of underwater weighing. In man, Siri has estimated that the error in percentage of fat calculated from density alone might be ±4% for wide variations postulated for *d fat* and for *d lean*. If total body weight is measured, the error of estimate of fat is reduced to ±2%, but these estimates of error rest on a tenuous basis of only a few determinations of *d fat* and *d lean*.

The present requirement calls for data on tissues, organs, and carcasses that relate specific gravity or density to chemical analysis of components. In turn, the chemical analyses require scrutiny since it may be mistakenly assumed that such analysis is subject to less error in replication than the indirect determinations. We have passed through the initial concept stage—the second period of more precise definition and scholarly exposition of limitations—to present quantitative analysis, which provides reliable statistical weighting of the various somolytic parameters involved in densitometry.

A. M. Pearson, Presiding

GENERAL DISCUSSION

G. C. PITTS I would like to make just one comment with respect to the anchor points of the regression line relating body density to body fat. I certainly agree with Captain Behnke that with no storage fat, the value of 1.100 is a good one. I would question whether the other anchor point of this regression line, 0.900, is a good one. Imagine a hypothetical individual who continues fat accretion *ad infinitum*. He is going to approach an asymptote that is not the density of pure fat, but will be the density of adipose tissue, containing about 80% of body fat, so that I would suspect that the value of 0.935 you quoted somewhat earlier might be close to the density of adipose tissue with 80% of body fat.

A. M. PEARSON Comment, Dr. Behnke?

A. R. BEHNKE This was considered very, very carefully by the Minnesota group, and it is true. As a consequence, we assume that the dry matter in adipose tissue does not change the 1.100 value appreciably, in that the real constant is the density of the lipid material.

A. M. PEARSON There will be a comment by Dr. Lesser.

G. LESSER I think Dr. Pitts himself may have provided a better answer for that question than any of us can give. I think he demonstrated quite well with his guinea pigs some years ago that what was laid down with changes in weight was, at one level of body-fat content, almost pure lipid, and that after a certain break point, perhaps at 25 to 30% of fat in guinea pigs, it appeared that new lean tissue was laid down as well. If I remember, your curve of adipose tissue showed that it was pure lipid being laid down. We do not have the amount of data that you have, but we feel that much the same thing occurs in humans. So, it may depend on what level of gain or loss or what level of body fat content one is talking about to be able to say whether that number (density) should be 0.900 or 0.934, at least insofar as differences are concerned, because if one subject is 25 to 30% fat, and one is comparing it with a heavier one, or if that individual gained fat, what is being laid down might well be 0.934. If one is talking about going from 10 to 15%, what is being laid down may be 0.900, so that it may not be a total linear relationship in the way that we lay down fat.

A. M. PEARSON One more question here.

M. F. BALL I am not known to most of you. I am part of Dr. Kyle's group in Washington. I would like to speak to the point of Dr. Siri's method of measuring helium. As Dr. Novak can comment also, measurement of and appreciation of the effect of temperature, carbon dioxide, and water-vapor pressure will significantly affect comparison and measurement of density by this technique. If you do not particularly take note of the effects of these three variables in your system it will not be comparable to underwater weighing, and you will have a significantly spuriously low measurement of body density.

A. M. PEARSON Thank you for the comment.

H. J. KRYSWICKI I have a question for Dr. Garrett. Why do you animal men insist on using water for measuring specific gravity of the carcass? We reported in 1959 (Dr. Allen, Roberts, and I) on the use of such organic solvents as kerosene and cellusol. There is no appreciable loss of fat, while the carcass leaves a little tissue, from 0.5 to 1 g—even a smaller piece than that. We worried about the leaching of the tissue of fat; but even then, none was there when we evaporated the kerosene to dryness.

A. M. PEARSON Would someone like to comment on that?

W. N. GARRETT I think the answer is very simple. We are working on a rather limited budget, and if we would try to sell the carcasses after being dipped in kerosene, I think we would run into some consumer problems, not to mention some problems with the USDA meat inspectors.

DILUTION METHODS

.

S. L. Hansard
Louisiana State University

DILUTION METHODS IN BODY-COMPOSITION STUDIES

The distinct advantage of being able to make periodic, rapid, and nondestructive quantitative measurements of yield in the growing or fattening animal is generally appreciated. Practical and specific application of this type of information in the various agriculture and medical disciplines is well recognized. It is anticipated, therefore, that during the progress of this symposium the relative merits and the status of the various methods currently being employed for partitioning body composition will be discussed and re-evaluated.

The most productive and promising procedures for quantitating body composition indirectly have been based on the dilution principle. This technique is not new, but it was recognized as a powerful tool for fundamental analyses and volume estimations long before radioisotopes were in common use. Its application in biological studies has been obvious where it is undesirable to disturb the body system or when it is impossible to isolate quantitatively or to measure unknown volumes directly.[12]

With the added glamour and increased sensitivity from atomic energy application, the "isotope-dilution" procedure has become widely accepted for the study of body processes and has contributed much to our present knowledge of body composition.[12,19] The principle of the method and its

simplicity are demonstrated in Figure 1. In short, the procedure consists of uniform incorporation of a small quantity of the labeled test substance into materials to be analyzed and sampling for analyses or isolating and measuring the isotope content in a known volume.

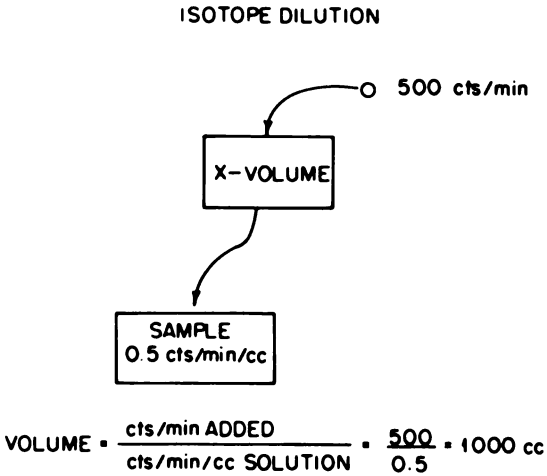


FIGURE 1 Use of the isotope-dilution principle for volume measurements.

Various modifications of this procedure have been employed in tracer studies for quantitating body compartments, for measuring the contribution of two or more sources to a product,[12] and for analytical methods involving the "inverse" isotope-dilution procedure[15,19] or the "double" isotope-dilution procedure.[1]

For dilution procedures in general, the experimental errors almost invariably lead to high values. Some of the more important sources of error include nonquantitative injection of test substance, accelerated metabolism or loss of label from the system, and inadequate mixing and sampling for analyses.

Hevesy and Hofer[13] introduced and pioneered the isotope-dilution technique in 1934 by measuring and tracing the fate of heavy water in the human body. However, its analytical value was not fully appreciated until Rittenburg and Foster[21] employed the method for separately measuring palmetic acid in animal fat and demonstrated the use of ^{15}N for the analysis of amino acids in body protein. Ussing[23] further revived interest in the procedure with his leucine-hemoglobin analyses and demonstrated its practical application to chemistry and biology. Neither space nor time permits a chronological annotation of the many reports in the literature dealing with this powerful proce-

dure. However, periodic reviews have established relevant developments that sustain our confidence in its adaptation for the solution of many of our problems relating to biological parameters. The first early critical discussion of this method was reported in 1941 by Flagg and Wiig.[7] Süe[22] made one of the initial reports on the use of a radioisotope in the isotope-dilution procedure with his isolation of ^{42}K and the subsequent calculation of specific activity in a mixture. In the same year, Moore[17] discussed total body water measurements, using deuterium oxide, and total body solids, using radiosodium and ^{42}K. Gest *et al.*[9] and Radin[19] presented equations for isotope-dilution analyses and have reviewed and discussed the validity of these procedures. Further reviews of the early developments and application of the isotope-dilution methods and the criteria of purity have been discussed by Yankwich[24] and by Berson.[2]

More recently, technique applications for separately measuring body fat, water, and fat-free components of body composition have been presented and evaluated in several symposia.[3,4,5] The use of these procedures for animal response measurements[11] in terms of animal growth and the differential measurement of body weight gains as pounds of fat, muscle, bone, and water for body calorimetry[8,10,20] and application for total body composition with farm animals have been discussed.[11,18,20] For the most part, however, more questions than answers have been presented, and the challenge for procedure perfection continues as a major problem in discussions of body composition.

The fundamental reference point for all indirect methods of body partition, of course, is provided by direct chemical analyses. This unique advantage and opportunity for both direct and indirect measurement procedures for defining structural as well as metabolic and physiological parameters in the same animal provides a real challenge to the animal scientist for leadership in method perfection. Current biological assumptions, range in data variability, and specific relationships to the whole animal, carcass, and empty-body analyses require confirmation to strengthen the link between *in vivo* and *in vitro* measurements.

In order to advance our knowledge of gross body composition, it is necessary that large numbers of normal animals be analyzed by the several indirect methods concurrently, specifying environment, age, condition, sex, breed, activity, conformation, and so on, and that these data be intercompared with the reference analytical values obtained by chemical analyses. Total body water or red-cell mass can be measured by many methods, all of which involve the dilution procedure.[12,16] Yet the range between laboratories indicates much to be gained by method and animal standardization.

Although results from the dilution procedure have been shown to agree favorably with composition data from other procedures,[6,14,16,18] much information is needed to support required biological assumptions, to account

for ranges in variability, and to confirm specific relationships in whole-body analyses.

Total body water calculations are based on the precept that water constitutes a constant fraction of the animal body mass. Yet reported fat-free water values for the various animal species range from 72 to 76% of the body weight.[2] The fact that body hydration continues to change during growth requires that consideration be given to more than one formula for estimating fat-free mass. Relationships of thyroid status and basal metabolic rate to red-cell mass need further clarification to establish method accuracy for predictions of lean body mass. We need more information on that composition being deposited and that being lost; we must determine the range of weight gains and weight reductions in which the mobile tissue will retain a constant composition and density; we need fat-composition values on tissues (cells and extracellular fluids) that account for differences in body weight and the "quality" of individual muscles. Although dilution procedures provide means for calculating total body fat, a suitable and sensitive direct method for measurements in the intact animal is needed.

It will be further evidenced in the discussions of this symposium that the dilution procedure is still a powerful tool in quantitating total body composition, for broadening our understanding of the basic and applied problems, and for procuring the needed comparative data for standardizing "biological constants" to strengthen our relationships between methods and between *in vivo* and the more tedious *in vitro* procedures.

REFERENCES

1. Armstrong, W. D., J. A. Johnson, L. Singer, R. I. Lienke, and M. L. Premer. 1952. Rates of transcapillary movement of calcium and sodium and of calcium exchange by the skeleton. Amer. J. Physiol. 171:641.
2. Berson, S. A. 1956. Some applications of isotope dilution techniques. Amer. J. Roentgenol., Radium Ther. Nucl. Med. 75:1059.
3. Brožek, J. [ed.] 1963. Body composition. Ann. N. Y. Acad. Sci. 110:1-1018.
4. Brožek, J. [ed.] 1965. Human body composition. VII. Symposia of the society for the study of human biology. Pergamon Press, New York.
5. Brožek, J., and A. Henschel [ed.] 1961. Techniques for measuring body composition. National Academy of Sciences–National Research Council, Washington, D.C.
6. Forbes, G. B. 1962. Methods for determining composition of the human body, with a note on the effect of diet on body composition. Pediatrics 29:477.
7. Flagg, J. F., and E. O. Wiig. 1941. Tracer isotopes in analytical chemistry. Ind. Eng. Chem., Anal. Ed. 13:341.
8. Garrett, W. N., J. N. Meyer, and G. P. Lofgreen. 1959. The comparative energy requirements of sheep and cattle for maintenance and gain. J. Anim. Sci. 18:528.

9. Gest, H., M. D. Kamen, and J. R. Reiner. 1947. The theory of isotope dilution. Arch. Biochem. 12:273.
10. Gnaedinger, R. H., A. M. Pearson, E. P. Reineke, and V. M. Hix. 1963. Body composition of market weight pigs. J. Anim. Sci. 22:495.
11. Hansard, Sam L. 1956. Tracer methods in mineral nutrition research. US-AEC-TID. 7512:31.
12. Hansard, S. L. 1963. Radiochemical procedures for estimating body composition in animals. Ann. N. Y. Acad. Sci. 110:229.
13. Hevesy, G., and E. Hofer. 1934. Elimination of water from the human body. Nature 134:879.
14. Kay, M., and A. S. Jones. 1962. The relationship between body density, body fat and body water in living pigs. Anim. Prod. 4:296.
15. Keston, A. S., S. Underfriend, and R. K. Cannan. 1946. Micro-analysis of mixture (AA) in the form of isotope derivatives. J. Amer. Chem. Soc. 68:1300.
16. Keys, A., and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:245.
17. Moore, F. D. 1946. Determination of total body water and solids with isotopes. Science 104:157.
18. Pearson, A. M. 1965. Body composition. *In* A. Albanese [ed.] Newer methods of nutritional biochemistry. Academic Press, New York.
19. Radin, N. S. 1947. Isotope techniques in biochemistry. Nucleonics 1(2):48.
20. Reid, J. T., G. H. Wellington, and H. D. Dunn. 1955. Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. J. Dairy Sci. 38:1344.
21. Rittenburg, D., and G. Foster. 1940. A new procedure for quantitative analysis by isotope dilution, with application to the determination of amino acids and fatty acids. J. Biol. Chem. 133:737.
22. Süe, P. 1946. Quantitative analysis by the variation of the specific activity of an added radioactive isotope. Nature 157:622.
23. Ussing, H. H. 1939. Analysis of protein by means of deuterium-containing amino-acids. Nature 144:977.
24. Yankwich, P. 1949. Radioactive isotopes as tracers. Anal. Chem. 21:318.

B. A. Panaretto
The Ian Clunies Ross
Animal Research Laboratory,
Prospect, Australia

ESTIMATION OF BODY COMPOSITION BY THE DILUTION OF HYDROGEN ISOTOPES

The scientific knowledge used to calculate the quantities of fat and protein in the bodies of living domestic animals has evolved from studies on carcass composition. Some early studies were made by Lawes and Bilbert,[44,45] von Bezold,[100] Moulton *et al.*,[65,66,67] and Moulton.[64]

Body-composition studies in man, on the other hand, developed without much analytical information on the composition of cadavers.[19,58] Some information has been obtained from patients who were clinically abnormal at death.[103] In spite of the small amount of this information, and relying often on extrapolation of results obtained from monogastric experimental animals to man (e.g., the analyses of dogs, monkeys, and rabbits for their water and elemental constituents Na, K, Cl, P and N by Harrison *et al.*[28]), clinicians have carried out a vast amount of research in the field of body composition in the healthy and in the sick.

Although composition of the carcasses of animals has since been studied in great detail (see reference 15, for example), these early observations provided the basis for present work. Attention was drawn early to the fact that fat animals contained proportionally small amounts of water.[64,69] The direct relation between nitrogen and water observed in carcasses in 1923 by Moulton[64]

provided an early clue to the suggestion that water is associated with maintaining the structure of proteins in tissues.[4,38]

It is unnecessary to make more than a passing reference to Moulton's concept of "chemical maturity," and it is sufficient to say that a large amount of evidence supports the generalization that the percentages of water, protein, and minerals approach constancy in the fat-free bodies of animals after "chemical maturity." Moulton[64] showed that animals are "chemically mature" after approximately 4.0% of the life-span has been expended. For his calculations, Moulton used time from conception to "chemical maturity" and an average figure for life-span derived from the most reliable sources then available.

Generally, then, the hypothesis derived from early investigations is that the animal body consists of two phases: the fat and the fat-free masses. The latter phase contains water in a fixed proportion (73 to 74%), and the fat-free dry matter is approximately 80% protein. If accurate measurements of water or fat can be made in the live animal, it follows that the body composition can be estimated with precision. Consequently, the measurement of body water by markers and the measurement of fat by fat-soluble gasses or specific-gravity techniques have constituted the major effort in the field for the last 20 years.

ESTIMATION AND CALCULATION OF DEUTERIUM OXIDE AND TRITIATED WATER SPACES

Deuterium was discovered in 1931 and became available in appreciable quantities for research in about 1934.[22]

By administering 50 to 100 ml of 99.5 to 99.8% D_2O in a suitable volume of carrier, an approximate concentration of 0.25 to 0.5 vol % deuterium oxide is obtained. Administration can be oral or parenteral. Blood samples are taken after the marker has come into equilibrium with the total body water. Water is obtained from the biological samples by vacuum distillation and is analyzed against standards for its content of marker by using either the falling drop method, described by Chien and Gregersen,[9] or mass spectroscopy, described by Solomon *et al.*[94] and Wentzel *et al.*[101] The dilution of the marker (the "space") is calculated and is corrected to allow for exchange between marker hydrogen ions and tissue hydrogen. The analytical errors when the above methods are used appear to be low. A standard deviation of 0.0012 vol %, in serial determination at a concentration of 0.305 vol %, which corresponded to ± 200 ml in total body water, was found for the falling drop method.[88,89] Wentzel *et al.*[101] reported a standard deviation of ± 2% between duplicates in their analyses with the mass spectrometer.

Tritium was discovered in 1939,[1] and until the widespread use of liquid scintillation counting, with the discoveries of Reynolds *et al.*[87] and Kallman and Furst,[37] analysis necessitated the conversion of the tritium to a gas (e.g., butane or hydrogen) that could be introduced into an ionization chamber, Geiger-Müller tube, or proportional counter. During the last 10 years, it appears to have become the analytical method of choice because of the tremendous growth of liquid scintillation counting. Briefly, 0.5 to 1.0 mc in a suitable volume is given to an organism in the weight range of 20 to 100 kg. Blood samples are taken after equilibration has been reached with the total body water. Water obtained by vacuum distillation is counted against standards in a toluene–ethanol mixture, containing scintillators, in a liquid scintillation counter. The "space" for the marker is calculated and corrected for hydrogen ion exchange. Three sources of error are involved in deriving the space: first, errors can arise from incomplete administration of the dose; second, there can be errors in pipetting while preparing the sample for counting; and third, there is the counter error. Overall, the analytical errors are of the order of ± 2% of total body water.

Finally, no correction has been made above for the amount of marker lost in the urine, sweat, and expired air from the animal between injection and equilibration. In ruminants housed at an environmental temperature of about 70° F, the equilibrium specific activity (6 hr) is probably within 1% of the value if mixing is instantaneous.[98] Hence, correction is not necessary unless spaces are calculated at unduly prolonged intervals.

BODY-COMPOSITION STUDIES WITH HYDROGEN ISOTOPES IN MAN

DEUTERIUM OXIDE IN BODY-COMPOSITION INVESTIGATIONS

The initial impulse to measure the mammalian body's water content with an isotope arose in a curious way. This was recorded in 1934 by Hevesy and Hofer:

> Shortly after the first application of radioactive isotopes as indicators the late H. J. G. Moseley and one of the present writers discussed the prospect opened by the introduction of this method when indulging in a cup of tea at the Manchester Physics Laboratory. The latter then expressed the wish that an indicator might be found that would allow one to determine the fate of the individual water molecules contained in the cup of tea consumed. Even a man of the vision of H. J. G. Moseley considered this hope to be a highly Utopian one.[32]

However, these authors obtained some heavy water and carried out two

experiments on themselves. They consumed the isotope and subsequently measured its concentration in the urine. After allowing for the amount of water lost by transpiration and evaporation (the weather was hot), they estimated the time an average water molecule spends in the body and also calculated the deuterium oxide space. In further studies, they calculated the deuterium oxide spaces in fishes and the exchange reactions between the tissue hydrogen of fish and the heavy water in its environment. Almost simultaneously with these reports, McDougall *et al.*[53] reported the absorption of deuterium oxide from the gut of two rats and calculated the space by assuming the complete distribution of the marker 1 hr after its injection.

A large amount of work then followed; measurements of a number of spaces thought to measure the total body water were made with a wide variety of substances, including urea, thiourea, and sulphanilamide. This proliferation of markers was probably due to the unavailability of heavy water and to lack of analytical facilities to measure it. For a variety of reasons, all substances investigated, apart from deuterium and tritiated water, have since been discarded, and interest in them is now historical.

The references given below in relation to the measurement of total body water with deuterium oxide and tritiated water (TOH) are not exhaustive, but they suffice to support the discussion. It is apparent that the use of deuterium oxide as a marker for body water was most popular between 1950 and 1958, and it is interesting to note that the subsequent decline in its use coincided with the rapid development of liquid scintillation techniques that so greatly facilitated the counting of tritiated water.

Generally, the type of research work with D_2O may be divided into five categories. First, the isotope was used to determine its volume of distribution, time taken to equilibrate with the various parts of body water, and turnover time.[12–14,16–18,21,23,27,33,36,49–52,59,80,89] Second, its volume of distribution was compared with other spaces used to estimate the total body water (e.g., antipyrine and D_2O spaces[17,35,48]; urea and D_2O spaces[7]). Third, early attempts were made to extend the use of body water measurements in pathological states, initially in states of edema[5,16,25,34,35,55,61,70] and leading through a series of investigations to those being carried out at the Harvard Medical School where the parameters are measured in a wide variety of diseases.[60,62] Fourth, the isotope was used in energy balance experiments.[13,46,61] Finally, a large amount of work was done on exchange between deuterium ions and tissue hydrogen ions. This work began with Krogh and Ussing[42] and was subsequently pursued by others.[12,33,93,99]

The above-mentioned lines of research established that the time taken for deuterium oxide to equilibrate with body water in healthy men was 1 hr,

whereas in edematous patients it was 6 hr.[70] Deuterium oxide spaces agreed well with estimates of total body water that were determined by desiccation of laboratory animals.[59] Indirect estimates of changes in tissue energy storage in health and disease could be made by using the total body water.[13,61] Measurements of body water turnover were made, and the exchange between deuterium and tissue hydrogen in animals was estimated to be about 5%.

THE REPLACEMENT OF DEUTERIUM OXIDE BY TRITIATED WATER TO ESTIMATE TOTAL BODY WATER

When first introduced,[71] TOH had little advantage over D_2O because both isotopes were difficult to measure. In the case of tritiated water, the marker was converted to a gas (e.g., hydrogen or butane) and was counted in an appropriate instrument such as an electronic reed vibrometer. Thus, at first, the pattern of research with tritiated water duplicated that of deuterium oxide.

Pace *et al.*[71] studied the distribution of tritiated water in two rabbits and one human. They reported that the time taken for the isotope to reach equilibrium with body water was 1 hr for the man and 30 min for the rabbits; and they showed that there was good agreement between tritiated water space and the total body water of the rabbits as determined by desiccation. Thompson[95,96] studied the distribution of tritiated water in body tissues of mice and rats. Tritium oxide concentrations in tissue water were similar in 13 organs in the rat.[96] Thompson[97] quoted similar observations by Pinson and Anderson, and by Myers *et al.*, in man. Pinson and Langham[81] studied the absorption of TOH from the gut of normal men, and they also determined the volume of distribution of the isotope. Tritiated water spaces in 20 healthy patients and 37 sick patients were compared with the antipyrine spaces, and the time required for TOH to equilibrate in these people was also investigated.[82] Siri[92] measured tritiated water spaces in 100 healthy men.

The extent to which exchange occurs between the radioactive marker and tissue hydrogen (2 to 5% of total body water) was established in a number of species.[20,71,73,79]

It first became advantageous to use TOH instead of deuterium oxide when liquid scintillation counting methods were developed to the stage where radioactivity could be reliably assayed in small samples.[29–31,91] This was a necessary phase before the isotope could be widely used in body-composition studies.[11,43] Although the biological properties of the isotopes were similar,[47] technological improvements conferred advantages upon the use of tritiated water for the investigation of body composition.

MEASUREMENT OF OTHER ISOTOPE SPACES IN BODY-COMPOSITION EXPERIMENTS IN HUMANS AND DEVELOPMENT OF THE MODERN CONCEPT OF BODY COMPOSITION IN CLINICAL MEDICINE

The main development at the stage when liquid scintillation counting became readily available was the great increase in the availability of radioisotopes of several elements (sodium, ^{24}Na; potassium, ^{42}K, and so forth) as well as isotope-labeled conventional markers (e.g., ^{131}I-inulin). A great deal of work was undertaken in the investigation of exchangeable ionic spaces as well as space measurements with isotope-labeled markers. To give but one example, extracellular fluid volume was measured by using a large number of materials (^{35}S-thiocyanate, ^{131}I-inulin, ^{82}Br, and ^{24}Na). The very multiplicity of these spaces emphasized the difficulties in interpretation that beset investigators in this field. Total body water measurements passed through a similar stage when several spaces (e.g., urea, thiourea, antipyrine, *N*-acetyl-4-aminoantipyrine, 4-aminoantipyrine, or sulphanilamide) were being investigated and compared.

The work of Moore *et al.*[62] illustrates the main emphasis of development of body-composition investigations in clinical medicine during the period 1946–1960.

> Our interest in this field began in 1941 after our first experiences with the challenge of severe surgical illness and with the advent to our laboratories, through the cyclotrons at Harvard and Massachusetts Institute of Technology, of radioactive sodium. With primitive target material crudely washed off the hot metal of the external-beam target-plate at a considerable retrospective hazard to the investigator, we measured the volume of body water available for sodium solution. These measurements were carried out in patients both well and those severely ill with burns and peritonitis . . . we returned to the work in 1944 and . . . conceived the measurement of total solids in solution by the same principle of isotope dilution (Moore 1946) . . . In any evolving field of human biology . . . evolution of method must temporarily yield to exploration of new areas using techniques that are satisfactory even if not ideal. During the period 1946 to 1954 we were mainly concerned with method(s). From 1954 to 1959 our emphasis was on the accumulation of biologic material from the study of man. In 1959 and 1960 the low dose modification and tritium were introduced. From 1960 to 1962 our primary emphasis has been on interpretation.[62]

It was obvious that these investigators were dissatisfied with imperfections in their methods; for example, Moore *et al.* (reference 62, pages 5–6) indicated that their choice of the "early" (i.e., soon after injection) bromide space to measure extracellular water (ECW) was "arbitrary." Cizek[10] drew attention to the fact that many investigators ignored gut water in ECW measurements; additional doubts in interpretation were raised by Bartley *et al.*[3] Despite these uncertainties, Moore *et al.*[62] have obtained clinically meaningful data

by estimating cellular hydration from the difference between the hydrogen isotope space and the bromide space. The "body cell mass," proposed by Moore [60] and Moore *et al.* [62] as a more useful parameter in body-composition studies than "lean body mass," can be calculated from the exchangeable potassium, given certain assumptions about tissue hydration; but its low-energy-requiring supporting structure, "extracellular tissue," is estimated by the extracellular fluid volume. In other words, the ratio of the body cell mass to its supporting tissue is best expressed by the ratio of intracellular to extracellular fluid volumes. The success of body-composition studies in the sick might yet prove to depend as much on deviations from normality in the diseased as any advantages obtained by using multiple compositional parameters. I am sure, however, that the last quotation shows clinicians to be aware of this possibility.

How did this work benefit the practice of clinical medicine? First, it allowed the calculation of total body water in health and disease. By using the formula of Pace and Rathbun, [72] fat could also be determined. [13,61,62] The fat-free weight of the body could then be calculated, and the importance and limitations of this parameter have been discussed. [56,57,60,62,68] Second, in conjunction with other measurements, these studies permitted estimates to be made of the state of cellular and extracellular hydration in both the healthy and the sick. As pointed out earlier, difficulties arise here because of variability in the estimation of ECW rather than in the hydrogen isotope space.

USE OF TRITIATED WATER FOR MEASURING THE BODY COMPOSITION OF DOMESTIC ANIMALS

DIFFERENT AIMS OF CLINICAL RESEARCH AND ANIMAL SCIENCE

With few exceptions, investigators in nonmedical fields have used a different approach to the study of body composition. The present limitations of "extracellular water" measurement in ruminants [76] and the need to investigate two other pools, namely, exchangeable sodium and potassium, have limited the development of multiparameter research. However, the extension of such investigations in adaptational studies with animals, to use but one example, is most certainly overdue. [74]

In body-composition studies in domestic animal experimentation, more emphasis has been placed on determining the composition of small but biologically significant changes in tissue energy storage. This has been investigated in *clinically healthy animals*, mostly ruminants, and a single parameter has been estimated in order to calculate energy loss or gain. The work has strong practical implications, since increased animal production from healthy animals

by improved selection and better nutrition forms a major part of animal research in many countries. In Australia, for example, a large research effort is devoted to the discovery of tropical and subtropical plants that will be assessed in the final analysis on their ability to promote body growth in cattle. It is for this reason that a technique for the indirect and precise measurement of the tissue energy storage in animals has been a matter of some interest.

MEASUREMENT OF TISSUE ENERGY STORAGE IN DOMESTIC ANIMALS BY DILUTION TECHNIQUES

Differences in the methods of approach to the problem of measuring tissue energy storage by dilution techniques were evident from the earliest stages. In reference to research in man, Brožek stated:

> Cyclic fluctuations along the acceptance–rejection continuum are not unusual in the history of scientific method. In the first stage the investigators . . . are apt to be enthusiastic. They are impressed by how good the first approximations are. In the second, the critical stage, the fact that they were only first approximations is likely to be stressed. The complexities of methodology, glossed over at first, are likely to be found overwhelming. The quantitative assumptions are questioned and the size of standard errors rather than typical values of biological "constants" are observed. It becomes clear, in time, that definitions must be sharpened, ambiguities of terminology reduced or eliminated, and quantitative assumptions replaced by factual data based on well-defined samples.[8]

It is quite clear that in the domestic animal sciences the field of body composition has experienced many of the vicissitudes outlined so well by Brožek, and probably the second stage of development has been reached. For example, the early enthusiasm engendered by the antipyrine experiments of Kraybill and his colleagues[39–41] has long since yielded to a more precisely defined approach to the problems. One of the main difficulties that investigators had to overcome was the volume of water in the rumen. The "empty bodies" of animals have been taken at different times as an eviscerated preparation or one that excludes only the contents of the alimentary canal. The "whole animal" sometimes refers to one of the above descriptions and other times is interpreted literally. Sometimes, owing to incomplete description, it is impossible to determine the meaning of the terms at all. Furthermore, uncertainty has arisen from the part that intestinal contents play in the relationships between total body water, fat, and protein, and in order to avoid this uncertainty, the empty body has become the reference structure in ruminants. Based on this, systems of analysis of animals have been suggested. Reid[83] and Reid *et al.*[86] showed how this problem could be attacked experimentally. They supported the initial hypothesis with a small amount of good

evidence from cattle and rabbits[84,85] but, in the light of more evidence, have shown the problem to be complicated and the methods to resolve it imprecise.[77,78,102]

ESTIMATION OF BODY COMPOSITION IN DOMESTIC ANIMALS BY THE DILUTION OF TRITIATED WATER

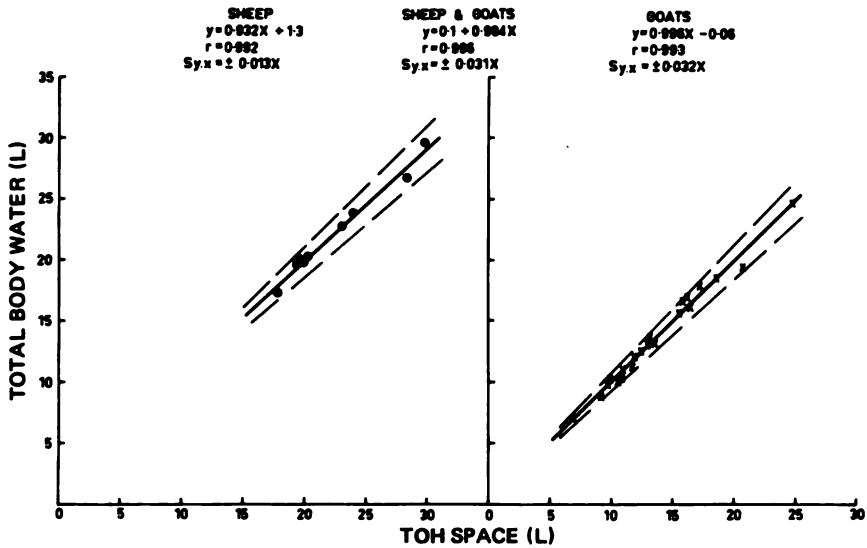
Tritiated water spaces in ruminants were first derived by Shumway *et al.*[90] and Till and Downes.[98] Both groups of investigators observed that equilibrium was reached throughout the body (including the rumen) approximately 6 hr after injection. It is interesting to note that TOH takes as long to equilibrate in the total body water in a ruminant as it does in an edematous human. Till and Downes[98] estimated that the equilibrium specific activity was within 1% of the value that would be obtained if there were instantaneous mixing of the dose with the total body water.

At this stage,* two questions arose. First, could TOH space be used to determine the body composition of ruminants and, if so, what were the errors involved? In order to answer these questions partially, a brief description is given of investigations in ruminants at our laboratory.[73,79]

To overcome, to some extent, the problem of fluid in the rumen, we deprived the animals of feed and water for 48 hr prior to injection. Methods of injection and analysis of samples for their radioactivity have been described.[79] After samples had been taken for measuring the tritiated water spaces, the animals were killed, and their bodies were analyzed for water, fat, protein, and ash. The total body water was taken as the volume of water in the whole animal, including that in the gut and its contents; the body fat, protein, and ash *excluded* only those amounts in the gut, that is, in the partly digested food and its liquid environment. The tritiated water spaces were calculated by dividing the dose of tritiated water injected (μc) by the specific activity of the serum water (μc/liter) and reduced by an amount equal to 3% of live weight to correct for hydrogen ion exchange.

Regressions of total body water, body fat, and protein as percentages of live weight on tritiated water as a percentage of live weight were calculated (Figure 1 a and b), and we suggested two methods for calculating the body composition of living ruminants based on these data. In the first, total body water and total body fat were calculated from the appropriate regressions. The fat-free dry matter was calculated by subtracting the sum of the water and fat from the live weight and was reduced by an amount equal to 1.5% of live

*Later work with TOH followed much the same pattern as outlined under deuterium oxide: measurement of the space,[2] comparison with antipyrine,[26] and comparisons of water turnover rate between species.



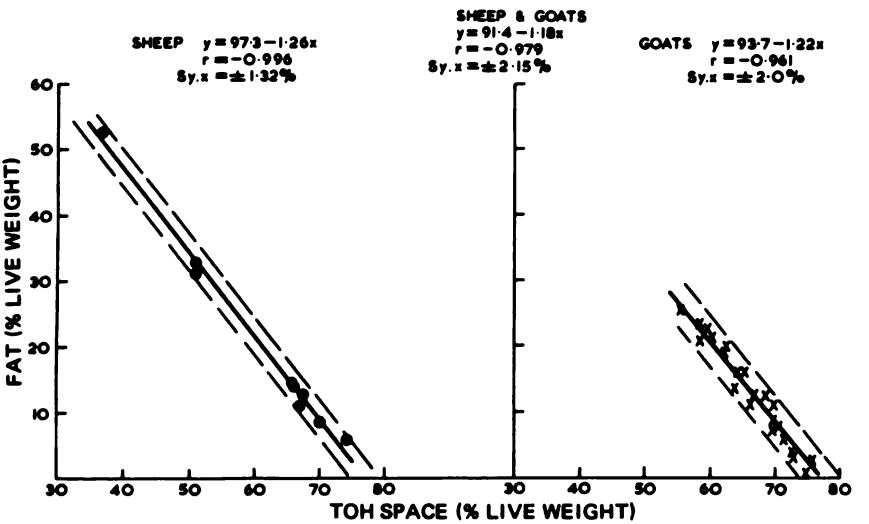


FIGURE 1a The regressions of total body water and of percentage of body fat on TOH spaces in sheep and goats. (Body water and fat were determined by direct analysis of the bodies of animals.)

weight in order to correct for undigested dry residues found in the gut of our experimental animals. The protein content of the resultant material was taken as 77% for sheep and 78% for goats.[73] Ash was obtained by difference.

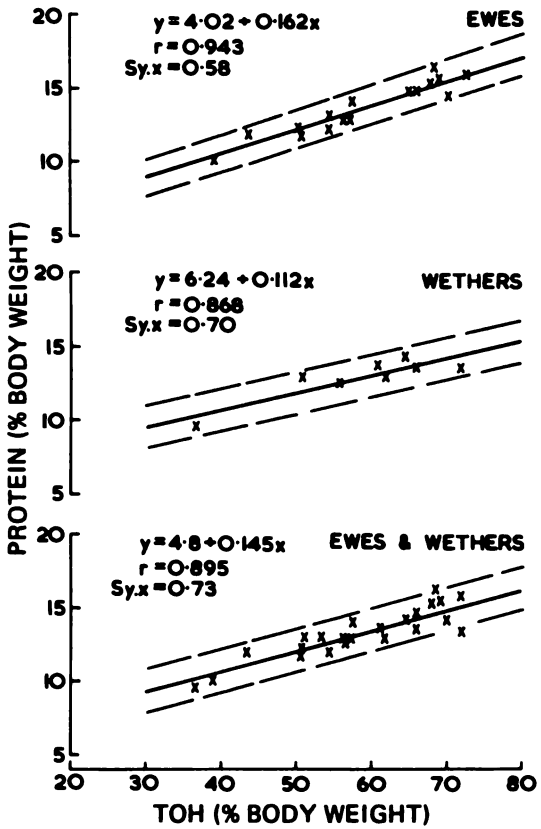


FIGURE 1b The regression of percentage of body protein on the TOH spaces in ewes and wethers. (Protein was determined by direct analysis of the bodies of animals.)

Alternatively, total body water and protein could be calculated from the tritiated water space. Ash was calculated by 0.2 x protein (kilograms). The resultant fat mass, corrected for dry solids in the gut, was obtained by difference. What are the errors in the procedure, and are they too great for our purposes? This is a most difficult question to answer. Blaxter and Rook[6] examined an indirect calorimetric method and suggested that it should have the same errors as energy balance studies from closed-circuit calorimeters where nitrogen and carbon balance were determined. Feed intake is known exactly in calorimetric measurements, but all other factors that apply to the free-ranging animal eating a similar food at pasture (e.g., the energy costs of muscular work and feeding and climatic effects) can only be derived indirectly.[24] The problem is not so much to demonstrate that the results given

by dilution techniques agree with indirect calorimetric methods in fasted, trained, and relatively immobile animals on known feed, but to derive methods that can be applied to free-grazing animals. This of course will necessitate methods to estimate the quantities of feed eaten. What then are the errors on the compositional side of this exercise in fasted animals under laboratory conditions? In our experiments,[73,79] the 95% confidence intervals for total body water predicted in an individual from tritiated water space was about 5% of live weight (LW) in goats and 2.7% in sheep. For groups, these intervals could be divided by $\sqrt{n}$. It was difficult to place confidence intervals on fat or protein (as a percentage of live weight) calculated from tritiated water spaces. However, a method for doing this has been to calculate the standard deviation (σy) of fat or protein (as a percentage of live weight) in the animals, together with correlation coefficients (r) between tritiated water spaces and fat or protein. The 95% confidence intervals for fat and protein predicted from tritiated water were then calculated by using 2 x standard error of estimate, that is, $\pm 2\,(\sigma y\sqrt{1-r^2})$. For fat (percentage of live weight), the interval was ± 4.0 for goats and 2.6% of live weight for sheep (Figure 1a); for protein, the interval was 1.5% of live weight for both ewes and wethers (Figure 1b). These intervals need to be examined in larger samples.

Morris *et al.*[63] were able to calculate the tissue energy lost by sheep during the 2 weeks after they had been shorn in a cold environment. They did this by calculating the simplest body-composition parameter—body solids (body weight minus tritiated water spaces)—and assumed a calorific value of 9 kcal/g for the tissue lost. Calculations of changes in tissue energy storage in these sheep, made by using the body weight and tritiated water spaces reported by Morris *et al.*,[63] and our scheme of analysis[75] gave results that agreed well with those derived by Morris and his colleagues. Finally, by assuming feed intake, these last authors estimated total energy used per day during the 13-day period.

FUTURE WORK

Three principal areas of research await development in the field of body composition in live animals.

First, the multiparameter approach that has been used in clinical medicine should be investigated in domestic animals. Indeed, such investigations may yet be forced on researchers if current dilution and densitometric methods used to calculate tissue energy storage prove inadequate. Second, laboratory comparisons have to be made between estimates of tissue energy derived from various methods of indirect calorimetry and those derived by using hydrogen

isotopes. Third, the extension of hydrogen isotopes to determine energy storage in free-grazing animals cannot be undertaken until the relations of the hydrogen isotope space to energy storage have been examined under the environmental and feed conditions of the experiment. In addition, methods to estimate feed intake in grazing animals require more work.

Finally, I would like to return to the work of Moore and try to look into the future as far as our own research is concerned. Moore[60] discussed the syndrome of "stressed starvation" in sick men; he emphasized the enormous erosion of body tissues in such situations and the changes in various body parameters. Similar situations exist in domestic animals, and we are studying these. We have studied nonpregnant sheep during undernutrition[75] and have shown that they survived until all measurable energy stores of fat were used, provided the animal was not stressed. However, the superimposition of conditions (e.g., cold) that create a sudden large demand for energy disturbs homeostatic mechanisms, and some animals succumb (Panaretto, unpublished data). Current work at our laboratories is concerned with the elucidation of the breakdown of normal mechanisms under conditions of stress.

REFERENCES

1. Alvarez, L. V., and R. Cornog. 1939. Helium and hydrogen of mass 3. Phys. Rev. 56:613.
2. Aschbacher, P. W., T. H. Kamal, and R. G. Cragle. 1965. Total body water estimations in dairy cattle using tritiated water. J. Anim. Sci. 24:430.
3. Bartley, W., R. E. Davies, and H. A. Krebs. 1954. Active transport in animal tissues and subcellular particles. Proc. Roy. Soc. (London), Ser. B. 142:187.
4. Berendsen, H. J. C. 1960. The structure of water in tissue, as studied by nuclear magnetic resonance. Wood's Hole Biol. Bull. 119:387.
5. Birkenfeld, L. W., J. Leibman, M. P. O'Meara, and I. S. Edelman. 1958. Total exchangeable sodium, total exchangeable potassium, and total body water in edematous patients with cirrhosis of the liver and congestive heart failure. J. Clin. Invest. 37:687.
6. Blaxter, K. L., and J. A. F. Rook. 1956. The indirect determination of energy retention in farm animals. J. Agr. Sci. 48:210.
7. Bradbury, M. W. B. 1961. Urea and deuterium oxide spaces in man. Brit. J. Nutr. 15:177.
8. Brožek, J. 1961. Body composition. Science 134:920.
9. Chien, S., and M. I. Gregersen. 1962. *In* W. L. Nastuk [ed.] Physical techniques in biological research. IV. Special methods. Academic Press, New York.
10. Cizek, L. J. 1954. Total water content of laboratory animals with special reference to volume of fluid within the lumen of the gastrointestinal tract. Amer. J. Physiol. 179:104.
11. Cooper, J. A. D., N. S. Radin, and C. Borden. 1958. A new technique for the simultaneous estimation of total body water and total exchangeable body sodium using radioactive tracers. J. Lab. Clin. Med. 52:129.

12. Edelman, I. S. 1952. Exchange of water between blood and tissues. Characteristics of deuterium oxide equilibration in body water. Amer. J. Physiol. 171:279.
13. Edelman, I. S., H. B. Haley, P. R. Schloerb, D. B. Sheldon, B. J. Friis-Hansen, G. Stoll, and F. D. Moore. 1952. Further observations on total body water; normal values throughout life span. Surg. Gynecol. Obstet. 95:1.
14. Edelman, I. S., and F. D. Moore. 1951. Body water, water distribution and water kinetics as revealed by the use of deuterium oxide. J. Clin. Invest. 30:637.
15. Ellenberger, H. B., J. A. Newlander, and C. H. Jones. 1950. Composition of the bodies of dairy cattle. Vt. Agr. Exp. Sta. Bull. 558.
16. Faller, I. L., E. E. Bond, D. Petty, and L. R. Pascale. 1955. The use of urine deuterium oxide concentrations in a simple method for measuring total body water. J. Lab. Clin. Med. 45:759.
17. Faller, I. L., D. Petty, J. H. Last, L. R. Pascale, and E. E. Bond. 1955. A comparison of the deuterium oxide and antipyrine dilution methods for measuring total body water in normal and hydropic human subjects. J. Lab. Clin. Med. 45:748.
18. Flexner, L. B., W. S. Wilde, W. K. Proctor, D. B. Cowie, G. J. Vosburgh, and L. M. Hellman. 1947. The estimation of extracellular and total body water in newborn human infant with radioactive sodium and deuterium oxide. J. Pediat. 30:413.
19. Forbes, R. M., A. R. Cooper, and H. H. Mitchell. 1953. The composition of the adult human body as determined by chemical analysis. J. Biol. Chem. 203:359.
20. Foy, J. M., and H. Schnieden. 1960. Estimation of total body water (virtual tritium space) in rat, cat, rabbit, guinea-pig and man and of the biological half-life of tritium in man. J. Physiol. 154:169.
21. Friis-Hansen, B. J., M. Holiday, T. Stapleton, and W. M. Wallace. 1951. Total body water in children. Pediatrics 7:321.
22. Glasstone, S. 1958. Source book on atomic energy. 2nd ed., pp. 218–220. Van Nostrand Co., Inc., Toronto, Princeton, New York, London.
23. Gotch, F., J. Nadell, and I. S. Edelman. 1957. Gastrointestinal water and electrolytes. IV. The equilibration of deuterium oxide (D_2O) in gastrointestinal contents and the proportion of total body water (T.B.W.) in the gastrointestinal tract. J. Clin. Invest. 36:289.
24. Graham, N. M. 1966. Predicting the maintenance requirements of sheep. Aust. Soc. Anim. Prod. 6:364.
25. Haig, C. P., and H. Schneiden. 1956. Virtual deuterium oxide space (total body water) in normal and protein deficient rats. J. Physiol. 131:377.
26. Hansard, S. L. 1964. Total body water in farm animals. Amer. J. Physiol. 206:1369.
27. Hardy, J. D., and D. L. Drabkin. 1950. The D_2O dilution space as a measure of total body water and the relation of body water to body size. Amer. J. Med. Sci. 219:108.
28. Harrison, H. E., D. C. Darrow, and H. Yannet. 1936. The total electrolyte content of animals and its probable relation to the distribution of body water. J. Biol. Chem. 113:515.
29. Hayes, F. N., and R. G. Gould. 1953. Liquid scintillation counting of tritium labeled water and organic compounds. Science 117:480.
30. Hayes, F. N., R. D. Hiebert, and R. L. Schuch. 1952. Low energy counting with a new liquid scintillation solute. Science 116:140.
31. Hayes, F. N., B. S. Rogers, and P. C. Sanders. 1955. Importance of solvent in liquid scintillators. Nucleonics 13:46.
32. Hevesy, G., and E. Hofer. 1934. Elimination of water from the human body. Nature 134:879.

33. Hevesy, G., and C. F. Jacobsen. 1940. Rate of passage of water through capillary and cell walls. Acta. Physiol. Scand. 1:11.
34. Hollander, V., P. Chang, and F. W. Co Tui. 1949. Deuterium oxide and thiocyanate spaces in protein depletion. J. Lab. Clin. Med. 34:680.
35. Hurst, W. W., F. R. Schemm, and W. C. Vogel. 1952. Simultaneous determination of total body water by antipyrine and deuterium oxide; evaluation of the methods on edematous subjects. J. Lab. Clin. Med. 39:36.
36. Hutchinson, D. L., A. A. Pleutl, and H. C. Taylor. 1954. The total body water and water turnover in pregnancy studied with deuterium oxide as isotopic traces. J. Clin. Invest. 33:235.
37. Kallman, H., and M. Furst. 1950. Fluorescence of solutions bombarded with high energy radiation. (Energy transport liquids). Phys. Rev. 79:857.
38. Klotz, I. M. 1962. Horizons of biochemistry. M. Kasha and B. Pullman [ed.] Academic Press Inc., New York and London. p. 523.
39. Kraybill, H. F., H. L. Bitter, and O. G. Hankins. 1952. Body composition of cattle. II. Determination of fat and water content from measurement of body specific gravity. J. Appl. Physiol. 4:575.
40. Kraybill, H. F., O. G. Hankins, and H. L. Bitter. 1951. Body composition of cattle. I. Estimation of body fat from measurement *in vivo* of body water by use of antipyrine. J. Appl. Physiol. 3:681.
41. Kraybill, H. F., E. R. Goode, R. S. B. Robertson, and H. S. Sloane. 1953. *In vivo* measurement of body fat and body water in swine. J. Appl. Physiol. 6:27.
42. Krogh, A., and H. Ussing. 1936. Exchange of hydrogen between free water and organic substances in the living organism. Scand. Arch. f. Physiol. 75:90. (Quoted by A. Keys, and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:245.)
43. Langham, W. H., W. J. Eversole, F. N. Hayes, and T. T. Trujillo. 1956. Assay of tritium activity in body fluids with use of a liquid scintillation system. J. Lab. Clin. Med. 47:819.
44. Lawes, J. B., and J. H. Gilbert. 1859. Experimental inquiry into the composition of some of the animals fed and slaughtered as human food. Phil. Trans. 149:494.
45. Lawes, J. B., and J. H. Gilbert. 1883. Supplement to former paper, Experimental inquiry into the composition of some of the animals fed and slaughtered as human food—Composition of the ash of the entire animals and of certain separated parts. Phil. Trans. 174:865.
46. Lee, J. S., and N. Lifson. 1960. Measurement of total energy and material balance in rats by means of doubly labeled water. Amer. J. Physiol. 199:238.
47. Liebman, J., F. A. Gotch, and I. S. Edelman. 1960. Comparison of tritium and deuterium oxides as tracers for body water. Circ. Res. 8:907.
48. Ljunggren, H. 1955. Measurement of total body water with deuterium oxide and antipyrine. Acta. Physiol. Scand. 33:69.
49. Ljunggren, H., D. Ikkos, and R. Luft. 1957. Studies on body composition. I. Body fluid compartments and exchangeable potassium in normal males and females. Acta Endocrinol. 25:187.
50. Ljunggren, H., D. Ikkos, and R. Luft. 1957. II. Body fluid compartments and exchangeable potassium in obese females. Acta Endocrinol. 25:199.
51. Ljunggren, H., D. Ikkos, and R. Luft. 1957. III. Body fluid compartments and exchangeable potassium in females with anorexia nervosa. Acta Endocrinol. 25:209.
52. London, I. M., and D. Rittenberg. 1950. Deuterium studies in normal man. II. Measurement of total body water and water absorption. J. Biol. Chem. 184:687.

53. McDougall, E. J., F. Verzar, H. Erlenmeyer, and H. Gaertner. 1934. Heavy water in the animal body. Nature 143B:1006.
54. MacFarlane, W. V., R. J. H. Morris, and B. Howard. 1963. Turnover and distribution of water in desert camels, sheep, cattle and kangaroos. Nature 197:270.
55. Mayer, J., and N. C. Hagman. 1953. Total body water and blood volume in hereditary obese hyperglycemic syndrome of mice. Proc. Soc. Exp. Biol. 82:647.
56. Miller, A. T., and C. S. Blyth. 1952. Estimation of lean body mass and body fat from basal oxygen consumption and creatinine excretion. J. Appl. Physiol. 5:73.
57. Miller, A. T., and C. S. Blyth. 1953. Lean body mass as a metabolic reference standard. J. Appl. Physiol. 5:311.
58. Mitchell, H. H., T. S. Hamilton, F. R. Steggerda, and H. W. Bean. 1945. The chemical composition of the adult human body and its bearing on the biochemistry of growth. J. Biol. Chem. 158:625.
59. Moore, F. D. 1946. Determination of total body water and solids with isotopes. Science 104:157.
60. Moore, F. D. 1962. Volume and tonicity in body water. Surg. Gynecol. Obstet. 114:276.
61. Moore, F. D., H. B. Haley, E. A. Bering, L. Brook, and I. S. Edelman. 1952. Further observations on total body water. II. Changes of body composition in disease. Surg. Gynecol. Obstet. 95:155.
62. Moore, F. D., K. H. Olesen, J. D. McMurrey, H. V. Parker, M. R. Ball, and C. M. Boyden. 1963. The body cell mass and its supporting environment. W. B. Saunders Co., Philadelphia, London.
63. Morris, R. J. H., B. Howard, and W. V. MacFarlane. 1962. Interaction of nutrition and air temperature with water metabolism in merino wethers shorn in winter. Aust. J. Agr. Res. 13:320.
64. Moulton, C. R. 1923. Age and chemical development in mammals. J. Biol. Chem. 57:79.
65. Moulton, C. R., P. F. Trowbridge, and L. D. Haigh. 1921. Studies in animal nutrition. I. Change in form and weight on different planes of nutrition. Mo. Exp. Sta. Res. Bull. 43.
66. Moulton, C. R., P. F. Trowbridge, and L. D. Haigh. 1922. Changes in proportions of carcass and offal on different planes of nutrition. Mo. Agr. Exp. Sta. Res. Bull. 54.
67. Moulton, C. R., P. F. Trowbridge, and L. D. Haigh. 1922. Changes in chemical composition on different planes of nutrition. Mo. Agr. Exp. Sta. Res. Bull. 55.
68. Muldowney, F. P. 1961. Lean body mass as a metabolic reference standard. *In* J. Brožek and A. Henshell [ed.] Techniques for measuring body composition. National Academy of Sciences–National Research Council, Washington, D.C.
69. Murray, J. A. 1922. The chemical composition of animal bodies. J. Agr. Sci. 12:103.
70. O'Meara, M. P., L. W. Birkenfeld, F. A. Gotch, and I. S. Edelman. 1957. The equilibration of radiosodium (Na^{24}) radiopotassium (K^{42}) and deuterium oxide (D_2O) in hydropic human subjects. J. Clin. Invest. 36:784.
71. Pace, N., L. Kline, H. K. Schachman, and M. Harfenist. 1947. Studies on body composition. IV. The use of radioactive hydrogen in measurement *in vivo* of total body water. J. Biol. Chem. 168:459.
72. Pace, N., and E. N. Rathbun. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158:685.

73. Panaretto, B. A. 1963. Body composition *in vivo*. III. The composition of living ruminants and its relation to the tritiated water spaces. Aust. J. Agr. Res. 14:944.
74. Panaretto, B. A. 1963. The estimation of body composition in living animals. Tech. Conf. Carcass Composition and Appraisal of Meat Animals Proc. D. E. Tribe [ed.] CSIRO, Melbourne, Australia.
75. Panaretto, B. A. 1964. Body composition *in vivo*. VI. The composition of ewes during prolonged undernutrition. Aust. J. Agr. Res. 15:771.
76. Panaretto, B. A. 1965. Body composition *in vivo*. VIII. Some physiological implications with respect to extracellular fluid volume arising from the distribution of thiocyanate in sheep. Aust. J. Agr. Res. 16:667.
77. Panaretto, B. A., and J. T. Reid. 1964. Body composition *in vivo*. IV. Comparison of the antipyrine and N-acetyl-4-aminoantipyrine spaces in goats. Aust. J. Agr. Res. 15:180.
78. Panaretto, B. A., and J. T. Reid. 1964. Body composition *in vivo*. V. The use of antipyrine and N-acetyl-4-aminoantipyrine for the estimations of gut water in living ruminants. Aust. J. Agr. Res. 15:195.
79. Panaretto, B. A., and A. R. Till. 1963. Body composition *in vivo*. II. The composition of mature goats and its relationship to the antipyrine, tritiated water, and N-acetyl-4-aminoantipyrine spaces. Aust. J. Agr. Res. 14:926.
80. Pinson, E. A. 1952. Water exchanges and barriers as studied by the use of hydrogen isotopes. Physiol. Rev. 32:123.
81. Pinson, E. A., and W. H. Langham. 1957. Physiology and toxicology of tritium in man. J. Appl. Physiol. 10:108.
82. Prentice, T. C., W. Siri, N. I. Berlin, G. M. Hyde, R. J. Parson, E. E. Joiner, and J. H. Lawrence. 1952. Studies of total body water with tritium. J. Clin. Invest. 31:412.
83. Reid, J. T. 1956. Body composition in feeding experiments. Cornell Nutr. Conf. Proc. p. 94-101.
84. Reid, J. T., C. C. Balch, and R. F. Glascock. 1958. The use of tritium, of antipyrine and N-acetyl-4-aminoantipyrine in the measurement of body water in living rabbits. Brit. J. Nutr. 12:43.
85. Reid, J. T., C. C. Balch, M. J. Head, and J. W. Stroud. 1957. The use of antipyrine and N-acetyl-4-aminoantipyrine in the measurement of body water and the intralumenal water of the gastro-intestinal tract of living cattle. Nature 174:1034.
86. Reid, J. T., G. H. Wellington, and H. O. Dunn. 1955. Some relationships among the major chemical components of the bovine body and their application to nutrition investigations. J. Dairy Sci. 38:1344.
87. Reynolds, G. I., F. B. Harrison, and G. Salvini. 1950. Liquid scintillation counters. Phys. Rev. 78:488.
88. Schloerb, P. R., B. J. Friis-Hansen, I. S. Edelman, B. B. Sheldon, and F. D. Moore. 1951. The measurement of deuterium oxide in body fluids by the falling drop method. J. Lab. Clin. Med. 37:653.
89. Schloerb, P. R., B. J. Friis-Hansen, I. S. Edelman, A. K. Solomon, and F. D. Moore. 1950. The measurement of total body water in the human subject by deuterium oxide dilution. With a consideration of the dynamics of deuterium distribution. J. Clin. Invest. 29:1296.
90. Shumway, R. P., T. T. Trujillo, J. A. Bennett, D. J. Mathews, and R. O. Asplund. 1956. Fat determination in live steers using tritium injection. West. Sec. Amer. Soc. Anim. Prod. Proc. 7:XXIV.

91. Simpson, J. D., and J. R. Greening. 1960. Preparation of tritiated water samples by distillation. Nature 186:467.
92. Siri, W. E. 1953. Fat, water and lean tissue studies. Federation Proc. 12:133.
93. Smith, P. K., J. Trace, and H. G. Barbour. 1936. The fate of deuterium in the mammalian body. J. Biol. Chem. 116:371.
94. Solomon, A. K., I. S. Edelman, and I. S. Soloway. 1950. The use of the mass spectrometer to measure deuterium in body fluids. J. Clin. Invest. 29:1311.
95. Thompson, R. C. 1952. Studies of metabolic turnover with tritium as a tracer. I. Gross studies in the mouse. J. Biol. Chem. 197:81.
96. Thompson, R. C. 1953. Studies of metabolic turnover with tritium as a tracer. II. Gross studies on the rat. J. Biol. Chem. 200:731.
97. Thompson, R. C. 1954. Biological applications of tritium. Nucleonics 12:31.
98. Till, A. R., and A. M. Downes. 1962. The measurement of total body water in the sheep. Aust. J. Agr. Res. 13:335.
99. Ussing, H. H. 1938. Exchange of H and D atoms between water and protein *in vivo* and *in vitro*. Scand. Arch. f. Physiol. 78:225.
100. von Bezold, A. 1857. Untersuchungen uber die Vertheilung von Wasser, organischer Materia und anorganischen Verbindungen im Tierreiche. Z. wiss. Zoöl. 8:487.
101. Wentzel, A. D., J. M. Iacono, T. H. Allen, and J. E. Roberts. 1958. Determination of heavy water (HDO) in body fluids by direct introduction of water into a mass spectrometer; measurement of total body water. Phys. Med. Biol. 3:1.
102. Whiting, F., C. C. Balch, and R. C. Campling. 1960. Some problems in the use of antipyrine, and N-acetyl-4-aminoantipyrine in the determination of body water in cattle. Brit. J. Nutr. 14:519.
103. Widdowson, E. M., R. A. McCance, and C. M. Spray. 1951. The chemical composition of the human body. Clin. Sci. 10:113.

H. Doornenbal
Canada Department of
Agriculture
Lacombe, Alberta, Canada

RELATIONSHIP TO BODY COMPOSITION OF SUBCUTANEOUS BACKFAT, BLOOD VOLUME, AND TOTAL RED-CELL MASS

Body-composition research has become of paramount interest because knowledge of the gross composition of fat, lean, and bone in both man and animals could have wide application in a variety of biological studies, including metabolic, nutritional, and dietary studies; drug therapy; and certain endocrinological experiments.

Techniques used for measuring body composition, with emphasis on the composition in man, have been reviewed by Brožek and Henschel.[2] Results of these techniques and procedures in determining body composition in man and in farm animals have been compiled by Brožek,[1] and certain techniques used to determine composition in live hogs have been reviewed by Hörnicke.[6] The techniques used in live animal composition research may be divided into two categories. One deals with measurements such as the ruler probe, leanmeter, ultrasonics, x-ray, and body measurements, where in each case, information on a specified part of the body is related to the body as a whole. These measurements have been termed "spot" measurements in contrast to those belonging to the second category, where attempts are made to determine one or more physiological units from which others may be predicted quantitatively.

The present report considers the spot measurement most widely used in live animal evaluation, that is, the thickness of the subcutaneous fat or back fat, and a physiological approach (blood volume and red-cell mass) as indicators of the chemically determined gross body composition of the pig.

MATERIAL AND METHODS

Body composition refers to the composition of the entire animal after removal of the blood, hair, toenails, and the contents of the digestive tract. The following parts were removed from the body and weighed individually: head, feet, tail, kidneys, spleen, liver plus gallbladder, mesentery, and the internal fat surrounding the kidneys. These items were combined, homogenized, and analyzed for gross chemical composition.

Removal of these parts from the body left the warm dressed carcass, which in turn was homogenized and analyzed for gross chemical composition. Details on the procedures used in the chemical analysis have been published elsewhere.[3]

The first section of this paper deals with data obtained during a study of the gross composition and the distribution of fat among external and internal fat depots in 36 market-weight pigs. The thickness of subcutaneous fat was measured on the live animal just prior to slaughter and again on the split, chilled carcass. The subcutaneous fat layer plus skin was removed on the left side along the lines shown in Figure 1. This side was then divided into the major cuts, untrimmed ham and belly, and trimmed shoulder and loin, plus the back fat trim (Figure 2).

Measurements of the thickness of the subcutaneous fat on the live animal with the ruler probe represent the average of three paired measurements, left and right, made by the method described by Rahnefeld.[8]

Measurements of the back-fat thickness on the split, chilled carcass represent the total of three measurements on each side, taken at the point of maximum thickness on the shoulder and at the points of maximum and minimum thickness on the loin. These three measurements were added for each side, representing total back-fat thickness, right side and left side.

The second part of this paper presents data on the interrelationships among total body protein, total blood volume, total red-cell mass, and body weight obtained during a course of studies in growth, development, and gross body composition in pigs ranging from 9 to 103 kg. Eleven weight groups of eight pigs each (four castrated males and four females) at approximately 11-kg intervals were used.

FIGURE 1 Site at which subcutaneous fat is removed from the loin and the shoulder.

Blood volume was determined by a standard dilution technique whereby a blood sample was labeled with radioactive chromium (^{51}Cr) and injected into the pig. Total red-cell volume was calculated from the total blood volume and the hematocrit values, multiplied by 0.96 to correct for trapped plasma.

RESULTS AND DISCUSSION

SUBCUTANEOUS, INTERNAL, AND TOTAL FAT

Thickness of the subcutaneous fat layer at various points is assumed by many to be highly and positively correlated with whole-body or carcass fatness.

FIGURE 2 The four major cuts and the subcutaneous fat removed from the loin and shoulder.

It was shown in a previous publication[4] that the correlation between carcass back-fat measurements and total chemically determined fat in the carcass of 50 females and 50 castrated males was low (0.78 and 0.50, respectively), even with a built-in part–whole correlation, as external and internal fat had not been separated. In the present study involving 36 market-weight pigs, average values for the back-fat measurements taken on the live animal and the carcass and the chemically determined fat content of the different fat depots are reported in Table 1.

The first five pairs of correlations in Table 2 relate the subcutaneous back-fat measurements on the live animal and on the carcass and the chemically

TABLE 1 Average Values and Standard Deviations for Back-Fat Thickness and Chemically Determined Fat of 36 Pigs[a]

Item	Males		Females	
	Mean	SD	Mean	SD
Live weight (kg)	89.30	1.73	89.84	2.21
Warm carcass weight (kg)	63.50	1.98	64.15	2.23
Live animal probe				
Total, right side (cm)	9.34	1.06	8.49	0.92
Total, left side (cm)	9.46	1.18	8.52	0.88
Carcass back-fat thickness				
Total, right side (cm)	10.55	1.16	9.93	1.01
Total, left side (cm)	10.78	1.24	10.10	1.00
Chemical fat				
In kidney fat (kg)	1.69	0.34	1.56	0.27
Total in right half (kg)	12.28	1.22	11.14	0.62
Total in left half (kg)	12.04	1.05	10.96	0.62
Weight of chemical fat				
In trim of loin (kg)	3.17	0.42	2.78	0.35
In trimmed loin (kg)	1.16	0.27	1.02	0.25
In trim of left side (kg)	4.01	0.50	3.46	0.45
In trimmed left side (kg)	8.03	0.81	7.49	0.50

[a] 19 males; 17 females.

determined fat content of the external and internal fat depots. Eight of these correlation coefficients (five for males and three for females) attained significance at $P \leqslant 0.01$. The highest correlations involve the external fat measurements taken on the live animal with the corresponding measurements taken on the chilled carcass (0.80 and 0.70 for males and females, respectively) and total probe on the left side with the weight of chemical fat in the back-fat trim of the left side (0.74 and 0.67 for males and females, respectively).

These results indicate that estimates of the thickness of the subcutaneous fat layer on the live animal show reasonably good relationships with estimates of back-fat thickness measured on the chilled carcass and that the live animal probe is useful in predicting chemically determined fat in the subcutaneous fat layer. The relationship between the live animal probe, average of right plus left side, and the chemical fat in the body was moderate for the males (0.67) but nonsignificant for the females (0.37), as illustrated in Figure 3.

The correlation of chemical fat in the subcutaneous fat layer with the total fat in the left side was moderate for both males and females (0.67 and 0.61, respectively) in spite of the built-in part–whole relationship between these two variables. When the external fat was separated from the total fat, this relationship was nonsignificant for both sexes, as shown in Table 2 and Figure 4.

TABLE 2 Phenotypic Correlation between Live Animal Back-Fat Measurements, Carcass Back-Fat Measurements, Chemical Back Fat, Chemical Fat in Internal Fat Depots, and Total Fat in Body of 36 Pigs[a]

Measurements Correlated	Males	Females
Probe, average right + left side (cm)–chemical fat in body (kg)	0.67	0.37
Probe, total right side (cm)–chemical fat in right side (kg)	0.68	0.18
Probe, total left side (cm)–chemical fat in trim left side (kg)	0.74	0.67
Probe, total right side (cm)–carcass back fat, total right side (cm)	0.80	0.76
Chemical fat in total trim left side (kg)–total chemical fat in left side (kg)	0.67	0.61
Chemical fat in total trim left side (kg)–chemical fat in trimmed left side (kg)	0.25	−0.14
Chemical fat in trim of loin (kg)–chemical fat in trimmed loin (kg)	0.19	−0.32
Chemical fat in total trim left side (kg)–chemical fat in kidney fat (kg)	0.41	0.16
Probe, average right + left side (cm)–chemical fat in kidney fat (kg)	0.15	0.09

SIGNIFICANCE LEVELS	@$P \leq 0.05$	i@$P \leq 0.01$
Males	$-r \geq 0.44$	$r \geq 0.56$
Females	$-r \geq 0.47$	$r \geq 0.59$

[a] 19 males; 17 females.

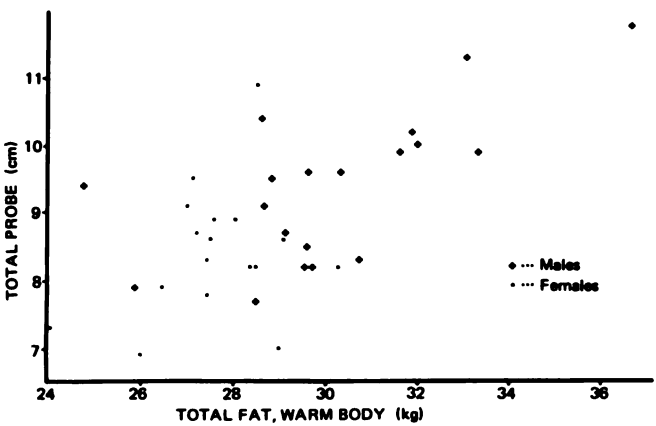


FIGURE 3 Relationship between live animal probe and the chemically determined fat in the entire body.

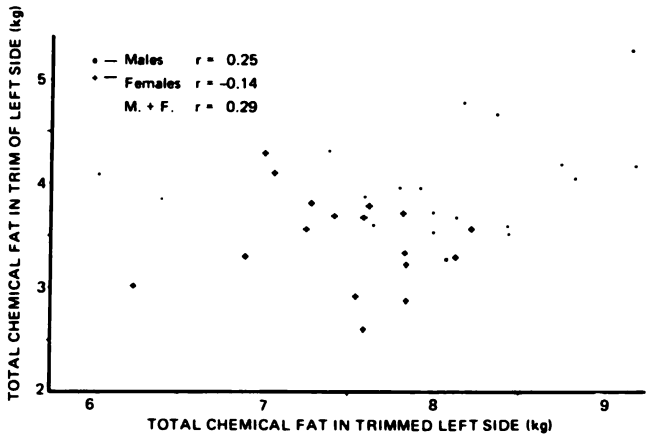


FIGURE 4 Relationship between the chemical fat in the subcutaneous fat layer over the shoulder and loin and the chemical fat in the remainder of the left side.

A similar correlation existed between the live animal probe, average of right and left side, and the chemically determined fat in the fat depot around the kidneys. This relationship is shown in Figure 5.

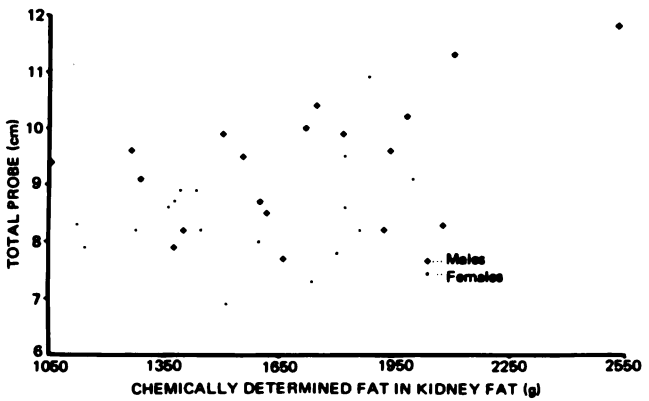


FIGURE 5 Relationship between live animal probe and the chemical fat in the kidney fat.

The results reported here clearly indicate that measurements of the subcutaneous fat layer on the live animal, or on the chilled carcass, and the weight of the chemical fat in the subcutaneous fat layer are not related to the internal fat depots, either the intermuscular plus intramuscular fat or the kidney fat.

Measurements of the thickness of the subcutaneous fat layer can be made with high repeatability. Therefore, errors in predicting total body fat or total

carcass fat are unlikely to be associated with inaccuracies in these spot measurements. However, it is unreasonable to expect that spot measurements can accommodate variation in the thickness of the subcutaneous fat layer over the entire body. In addition, total body fat and total carcass fat include internal fat depots, which are not measured at all by these subcutaneous fat measurements.

Presumably, these reservations would apply to all techniques for spot measurements of the subcutaneous fat layer, including ultrasonic and radiographic measurements. In fact, using cutout yield as an end point, Martin and Fredeen[7] concluded that x-ray measurements of back-fat thickness in the live animal were not superior to the simpler probe method.

The nonsignificance of the phenotypic correlation reported in the current work between the subcutaneous fat depot and the internal fat depots explains results reported elsewhere,[4] in which animals of the same sex and weight and with the same amount of total chemical fat in the carcass differed in total thickness of the subcutaneous fat layer on the chilled carcass by more than 1 inch; whereas pigs of the same sex and weight and with identical back-fat thickness differed in total chemical fat by as much as 15 lb, which represented 10% of the average carcass weight. Similar variability was found among the 36 animals reported on here.

Because of the many physiological similarities between man and the domestic pig, errors encountered in predicting body composition in the human, when simple anthropometric measures are used, particularly skinfold thickness, may well be due to the same limitations as those outlined for the pig.

BLOOD VOLUME, RED-CELL MASS, AND BODY WEIGHT

It has been well established that the oxygen consumption of mammals depends on the amount of metabolically active tissue in the body and is not necessarily correlated with body weight, since adipose tissue requires little oxygen. One can, therefore, expect a high correlation between the oxygen carriers (total blood volume or red-cell mass, or both) and the oxygen consumer (lean body mass). In this work, the protein mass has been taken to be the oxygen-consuming tissue and, because of the analytical procedure employed, consists of everything remaining in the body after all body moisture, total fat, and all minerals have been removed. It includes the protein in the cellular scaffolding structure of adipose tissue. Average values for blood volume, red-cell mass, body weight, and the gross composition of the body are reported in Table 3.

Support for the basic hypothesis appears to be provided by Figure 6. Blood volume per unit of body mass (top) decreases as the live weight increases, be-

TABLE 3 Average Blood Volume, Red-Cell Mass, and Gross Composition of 88 Pigs Ranging from 9 to 103 kg of Live Weight

Group Live Weight[a] (kg)	Blood Volume (cc)	Red-Cell Mass (cc)	Total Body Protein (kg)	Total Body Fat (kg)	Total Body Ash (kg)	Total Body Moisture (kg)
10.5	669	262	1.46	1.21	0.31	6.06
22.4	1,363	509	3.07	3.55	0.62	12.32
34.2	2,102	803	4.68	6.30	0.95	17.79
45.5	2,616	1,000	6.18	9.60	1.24	22.56
52.7	2,947	1,211	7.13	12.73	1.42	25.67
65.3	3,571	1,565	8.66	17.33	1.68	30.63
69.1	3,907	1,740	9.10	19.17	1.75	32.08
76.9	4,154	1,853	10.03	22.78	1.95	34.74
83.6	4,250	1,901	10.60	26.33	2.11	36.65
91.2	4,610	2,222	11.52	30.09	2.31	39.40
99.2	4,900	2,353	12.19	35.86	2.43	40.82

[a]Average of eight pigs per weight group; four castrated males and four females.

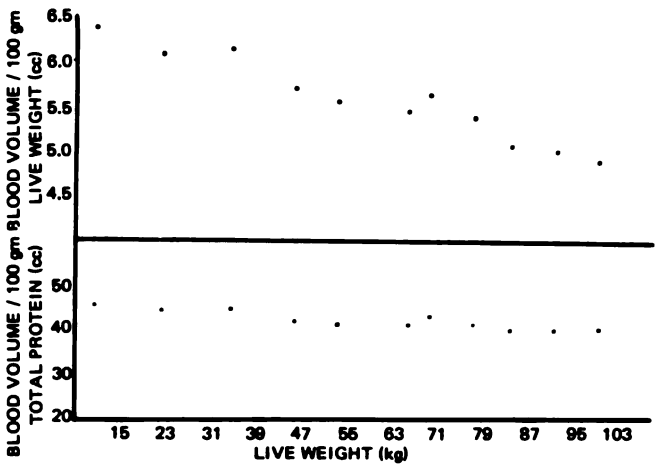


FIGURE 6 Relationships between live weight and blood volume per unit of live weight (top), and live weight and blood volume per unit of body protein (bottom).

cause of the decrease in protein mass or the increase in body fat per unit of body mass, as shown by the data of Table 3. On the other hand, with blood volume expressed per unit of protein mass, this ratio is nearly constant over a live-weight range of approximately 43 to 103 kg. The somewhat higher ratios

for the very immature groups may be partially explained in terms of a higher metabolic rate at this age. The same relationship with live weight existed between red-cell mass per unit of body weight and red-cell mass per unit of protein mass.

The close relationship between total protein in the body and total blood volume is illustrated further in Figure 7. The correlation of 0.97 between

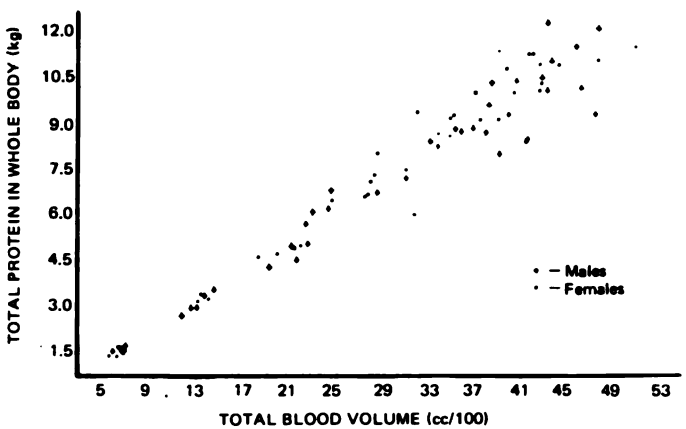


FIGURE 7 Relationship between total body protein and total blood volume.

these two variables compares favorably with correlations reported by other workers employing other physiological measurements. However, an important point to be considered in this connection is body weight. In many investigations dealing with body composition of man and domestic animals, subjects that vary considerably in body weight have been used. Many of these studies established highly significant correlations between certain parameters and body composition. However, frequently the simplest measurement—body weight—was ignored. Where data on body weight were supplied and relationships could be calculated, invariably body weight itself was as good an index of body composition, or even better, than the parameters under investigation.

Similar results were obtained in our studies when the entire weight range of 9 to 103 kg was considered.

The data in Table 3 show that blood volume, red-cell mass, and body protein increase with an increase in body weight. The close associations of total body protein ($r = 0.99$) and blood volume ($r = 0.97$) with live weight are illustrated in Figures 8 and 9.

Over the entire weight range of 9 to 103 kg, multiple regression analysis of the data, with total body protein in kilograms (Y) as the dependent variable,

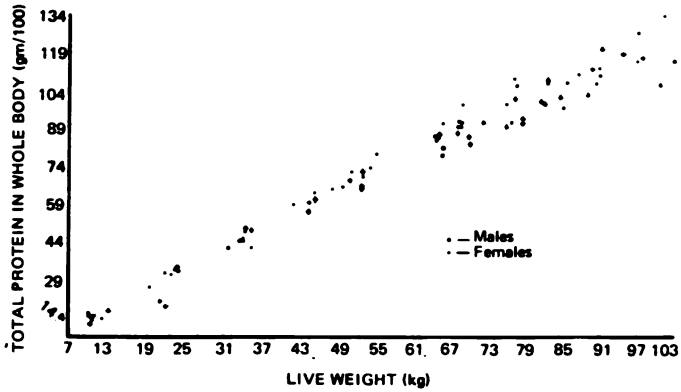


FIGURE 8 Relationship between total body protein and body weight.

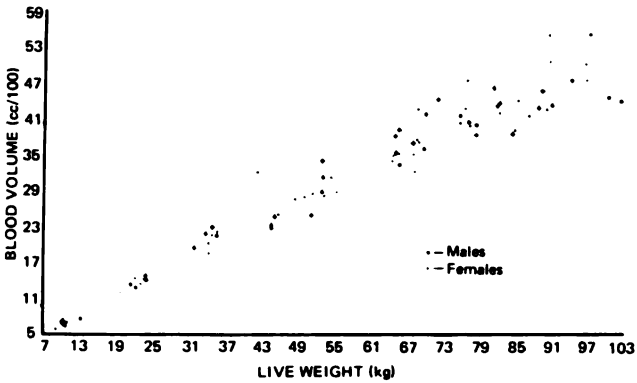


FIGURE 9 Relationship between total blood volume and body weight.

and body weight in kilograms (X_1) and total blood volume in cubic centimeters (X_2) as the two independent variables, explained for both sexes virtually all the variance observed in total protein ($R^2 \times 100 = 98\%$). Body weight accounted for almost all the variance. Over such a wide weight range it can be expected that any body measurement or physiological entity has a high relationship with body weight simply because the main physiological event taking place in a developing animal is an increase in the size, weight, and volume of all components of the body. Expressing total body protein as a percentage lowered the variation attributed to body weight to approximately 60% ($r = 0.77$) of the variance in protein percentage.

In an attempt to restrict the effect of body weight further, the data were subdivided, covering a weight range of 81 to 103 kg. The coefficient of deter-

mination (R^2 x 100) for this restricted weight range was 58%, with 8% ($P <$ 0.1 and $>$ 0.05) explained by blood volume. The weight range involved is still large (21.4 kg) and, as such, body weight is still of major importance in explaining the variation in total body protein. However, it does establish that the precision in predicting total protein in the body can be improved by the use of blood volume determinations when the weight range is restricted. When red-cell mass was substituted for blood volume as in independent variable in the multiple regression analysis, the results were, for practical purposes, identical to those obtained when blood volume was used.

Because one of the objectives in swine improvement is to differentiate between leaner and fatter individuals at a similar body weight, high correlations involving total body protein and body weight, computed from animals varying considerably in body weight, are of little importance.

Accordingly, we are now conducting an experiment to evaluate the use of blood volume or total red-cell mass, or both, for differentiating between animals over a narrow weight range with animals of 90 ± 5 kg of live weight. Preliminary results to date, based on a small number of the animals under study, indicate that the relationships involving total body protein and the physiological measurements in question are not high enough to be of satisfactory predictive value. (This seemed assured by the antithesis to the previous paragraph.)

However, in these preliminary results it has been observed that total body protein and blood volume or red-cell mass generally increase or decrease simultaneously. Occasionally, however, total body protein will be lower compared with other animals, while total blood volume or red-cell mass is considerably higher. The reason for this variability may well be the differences in degree of excitement among animals during the injection of labeled cells and the subsequent drawing of blood samples.

The effects of exercise, excitement, age, and certain drugs on blood volume and venous hematocrit have been well documented. The work by Turner and Hodgetts[9,5] with sheep is of particular interest. These workers found that in a conscious sheep, in quiet surroundings, the spleen may contain up to one seventh of the total blood volume and one fourth of the total red-cell volume. In this connection it was pointed out[5] that techniques to determine red-cell volume, such as ^{51}Cr-labeling, may be subject to error because a considerable portion of the total red cells (on the order of 10%) was found to be withheld from free "mixing" in some animals. This was readily overcome by intravenous administration of adrenalin.

Hodgetts[5] also illustrated the effect of fluctuations of the jugular hematocrit upon blood volume and red-cell volume of a hypothetical "average" sheep under four sets of conditions for which calculations had been made. The conditions

involved were: moderate excitement of the untrained animal, placidity induced by gentle restraining and soothing, anesthesia, and simulated excessive excitement and splenic contraction induced by intravenous injection of adrenalin. The values ranged from 25 to 40 for the jugular hematocrit, from 59.5 to 74.3 ml of blood/kg of body weight, and from 14.9 to 29.7 ml of red cells/kg of body weight between anesthetized and adrenalin-injected animals.

On the basis of these results obtained with sheep, the relationship between total body protein and blood volume or red-cell mass in pigs may become considerably higher if the variability in degrees of excitement is removed by injection of epinephrine or by the use of a proper tranquilizer that would not affect the spleen.

REFERENCES

1. Brožek, J. [Cons. Ed.] 1963. Body composition. Parts I and II. Ann. N. Y. Acad. Sci. 110.
2. Brožek, J., and A. Henschel [ed.] 1961. Techniques for measuring body composition. National Academy of Sciences–National Research Council, Washington, D.C.
3. Doornenbal, H., and A. H. Martin. 1965. The evaluation of blood volume and total red cell mass as predictors of gross body composition in the pig. Can. J. Anim. Sci. 45:203.
4. Doornenbal, H., and A. H. Martin. 1966. The association between commercial cut-out yield and gross chemical composition in market pigs. Anim. Prod. 8:445.
5. Hodgetts, V. E. 1961. The dynamic red cell storage function of the spleen in sheep. III. Relationship to determination of blood volume, total red cell volume, and plasma volume. Aust. J. Exp. Biol. Med. Sci. 39:187.
6. Hörnicke, H. 1959. Methoden zur Bestimmung der Körper-zusammen-setzung lebender Schweine. Ph.D. thesis, Tierärzlichen Hochschule, Hannover.
7. Martin, A. H., and H. T. Fredeen. 1966. Radiography of the live animal as a technique for predicting carcass characteristics in swine. Can. J. Anim. Sci. 46:83.
8. Rahnefeld, G. W. 1964. Probing for backfat thickness in hogs. Can. Dept. Agr. Publ. 1211.
9. Turner, A. W., and V. E. Hodgetts. 1959. The dynamic red cell storage function of the spleen in sheep. I. Relationship to fluctuations of jugular haematocrit. Aust. J. Exp. Biol. Med. Sci. 37:399.

Alexander P. Remenchik
Loyola University
Stritch School of Medicine

Charles E. Miller
Argonne National Laboratory

W. V. Kessler
Purdue University

ESTIMATES OF BODY COMPOSITION DERIVED FROM POTASSIUM MEASUREMENTS

Several investigators have suggested that body potassium may be used as an estimator of body solids, cell mass, muscle mass, lean body mass, total body fat, and in conjunction with body water measurements as an estimator of muscle mass, adipose tissue, and muscle-free lean tissue.[1,6,12,23,25] Each of these investigators has made crucial assumptions while formulating his model, and for man it has been impossible to evaluate directly the validity of the models that have been proposed. Our purpose is to review certain evidence bearing on these assumptions, to examine the appropriateness of these models in view of this evidence, and to discuss the use of body potassium measurements in clinical medicine.

POTASSIUM AS AN ESTIMATOR OF FAT CONTENT OF MAN

Since we cannot dissect and dissolve the humans we study and thus validate our indirect techniques, we must make inferences about the validity of specific techniques for man from validation studies of animals. One selects

a method that has been shown to be valid for animals, assumes it is valid for man, formulates specific predictions for man predicated on this assumption, and designs some experiments to test these predictions. For the evaluation of potassium as a predictor of body fat, we elected to use body water as an estimator of fat-free tissue. Our review of the data suggested to us that body water is a reliable estimator of fat-free tissue. Keys and Brožek[8] stated that their analysis of the data of Pace and Rathbun[14] indicated that the water content of lean tissue was dependent on fatness. However, this conclusion was based on erroneous calculations (J. Brožek, personal communication). As a matter of fact, it is not appropriate to calculate a simple summary statistic for the data of Pace and Rathbun, as is readily evident from an examination of a plot of their data (Figure 1). It is apparent that one cannot define an elliptical

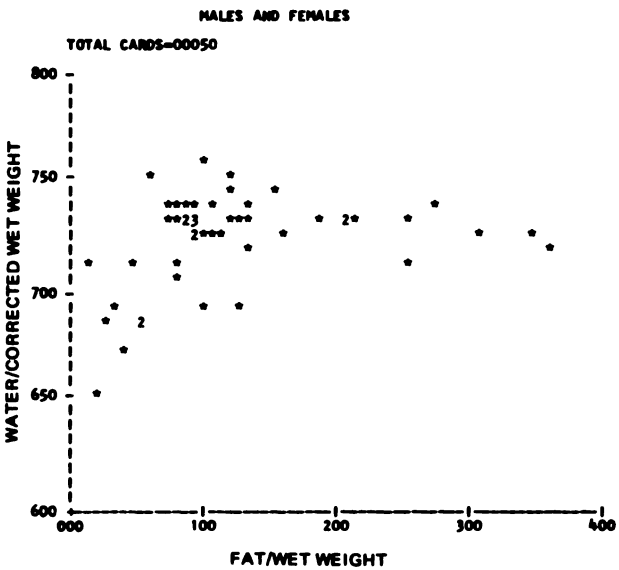


FIGURE 1 Computer printout of percentage of water x 10 (ordinate) versus percentage of fat x 10 (abscissa) for male and female guinea pigs. Data of Pace and Rathbun.[14]

field for these distributions. This is still the case if one separates the sexes (Figures 2 and 3). Pitts,[16] after reviewing his own observations and the data reported from other laboratories, concluded that the water content of lean tissue is not influenced by the fat content of the carcass. We agree with his interpretation of the data. The variance of the water content of fat-free tissue of different species is remarkably small, as illustrated in Figure 4. One may infer from these data that the water content of fat-free tissue is a fundamental

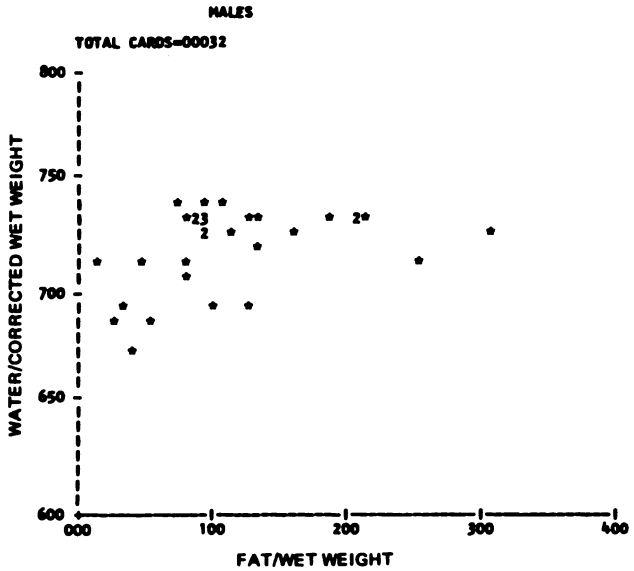


FIGURE 2 Computer printout of percentage of water x 10 (ordinate) versus percentage of fat x 10 (abscissa) for male guinea pigs. Data of Pace and Rathbun.[14]

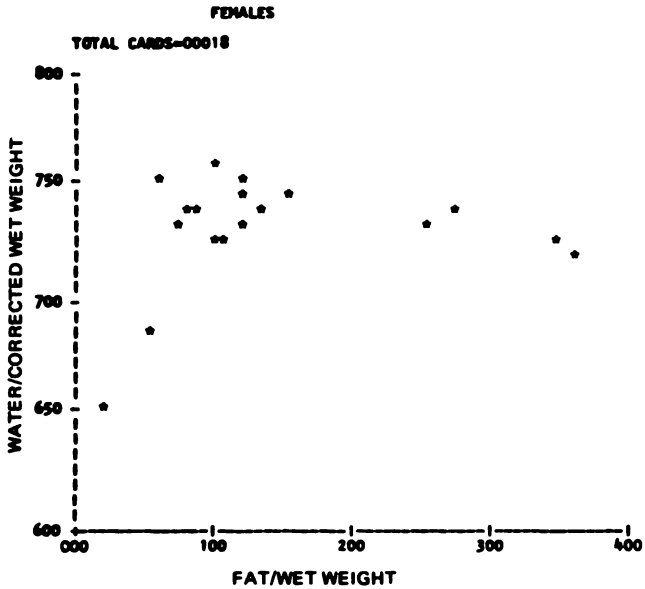


FIGURE 3 Computer printout of percentage of water x 10 (ordinate) versus percentage of fat x 10 (abscissa) for female guinea pigs. Data of Pace and Rathbun.[14]

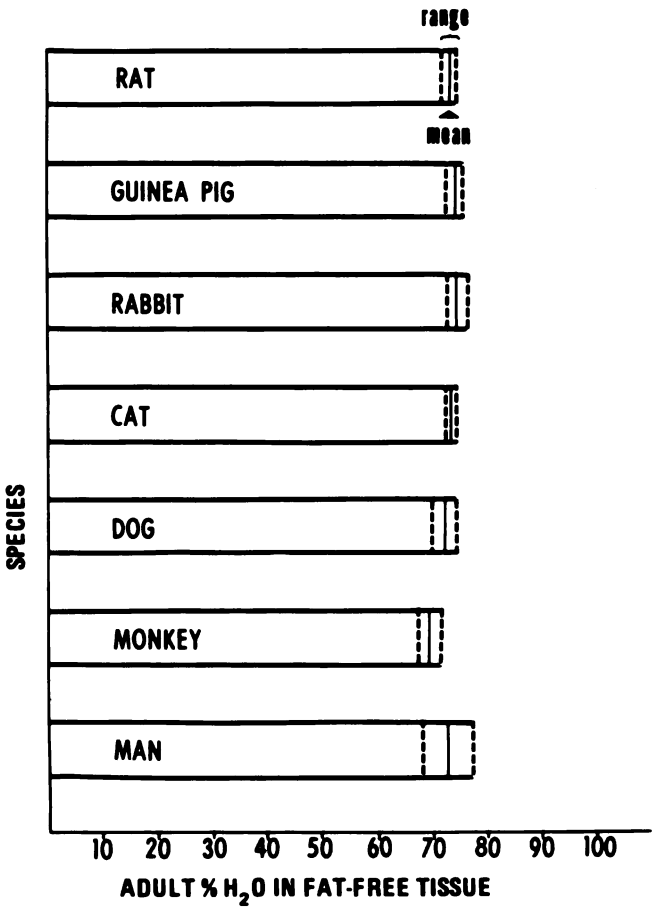


FIGURE 4 Water content of fat-free tissue of different species. Compiled by Pace and Rathbun.[14]

biological constant for any species. Crucial, of course, to this discussion are data with respect to the composition of fat-free adipose tissue of man. A limited number of analyses of adipose tissue (A. P. Remenchik, unpublished data) have indicated that the water content of fat-free adipose tissue is approximately 70% (Figure 5), whereas the potassium content is approximately 25 mEq/kg (Figure 6). Thus, the water content of fat-free adipose tissue is approximately that of other tissue, but the potassium content is very much

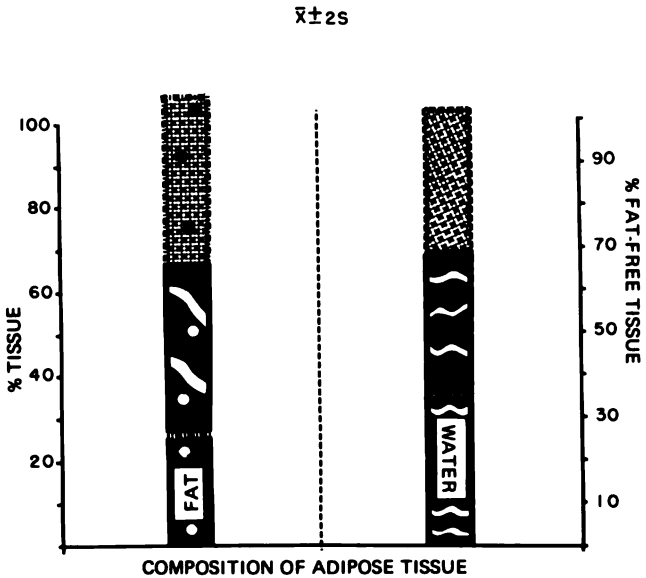


FIGURE 5 Water and fat content of edipose tissue (A. P. Remenchik, unpublished data).

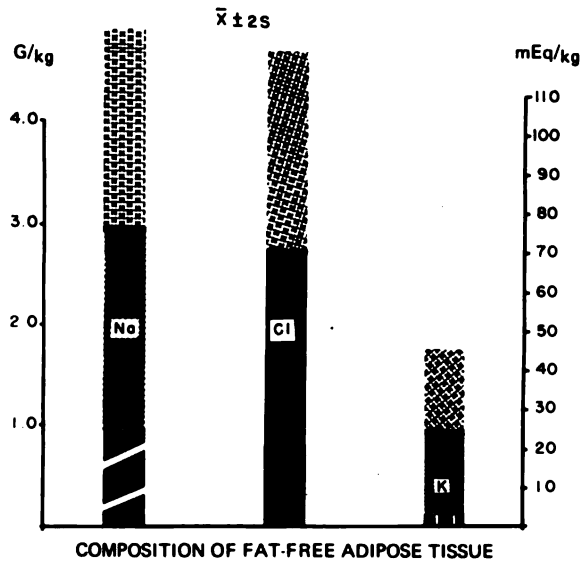


FIGURE 6 Sodium, potassium, and chloride content of fat-free edipose tissue (A. P. Remenchik, unpublished data).

less than that of muscle and viscera.* We would predict from these data that the specific gravity of fat-free adipose tissue is less than the specific gravity of fat-free eviscerated carcass, and, indeed, Pitts has reported this is the case.[16] Therefore, we predicted that potassium estimates of fat calculated by the method of Forbes *et al.*[6] would overestimate the fat content of man, and this is indeed so, for

$$F = {}^{40}K_B/68,$$

as illustrated in Figure 7 for the 8 by 4 crystal technique, and in Figure 8 for the liquid scintillation counter technique. For each subject, fat content estimated from potassium lies above the line, $y = x$. This overestimate is also the

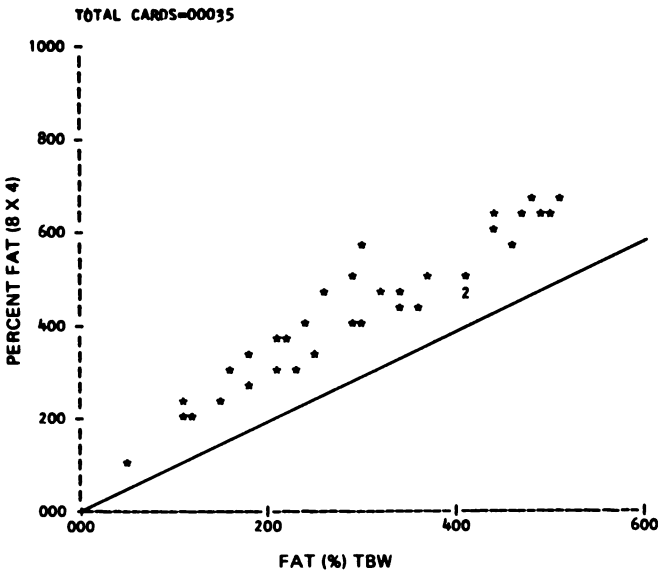


FIGURE 7 Computer printout of percentage of body fat x 10 calculated from potassium measurements (ordinate) versus percentage of body fat x 10 calculated from total body water measurements (abscissa). Potassium measured by 8 by 4 crystal technique (Argonne National Laboratory).

*After this paper was presented at the conference, Dr. Gilbert Forbes brought to our attention some data he had reported in a footnote in an article of his.[5] He stated: "Analysis of 12 samples of adipose tissue from exsanguinated rat, dog and rabbit revealed 41–93% fat (average 80%); on a fat-free basis, water content was rather uniform (81%); sodium, chloride and potassium concentrations were 122, 134 and 68 mEq/L of water, respectively." It is evident from these data that the potassium:water ratio of fat-free adipose tissue is < 1 in contrast to a ratio > 1 for muscle and viscera.

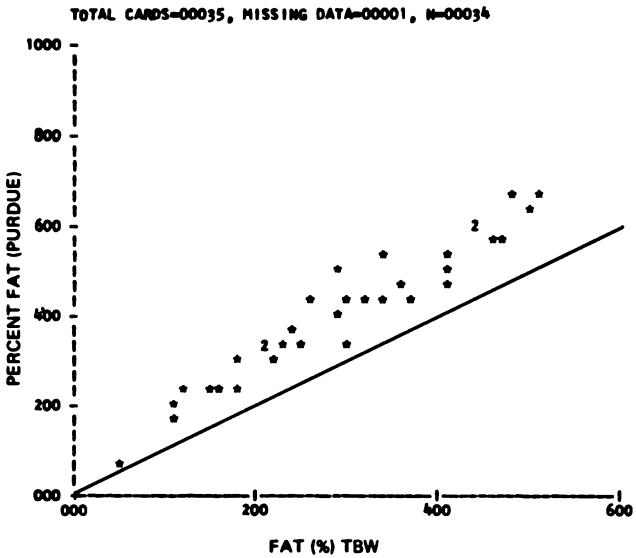


FIGURE 8 Computer printout of percentage of body fat x 10 calculated from potassium measurements (ordinate) versus percentage of body fat x 10 calculated from total body water measurements (abscissa). Potassium measured by liquid scintillation counter technique.

case for potassium determined by the other techniques. The diluting effect of fat-free adipose tissue can be illustrated in another way. If we compute the lean body mass content of potassium and plot this against percentage of body fat, we observe a significant negative regression (Figure 9). The mean potassium content of fat-free lean tissue obtained by extrapolation of the regression lines calculated for each technique to 0% fat content is 2.55 g/kg (Table 1). This is less than the mean of 2.68 g/kg obtained from analysis of cadavers (Table 2).[19] The discrepancy may be due to the fact that body water is overestimated by tritium-dilution techniques. In a footnote in his article Pitts stated:

> On human data from various sources, a significant coefficient of correlation between fatness and FFBW has not been encountered. While this circumstance suggests that the factors producing such a correlation in guinea pigs and steers are nonoperative in man, many additional and uncontrolled variables in man may mask any correlation which might otherwise be present.[16]

We have confirmed his expectations for man by plotting amount of fat versus lean body mass for men and for women (Figures 10 and 11). What can we say about fat estimates from potassium? Their utility depends upon the question. If we ask for an absolute estimate of fat, it is evident that we are probably unable to provide this estimate by potassium measurements.

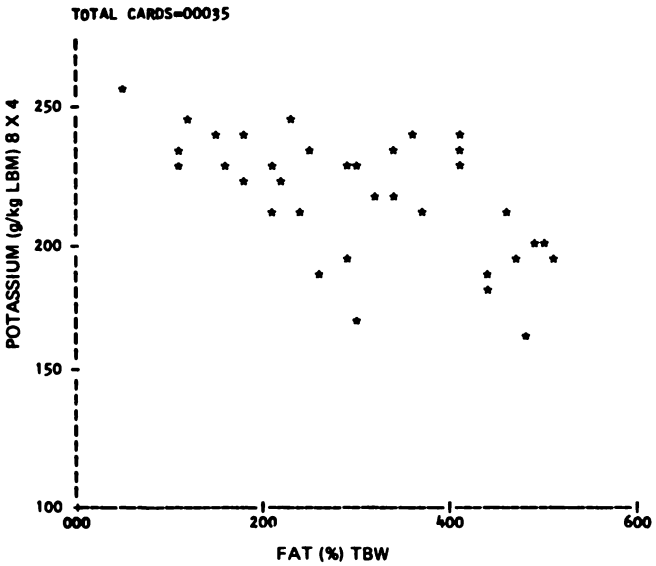


FIGURE 9 Computer printout of (grams of potassium per kilogram of lean body mass) x 100 (ordinate) versus (percentage of fat) x 10 from total body water measurements (abscissa). Potassium measurements by 8 by 4 crystal technique.

TABLE 1 Potassium Content of Lean Body Mass Calculated by Computing Intercept of Regression Line for Plot of Individual $K_{B/LBM}$ versus Percentage of Fat (from total body water measurements)

Technique of Measurement	$K_{B/LBM}$ @ 0% Fat (g/kg)
$^{42}K_{B/0.91}$	2.49
$^{40}K_{B}$ (Purdue)	2.59
$^{40}K_{B}$ (8 x 4 crystal)	2.50
$^{40}K_{B}$ (log crystal)	2.61

Do we want to rank order the population with respect to fat content? The data indicate that any estimate of potassium will do so, with a high degree of precision. However, for this purpose we can do almost as well by some simple techniques, as illustrated in Figures 12 and 13, which are plots of fat content versus body weight for females and for males, respectively.

TABLE 2 Potassium Content of Man Obtained by Direct Analysis of Cadavers

Reference	Sex	Age	K, Fat-Free Basis (g/kg)
Shohl[22]	–	–	2.61
	–	–	2.66
Widdowson[26]	M	25	2.78
	F	42	2.84
	M	48	1.31
Forbes[7]	M	46	2.60
	M	60	2.60

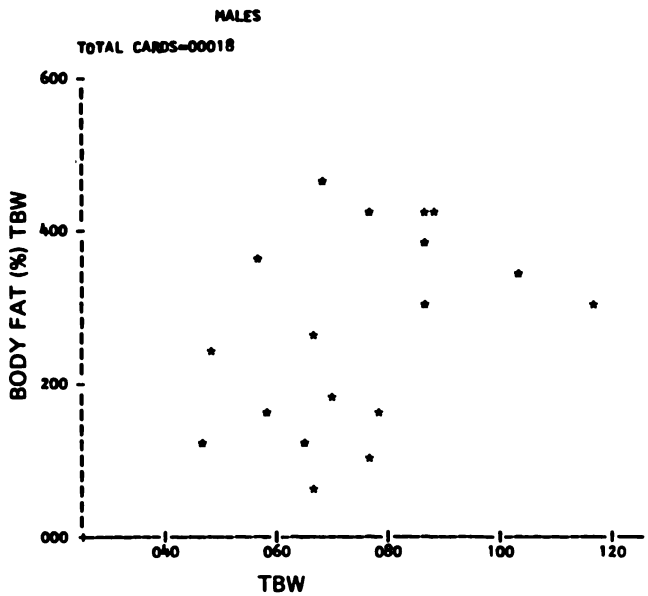


FIGURE 10 Computer printout of percentage of body fat x 10 calculated from total body water measurements versus lean body mass calculated from total body water measurements (males).

POTASSIUM AS AN ESTIMATOR OF FAT-FREE TISSUE OR MUSCLE MASS

Can we use potassium as an estimator of fat-free tissue or muscle mass? Pearson,[15] in a recent review, cautiously concluded that "present methods

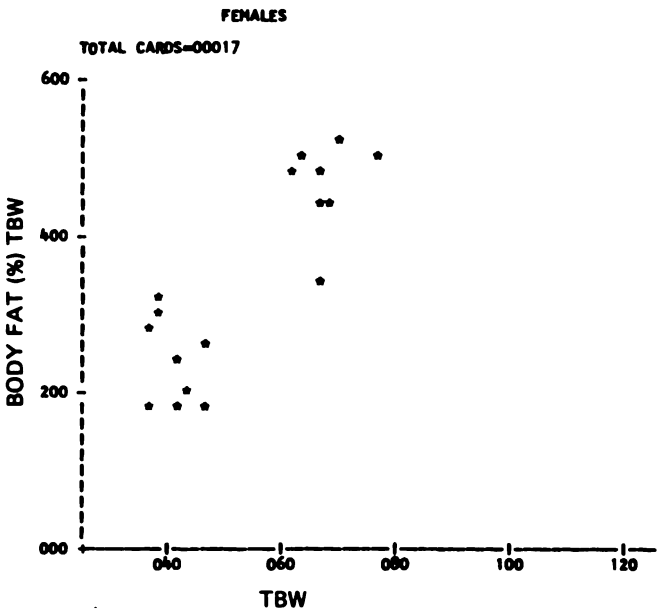


FIGURE 11 Computer printout of percentage of body fat x 10 calculated from total body water measurements versus lean body mass calculated from total body water measurements (females).

do not provide precise compositional values." We agree and would like to review why potassium cannot be a reliable estimator. Investigators proposing these models have assumed that the concentration of potassium in muscle is invariate and identical from muscle to muscle and from individual to individual. They have assumed that the potassium content of other tissue is identical for all individuals. Neither of these assumptions is reasonable.[4] The potassium content of different muscles may differ by as much as 30%, as reported by Lawrie and Pomeroy.[9] In addition, the potassium content of other tissue may vary radically, as has been shown for the sheep erythrocyte.[13] The large variance of potassium for muscle from clinically healthy subjects is illustrated in Figure 14, which summarizes data accumulated by several laboratories[2,10,24,27] (Remenchik, unpublished data). Relating potassium to intracellular water does not improve the situation (Figure 15). What is the situation if one analyzes muscle from patients who are ill? It is evident that the variance is large when one examines potassium expressed as potassium per kg of fat-free muscle (Figure 16), or potassium per liter of intracellular water (Figure 17).

This conclusion can also be deduced from an examination of the potassium content of fat-free tissue of patients selected for study when we assumed that

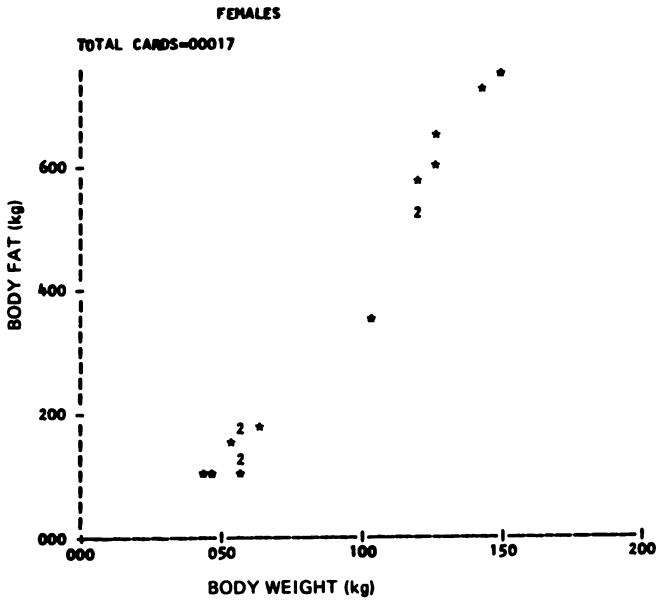


FIGURE 12 Computer printout of body fat in kilograms x 10 (ordinate) versus body weight in kilograms (abecissa) (females).

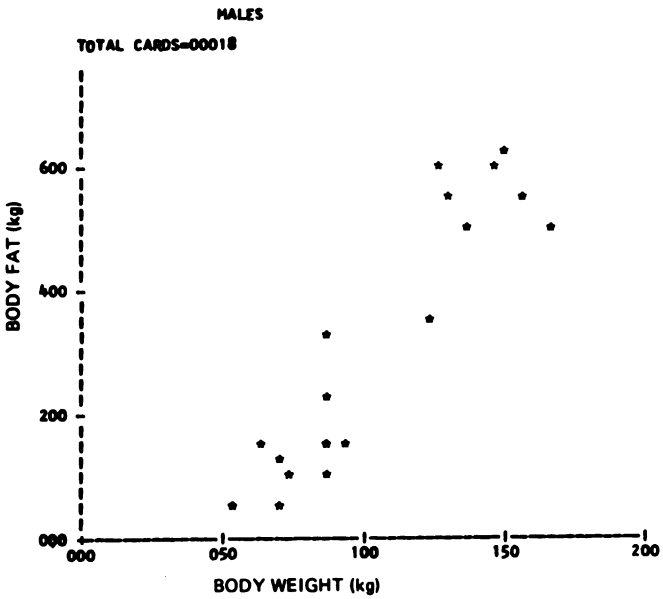


FIGURE 13 Computer printout of body fat in kilograms x 10 (ordinate) versus body weight in kilograms (abecissa) (males).

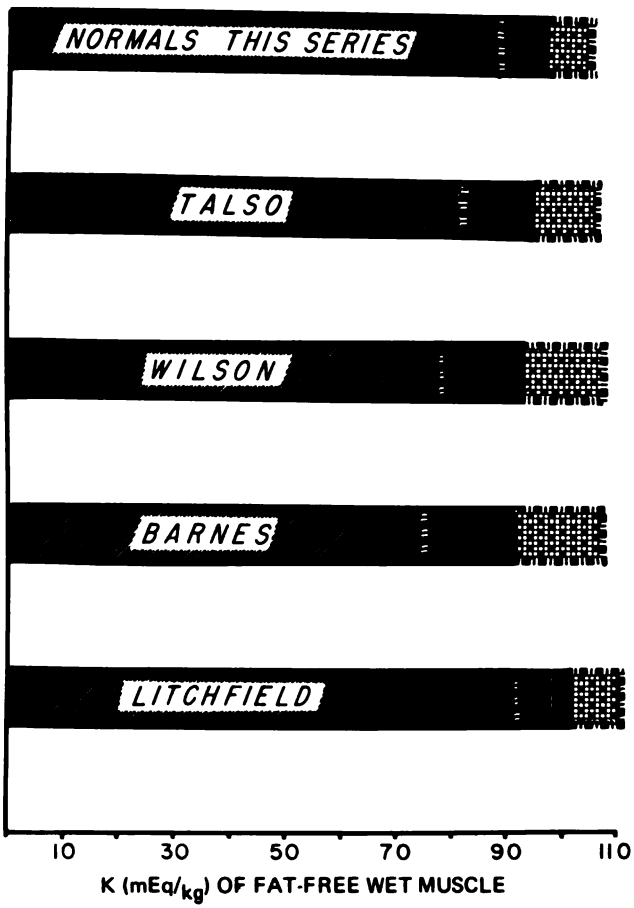


FIGURE 14 Potassium content (milliequivalents/kilogram) of fat-free muscle[2,10,24,27] (A. P. Remenchik, unpublished data).

the potassium content of lean tissue was invariate. The large variance is evident in Figure 18. Finally, we would like to state that the exchangeable fraction we reported previously[19] is the same as that reported for clinically healthy subjects by Miller *et al.* (this volume, page 350), thus confirming our view that Moore's contention[11] that potassium is 100% exchangeable is wrong. We conclude that the cell-mass model of Moore *et al.*[12] and the three-component

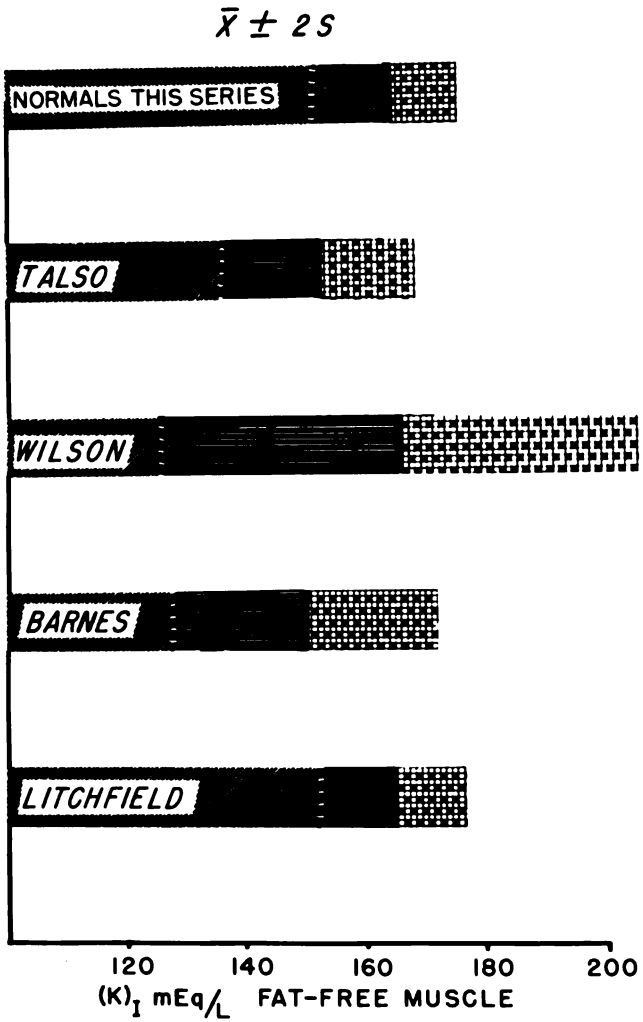


FIGURE 15 Potassium content (milliequivalents/liter of intracellular water) of muscle[2,10,24,27] (A. P. Remenchik, unpublished data).

model of Anderson[1] are not appropriate models for body-composition work. It should be evident from this analysis why Anderson could not explain potassium:water ratios.[1]

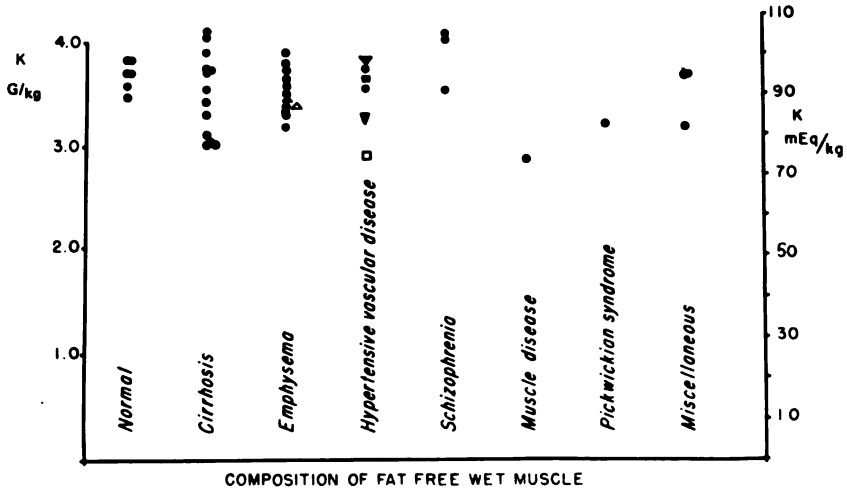


FIGURE 16 Potassium content (milliequivalents/kilogram) of fat-free muscle from patients with different diseases (A. P. Remenchik, unpublished data).

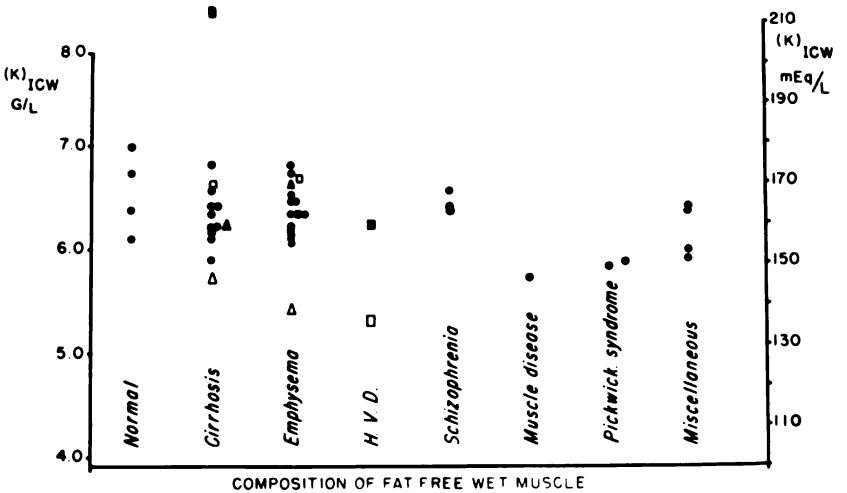


FIGURE 17 Potassium content (milliequivalents/liter of intracellular water) of muscle from patients with different diseases (A. P. Remenchik, unpublished data).

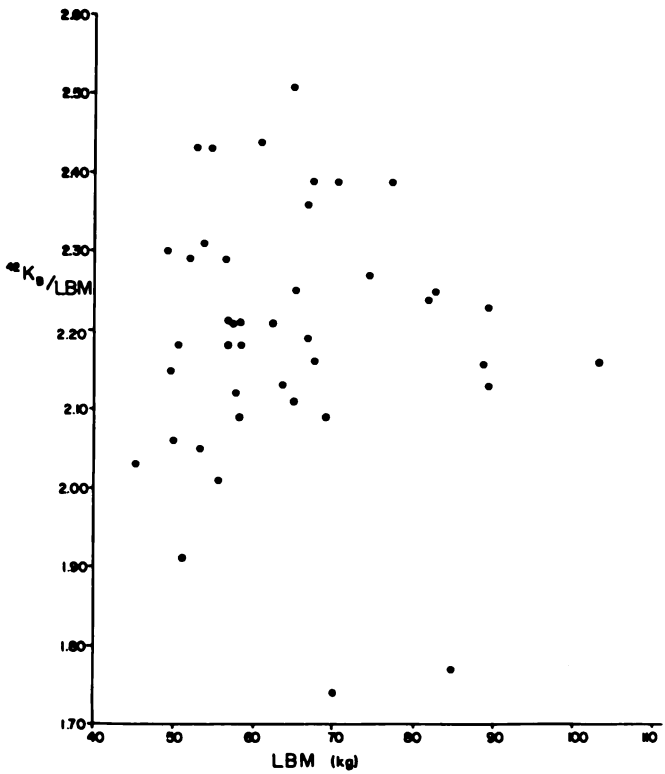


FIGURE 18 Potassium content (grams/kilogram) of lean body mass (ordinate) versus lean body mass (abscissa). Data of patients hospitalized for diseases that presumably did not affect potassium metabolism.[19]

CLINICAL APPLICATIONS OF POTASSIUM MEASUREMENTS

Does this mean that potassium measurements are useless? No, it does not. If one knows one is studying a clinically healthy population and that the experimental procedure is not going to affect the potassium concentration of fat-free tissue, then it is easy to show that the potassium content of lean tissue is stable and reproducible. Figure 19 illustrates data derived from a study of an ambulatory population.[21] Replicate measurements are illustrated by the

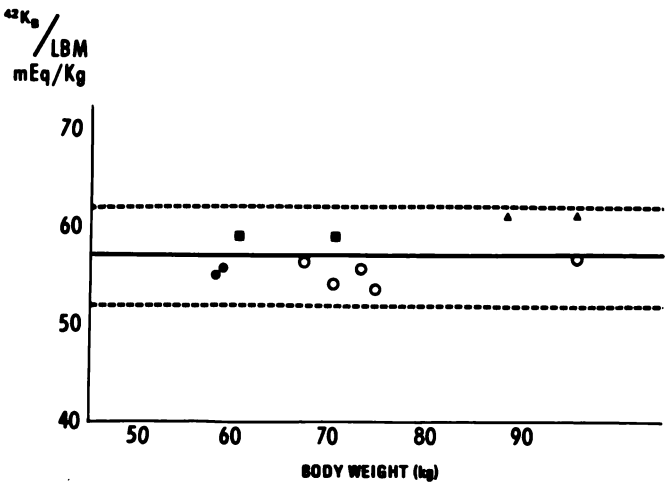


FIGURE 19 Potassium content (milliequivalents/kilogram) of lean body mass (ordinate) versus body weight of embulatory schizophrenic subjects.

solid circles, squares, and triangles. Note that the potassium content of fat-free tissue did not change much, despite a change in weight, and this is because predominantly fat-free tissue was added. As we mentioned earlier, changes in the body content of potassium do reflect changes in the potassium content of muscle. Potassium-depleting diuretics were administered to a subject with hypertension. Body potassium measurements were made, and a muscle biopsy was done before these medications were administered and after the subject had ingested the diuretics for 4 weeks. The data are illustrated in Figure 20, and it is evident the subject's body potassium was significantly reduced. The changes in $^{42}K_B$ reflected changes in muscle content of potassium and not of muscle mass or cell mass.[20]

Another illustration of the clinical usefulness of body potassium measurements in conjunction with other dilution techniques is apparent in Figure 21. It illustrates changes in body composition of a patient with acute poliomyelitis. The metabolism of potassium in this boy was similar to the metabolism of women with this disease, and different from that of men.[17] Clinically, his course resembled the clinical course of women. One can use the technique to study the effect of pharmacological agents on the potassium content of man. Figure 22 illustrates some measurements of a group of hypertensive subjects ingesting a diuretic.[18] The agent depleted them of potassium.

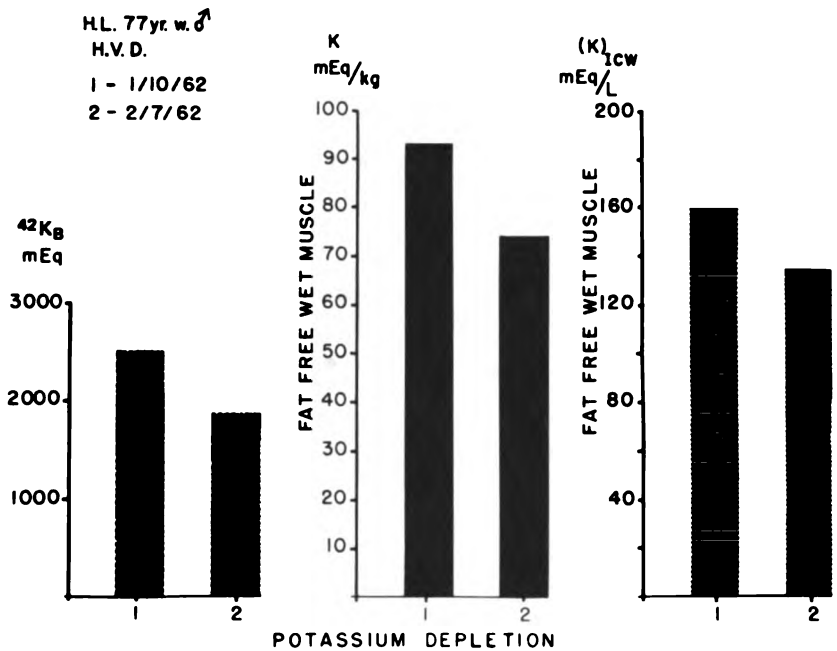


FIGURE 20 Changes in body content of potassium and muscle content of potassium after administration of diuretics.

We wanted to make some comments here with respect to relationships between anthropometric data we have accumulated, and potassium and fat, but the limited time precludes further discussion. We remember a presentation at another conference during which the speaker stated that if you have more salt water you have more salt and more water,[3] and so Figures 23 and 24 illustrate the fact that if you have more fat, then you are fatter, irrespective of sex. Needless to say, this observation is independent of the technique used to estimate fat content.

This investigation was supported in part by Public Health Service Research Grant RH 00283, Division of Radiological Health, General Research Support Grant 1S01-FR-5368, and by the American Medical Association Education and Research Foundation grant-in-aid for research project AMA-ERF 149, the United States Atomic Energy Commission, and the Department of Bionucleonics, Purdue University.

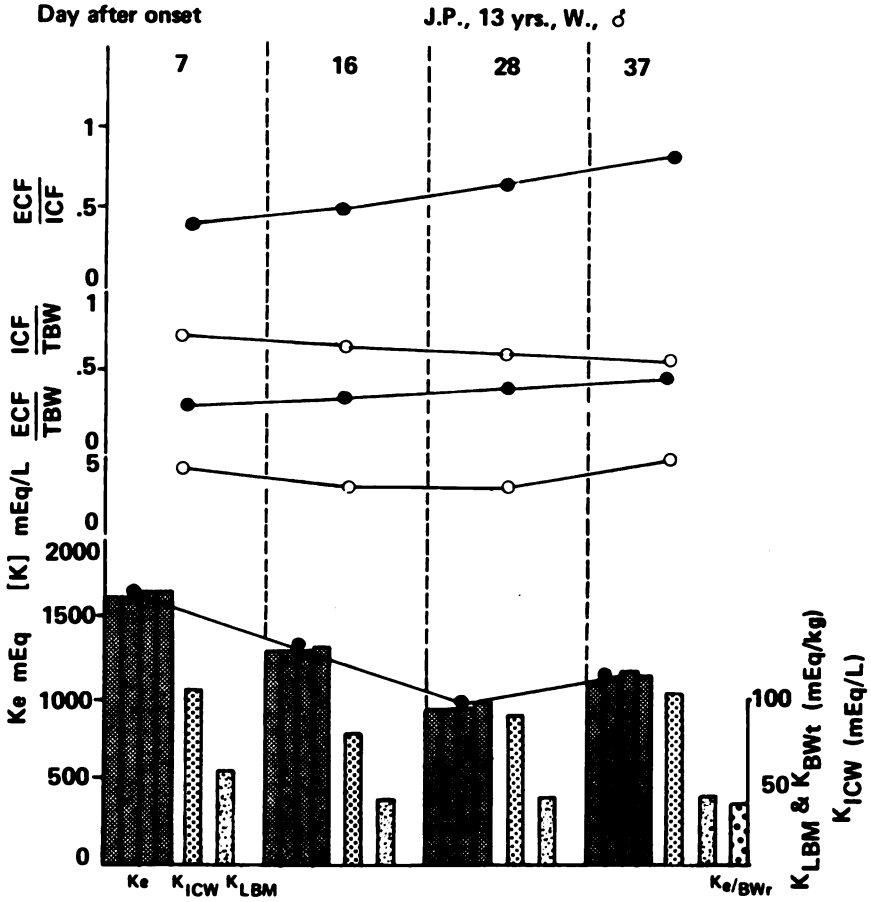


FIGURE 21 Changes in total body potassium (K_e), intracellular concentration of potassium (K_{ICW}) and lean body mass content of potassium (K_{LBM}) during the course of poliomyelitis. The measurements were made 7, 16, 28, and 37 days, respectively, after the onset of the disease.

The authors would like to thank Arthur Johnson, Jack Bechtel, and Harold Schoolman of the Biostatistics Research Support Center, Veterans Administration Hospital, Hines, Illinois, for assistance with the design of some of these experiments and with analysis of the data; Joanna Wheeler, Research Coordinator, Veterans Administration Hospital, Hines, Illinois, for general assistance during the course of this study; and Herta Spencer, Chief, Metabolic Service,

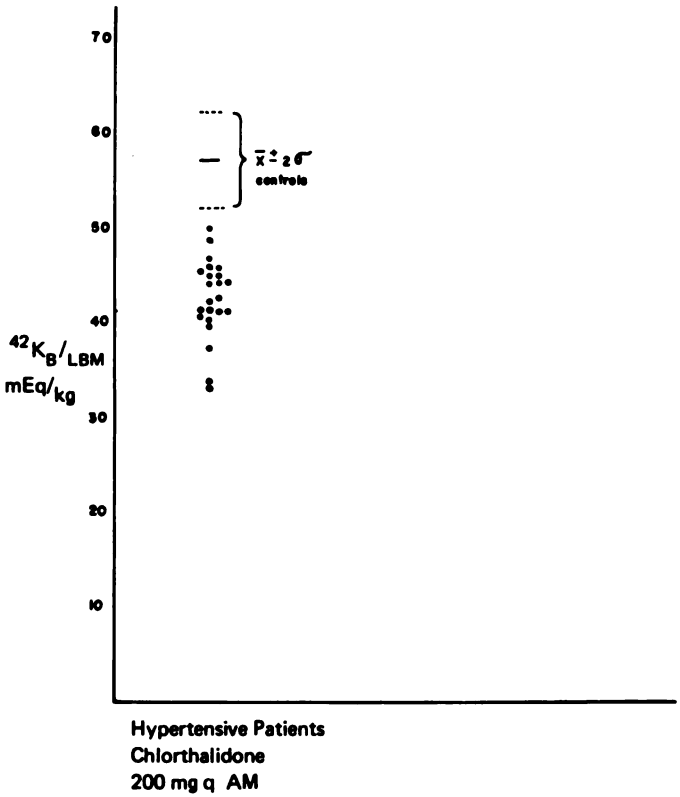


FIGURE 22 Potassium content of lean body mass of subjects to whom potassium-depleting diuretics were administered.

and her staff, Veterans Administration Hospital, Hines, Illinois, for cooperating with us by hospitalizing and managing the obese patients who starved. Some of these data were obtained during the tenure of a clinical investigatorship (APR) in the Veterans Administration Hospital, Hines, Illinois.

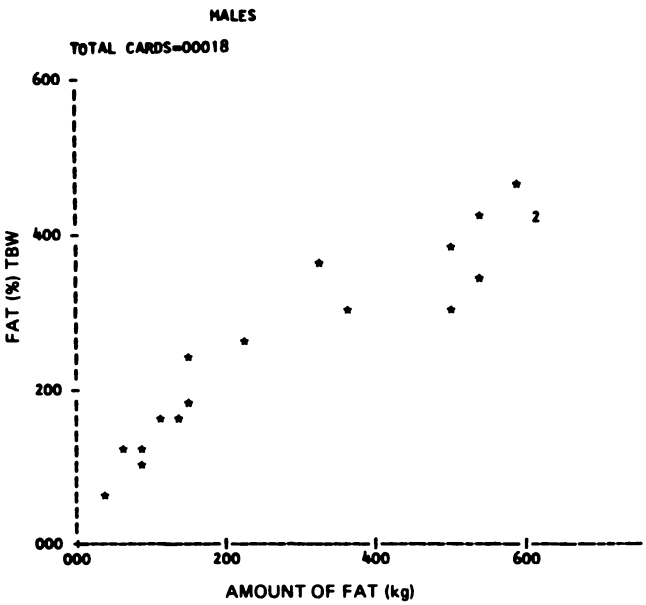


FIGURE 23 Computer printout of percentage of fat x 10 (ordinate) versus kilograms of fat x 10 (abscissa). Data calculated from total body water measurements (males).

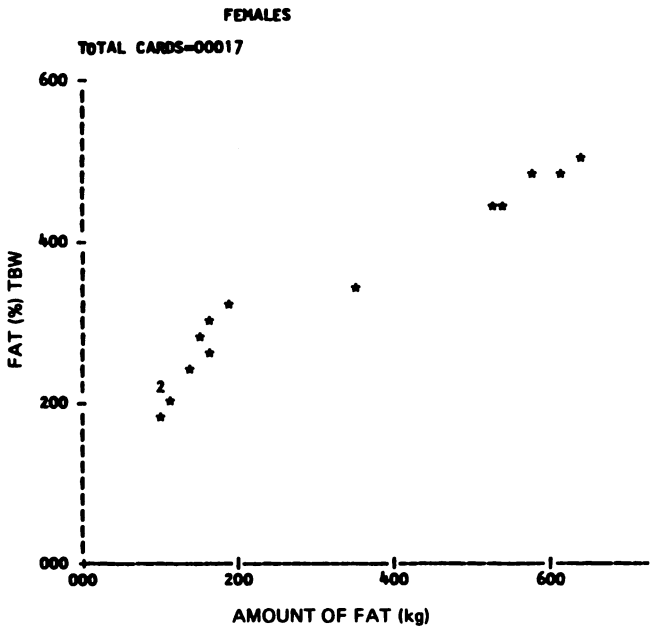


FIGURE 24 Computer printout of percentage of fat x 10 (ordinate) versus kilograms of fat x 10 (abscissa). Data calculated from total body water measurements (females).

REFERENCES

1. Anderson, E. C. 1963. Three component body composition analysis based on potassium and water determinations. Ann. N. Y. Acad. Sci. 110:189.
2. Barnes, B. A., E. B. Gordon, and O. Cope. 1953. Skeletal muscle analyses in health and in certain metabolic disorders. I. The method of analysis and the values in normal muscle. J. Clin. Invest. 32:214.
3. Boling, E. A. 1963. Changes in body composition during illness and convalescence. Ann. N. Y. Acad. Sci. 110:978.
4. Flear, C. T. G., R. G. Carpenter, and I. Florence. 1965. Variability in the water, sodium, potassium and chloride content of human skeletal muscle. J. Clin. Pathol. 18:74.
5. Forbes, G. B. 1962. Methods for determining composition of the human body. Pediatrics 29:477.
6. Forbes, G. B., G. Gallup, and J. B. Hursh. 1961. Estimation of total body fat from potassium-40 content. Science 133:101.
7. Forbes, G. B., and A. M. Lewis. 1956. Total sodium, potassium and chloride in adult man. J. Clin. Invest. 6:596–600.
8. Keys, A., and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:245.
9. Lawrie, R. A., and R. W. Pomeroy. 1963. Sodium and potassium in pig muscle. J. Agr. Sci. 61:409.
10. Litchfield, J. A., and R. Gaddie. 1958. The measurement of the phase distribution of water and electrolytes in skeletal muscle by the analysis of small samples. Clin. Sci. 17:483.
11. Moore, F. D. 1963. Discussion. Ann. N. Y. Acad. Sci. 110:211.
12. Moore, F. D., K. H. Olesen, J. D. McMurrey, H. V. Parker, M. R. Ball, and C. M. Boyden. 1963. The body cell mass and its supporting environment. W. B. Saunders, Philadelphia.
13. Mounib, M. S., and J. V. Evans. 1960. The potassium and sodium contents of sheep tissues in relation to the potassium content of the erythrocytes and the age of the animal. Biochem. J. 75:77.
14. Pace, N., and E. N. Rathbun. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158:685.
15. Pearson, A. M. 1965. Body composition, Vol. II, p. 1–40. *In* A. A. Albanese [ed.] Newer methods of nutritional biochemistry. Academic Press, New York.
16. Pitts, G. C. 1962. Density and composition of the lean body compartment and its relation to fatness. Amer. J. Physiol. 202:445.
17. Remenchik, A. P., J. Dyniewicz, and J. Schoenberger. 1959. Changes in body composition during the course of acute anterior poliomyelitis. J. Lab. Clin. Med. 53:195.
18. Remenchik, A. P., and L. C. Johnston. 1966. Potassium depletion produced by administration of chlorthalidone to nonedematous patients with arterial hypertension. Amer. J. Med. Sci. 252:171.
19. Remenchik, A. P., and C. E. Miller. 1962. The measurement of total body potassium in man and its relation to gross body composition. *In* Whole body counting, pp. 331–339, IAEC Vienna.

20. Remenchik, A. P., C. E. Miller, P. J. Talso, and E. O. Willoughby. 1966. Depletion of body potassium by diuretics. Circulation 33:795.
21. Remenchik, A. P., and P. J. Talso. 1965. Body composition of schizophrenics. Arch. Gen. Psychol. 13:444–446.
22. Shohl, A. T. 1939. Mineral metabolism. Reinhold Publishing Corporation, New York.
23. Talso, P. J., C. E. Miller, A. J. Carballo, and I. Vazquez. 1960. Exchangeable potassium as a parameter of body composition. Metabolism 9:456.
24. Talso, P. J., N. Spafford, and M. Blaw. 1953. The metabolism of water and electrolytes in congestive heart failure. I. The electrolyte and water content of normal human skeletal muscle. J. Lab. Clin. Med. 41:281.
25. Van Dobeln, W. 1962. Estimation of muscle mass of the human body from ^{40}K determination. Medd. Flygbl. Nav. Med. Namnd. 11:1.
26. Widdowson, E. M., R. A. McCance, and C. M. Spray. 1951. The chemical composition of the human body. Clin. Sci. 10:113–125.
27. Wilson, A. O. 1955. Electrolyte content of muscle samples obtained at surgical operations. Brit. J. Surg. 43:71.

S. L. Hansard, Presiding

GENERAL DISCUSSION

S. L. HANSARD The papers in this session will be discussed by Dr. G. P. Lynch, and following his comments, we will have an open discussion.

G. P. LYNCH I prepared some general remarks concerning dilution methods, but since we are approaching the hour of lowered blood-glucose levels, I think I had better restrict myself to one or two comments and then open the discussion to the audience.

I fully agree with Dr. Panaretto that perhaps the tritium-dilution method predicts body water as accurately as any we have today. This is particularly true when we think of applying these methods to farm animals. One thing that he did omit, however, is the relatively short biological half-life of this isotope. I think when people think of using the tritium-dilution technique, they automatically think of the 12½-year half-life of the isotope and immediately forget that biologically this isotope has a half-life of 4 or 5 days for sheep and cattle and about 10 days for humans. Is Dr. Panaretto here? Did you do any studies on this question with your sheep and goats?

B. A. PANARETTO No, but Till and Downs did. I omitted turnover rates specifically for one reason. Although this is the subject of the school of re-

search in Australia, headed by Professor McFarlane (who is trying to show that the turnover rate is somehow associated with the productivity of the animal), I feel that turnover rates using tritiated water in animals have been used very unwisely. There is a school that says, "Ah, there's a desert rat; he lives in the desert; therefore, he turns over his water very, very slowly. Now let's prove this." So they get a desert rat and he is given nothing to drink, and they compare him with guinea pigs, which have as much water to drink as they like. Of course, they are different, because if the desert rat turned over his water quickly he would die. So I purposely omitted turnover rate, but I feel if you are going to start talking about turnover rates of tritiated water and water in the body, then you must be fairly careful about food conditions, environmental temperatures, and a thousand and one other things. These people who are trying to do other things with turnover rates, I feel, ought to be fairly cautious.

G. P. LYNCH The point that I was trying to make is: isn't this really an advantage of this particular method, rather than a disadvantage?

B. A. PANARETTO Yes.

G. P. LYNCH Then in looking at the half-life of the isotope, let us consider the biological half-life.

B. A. PANARETTO Yes, it is gone in a few days. In fact, if you take a preinjection blood sample, you can use tritium at 24-hour intervals.

G. P. LYNCH Another point I would like to mention is one that Dr. Doornenbal touched on rather briefly. This concerned the splenic and thyroid relationships to the red-blood-cell volume. In this, he proposed that he would use tranquilized hogs in his future experiments. I had some experience in this line in my project of drowning hogs a few years ago, and I feel that it is virtually impossible to put a hog into a tranquil or docile state. I believe if you combined a tranquilizer with anesthesia, you would approach this condition a little more closely than any other way, but it is still quite a formidable task.

Many of you are going to have questions on Dr. Remenchik's work. The problem of potassium content of muscles is well known, has been well described, and is particularly important as far as muscle biopsy is concerned, but does it affect the dilution or whole-body counting methods? The red-blood-cell potassium content of sheep represents a problem when you are using dilution techniques, particularly, I think this could be accounted for prior to the beginning of the experiment, however, by accurately analyzing for this element. The biggest thing that I got out of Dr. Remenchik's talk was the point, also emphasized by Dr. Behnke, that we need to approach more nearly con-

trolled conditions for our subjects. This is being emphasized more and more as the conference progresses.

Another aspect of the conference that seems to be coming to light is that one of the approaches we should be looking at a little more thoroughly is the marriage of one method of analysis to that of the other, so that we can predict several body components. I mention it again for re-emphasis.

S. L. HANSARD The papers presented in the session on dilution methods are now open to audience discussion.

M. A. FLYNN I have been impressed with the fact that two investigators castrated their males before they compared them with females. Now in human studies, we do not have this privilege. We are perfectly happy to accept humans intact.

S. L. HANSARD Is there another question or comment?

N. PIERSON The red-cell volume is a very respectable measurement with a very small standard deviation. (This is directed to Dr. Doornenbal.) The total blood volume, though, is a very unrespectable measurement when you estimate it from the red-cell mass and not by measuring the plasma volume separately. While those lines look quite nice, why not take the red-cell mass that you are taking the trouble to measure (a lot more trouble, incidentally, than measuring the plasma volume)? Why not make your correlations between red-cell mass and protein in weight, rather than the total blood volume, which is a derivative, and since the venous hematocrit varies so much from one place in the body to another, and an unrespectable derivative?

H. DOORNENBAL They were identical. I could just as well have taken the red-cell mass. These were, in this particular instance, identical correlation relationships. There is no difference, and probably I should have taken the red-cell mass in plotting our graphs, but it would not make any difference. I may just make a comment on the whole hypothesis. Muldowney has indirectly proved this to be quite a correct estimate of lean body mass in man. I still feel that his hypothesis is probably right. It is just the application to individual animals and refinement of the technique, which is a problem when working with pigs. They are difficult animals to handle, and we need standardization, but I do not know how we would do it. Some form of standardization for just a half-hour period is all that is required. We all know it is difficult, but standardization may show a different light of the application of this technique to composition studies.

S. L. HANSARD I have one question. Did you plot this as a percentage of body weight or total nitrogen versus total body mass?

H. DOORNENBAL No, it was plotted as totals; total blood volume or red-cell mass with total protein as weight.

G. M. WARD I have a comment and a question. Not having worked previously with pigs, we stumbled on a good means of tranquilizing them, which Dr. Johnson may mention this afternoon. When they are flopped bottom side up, they are very quiet and cooperative.

I have a question for Dr. Remenchik. He hastily went over graphs showing potassium on an intercellular water basis, and I was wondering how the intercellular water was calculated.

A. P. REMENCHIK That was a self-laid space with samples being taken every hour for 5 hours and then extrapolated back to "0" time, and repetitive measurements of subjects give standard deviations of 4 or 5%. They were not single shots, which I think are useless. Anybody who does single-shot spaces is wasting our time.

B. A. PANARETTO May I ask a question? How do you measure intercellular volumes?

A. P. REMENCHIK You are absolutely right. I should be saying intercellular space. We should use pragmatic definitions or methodological definitions.

B. A. PANARETTO Have you looked at the concentration of the marker in the gut at the same time that the extracellular marker was determined?

A. P. REMENCHIK What do you mean by look?

B. A. PANARETTO Have you done any analyses?

A. P. REMENCHIK Do you mean in humans?

B. A. PANARETTO There are stomach tubes, aren't there?

A. P. REMENCHIK Yes.

B. A. PANARETTO Well, have you done the analysis?

A. P. REMENCHIK No.

B. A. PANARETTO That answers my question.

A. R. BEHNKE In work using radiation as a tool, with certain species, particularly the dog, the Radiological Defense Laboratory found it necessary to tie off the spleen. The spleen has a large reservoir of cells (I do not know about other species), but this would be a source of large error. If the animal is excited, adrenalin would squeeze out these cells, and one might do these

other things under conditions that excite adrenalin output to get these cells mobilized. I certainly agree that the plasma volume, which is a routine measurement, should be done along with the other measurements.

Then, Dr. Remenchik, when you measure sodium exchangeable and potassium exchangeable relative to body water, what is the K value, and how constant is it?

A. P. REMENCHIK For the clinically healthy subjects, it is very constant. Unfortunately, it is rather difficult to persuade people to participate in these kinds of studies unless you are in the military service.

A. KODOMA I wonder if any of the speakers would like to comment on the ethanol-dilution method reported by the Australian workers on sheep and by the British workers on humans. The Australian workers indicated that it is the easiest method and as accurate as the antipyrine method.

B. A. PANARETTO I would rather not comment. I cannot get a decent method for analyzing ethanol in blood. There must be one, because of the police court cases. No one I know is using it in Australia now. In fact, I do not know of anybody who is using it in the world, but that does not mean it is wrong.

D. LISTER I can only say that the work on ethanol was done by Dr. Southgate, and I think the thing was that he was unable to find a good zero-time point. This was the whole problem.

E. M. WIDDOWSON I would just like to come back to the question of the constancy or not of the water and of the potassium in the lean body mass. I agree with all of you that, for all practical purposes, it is fine; it is more or less constant because we have rather the same proportions in liver, muscle, bone, and so on, but when you come down to saying that this is a biological constant, that is when I quarrel with you. I know it is now two-to-one, but I still stick to my point, and I think you are arguing against yourself because you have shown how it varies from muscle to muscle. The potassium also varies from one part of the skin to the other. The skin of the abdomen is much more cellular than the skin of the back, and it has a much higher potassium concentration, so we know that different parts of the body all contribute different proportions to the body weight in different individuals. So this cannot be a biological constant.

A. P. REMENCHIK Thank you, Dr. Widdowson, perhaps you are right; it may not be the proper choice of words. I mean that I hope you will appreciate the fact that I say some of these things to provoke some discussion, which I have. Anyway, I think it is more constant than potassium. The point I am

trying to make is that the variation of water in lean tissue is considerably less than the variation of potassium. Potassium is much more labile ion in the body. Would you agree with that?

E. M. WIDDOWSON Yes, but I think that what I say about potassium I say also about water; that is also not a biological constant, but it may be more constant for practical purposes than potassium.

A. P. REMENCHIK Potassium is more labile than water, right? Would you agree to that?

E. M. WIDDOWSON It is more variable.

A. P. REMENCHIK More variable than water; all right. So, as a result, because of this variability from tissue to tissue, we cannot make potassium measurements and from them assume a certain constant for the body and predict size of body compartments. That is all I am saying. I am trying to say that the measurement of potassium is an exceedingly important measurement. This is a very important ion; some work I have done, the results of which have been published, shows that potassium depletion produces changes in the levels of ATP, AMP, and ADP in the erythrocyte. These are exceedingly important and interesting changes, so it is an important ion, and it is important to measure. We can measure the total amount, but we cannot use it to predict sizes of compartments. That is the point I am trying to make.

E. M. WIDDOWSON Now, we come together over the fact that potassium is important.

S. L. HANSARD Is there any other question or comment.

A. R. BEHNKE I would like to make a comment. No conference like this has a happy ending–I know we are not yet finished, but it seems to me that there should be something on which we have a high correlation to go away with, and I think we should plot the inconstancy of potassium against the inconstancy of body water.

S. L. HANSARD Would it be acceptable to Dr. Remenchik to qualify his statement about the constancy of body potassium in relation to body water by saying that potassium is more variable than intercellular water rather than the unqualified statement of body water. This would be more in agreement with the statement that the intercellular water for the species at a given age is more or less constant, and erratic changes that take place are really in the extracellular water.

A. P. REMENCHIK I think what you are saying is that you and Dr. Panaretto disagree. That is how I interpret it, but I would agree with you; yes.

MILLER I want to make one comment that may be out of line here. The question was asked by the discussant if Dr. Panaretto had checked the turnover rate of tritium. I have been making a lot of measurements on the retention of isotopes in animal versus age, and I have a reputation for one bad habit. I do not measure the animal today, next week, next month, next year, but I measure it every day and have hundreds of measurements on it. Where you, or someone else, can draw a nice straight line through this, we have 18 or 20 points between each two that he has, and we find that the retention of different radioisotopes in an animal varies greatly according to the age at which he was given it and the length of time that he has it. Someone traced cesium in mice recently, and it did not follow a power function. It will follow one exponential awhile, and suddenly it will break and change to another exponential, and then it will break and change to still another exponential, and this is not the sum of exponentials. It is simply one exponential, then another. Something goes on, and these breaks depend upon the age of the animal when you gave it the isotope. If you are essentially doing retention of tritium in animals, and if you have not done this, perhaps it should be investigated as a function of the age of the animal. As I mentioned, this man from the University of Utah traced cesium in mice, and his data demonstrate exactly the same thing. The mice excreted the cesium at a certain rate for 15 days, and at 15 days there was a break; then it went along to a certain point, and at that point, there was another break. It followed the data we had obtained on our animals of that age perfectly. It is not an artifact; I have no explanation for this. But, if you are checking turnover rates on tritium, be a little suspicious of the age of the animal, and how long he has had it. Maybe it will not come into play at all, but be forewarned.

S. L. HANSARD That is one of the rhythms of nature, I guess.

WHOLE-BODY COUNTING

G. M. Ward
Colorado State University

INTRODUCTION TO WHOLE-BODY COUNTING

My purpose in introducing this session on whole-body counting is to acquaint you with the available types of whole-body radiation detection devices. The emphasis will be upon their application to body-composition research with domestic animals. Four symposia[1-4] on whole-body counters have been conducted in recent years, but with almost exclusive emphasis on applications to man.

The emphasis of this conference is upon applications to animal research. In the calibration of whole-body counters, animal research workers have an enormous advantage over those in the medical field. They can slaughter their subjects and by chemical analysis determine the precision of their estimates from whole-body counting. Animal research is utilizing techniques developed for human uses but, in turn, it is anticipated that much information of use in medicine will be gleaned from experiments on whole-body counting of animals.

Whole-body counters as we know them today were developed in the 1950's for monitoring personnel working in the rapidly developing field of nuclear technology. Whole-body counters are expensive to build, and an even greater cost has been involved in the talent and time required for the design, development, and calibration of these instruments. The costs probably could have

been borne only by our large national laboratories. It is a benefit of AEC-sponsored research that these counters have become available to evaluate body composition. It is doubtful that the livestock industry ever would have allotted the resources necessary for developing whole-body counters solely for evaluating body composition of meat animals.

Estimating the body composition of a living subject by whole-body counting is feasible only because of the direct relation of potassium to lean body mass and its indirect relation to fat. Potassium is the only single element found in body tissue in significant amounts that has any predictive value of body composition. Potassium is found predominantly in the intracellular space, and thus total body potassium is indicative of total body cell mass. Potassium is not found in fat, and thus if potassium is present in the fat-free tissue as a constant percentage, then a value for total body potassium can be converted to a weight of lean body mass (LBM). It follows then that body weight minus LBM equals body fat. Please note that fat-free tissue and LBM are used synonymously.

The properties of ^{40}K that confer a unique value for estimating total body K and hence body composition are:

1. ^{40}K is an isotope of, and hence is metabolically identical to, the only element (potassium) that appears to have value in determining lean body mass.
2. ^{40}K has a long physical half-life (3×10^9 years), and it is uniformly distributed with all stable potassium in nature to the extent of 0.012%, which is a high enough concentration to make possible the external detection of the isotope in the living animal.
3. ^{40}K disintegrations produce gamma rays of high energy (1.46 MeV). Whole-body counting requires gamma-ray emission and the energy of gamma ray must be reasonably high.

A whole-body counter consists of a chamber shielded usually by 4 to 8 inches of steel against environmental or background radiation. Shielding is necessary in order to measure the low levels of radiation resulting from radionuclides such as ^{40}K.

Gamma rays produced by disintegration by ^{40}K are detected by scintillators. Two types of scintillators are in general use: the sodium iodide crystal detector and the liquid or plastic scintillation detector. The major advantage of the sodium iodide crystal detector is its high-energy resolution, which allows gamma-ray spectrometry. The advantage of a liquid or plastic scintillator is that, being of lower cost, it may be used in configurations that give better counting geometry (2-pi or 4-pi) and consequently shorter counting times. A sodium iodide crystal is the detector of choice if several radionuclides

are present with different gamma-ray energies. Liquid scintillators are favored where interfering nuclides are not important and rapid counting is important.

We are fortunate in having here two men who were pioneers in the development of these types of whole-body counters and who represent the two national laboratories responsible for much of the development in this field: Dr. C. E. Miller of the Argonne National Laboratory, who is a pioneer in the field of crystal-type counters, and Dr. E. C. Anderson of Los Alamos whose discussion of liquid scintillation counters is published in these proceedings.

REFERENCES

1. Meneely, G. R., and S. M. Linde [ed.]. 1965. Radioactivity in man (Northwestern University, September 1962). Charles C Thomas, Springfield, Illinois.
2. Whole body counting. 1962. (Vienna, June 1961.) International Atomic Energy Commission, Vienna.
3. Meneely, G. R., and C. C. Thomas, [ed.]. 1961. Radioactivity in man. Charles C Thomas, Springfield, Illinois.
4. Allsop, C. B. [ed.]. 1956. The measurement of body radioactivity. Brit. J. Radiol., Suppl. 7.

E. C. Anderson
Los Alamos
Scientific Laboratory
University of California

ORGANIC SCINTILLATION DETECTORS AND THEIR USE IN THE STUDY OF BODY COMPOSITION

A major objective of this conference is the assessment of the validity of methods for determining body composition. It seems appropriate, therefore, in a discussion of the characteristics of organic scintillation detectors to focus attention on their accuracy and to review the various sources of error that can affect their performance. This paper, therefore, contains only a brief discussion of the design of these counters and is concerned primarily with the magnitude of the errors to be expected and with general methods of evaluating and controlling these errors. The data are derived from the measurement of normal human subjects in 4-pi counters—a favorable situation in which the errors are comparatively small. An attempt is made to indicate what might be expected in counters of more limited geometry and with larger samples.

BASIC DESIGN OBJECTIVES

The natural radioactivity of potassium (due to the isotope ^{40}K) offers an attractive method for the nondestructive measurement of the total amounts of that element in large samples, especially living animals.[6,12,14,15] The

gamma rays emitted have an energy of 1.46 MeV, and their penetrating power is sufficient to permit escape from massive samples without prohibitive self-absorption. However, the concentration of potassium in the normal body is very low. In terms of specific radiation, the gamma-ray emission rate is only about 0.4 min^{-1} g^{-1} for an adult human male with 150 g of K in a body weight of 70 kg. Measurement is, therefore, an extremely low-level counting problem. (For comparison, natural radiocarbon in the human body has a specific activity of 2.5 betas min^{-1} g^{-1}, about 6 times higher.) Only the very large mass of sample available makes it possible to measure animal potassium in a reasonable counting time, and there is a real incentive to increase the counting efficiency. The large organic scintillation counter is designed with this objective primarily in mind. A geometrical efficiency of 94% may be obtained for a sample 1.5 m in length. The total efficiency for a point source of ^{40}K can be as high as 77% in a wide energy band and 36% in an operational potassium band, with energy discrimination against ^{137}Cs, ^{65}Zn, and ^{60}Co.[18] As a result of this high efficiency, human potassium can be measured to a precision of ± 4 g (one standard deviation) in 3 min of counting time.

A very important "by-product" of high counting efficiency is a greatly decreased dependence of counting rate on the location of the radioactivity within the sample. This matter is discussed in greater detail below.

To obtain a high counting efficiency, it is necessary that the detector intercept and absorb a large fraction of the radiation emitted by the sample. Enclosing a sizable animal more or less completely is technically impossible with the best (in terms of energy resolution) gamma-ray detectors (the "solid-state" silicon or germanium semiconductors) and is prohibitively expensive with the second-best detector (the sodium iodide crystal). Organic scintillators (liquid or plastic solutions) rank as third best for gamma spectrometry because of their limited energy resolution and low stopping power but are economically feasible and, if the objective is the rapid counting of simple, known mixtures of gamma activities, some energy resolution can be sacrificed to reduce counting time and to increase accuracy.

DESCRIPTION OF A COUNTER

HUMCO I, a typical organic scintillation detector of the size commonly called a human counter, is shown in schematic cross section in Figure 1. In counters of this type, the subject is contained in a cylindrical tube that may be 46 to 56 cm in diameter and 1.8 m long, surrounded by a layer of scintillator 10 to 30 cm thick in various models. Since the detector almost completely fills the solid angle of 4-pi steradians, counters of this geometry are often called 4-pi systems. (Similarly, a half cylinder is called 2-pi.)

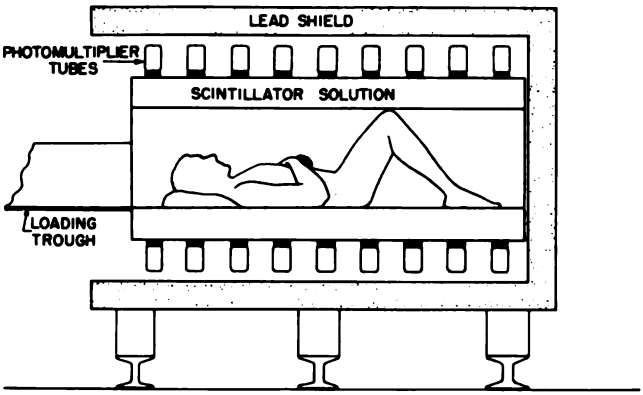


FIGURE 1 Schematic cross section of a 4-pi liquid scintillation human counter (HUMCO I).

HUMCO II, the Los Alamos human counter facility,[4] is shown in Figure 2. The data used in this paper were obtained largely with this counter. The detector tank containing the liquid scintillation solution (PPO plus POPOP in a

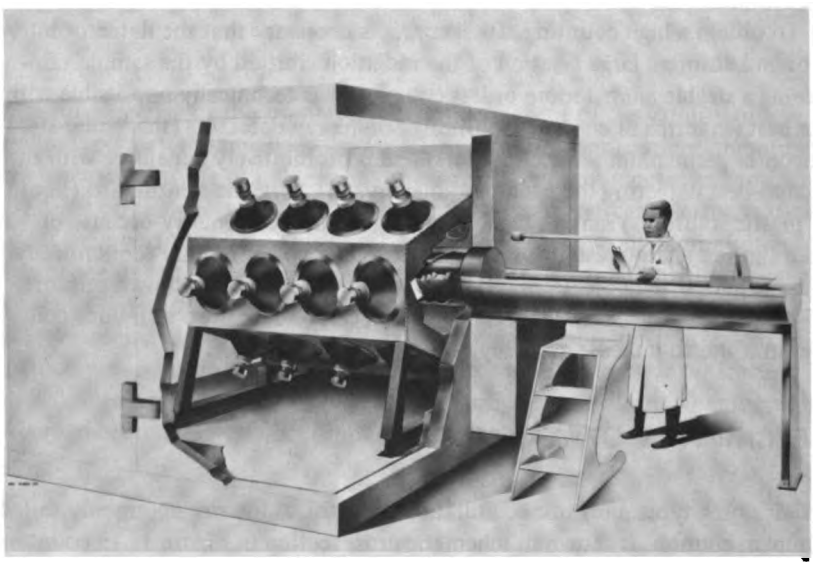


FIGURE 2 The Los Alamos human counter II (HUMCO II).

commercial paint solvent, TS-28)[18] is visible through the open door of the 100-ton steel room (walls 18 cm thick). The subject is transported into the counting well by a motorized sling-and-trough system. The scintillator has a minimum thickness of 30 cm and a total mass of more than 1,000 kg. Scintillation light is collected and amplified by 24 multiplier phototubes (DuMont K-1328) with photocathodes 16 in. in diameter. A multichannel analyzer is available for energy calibration and testing, but routine measurements are made with six single-channel analyzers and scalers covering the energy range from 0.1 to 2.7 MeV, the potassium channel extending nominally from 1.15 to 1.68 MeV (energy scale based on maximum double-Compton scattering).[18] Total counts in each channel, counting time, and auxiliary information (such as sample identification number, sample weight, date, and clock time) are punched directly onto IBM cards at the end of each automatically timed count. All data processing and analysis are done by digital computer.

The energy resolution of HUMCO II is adequate to give complete separation of the ^{40}K double-Compton peak from ^{137}Cs, as shown in Figure 3. For a detailed discussion of theoretical and attainable resolution in detectors of various sizes and geometries, see Van Dilla and Anderson.[18] Even with considerably poorer resolution than this, ^{137}Cs is no problem, but ^{226}Ra daughter products have spectra that overlap the spectrum of ^{40}K. Some fission products, especially $^{140}Ba/^{140}La$, also overlap.

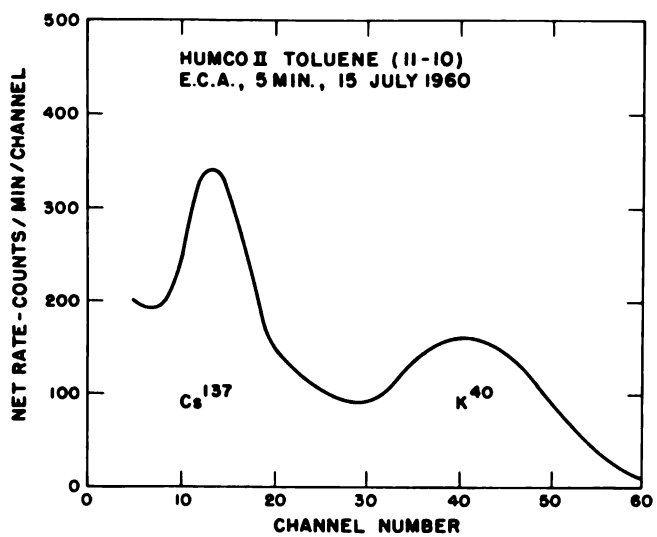


FIGURE 3 Net gamma-ray spectrum of a human subject as measured with HUMCO II.

SOURCES OF ERROR IN TOTAL BODY POTASSIUM DETERMINATIONS

It is of fundamental importance in any investigation to establish the nature and magnitude of the errors of the analytical methods. This is particularly true in attempts to determine body composition where a high degree of accuracy is required, because the natural homeostasis of the organism limits the range of variability of any parameter, often severely.

The present paper does not consider the question of the reliability of the biological models used to analyze the primary data, although this is, indeed, a point of equal importance. Clearly, it is not possible to reach a decision as to the adequacy of a method nor as to the accuracy required independently of the subsequent calculations to be made with the data. A basic question to be decided in formulating a model for interpreting total potassium data is whether the potassium is to be considered as correlated with a single physiological component, or whether it is to be distributed among two or more biologically significant and independent entities.[1,19] If the latter, then what additional parameters (water, density, and other electrolytes, for example) will be simultaneously determined? The degree of sophistication of the model will be determined by the ambition of the objective. Crude correlations can be based on approximate models; specific conclusions require that the model display at least some of the natural complexity of the subject. Thus, in a manner typical of scientific progress in general, one proceeds by successive approximations as theory and experiment develop together.

It is already clear that the brilliant approximations of the early investigators of body composition (the simple two-component models of body composition)[5,10] must be refined and extended if comparable progress is to continue. This means that the requirements for precision and accuracy on current experimental techniques are more stringent than in the past, and correspondingly closer attention must be paid to the several sources of error.

Several independent sources of error can affect the accuracy of potassium determination by whole-body gamma counting. These errors are common to the sodium iodide spectrometer and the high-geometry organic scintillator, but their relative importance may vary in the two systems. They are discussed here only in connection with the latter detectors, but *mutatis mutandis* may be applied also to crystals. The different error sources can be identified and evaluated by different methods, and their control or correction is accomplished (or attempted) differently. The primary sources of error are counting statistics, instrument instability, contamination, and calibration errors: efficiency and background depression. The first two cause random errors and

thus affect precision (i.e., reproducibility) but do not introduce a consistent bias into the results. The third may be more or less random in its magnitude and frequency, but contamination of the sample is, of course, a consistent error in that it can only make the result too high. Erratic background variations due to contamination of the detector or shield can cause either random or consistent errors, depending on their pattern of incidence. Calibration errors are consistent and may be more serious, particularly in that they are harder to detect and the experimenter may be misled by a high degree of reproducibility.

COUNTING STATISTICS

Counting statistics, the simplest of the error sources, is well understood theoretically, and can be predicted *a priori* from first principles (that is, the standard deviation associated with the observation of N random counts is equal to the square root of N). It is also perhaps the least important with high-efficiency detectors, because even a counting period of a few minutes is sufficient to reduce the counting error to the level at which it may cease to be limiting and other factors take over. This is not always the case, however, and if other sources of error are minimized, the counting statistical limit can be pushed surprisingly far. Thus, in the determination of human potassium with a 4-pi counter, under "routine" conditions a 3-min counting time was expected to give a counting error (one standard deviation) of ± 3 g of K; whereas the observed scatter of repeated measurements on a standard phantom corresponded to ± 5 g of K.[2] Clearly, other sources of random error were contributing ± 4 g of K and thus were somewhat larger than counting statistics alone. By refining the measurement technique, which meant primarily the measurement of background before and after every sample, it was possible to reduce the error to ± 3 g of K and even to demonstrate a reproducibility of ± 1 g of K for 30-min counting times, in agreement with the theoretical limit.

The theoretical error based on counting statistics is, therefore, a firm limit to the precision attainable but is a goal that may or may not be reached. It is important to know what the limit is for a given system so that one will not attempt demonstrably impossible experiments or waste time attempting to improve other components or procedures if counting statistics are, in fact, limiting. Evans (reference 9, Chapter 26) has published an excellent discussion of the laws of counting statistics. While sometimes called nuclear decay statistics, the relevant laws are, in fact, more general than this and apply to any counting process in which the probability of scoring another event is independent of previous events.

INSTRUMENT INSTABILITY

Included in instrument instability are factors that influence the precision of repeated measurements on samples known to be invariant. The inherent factors on which this stability depends are:

1. The characteristics of the scintillator as a transducer of high-energy radiation into visible light.
2. The collection, conversion, and amplification factors of the multiplier phototubes.
3. The stability of the phototube high-voltage supply (±0.002% is necessary).
4. The noise level of the system.
5. The gain of the electronic amplifiers.
6. The discrimination of the pulse-height analyzers, which select for recording only those events corresponding with the desired gamma-ray energy.
7. The reliability of the scaling system that tallies the selected events.

Maintenance, diagnosis, and repair of scintillation counters are so abstruse that they are best entrusted to a specialist. However, the present state of the art has advanced to the point where long trouble-free periods of operation can be expected, and it is sufficient that the user perform the routine tests that assure him of satisfactory performance or else signal the necessity of expert assistance. For this reason, we will not enter a detailed discussion of the factors that affect stability but will be content with a few general remarks.

By addressing our attention to the reproducibility of repeated measurements, we are concerning ourselves primarily with random, short-term errors. Longer, consistent drifts are best treated as a problem in accuracy and are discussed below. Thus, changes in the scintillator, being most likely irreversible and slow, are unlikely to be relevant in the present context.

Instrument instability is almost certain to be electronic, although the difficulties may be either internal or external (the latter including temperature fluctuations and electrical noise in the power lines). The use of standard monitor devices such as precision potentiometers on the high-voltage supplies, oscilloscope display of amplifier outputs, precision pulse generators to test amplifier gain, and discriminator settings should be routine, and a log book recording daily observations of pertinent operating data can be very valuable in helping to identify the onset of trouble and in deciding *a posteriori* whether a given set is to be considered reliable.

The best method for quantitative evaluation of instrument stability is the frequent measurement of stable, inanimate reference samples that resemble

TABLE 1 Precision of Repeated Measurements of Potassium Content of Control Milk Sample

Period	Number of Determinations	Average Potassium (g)	Observed Standard Deviation (g)
1965			
November	19	359.3	5.1
December	18	355.8	4.8
1966			
January	17	356.0	6.8
February	12	355.1	4.4
March	13	356.1	1.9
April	12	356.3	3.0
May	12	356.0	4.3
June	11	355.0	3.3
July	12	353.7	4.6
August	10	355.9	4.0
September	12	355.6	3.3
October	13	354.6	1.8
November	12	354.8	3.4
Entire period	173	355.9	4.4

the experimental objects to a reasonable* approximation. These samples are in addition to the calibration standard which provides a frequent check of a point on the calibration (efficiency versus weight) curve. The apparent activity of this standard is not a useful parameter because it is used as a normalization point. (The electronic settings necessary to maintain constancy of the standard are an indication of drift but are not interpretable as a precision index.) The reference samples are routinely measured with sufficient frequency to provide an adequate sampling of counter behavior. Statistical analysis of these data can then give a quantitative evaluation of counter precision as a function of time for samples of the type represented.

An example of results obtained with such a reference sample is given in Table 1. The sample is 23 kg of nonfat dry milk solids measured several

*"Reasonable" is deliberately vague, since the requirement depends directly on the precision required and on the variability of the experimental samples. The effect of instrumental changes on the counting rates of the reference and experimental samples should be comparable. The former may well contain more potassium, so that their precision is greater.

times weekly. The potassium content of 356 g is about 3 times larger than that of an adult subject, so statistical precision is greater. Data are averaged over 1-month periods, and the standard deviation is calculated from the scatter about the monthly mean. The resulting value of 4.4 g is somewhat larger than the 3.0 g expected if counting statistics were the only error.

Another reference "sample" is the null sample (i.e., background). The high background characteristics of 4-pi human counters make a major contribution to the error in the net activity, and a high degree of stability is required (e.g., ± 2% precision on human ^{40}K may require ± 0.7% precision on background). Background can, of course, vary for reasons other than instrumental ones (contamination, cosmic-ray intensity changes), and detailed records of its level are useful in mirroring and providing evidence about these effects as well.

The nonstatistical fluctuations in background and reference samples will probably be insignificant for short counting times where the statistical errors are large. As counting time is lengthened and the error decreased, a point may be reached at which the observed standard deviation of a series of counts ceases to follow the expected square root of time dependence. The coefficient of variation (i.e., fractional standard deviation) will reach a minimum and may actually get worse for longer counting times. It is important to know at what level this occurs. If the counter is capable of automatic operation, this is easily accomplished by letting it run a long series of repeated backgrounds (or samples) using a short counting time. The result of using a longer counting time can then be calculated by summing the appropriate number of actual determinations, and the precision of these hypothetical longer counts can be calculated from their variance about their own mean. When this is done, the results may resemble those given in Figure 4, which were obtained with HUMCO II. The solid line is the theoretical expectation if counting statistics were the only source of error; the points are derived from the experimental data (see reference 2, Table VII) The significant feature is that the coefficient of variation of background follows counting statistics down to 0.15% (for ½-hr counting times). For longer times the error rises, so that for a 9-hr count it is 0.25% compared with the theoretical 0.03%. The maximum profitable uninterrupted counting time varies depending on the stability of the system.

If higher precision is necessary, then sample, background, and calibration must be alternated over a longer period so that the additional error can be averaged out. The precision actually attained must be evaluated from the scatter of repeated counts. This procedure clearly becomes very tedious and time-consuming, so, on a routine basis, it is not feasible to gain a great deal by this method. Improvement of the system by control of the source of the trouble may be necessary.

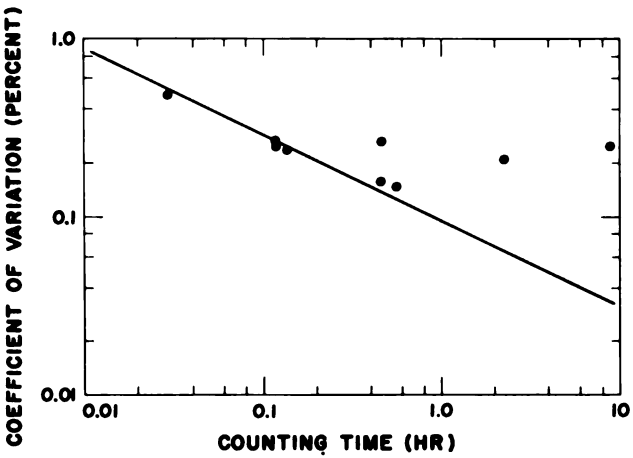


FIGURE 4 Stability of background of HUMCO II. The observed coefficient of variation (100 times the ratio of the standard deviation to the mean) for a series of repeated background determinations is plotted as a function of duration of each measurement. The solid line is theoretical expectation, and the points are experimental. Data from Anderson (reference 2, Table VII).

CONTAMINATION

The natural potassium radioactivity of animals is not large compared with the levels of other radioactivity in the environment and, in spite of energy discrimination, significant errors can be caused by contamination. The offenders are primarily the natural radioactivities but can also include tracers or leaky sources used elsewhere in the vicinity and, to a lesser extent these days, fallout from nuclear weapons testing. The contamination may affect the counting facility or the sample, or both. The organic scintillator, with its limited energy resolution and wide counting bands, is less tolerant of contamination than is the crystal spectrometer.

Contamination of the counter is easier to detect than contamination of the sample, since background changes are easily noted if proper records are kept. Perhaps the most likely source of counter contamination is from the animal being measured, but airborne gases and dusts can also contribute. Disposable liners and paper or plastic covers are probably the best protection, but frequent background measurements are necessary to give warning when decontamination is required.

Contamination of the sample results, of course, in a consistent error and can lead only to overestimation of the potassium. It is hard to identify, especially at low levels. If the contamination level is not constant, the variable portion can be detected by the variation of a series of measurements on the animal. The interpretation is complicated by possible true variation in the potassium content as well as by a possible invariant but positive lower limit on the contamination itself. Gamma-ray spectrometry can, in principle, identify the nuclides being measured and distinguish ^{40}K from the other possibilities. In practice, however, an amount of activity sufficient to cause a significant error may not be identifiable in this manner. (Quantitation, being based on the summation of a wide energy band of counts, generally has better statistical precision than identification, which requires the use of narrow bands.) However, even with an organic scintillator of limited resolution, warning of trouble can be obtained sometimes by measuring the activity in other energy bands in addition to the ^{40}K Compton peak. An estimate of the probable level of difficulty can sometimes be obtained by special experiments such as repeated washing of an animal exposed to typical external contamination, separate measurement of the contents of the rumen and gastrointestinal tract, and study of the effect of especially clean environment or diet, or both. It is probably useless to attempt to generalize because each problem will have its own solution, depending on the accuracy and sensitivity required and on the detailed environment.

With respect to contamination by fallout of fission products from weapons testing, the situation is more favorable now than it has ever been. Atmospheric detonation of nuclear explosives is no longer practiced by the major powers, and as a result the contamination of the biosphere has declined. The situation for ^{137}Cs in people is shown in Figure 5,[13] which is a plot of the body burden

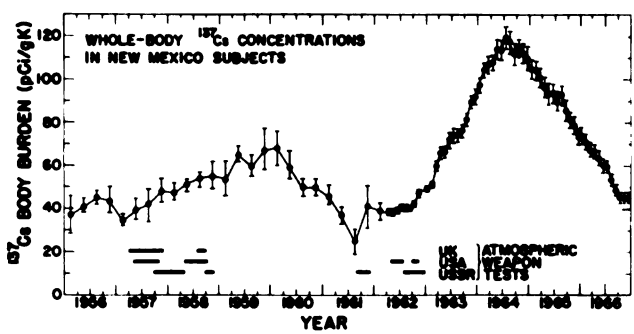


FIGURE 5 Average body burden of ^{137}Cs (relative to potassium) in human subjects (New Mexico) for the period 1956–1966.

(in pCi/g of K)* for a group of New Mexico subjects over the 10-year period through 1966. The concentration of this nuclide has dropped by a factor of 3 since its peak in 1964. Interference with potassium determination has actually declined by more than this, since present fallout is largely "old" (i.e., the radioactivity produced about 5 years ago) and the shorter-lived fission products such as $^{140}Ba/^{140}La$ and $^{95}Zr/^{95}Nb$ have now decayed. Contamination by these activities was greater from the testing of small weapons in Nevada than from large tests in the Pacific and was probably responsible for a significant increase in the error of human potassium determinations in 1956 and 1957, as illustrated in Table 2. The data here are the results of repeated measurements of the

TABLE 2 Yearly Averages for Total Potassium Measurements on a Control Subject

Year	Number of Determinations	Average Weight (kg)	Average Potassium[a] (g)
HUMCO I			
1956	71	65.7	135.7 ± 11.7
1957	26	65.2	133.3 ± 9.8
1958	24	65.3	134.9 ± 5.3
1959	11	66.4	133.0 ± 5.6
1960	8	65.4	130.3 ± 3.5
1961	14	69.0	140.7 ± 4.6
1962	20	70.4	139.9 ± 6.3
HUMCO II			
1962	52	70.0 ± 0.6	140.3 ± 4.6
1963	53	66.3 ± 2.8	136.7 ± 4.5
1964	44	63.5 ± 1.0	135.0 ± 3.6
1965	48	60.5 ± 0.9	138.6 ± 3.8
1966	41	61.0 ± 0.9	141.7 ± 4.7

[a]The tabulated error is the standard deviation of a single determination as calculated from the scatter of the data about the yearly mean.

potassium in a control subject, and the standard deviation given in the last column (calculated from the scatter of the individual results about the yearly mean) includes true biological changes as well as random errors. The errors of 12 and 10 g of K in the first 2 years are in striking contrast with those of 4 and 5 g of K attained later. Some of the gain is perhaps due to improved techniques, but the observed presence of $^{140}Ba/^{140}La$ in foods during 1957[3] supports the conclusion that it probably contributed to high ^{40}K results in people during this period. It is interesting to note, however, that J. E. Johnson, G. M. Ward, and L. B. Sasser report $^{140}Ba/^{140}La$ from Chinese nuclear tests (see page 317).

*pCi = picocurie (2.2 disintegrations per minute).

A more enduring source of contamination is the natural radioactivity of the environment, especially that of ^{226}Ra and its short-lived daughter products. The normal body burden of these nuclides is extremely low[17] because they are chemically rejected by the food chain. The problem is, therefore, one of external surface contamination or of material in transit through the gastrointestinal tract. The contaminant may be dust and dirt, in which case it will have the very long half-life of the radium (or uranium) parent activity. It may also be gaseous radon emanating from radium in the soil (or in pumice blocks or bricks), in which case the half-life can range from 3.8 days (radon itself) to 20 minutes (the gamma-emitting daughter products). The radon content of laboratory air is often extremely variable, as it depends on how much is baked out of the surrounding soil and how much is trapped by atmospheric inversion layers.

Potassium is also a prominent component of the natural radioactivity of the environment. Here energy discrimination is, of course, of no help, except insofar as the potassium is accompanied by other gamma emitters whose presence can be detected by this means. Maximal cleanliness and decontamination procedures are really the only cure (e.g., by a special feeding program for ruminants prior to counting).

CALIBRATION ERRORS

Efficiency

Gamma radiation is significantly scattered and absorbed by any sample whose mass exceeds a few kilograms. All counters thus require an empirical efficiency calibration based on the experimental determination of the counting rate per gram of K as a function of sample mass. It is also necessary to consider whether mass alone is enough to determine the efficiency or whether sample shape must also be taken into account. In addition, one must estimate how large an error might result from variations in the pattern of distribution of the activity through the sample.

Calibration can be accomplished by construction of phantoms of known composition, by introduction into the subject of known amounts of ^{42}K (isotopic with ^{40}K and having a similar gamma energy), and by chemical analysis of samples after counting. In addition, theoretical calculations of efficiency, while difficult for many situations, can help delineate critical areas and parameters.

A typical potassium calibration curve for a 4-pi organic scintillator (HUMCO I) used for human counting is shown in Figure 6.[2] The circles were determined by ^{42}K ingestion and the triangles by sugar phantoms (cylindrical) containing KCl. Note the excellent agreement between the two

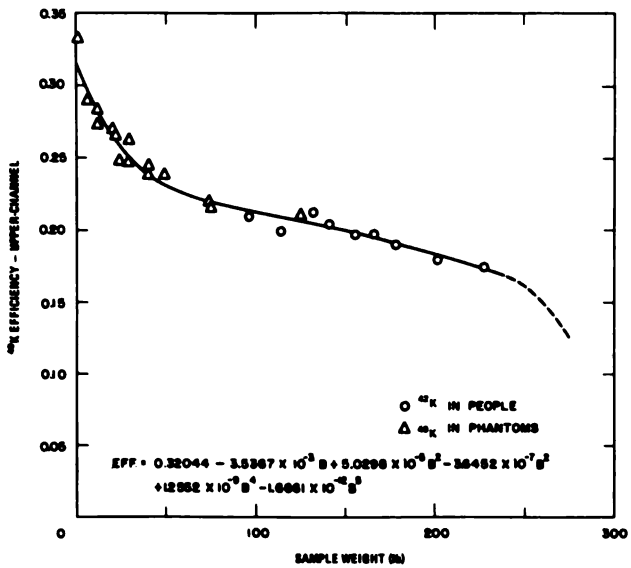


FIGURE 6 Potassium efficiency calibration of HUMCO I (the fraction of ^{40}K gamma rays detected as a function of weight of the sample). The equation is the least squares best fit used in computer processing of the data.

methods. The determination of such curves is discussed by Dean.[8] For human counting to a precision of a few percent, gross body weight is the only parameter needed to define the efficiency curve of a 4-pi counter. This experimental observation can be confirmed by the following theoretical analysis, which also serves to derive the shape of the curve in Figure 6. The basis of the analysis is to approximate the human body of weight, W (kg) by a cylinder of unit density whose length is equal to the subject's height, h (cm), and whose radius, r (cm), is such that

$$1{,}000\ W = \pi r^2 h. \tag{1}$$

The ratio $k = h/r$ defines the shape of the cylinder. If k is large and if the counter is sufficiently long, then the counting efficiency will depend only on r, whereas sample weight depends on both r and k. Therefore, one can construct a family of curves for efficiency versus weight, each one for a different k.

Interesting values of k can be deduced from the regression of height on weight (reference 16, page 181) by using the formula

$$h\ (\text{cm}) = 40.9\ W^{0.35}\ (\text{kg}) \tag{2}$$

for the healthy male population over the weight range 20 to 70 kg. If the exponent were 0.33, then all model cylinders would have the same proportions with a constant height-to-radius ratio k. Solving Equations 1 and 2 for radius gives

$$r\,(\text{cm}) = 2.79\ W^{0.325}\,(\text{kg}). \qquad (3)$$

Using this equation, one finds that for the male population the average k varies only from 16.1 at $W = 40$ kg to 15.5 at 80 kg.

The gamma-ray flux leaving a cylinder from a uniformly distributed source can be calculated by using the theoretical analysis of Case *et al.*[7] [Their treatment was intended for neutron diffusion but is based only on geometry and exponential absorption and, hence, is equally applicable to gamma rays; see also Price *et al.* (reference 11, Chapter 5)]. The cylinder radius is expressed in units of mean free path which is the reciprocal of the linear absorption coefficient. For 1.46-MeV gamma rays in water, the latter is 0.06 cm^{-1} (reference 9, page 714) when the total attenuation coefficient is used (i.e., by assuming that any gamma ray that is scattered is not detected; this is the case when the detector accepts only those gammas still retaining their full initial energy). The efficiency versus weight curve is thus constructed by calculating the radius of the equivalent cylinder from Equation 3, dividing by the mean free path (16.7 cm), and looking up the collision probability (P_c) (reference 7, Table 4). Then $(1 - P_c)$ is the fraction of gammas emerging from the cylinder without energy loss. For comparison with experiment, this must be multiplied by the fraction of the gammas that pass through the counter and are detected. This is most easily done by normalizing the curve at one point. The result is shown in Figure 7, in which the solid line is the calculated result normalized at 60 kg to the broken line which is the experimental efficiency curve for HUMCO II.[8] The agreement is excellent (within 1 or 2%) except at large masses, where the experimental curve is depressed by reduced counter efficiency as more of the sample extends into the regions of lower geometrical efficiency near the ends.

The principal value of the theoretical curve is that it makes it possible to estimate variations in counter efficiency which would result from changes in sample shape. For this calculation, we abandon the "average shape" given by Equation 3 and instead solve Equation 1 for r of the equivalent cylinders, using the measured height and weight of appropriate population extremes. The rest of the calculation of efficiency proceeds as above.

According to Spector (reference 16, page 176), the 95% limits on height and weight for American adult white males are 164 to 191 cm and 55 to 86 kg, respectively. Making the pessimistic assumption of pairing the minimum height with maximum weight and vice versa, one can conclude that for the great majority of that population the value of k should lie between 12 and 20.

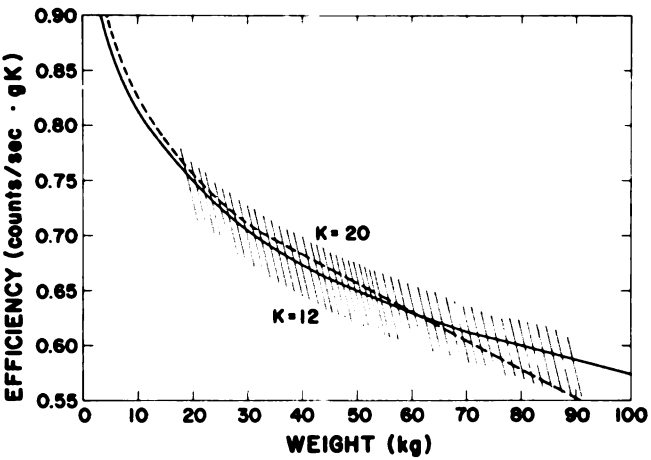


FIGURE 7 Comparison of theoretical efficiency curve (solid line) with experimental curve (broken line) for human potassium counting with 4-pi detector. Limits of the cross-hatched band correspond to cylinders with length-to-radius ratios of 12 and 20, respectively.

The cross-hatched area of Figure 7 covers the efficiency-weight range expected on this basis. Even on this extreme assumption, the maximum variation of counting efficiency from the mean would give a potassium error of only ±5%. The actual effect would be much smaller and would be very difficult to establish experimentally.

These calculations apply only to 4-pi systems in which radiation is detected no matter on which side of the subject it emerges. For systems of other geometries, a different and generally more complicated model must be used. Other tables and functions that may be useful are found in Case *et al.*[7] For intractable cases that cannot be represented by a simple approximation, a Monte Carlo calculation will be necessary. A computer code similar to that of Zerby and Moran,[20] designed for the inverse problem of calculating absorption of external gamma rays, could be used.

The above analysis assumes that the source is uniformly distributed throughout the sample. If the concentration is nonuniform but the pattern of distribution is similar in all samples, then the deduced potassium concentrations may be in the correct ratios to one another even though they all suffer a consistent error. However, if the distribution pattern changes, or if one compares samples with differing patterns, the errors become more serious.

We can attempt to estimate the magnitudes of the effects of nonuniform distribution by the following simplified, one-dimensional model (Figure 8).

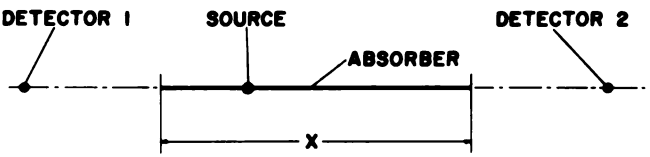


FIGURE 8 Simple one-dimensional model for estimating effect of self-absorption on counting efficiency as a function of source position.

A point source is located in an absorber of length x whose linear absorption coefficient is μ. Detectors 1 and 2 measure the unabsorbed radiation emerging. We now ask: what is the ratio of the minimum to maximum signal detected as the source is moved along the absorber? This ratio we will call the minimum relative efficiency (MRE). We consider two cases:

Case A: Only one detector is present. In this case, the maximum signal (I_o) is observed when the source is at the end of the absorber nearest the detector and the minimum signal ($I_o e^{-\mu x}$) when the source is at the other end. Therefore,

$$\text{MRE} = e^{-\mu x}. \tag{4}$$

Case B: Both detectors are present. When the source is at either end of the absorber (best case), one detector sees an intensity I_o and the other an intensity $I_o e^{-\mu x}$ for a total signal $I_o\,(1 + e^{-\mu x})$. In the worst case, the source is at the middle of the line, and the total signal is $2\,I_o e^{-\mu x/2}$. Therefore,

$$\text{MRE} = \frac{2\,e^{-\mu x/2}}{(1 + e^{-\mu x})}. \tag{5}$$

Note that we are not considering the over-all effect of absorption on efficiency but are asking what is the maximum possible change due to non-uniform distributions of activity in a given sample. If the MRE approaches unity, then there can be little or no change in the signal strength as the source is moved along the line. By using the approximation in Equation 5 that $e^{-\mu x} = 1 - \mu x$ when μx is small (i.e., less than, say, 0.4), one can see that in Case B (two detectors) the MRE is identically unity (i.e., counting efficiency is completely independent of source position). For Case A, the similar approximation gives

$$\text{MRE} = 1 - \mu x.$$

Using 0.06 cm^{-1} as the linear absorption coefficient (total attenuation), one can solve Equations 4 and 5 for the value of MRE as a function of absorber length for the two cases. The results are shown in Figure 9; the lower

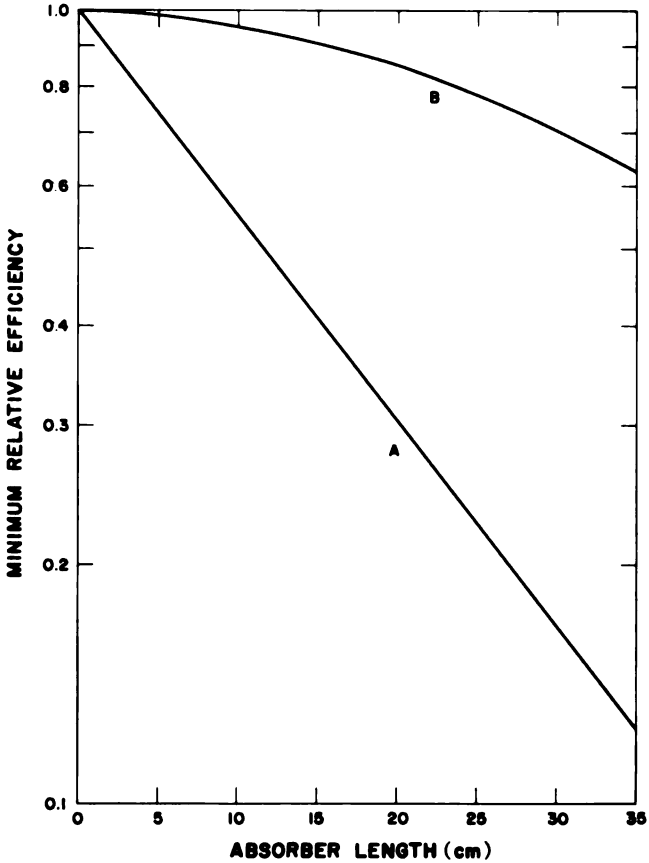


FIGURE 9 Ratio of minimum to maximum counting efficiency (minimum relative efficiency) for linear geometry of Figure 8 (*A*, one detector; *B*, two detectors).

curve is for Case A ("single-sided" detection), and the upper curve for Case B ("double-sided" detection). At the equivalent cylindrical radius for the adult male of 11 cm, the length of the one-dimensional approximation would be taken as 22 cm. Here the values of MRE are 0.81 and 0.27, indicating that, if 10% of the activity were moved from the most favorable to the least favorable position, the changes in detected signals would be 1.9% in Case B and 7.3% in Case A. The latter is about 4 times as sensitive as the former to relocation of potassium.

These numerical results cannot be applied directly to an actual case because of the complications of three-dimensional geometry, scattering, and energy

dependence of counting efficiency. The interesting cylindrical approximation cannot readily be made because of computational complexity. However, a spherical approximation is relatively straightforward. Case *et al.*[7] have shown that the fraction F of an isotropic incident flux absorbed by any convex body is given by

$$F = \frac{4V}{LS} P_o, \tag{6}$$

where V is the volume, S the surface area, L the mean free path, and P_o the escape probability from the body. For the sphere, this becomes

$$F = \frac{4a}{3L} (1.0 - P_c), \tag{7}$$

where a/L is the radius in units of the mean free path and P_c is the collision probability (reference 7, Section 10.2, Table 3). For a point source on the surface of a spherical absorber surrounded by a 4-pi detector, the flux in one hemisphere will be unabsorbed and that in the other will have an intensity $(L - F)$ after transmission through the sphere. The average flux for the best case is, therefore, $(2 - F)/2$. For a point source in the center of the sphere (the worst case), the flux is attenuated equally in all directions to a value $e^{-\mu a}$. The ratio between these quantities is the MRE for this model and is plotted as curve B in Figure 10. For a single, small-angle detector on one side of the sphere, the MRE is $e^{-2\mu a}$, which is curve A in the same figure.

The expectation for a human subject in a 4-pi counter (curve B) would lie between the value for a 70-kg sphere (a = 26 cm, MRE = 0.36) and the value for a sphere with radius equal to that of the equivalent cylinder (a = 11 cm, MRE = 0.73). The error resulting from moving 10% of the potassium from the best to the worst position is, therefore, between 2.7 and 6.4%. Clearly, samples as large as a normal man are on the verge of a serious sensitivity to variations in the distribution pattern of potassium. For large animals, the problem should be analyzed in more detail with more realistic models (e.g., in the cylindrical or prolate spheroid approximation by numerical or graphical integration or by a Monte Carlo calculation).

Background Depression

Another source of consistent error in calibration is the reduction of the background rate of the counter as a result of absorption of environmental gamma rays by the sample. The magnitude of this effect, ranging from negligible to serious, depends on the mass of the sample, the nature of the detector, and

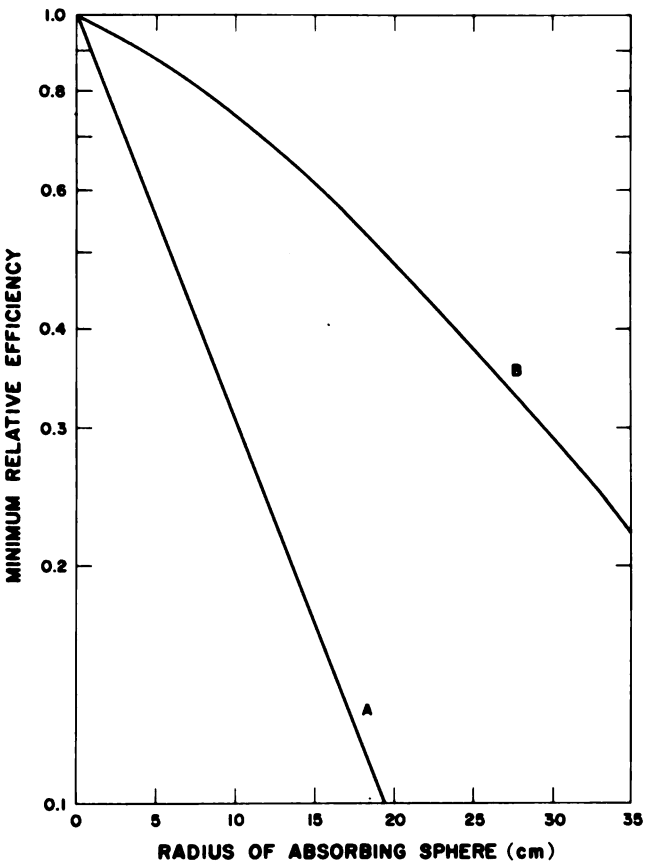


FIGURE 10 Minimum relative efficiency for a spherical absorber as a function of the radius of the sphere (*A*, single, small-angle detector; *B*, 4-pi detector).

the quality of the background radiation. Gamma rays from the external environment can be reduced to a negligible level by massive shielding, and some shielding materials are sufficiently clean that they do not contribute significantly to background. Any material, however, is a source of secondary gamma rays generated by the penetrating component of the cosmic radiation which cannot be stopped except by many feet of material. Even the best shield, unless buried far underground, will have a gamma-ray background inside that will contribute a significant fraction of the residual counter background. When a massive sample is introduced into the counter, it will absorb some of this background, thus introducing a potential error proportional to

sample mass and independent of sample activity. Therefore, correction for this effect cannot be combined with the efficiency calibration.

The situation is illustrated in Figure 11 for three possible counter geometries. Cosmic rays, which are heavily collimated about the zenith direction,

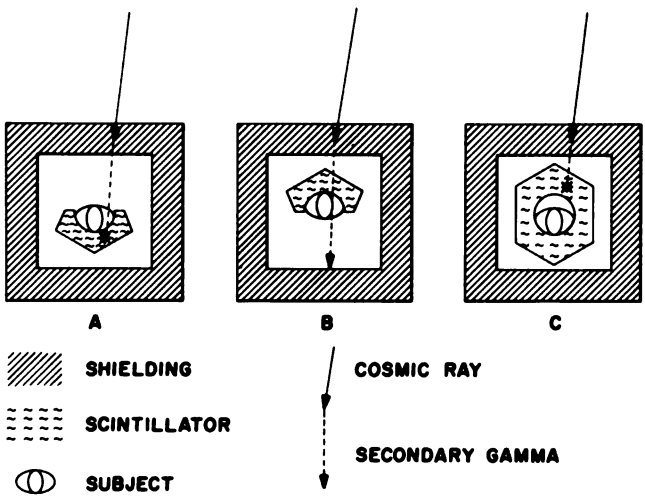


FIGURE 11 Schematic illustration of background depression due to absorption of secondary gamma rays by the subject.

produce secondary gamma rays in the shield, and a majority of these secondary radiations are projected in the forward direction. In Case A, a horizontal 2-pi system, the number of secondary gamma rays reaching the detector will be reduced on interposition of the sample. In Case B, in which the counter is inverted with the sample below, absorption in the latter is without effect, since the gamma rays from above have already traversed the counter. Absorption of gamma rays from below will still result in a background depression, but these gamma rays are less numerous, and the effect will be smaller. There may also be some partial compensation as a result of backscatter: a gamma ray from above, which passed the counter without producing a count, may be reflected back by the sample, thus *raising* the background. This geometry is somewhat less favorable, however, in that gravity does not press the sample down into the counter in a reproducible position of maximum efficiency.

Case C represents a 4-pi counter. If the scintillator is thick enough (e.g., 30 cm) that a majority of the gamma rays have already produced a scintillation before reaching the sample (even though they are not completely ab-

sorbed), then background depression may be negligible, as is the case with human subjects in HUMCO II. If the scintillator is thin (e.g., 15 cm or less), then the counter resembles Case A, and a correction for background depression is necessary (e.g., HUMCO I).

The need for this correction must be determined experimentally, and measurements of sufficient precision can be difficult. For illustration: in the Los Alamos human counter, an error of 3 g of K (or 2% in an adult male subject) corresponds to a change in background rate of 0.7%. Very precise measurements with completely nonradioactive samples (sugar is most convenient) are necessary to establish the calibration for such small effects as a function of sample mass. Adsorption of airborne radioactivity to the surface of the sample by static electric charges can often completely override the small effect of background depression. In practice, one should decide on the accuracy desired (in grams of K) and multiply this by the counting efficiency (counts per minute x grams of K) for the heaviest sample to be measured. The product is the precision with which the correction for background depression must be measured.

ACCURACY ATTAINABLE

The question of absolute accuracy which should be attainable in a given situation depends strongly on the characteristics of the detector and the size and nature of the sample. For example, it is very difficult to do as well in measuring large animals or abnormally large people as was possible in measuring normal people with a very large 4-pi counter such as HUMCO II. The results obtained in that case might, therefore, be taken as a probable upper limit.

Evaluation of absolute accuracy is not easy since it requires an independent determination of high accuracy. Construction of phantoms of known composition is tedious and also leaves open the question of how well geometrical distribution was reproduced. Chemical analysis of an entire large sample is difficult at best and is especially so where the human body is concerned. The procedure used to evaluate the performance of HUMCO II was, therefore, based on two less-direct comparisons: first, comparison with exchangeable potassium as determined by ^{42}K isotope dilution, and second, comparison with the results of other laboratories. The first suffers from the possible difficulty that a portion of body potassium may be nonexchangeable and the second from the objection that there may be similar, consistent errors in all the measurements. In both cases, the total discrepancy is a result of all errors in the determinations compared and, therefore, constitutes only an upper limit on the error of any one method. Granting these limitations, the comparison is nevertheless useful as a guide.

The methods and numerical results are described elsewhere,[2] and only a summary is given here. In the ^{42}K comparison, the average ratio of exchangeable potassium to total potassium for five subjects was 0.964, with a range of 0.92 to 0.99 and a standard deviation of 0.03. In view of the difficulties and probable bias of the dilution method, the conclusion that the absolute error of the counting method is less than 3% appears justified.

When three subjects were measured in five other laboratories (three sodium iodide crystals, one 2-pi liquid scintillation counter, and one plastic counter), the average of 10 determinations was 0.987 for the ratio of the Los Alamos result to that of the independent laboratory, with a standard deviation of 6%. A similar comparison was based on measurements of the average potassium content of groups of people of a given sex and age (a parameter shown to have a standard deviation of 10 to 12%).[18] The six other methods included one ^{42}K dilution, four sodium iodide spectrometers, and one 2-pi liquid scintillator. The ratios to the Los Alamos results ranged from 0.923 to 1.161, with a mean of 1.023 and a standard deviation of 0.08. (The ^{42}K dilution result gave 1.012.) The interlaboratory comparisons with other counters are, therefore, less encouraging than isotope dilution in their estimate of possible accuracy, suggesting that absolute errors of 7% are common. It may be significant that most of the other counters were of low geometrical efficiency and thus perhaps offered a more difficult calibration problem. Too much confidence should not be placed on the agreement with ^{42}K, in view of the discrepancies between counter potassium and exchangeable potassium reported at this conference by A. P. Remenchik (see page 231).

SUMMARY AND CONCLUSIONS

For the measurement of total potassium content of normal human subjects, large organic scintillation counters are capable of a precision of ± 3 g of K (one standard deviation) under routine conditions. With special care, a precision of 1 g of K has been approached. The absolute accuracy may be comparable under favorable conditions, but the error can be larger than 8%.

Sources of error include counting statistics, instrument instability, contamination, and calibration errors. General methods of detecting and evaluating these errors are discussed, and some results obtained with human counters are given.

For the measurement of ^{40}K in normal human subjects in a 4-pi counter, the subject's weight is the only parameter necessary to define counting efficiency to an accuracy of a few percent, and nonuniformity of potassium distribution is not likely to cause significant errors. Methods of extending these

estimates to larger animals are indicated. Animal measurements are undoubtedly more difficult because of the more serious problem with control of contamination and, in some cases, because of the larger mass.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

REFERENCES

1. Anderson, E. C. 1963. Three-component body composition analysis based on potassium and water determinations. Ann. N. Y. Acad. Sci. 110:189.
2. Anderson, E. C. 1965. Determination of body potassium by four-pi gamma counting, p. 211. *In* G. R. Meneely and S. M. Linde [ed.] Radioactivity in Man. II, Charles C Thomas, Springfield, Ill.
3. Anderson, E. C., R. L. Schuch, W. R. Fisher, and M. A. Van Dilla. 1958. Barium-140 radioactivity in foods. Science 127:283.
4. Anderson, E. C., R. L. Schuch, V. N. Kerr, and M. A. Van Dilla. 1961. HUMCO II: A new four pi liquid scintillation counter, p. 31. *In* G. R. Meneely [ed.] Radioactivity in Man. Charles C Thomas, Springfield, Ill.
5. Behnke, A. R. 1941/1942. Physiologic studies pertaining to deep sea diving and aviation, especially in relation to the fat content and composition of the body. Harvey lect., Ser. 37:198.
6. Burch, P. R. J., and F. W. Spiers. 1953. Measurement of the gamma radiation from the human body. Nature 172:519.
7. Case, K. M., F. deHoffmann, and G. Placzek. 1953. Introduction to the Theory of Neutron Diffusion. Los Alamos Scientific Laboratory, Los Alamos, N. M. Sec. 10, p. 17 ff. (Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C.).
8. Dean, P. N. 1965. Experimental techniques for the high precision calibration of whole body counters, p. 94. *In* G. R. Meneely and S. M. Linde [ed.] Radioactivity in Man. II. Charles C Thomas, Springfield, Ill.
9. Evans, R. D. 1955. The Atomic Nucleus. McGraw-Hill Book Company, New York.
10. Keys, A., and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:245.
11. Price, B. T., C. C. Horton, and K. T. Spinney. 1957. Radiation Shielding. Macmillan, New York.
12. Reines, F., R. L. Schuch, C. L. Cowan, F. B. Harrison, E. C. Anderson, and F. N. Hayes. 1953. Determination of total body radioactivity using liquid scintillation detectors. Nature 172:521.
13. Richmond, C. R., and J. E. Furchner. 1967. Cesium-137 body burdens in man: January 1956 to December 1966. Radiat. Res. 32:538.
14. Sievert, R. M. 1951. Measurements of gamma-radiation from the human body. Ark. Fys. 3:337.
15. Sievert, R. M. 1956. Untersuchungen uber die Gammastrahlung des menschlichen Korpers. Strahlentherapie. 99:185.
16. Spector, W. S. 1956. Handbook of Biological Data. W. B. Saunders Co., Philadelphia, Pa.

17. United Nations. 1958. Report of the Scientific Committee on the Effects of Atomic Radiation, General Assembly Official Records: 13th Session, Suppl. No. 17 (A/3838), New York, p. 56.
18. Van Dilla, M. A., and E. C. Anderson. 1962. Human counters using liquid scintillators, p. 41. *In* Whole-body counting. International Atomic Energy Agency, Vienna.
19. von Dobeln, W. 1962. Estimation of muscle mass of the human body from potassium-40 determination. Medd. Flygbl. Nav. Med. Namnd. 11:1.
20. Zerby, C. C., and H. S. Moran. 1960. *In* Proceedings of the Total Absorption Gamma-Ray Spectrometry Symposium, Gatlinburg, Tenn. (May 10–11), p. 80–88 and 113–119.

FACTORS AFFECTING POTASSIUM-40 MEASUREMENT IN THE WHOLE BODY AND BODY COMPONENTS

T. G. Lohman
University of Illinois

W. J. Coffman
University of Missouri

A. R. Twardock
University of Illinois

B. C. Breidenstein
Wilson and Co.

H. W. Norton
University of Illinois

Precise and accurate measurements of ^{40}K by whole-body counting require careful standardization of the instrumentation and detailed calibration of factors that affect ^{40}K measurements. Routine electronic adjustments are necessary to maintain detection efficiency close to a reference value.[3] Time is required for photomultiplier tubes to stabilize after exposure to high scintillation rates.[8] Standards representative of the size and shape of the sample to be assayed are advantageous in accounting for variation in detection efficiency.[16] Long-term fluctuations in detection efficiency are caused by changes in the performance of photomultiplier tubes and of electronic components and by oxygen absorption into the scintillator fluid.[16]

Calibration of counters relates sample weight to detection efficiency and to background depression. There is considerable evidence that several other factors should be included. Remenchik and Miller[15] reported that body potassium estimated from whole-body ^{40}K count with a crystal detector appears to underestimate the potassium in obese subjects, as determined by the ^{42}K dilution technique. Uniformly distributed sources of radioactivity are not sufficient for calibration for highly accurate results.[14] Von Döbeln[18] suggested that the distribution of potassium throughout the lean body mass varies among

individuals and that estimating body composition from potassium involves a determination of three body components: muscle lean, muscle-free lean, and adipose mass. Anderson[2] showed that variation in the potassium-to-water ratio in humans can be accounted for by von Döbeln's three-component system. Breed and muscle differences in potassium content of porcine muscle[5] and of bovine muscle[6] have been suggested as additional sources of variation in estimates of body composition. Gastrointestinal radioactivity in cattle fed roughage diets was highly variable and greatly affected the precision of the relation between whole-body ^{40}K count and carcass lean muscle mass.[12]

Thus, several factors need to be considered in interpreting ^{40}K measurements and estimates of body composition. This paper describes some of the counter-induced and sample-induced variables that affect ^{40}K measurements in which liquid scintillation whole-body counters are used.

PROCEDURE

COUNTER-INDUCED VARIABLES

Observations from whole-body counters at the Universities of Missouri and Illinois were used to study counter-induced variables affecting ^{40}K measurements. Missouri results are from a 2-pi liquid scintillation modular detector employing six 16-gauge (1.52-mm) stainless steel detector tanks with a 40.5-cm photomultiplier tube (DuMont K-2128) mounted on the back of each tank. The six tanks are arranged in pairs to form a half cylinder with a diameter of 61 cm. Each tank is filled with 105 liters of scintillation fluid, composed of a toluene equivalent solvent and the scintillators PPO and POPOP. The detector, 2 m long, can be moved vertically and horizontally. It is contained within an 80-metric-ton steel chamber with walls 10 cm thick; the interior dimensions of the chamber are 2.7 m high, 2.7 m wide, and 5.5 m deep. In the Illinois 4-pi liquid scintillation counter two separate detector tanks (1.8 m long) are used, with six photomultiplier tubes mounted on each tank.[16]

Day-to-day variation in detection efficiency was studied by using ^{137}Cs in aqueous solution in a sealed plexiglass tube 2.8 m long by 1.2 cm in diameter. About 2 μc of ^{137}Cs was used for the Missouri standard, and 0.5 μc for the Illinois standard. Three ^{137}Cs counts, each preset for 900,000 counts (total counting time was 2.7 min), and one 1-min background count were taken with the 2-pi counter. Three 1-min counts on the ^{137}Cs standard and one 1-min background count were made with the 4-pi counter. Counts were taken for each of 10 days by both counters, and observed and nuclear decay coefficients of variation were calculated.[16]

Variation in the potassium mass of a human subject was also observed by each counter by daily counts over a period of 10 days. Three subject counts, each preset for 50,000 counts (total counting time was 11.5 min), and one 5-min background count were taken with the 2-pi counter, and two 3-min subject counts and two 3-min background counts were taken with the 4-pi counter. Both subjects were counted in street clothes but without shoes. A 54-kg standard (5.4 kg of KCl in 48.6 kg of sugar) was counted before each subject count at the 4-pi counter to enable correction for variation in detection efficiency. A 65-kg standard (2.7 kg of KCl in 62.3 kg of water) was used at the 2-pi counter.

SAMPLE-INDUCED VARIABLES

Observations from the whole-body counter at Illinois during a 2-year period (1963–1964) were used to study sample-induced variables affecting ^{40}K measurement. Separate counts were made on the whole body, on the carcass, and on carcass and noncarcass components of 90 steers. Thirteen additional carcasses were counted during this period.

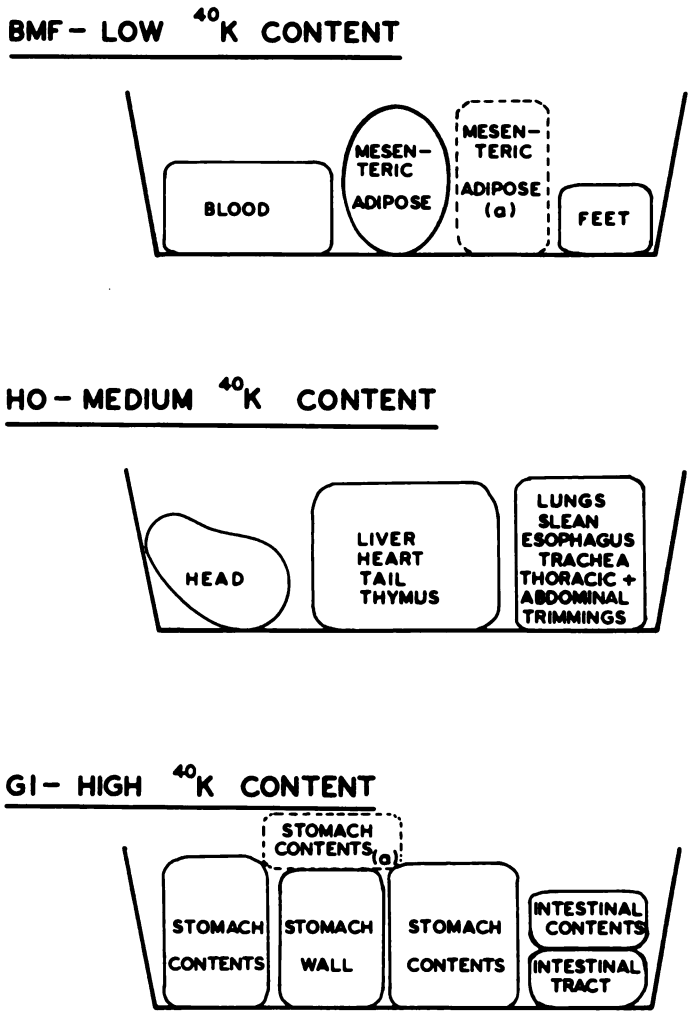
During the first year (phase I) close attention was given to sources of variation in whole-body counting other than those caused by differences in carcass composition. The counting procedures were then modified to eliminate or reduce these sources of variation, and during the second year (phase II) the whole-body counting method was evaluated under more-controlled conditions.

Counting procedures of phases I and II for whole-body and carcass ^{40}K counting were those described by Twardock *et al.*[16] and Lohman *et al.*[12] Use of standards representative of the size and shape of the various samples counted and the feeding of a diet (oats) low in radioactivity were the principal changes from phase I to phase II.

Each steer was slaughtered the day after the whole-body count. Noncarcass components were usually counted within 24 hours after slaughter. The components were placed in polyethylene bags for counting (Figure 1), and were counted in four groups based on anatomical function and potassium content.

Except for the hide, each group was counted in an aluminum container centered on a stretcher, with the detector tanks in the 4-pi position. For each steer, the components were placed in the same order in the container.

Two positions (*A* and *B*) of the detector tanks were used for the whole-body count.[16] Count variation due to vertical movement of the steer relative to the tanks was minimized with the tanks open an equal distance at top and bottom (56 cm), and this position (*B*) was used in phase II in addition to that used in phase I (*A*). The contribution of other radionuclides to the ^{40}K channel



(a) FOR MOST OF THE HEAVIER STEERS TWO BAGS WERE USED TO CONTAIN MESENTERIC ADIPOSE AND THREE BAGS FOR STOMACH CONTENTS.

FIGURE 1 Noncarcass components.

count-rate was reduced in phase II by raising the pulse-height analyzer settings from 0.8 through 1.6 MeV, used in phase I, to 0.9 through 1.9 MeV. The hide was counted with the detector tanks in position *A*, and was spread on a rack

fitted on the cattle cart. This counting position could not easily be calibrated. Potassium values for the hide were estimated from the hide mass rather than ^{40}K count; a constant potassium content (0.1%)* was assumed.

The noncarcass components with a low potassium content—blood, mesenteric fat, and feet (BMF)—were counted for two 4-min counts, with 4-min background counts immediately before and after each sample count. The remaining two groups of components, head and organs (HO) and gastrointestinal tract and contents (GI), were each counted for two 2-min counts, with 2-min background counts before and after. The aluminum container and stretcher were counted for two 4-min counts on each day of use.

Within 7 to 15 days after slaughter the right side of each carcass was separated into a standard trimmed lean (STL), bone (CB), and adipose mass (CA). The fore and hind lean portions were separately ground through a 1-cm breaker plate before being counted. After thorough mixing, each was placed in a polyethylene bag (7 to 20 kg/bag) and counted in the aluminum container for two 4-min counts for each portion. Bone and fat were also counted separately for three 4-min counts. The bones were distributed throughout the aluminum container. Adipose tissue was placed in polyethylene bags as was the lean. The counting of each carcass component was preceded and followed by a 4-min background count.

For carcass and noncarcass component counts, a 27-kg standard composed of 2.7 kg of KCl in 24.3 kg of sugar (contained in polyethylene bags) was counted in the aluminum container on the stretcher during the last two thirds of phase I to enable adjustment for changes in counter efficiency. In phase II, a standard containing 3.6 kg of KCl in 32.8 kg of sugar was counted for each carcass and noncarcass component count.

The counter was calibrated for the carcass and noncarcass components by using KCl in sugar and KCl in water over the range of weights of the components. The aluminum container and stretcher were used to contain the phantoms. There was a significant quadratic regression of 4-pi detection efficiency on sample mass for the phantoms containing KCl in water. Detection efficiency appeared to be independent of the carrier used (water versus sugar) over the weight range studied (Figure 2). Background depression was determined in the 4-pi position under conditions similar to those used for determining detection efficiency. Distilled water was used as the nonradioactive mass.

Because of the difficulty of constructing phantoms resembling carcass quarters, the counter was not calibrated directly for carcass ^{40}K detection efficiency. Potassium data obtained from the carcass components were used for the in-

*The value of 0.1% potassium was derived from the ^{40}K count of two hides in the aluminum container.

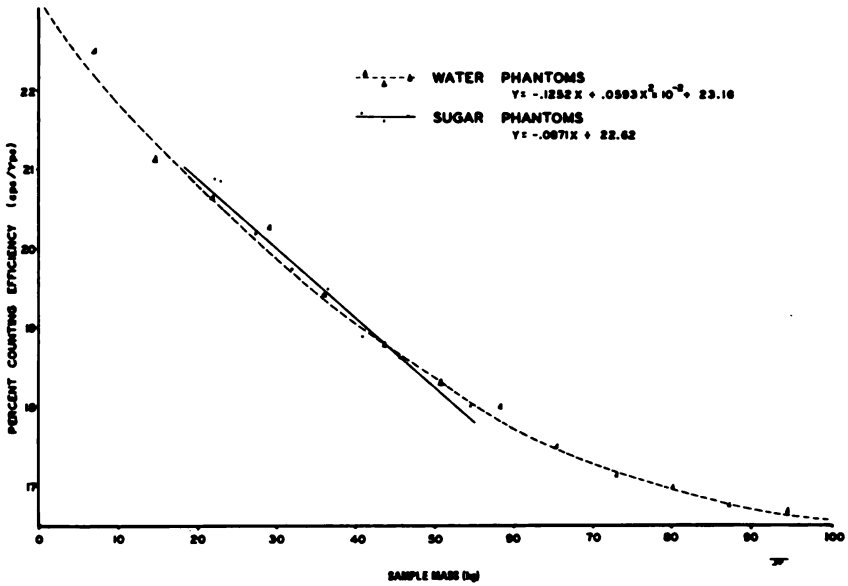


FIGURE 2 ^{40}K 4-pi counting efficiency.

direct calibration of intact carcasses. The calibration equation was determined from the regression of carcass component potassium on carcass count and mass.

Whole-body potassium was estimated from the ^{40}K count of the live steers and the calibration equations relating ^{42}K *in vivo* detection efficiency and phantom background depression to body mass.[16] In the component parts, potassium was estimated from the component ^{40}K counts and calibration equations relating ^{40}K phantom detection efficiency and background depression to phantom mass. The general form of the calibration equation relating ^{40}K count to potassium mass has been described by Anderson.[1]

PRESLAUGHTER TREATMENT

The steers used and their preslaughter treatment were described by Madamba.[13] Four breed types (Holstein, Angus, Angus × Holstein, and Charolais × Angus) were used in a 4 × 4 × 2 × 2 factorial experiment. The cattle were slaughtered at one of four preset weights (305, 385, 465, or 544 kg). They were individually fed *ad libitum* one of two pelleted diets containing different levels of roughage and exhibiting different levels of gamma-emitting radioactivity. Half were implanted with 24 mg of diethylstilbestrol.

Upon reaching the preset weight, body measurements were taken on each steer, including height of withers from the ground to top of shoulders, length from shoulder top to pin and from point of shoulder to pin, width of shoulder from left to right junction of scapula and humerus, width of round from left to right hip bone, heart girth, and circumference of paunch.

SLAUGHTER PROCEDURE

After whole-body counting, steers were slaughtered and dressed. Blood was collected by bleeding the hoisted steer from the jugular vein. Head, hide, and feet were separated from the carcass and weighed. The viscera were divided into 18 components and put into several polyethylene bags for ^{40}K counting.

Chilled masses of the carcass sides were taken after they had been separated into forequarters and hindquarters and hung in a cooler for 48 hours at 1° C. Standard trimmed lean was sampled for chemical analysis according to the procedures of Lohman *et al.*,[12] and the total fat-free carcass lean muscle mass (CLMM) was calculated for each steer.

Data were analyzed by the method of least squares.[4]

RESULTS AND DISCUSSION

COUNTER-INDUCED VARIABLES

Variation in count rate caused by randomness of radioactive disintegration, termed nuclear decay variability,[16] affects the precision of ^{40}K measurements. It depends on the total count for sample and for background. Reported values of the nuclear decay coefficient of variation (NDCV) of sample measurements in body-composition studies range from 1 to 12%, mostly greater than 3%.[9] Our counting times were chosen to reduce this source of variation to below 2% where practical. The NDCV's were calculated for all samples counted (Tables 1 and 2).

Day-to-day fluctuations in detection efficiency were studied for both counters by counting a ^{137}Cs standard and a human subject over a period of 10 days. A significantly greater ($P < 0.05$) variation in detection efficiency was observed by both counters than could reasonably be ascribed to the NDCV for counts of the ^{137}Cs standard (Table 1). The observed variability was probably caused by electronic-instrument drift and photomultiplier-tube instability.

The variation of 10 counts with the 4-pi counter on a human subject was significantly ($P < 0.05$) greater than that expected from nuclear decay. The

TABLE 1 Whole-Body Mean Counts and Coefficients of Variation

Sample and Counter	Mean	Sample Counting Time (min)	Coefficient of Variation	
			Observed	Nuclear Decay
^{137}Cs standard				
2-pi counter (cps)	15,834	2.76	0.59	0.066
4-pi counter (cps)	5,622	3.000	0.17	0.10
Human subject (potassium mass)				
2-pi counter:				
10-day period (g)	140.0	11.47	1.8	2.0
4-pi counter:				
10-day period (g)	167.2	6.000	2.7	1.7
2-year period (g)	174.4	6.000	4.2	1.6

observed CV was markedly reduced almost to half its original value of 2.7% upon excluding one observation, but no reason can be given for excluding this datum except that it appeared to be in error. Close agreement was found between the observed CV and NDCV for the 2-pi counter.

A coefficient of variation of 4.2% was observed for eight counts taken over a 2-year period as compared with 2.7% for ten counts on the same subject taken in a 10-day period with the 4-pi counter. Anderson[3] reported an observed CV of 3.7% as determined from 30 or more counts on each of 5 human subjects over a 7-month period. The NDCV was 2.0%.

Long-term fluctuations in detection efficiency have been reported with coefficients of variation up to 4.1%. Detection efficiency of large phantoms shows a greater sensitivity to electronic instrument drifts than that of smaller ones because of the shape of the ^{40}K spectrum. Thus, the use of standards representative of the size and mass of samples that are measured is essential to account accurately for variations in detection efficiency on a given day. Measurement of detection efficiency variation depends on the size and shape of a standard, not only because the shape of the spectrum is affected, but also because geometry affects the output of individual photomultiplier tubes.[16]

SAMPLE-INDUCED VARIABLES

Whole-Body ^{40}K Count

The importance of removing external radiocontamination has been shown for sheep and for cattle. In sheep the main contaminant caused by the accumulation of suint in the wool is potassium, and several fallout nuclides were found

TABLE 2 ^{40}K Count-Rate and Nuclear Decay Coefficient of Variation (NDCV) Associated with Body Components

Item	Phase I (37 steers) Mean (cps)	Counting Time (min)	NDCV (%)	Phase II (44 steers) Mean (cps)	Counting Time (min)	NDCV (%)
Whole body[a]	209	4	0.9	153	4	1.2
Carcass side[b, c]	187	8	1.3	161	8	1.5
Standard trimmed lean[c]	157	8	0.8	143	8	0.9
Carcass bone[c]	41	12	2.6	42	12	2.5
Carcass adipose tissue[c]	14	12	7.5	17	12	6.2
Blood, mesenteric fat, and feet[c]	17	8	6.6	16	8	7.2
Head and organs[c]	50	4	3.3	41	4	3.9
Gastrointestinal contents and tract[c]	131	4	1.3	49	4	3.4
Counting equipment[d]	8	8	13.5	8	8	13.5

[a] Detector position A.
[b] NDCV calculated from two 2-min counts on right forequarter and two 2-min counts on right hindquarter.
[c] 4-pi detector position.
[d] Empty aluminum container on stretcher used for counting all body components.

in hair clippings from cattle. Washing cattle reduced the ^{40}K channel count rate by only 4.8%;[16] the total body potassium of sheep was lowered 35% by shearing and washing.[10]

By far the most important source of variation in whole-body potassium in ruminants is the diet. An average of 22%, ranging from 10 to 30%, of the whole-body count originated from the GI tract and contents of steers in phase I. These steers were fed one of two pelleted diets containing 15% or 60% alfalfa meal.[12] Considerable amounts of $^{106}Ru/^{106}Rh$, $^{95}Zr/^{95}Nb$, and ^{54}Mn occurred in the rumen contents,[16] and a portion of gamma rays from these radionuclides are counted in the ^{40}K channel by liquid scintillation counters.

Two spectra obtained from an NaI(T1) crystal detector 12.7 cm in diameter are shown of a reconstructed steer, one with the GI contents and tract in the steer, the other without (Figure 3). The method of reconstruction

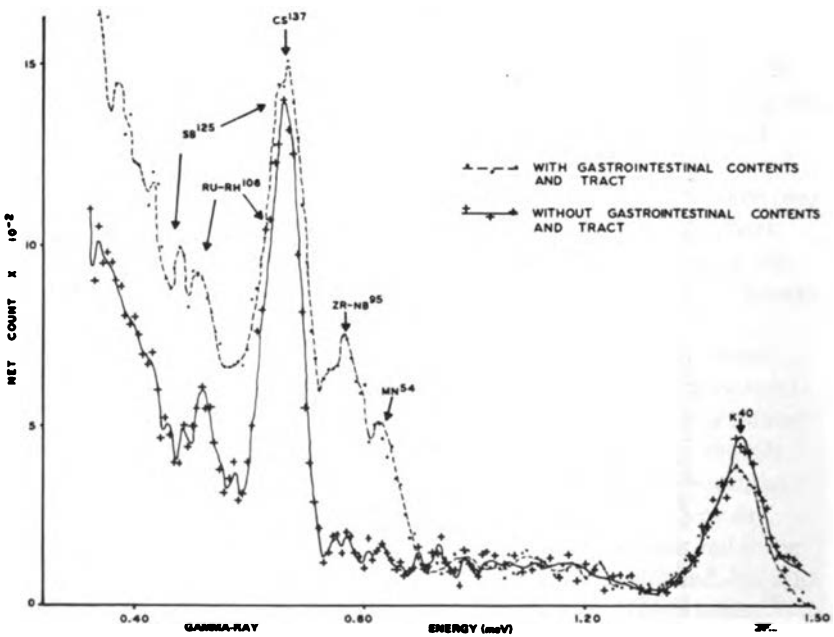


FIGURE 3 Gamma-ray spectra of phase I reconstructed steer.

was described by Lohman *et al.*[12] ^{137}Cs and ^{40}K were predominant in the empty body, with small amounts of $^{106}Ru/^{106}Rh$. When the GI was included, ^{125}Sb, $^{95}Zr/^{95}Nb$, and ^{54}Mn were present. A large portion of the fallout

nuclides of the whole-body spectrum were traced to the GI, in agreement with results of others.[17] In addition to the above radionuclides, $^{141,144}Ce/^{141,144}Pr$, ^{131}I, and $^{140}Ba/^{140}La$ have been reported in the gamma-ray spectra of cattle.[7]

The distribution of potassium throughout the body is given in Table 3 for each phase. In phase II, when the low-radioactivity diet was fed, GI potassium was only 11% of total body potassium, and was less than half the GI potassium of phase I steers, based on the count in the potassium channel. The standard deviation was reduced to one fifth of the phase I level, showing that this source of variation can be effectively reduced by feeding diets low in radioactivity prior to whole-body counting. In both phases, CB had considerable amounts of potassium; the potassium concentration (grams per kilogram) approached that of the STL and was considerably higher than reported by Kirton *et al.*[10] in sheep bone and Kulwich *et al.*[11] in beef round bone.

The effect of variability in GI radioactivity on the whole-body ^{40}K count was further verified by feeding two steers the high-roughage pelleted diet plus hay for 10 days followed by a diet (oats) low in radioactivity. A heifer was fed a concentrate diet plus hay throughout the trial. Variation in radioactivity of the hay from bale to bale proved to be an important source of variation in the ^{40}K counts. Hays fed after the first 2 days contained only 60 or 40% of the ^{40}K-channel count of that fed the first 2 days. After 10 days, the whole-body count had decreased 13% in the heifer, 11% in steer 1, and 6% in steer 2 (Figure 4).

The oat diet was fed to the two steers for an additional 7 days as the sole source of nutrition, and the count was further lowered by 4% in steer 1 and by 6% in steer 2. The heifer continued to consume hay during this period, and changes in the radioactivity of hay may have caused the fluctuations observed in her count. Refeeding the high-roughage diet and a high-radioactivity hay for 12 days raised the whole-body counts of the two steers to their previous levels. After a final 9-day period in which all animals were fed the concentrate diet and a low-radioactivity hay, steers 1 and 2 decreased in whole-body count by 5% and 11%, respectively (Figure 4).

Variation in the whole-body ^{40}K count also comes from variations in positioning the steer in the counter and in accounting for variability in the detector efficiency. The steer x day interaction mean square of whole-body ^{40}K count was significantly greater than the nuclear decay variance.[12] By changing the position of the detector tanks, position *B* was found to be less sensitive to the location of the sample.[16] Based on counts obtained from 44 steers in detector positions *A* and *B* on each of 2 days, the experimental counting error based on

TABLE 3 Distribution of Mass for Potassium and Body Components

Item	Phase I (37 steers)				Phase II (44 steers)			
	Potassium Mass (g)		Component Mass (kg)		Potassium Mass (g)		Component Mass (kg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Standard trimmed lean	505.0	87.6	154.1	28.3	464.6	73.4	140.4	27.5
Carcass bone	112.2	19.9	38.8	7.8	112.9	28.0	35.8	6.7
Carcass adipose tissue	34.8	13.2	49.8	25.1	37.4	15.2	52.6	27.6
Blood, mesenteric fat, and feet	26.4	6.2	39.3	11.1	23.3	7.8	36.4	9.6
Head and organs	75.1	10.0	30.4	4.7	65.8	9.0	28.0	4.2
Gastrointestinal contents and tract	227.9	104.5	55.2	12.5	90.2	20.0	57.1	9.3
Hide[a]	30.5	–	30.5	5.2	30.4	–	30.4	5.2
Sum of Parts	1,012.0	213.7	398.2	79.4	824.6	132.3	380.8	75.4

[a]Potassium values based on 0.1% potassium concentration in the hide.

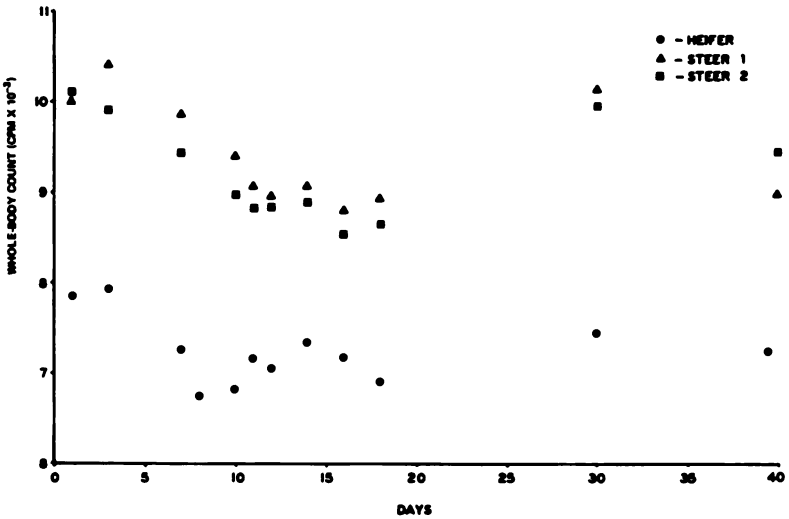


FIGURE 4 Effect of diet on whole-body count.

one 4-min count was 2.5% for whole-body potassium as determined for position *A* and 1.7% for position *B*, the NDCV being 1.3%. These results agree with earlier results[12] indicating appreciable sources of variation of experimental origin in whole-body ^{40}K count in addition to nuclear decay variability.

Carcass ^{40}K Count

Total carcass potassium was calculated as twice the sum of potassiums found in the right-side carcass components (hereafter called carcass component potassium), which in turn were calculated from the component ^{40}K counts and ^{40}K calibration by using KCl-in-water phantoms. The regression of carcass component potassium on intact carcass ^{40}K count and mass within phase was used as the calibration equation for intact carcasses. The two independent variables together accounted for 95.3% and 89.0% of the variation in carcass component potassium in phases I and II, respectively (Table 4). While there was no significant difference between phases in the regression coefficients, there was a significant difference in the constant term (because phase I carcasses were counted at a slightly higher efficiency than phase II carcasses) and in the standard error of estimate (SE), which was considerably greater in phase II. Accounting for differences in breed type, the quadratic effect of

TABLE 4 Relation between Carcass Count and Mass and Carcass Component Potassium

Independent Variables	Dependent Variable (Carcass Component Potassium)					
	Phase I (47 steers)[d]		Phase II (56 steers)[d]			
	b	Sb	b	Sb	b	Sb
Carcass ^{40}K (cps)[a]	2.26*	0.23	1.81*	0.32	–	–
Carcass ^{40}K (cps)[b]	–	–	–	–	2.08*	0.31
Carcass mass (kg)[c]	2.05*	0.21	2.42*	0.27	2.08*	0.28
Constant term	−24.5		36.8		29.2	
$R^2 \times 100$	95.3		89.0		90.3	
SE (kg)	24.8		36.8		34.6	
SE (%)	3.8		6.0		5.6	

[a] Right side of carcass uncorrected for changes in counter efficiency.
[b] Right side of carcass corrected for changes in counter efficiency.
[c] Right side of carcass.
[d] Asterisk indicates $P < 0.05$.

carcass mass, or the trimmed carcass adipose mass, did not significantly decrease the SE in either phase.

In phase I the SE was 3.8%. The NDCV of the intact carcass count was 1.4%, and the NDCV for the carcass component potassium was 1.6%. These two sources of variation accounted for about 31% of the total deviation mean square. In phase II the SE was 6.0%. Correcting carcass counts for changes in counter efficiency had little effect on the SE (Table 4). Thus, appreciable variation was found between the intact carcass ^{40}K count and mass and carcass component potassium, particularly in phase II.

The relation of the right- to left-side intact carcass ^{40}K count and potassium was studied to provide a better estimate of the within-day experimental errors in carcass ^{40}K counting. The SE estimated from differences between left and right sides was 1.7% for intact carcass ^{40}K count and 1.1% for intact carcass potassium, not significantly different from the NDCV. These results indicate that the intact carcass count was highly repeatable from side to side and that little variation was observed above that contributed by nuclear decay variability. However, these values are not estimates of total experimental error because they are based on counts taken within a short period of time.

The intact carcass ^{40}K count, carcass mass, breed type, and phase accounted for 96.8% of the variation in CLMM with a SE of 3.2% (Table 5). When intact carcass ^{40}K count was replaced by carcass component potassium, 4.5% less of the variation in CLMM was accounted for, the SE being 5.0%. These results indicate that measured gamma radiation from the intact carcass was a more precise indication of carcass composition than the total measured for carcass components.

TABLE 5 Relation of CLMM to Carcass Measurements and Breed Type (103 Steers)

Independent Variable	Dependent Variable: CLMM[a] b	Sb	CLMM[a] b	Sb
Intact carcass ^{40}K (cps)	0.521*	0.033	–	–
Carcass component potassium (g)	–	–	0.139*	0.016
Carcass mass (kg)	0.360*	0.029	0.239*	0.062
Breed-type comparisons				
1	0.88*	0.28	1.56*	0.40
2	−1.15*	0.36	−2.74*	0.53
3	0.49	0.61	0.46	0.95
Phase	−1.16*	0.53	–	–
Constant term	−1.13		16.78	
$R^2 \times 100$	96.8		92.3	
SE (g)	4.3		6.6	
SE (%)	3.2		5.0	

[a]Asterisk indicates $P < 0.05$.

To determine the influence of carcass ether extract mass on carcass ^{40}K count, the regression of carcass count on carcass ether extract was adjusted for differences in CLMM, carcass mass, breed type, and phase. The partial regression coefficient found was negative and significant ($P < 0.05$), which indicates that larger amounts of carcass ether extract were associated with a smaller carcass count. However, the effect was not large: an increase of 5 kg ether extract (9% of the mean) corresponded to a decrease in the predicted ^{40}K of 3 counts per second (cps), 1.6% of the mean. Also, the coefficient of determination (CD) was decreased by only 1%, from 94.1 to 93.1, when ether extract was omitted from the analysis.

Regression of Sum-of-Parts on Whole-Body Potassium

The total potassium from counts on separate components had a mean value of 1,012.0 g in phase I and 824.6 g in phase II (Table 3). The mean potassium values derived from the whole-body ^{40}K count were 1,131.9 and 898.9 g–12% higher in phase I and 9% higher in phase II. These results contrast with those reported by Kirton *et al.*,[10] who found the sum-of-parts potassium 14% greater than the whole-body potassium in sheep, based on ^{40}K counting. From the regression of intact carcass plus noncarcass potassium on whole-body potassium, CD's of 94.0% and 87.4% were found in phases I and II, respectively, with SE's of 5.2% and 5.5%. Larger CD's were observed when carcass

component potassium replaced intact carcass potassium. Thus, in each phase, considerable variation in the sum-of-parts potassium could not be accounted for by the whole-body potassium.

Whole-body potassium was determined by using calibration factors from a separate study involving 19 cattle, all of beef breeding.[16] Body mass accounted for 95.7% of the ^{42}K detection efficiency variation in position *A*, and 89.3% in position *B*. Body measurements, including length, width, height, and circumference, did not account for significant additional variation in ^{42}K detection efficiency.

The effect of body conformation on whole-body potassium was studied further in an attempt to account for the variation in the relation of the parts to the whole. Body measurements (length, width, height, and circumference) of phase II steers were used.

Whole-body potassium accounted for 87% of the variation in the intact carcass plus noncarcass potassium. With the addition of nine body measurements, each as an independent variable, the variation accounted for increased by 9% (Table 6). Of nine measurements (including body mass) only partial regression coefficients for body mass and length were significant ($P < 0.05$). The use of body mass and length in addition to whole-body potassium accounted for as large a proportion of the variation as was accounted for by all nine measurements in sum-of-parts potassium.

TABLE 6 Relation of Sum-of-Parts Potassium to Whole-Body Potassium and Body Measurements (44 Phase II Steers)

Independent Variable	Dependent Variable: Intact Carcass plus Noncarcass K		
	R^2 or $R^2 \times 100$[b]	SE (kg)	SE (%)
Body mass (kg)	91.1	38.1	4.6
Whole-body potassium (g)[a]	87.4	45.2	5.5
Whole-body potassium plus:			
Nine body measurements	96.2	27.9	3.4
Two body measurements[c]	95.3	28.3	3.4
Three breed-type comparisons	95.8	27.0	3.3
Three breed-type comparisons and body mass	96.9	24.0	2.9
Three breed-type comparisons and body adipose mass	96.2	26.2	3.2

[a] Based on mean of counts on 2 days with detector position *A*.
[b] All coefficients of determination significant, $P < 0.05$.
[c] Shoulder point to pins and body mass.

The effect of breed type on the relation between whole-body and sum-of-parts was also studied. The three breed-type comparisons were all significant, and the variation that was accounted for, above that by whole-body potassium alone, was 8.4% for sum-of-parts potassium (Table 6). The SE was 3.3%, and by including body mass as an additional independent variable, the SE was lowered to 2.9%. Of the remaining variation unaccounted for, almost half was due to experimental errors in the whole-body count and in the NDCV for the intact carcass plus noncarcass potassium. Thus whole-body potassium, when adjusted for differences in body conformation or breed type, was found to be closely related to the intact carcass plus noncarcass potassium.

The discrepancy between the mean whole-body potassium for the 44 steers and that of the mean potassium for carcass plus noncarcass potassium was further studied by comparing values in phase II within breed type (Table 7). Close agreement was observed between the mean whole-body potassium and mean sum-of-parts potassium for the Angus; however, as the breed type changed from beef to dairy the ratio of whole-body to sum-of-parts potassium progressively increased. Adjusting for differences in the intact carcass plus noncarcass potassium, the Holstein, Angus x Holstein, and Charolais x Angus averaged 15%, 12%, and 5% higher whole-body potassium values than the Angus (Table 7). The only nonsignificant difference was between Charolais x Angus and Angus. Since the ^{42}K whole-body counting efficiency calibration was with beef cattle, and since calculation of all whole-body potassium values were based on this ^{42}K calibration, these results appear logical.

Mean length, width, and mass of the four breed types are given in Table 7, with the standard deviations. There is some correspondence between breed type and body measurements, but much variation was observed within each type. However, when body length was used in addition to breed type and whole-body potassium, there was no significant increase in the variance accounted for in sum-of-parts potassium.

Body fat was estimated by combining the trimmed carcass adipose mass with the mesenteric adipose mass. Little additional variation was accounted for in the sum-of-parts potassium when adipose mass was included as an independent variable with whole-body potassium and the breed-type comparisons (Table 6).

The magnitude of the adipose mass regression coefficient when the intact carcass plus noncarcass potassium was the dependent variable was 1.7 times greater than its standard error, and its sign was positive, which suggests that large amounts of adipose tissue tend to cause whole-body potassium to underestimate the sum-of-parts potassium. This observation agrees with results of Remenchik and Miller,[15] who reported underestimations of potassium in obese human subjects based on measurement of ^{40}K with a crystal detector. Because

TABLE 7 Body Measurements Within Breed Type (Phase II)

Item	Holstein (10 Steers)		Angus x Holstein (14 Steers)		Charolais x Angus (8 Steers)		Angus (12 Steers)	
Whole-body potassium (g)[a]	954		925		871		845	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Whole-body potassium (g)								
Detector position *A*	955	167	905	146	856	141	874	139
Detector position *B*	939	173	890	161	838	144	863	155
Intact carcass plus noncarcass potassium (g)	824	142	811	127	808	112	853	129
Body mass (kg)	385	77	399	84	384	73	416	77
Body length, shoulder point to pins (cm)	144.0	7.8	135.3	7.6	129.7	8.7	130.0	5.8
CLMM (kg)	132.5	24.9	128.9	20.1	122.3	21.4	125.6	21.9

[a]Based on the mean of counts on 2 days with detector position *A* adjusted for differences in intact carcass plus noncarcass potassium.

the cattle in this study were not obese, the effect of body adipose mass was not pronounced.

Regression of Whole-Body Potassium on Component Potassiums

Regression of whole-body potassium on the potassiums of various component parts, each as an independent variable, was studied to determine which components had a significant effect on whole-body potassium. In phase I, STL and GI potassium were significantly related to whole-body potassium (Table 8). The STL potassium and two breed-type comparisons were significant for both detector positions of phase II, whereas breed-type comparison 1 was significant only for position *A*. The general agreement between positions *A* and *B* in regression coefficients for whole-body potassium on component potassiums indicate no major difference between detector positions in the contributions of components to the whole-body potassium. The relatively large standard errors of the regression coefficients for most

TABLE 8 Relation of Whole-Body Potassium to Potassium of Component Parts

	Dependent Variable (Whole-Body Potassium)[a]					
	Phase I (37 steers)		Phase II (44 steers)			
Independent Variable	Detector Position *A*[b] b	Sb	Detector Position *A*[b] b	Sb	Detector Position *B*[b] b	Sb
Potassium (g)						
Standard trimmed lean	1.51*	0.20	1.39*	0.18	1.48*	0.19
Carcass bone	0.64	0.63	1.05	0.54	1.14	0.60
Carcass adipose	1.14	0.83	−0.40	0.88	−0.34	0.96
Blood, mesenteric fat, and feet	1.98	2.52	2.39	1.73	2.51	1.91
Head and organs	2.83	1.81	−0.14	1.53	0.22	1.68
Gastrointestinal contents and tract	0.70*	0.12	0.78	0.53	0.61	0.58
Breed type						
1	8.76	6.43	11.25*	5.52	10.61*	6.07
2	−12.56	7.89	−19.63*	6.43	−18.95*	7.08
3	4.53	14.55	25.28*	11.75	26.41*	12.94
Constant term	−166.9		32.7		−47.8	
R^2 x 100	97.3		92.2		91.7	
SE (g)	46.9		46.3		51.0	

[a] Based on mean of counts on 2 days.
[b] Asterisk indicates $P < 0.05$.

TABLE 9 Relation of Whole-Body Potassium to Carcass and Noncarcass Potassium

Independent Variables	Dependent Variable (Whole-Body Potassium)[a]							
	Phase I (37 steers)[b]				Phase II (44 steers)[b]			
	b	Sb	b	Sb	b	Sb	b	Sb
Potassium (g)								
Standard trimmed lean	1.60*	0.19	–	–	1.31*	0.16	–	–
Intact carcass	–	–	1.54*	0.11	–	–	1.21*	0.06
Muscle-free lean[c]	1.39*	0.46	–	–	1.02*	0.33	–	–
Gastrointestinal contents and tract	0.75*	0.12			1.04*	0.41*		
Noncarcass	–	–	0.73*	0.09	–	–	0.82*	0.18
Breed type								
1	9.66	4.79	18.97*	3.88	13.50*	4.61	17.94*	2.74
2	−10.01	7.16	6.39	5.60	−19.00*	5.67	−16.34*	3.76
3	10.70	13.51	11.29	11.35	25.59*	11.04	24.29*	6.87
Constant term	−149.9		−127.1		−17.7		−18.9	
R^2 x 100	97.0		97.8		92.2		96.3	
SE (g)	47.6		40.0		44.3		30.1	

[a] Based on 1 day's count in phase I and on the mean of counts on 2 days in phase II with detector position A.
[b] Asterisk indicates $P < 0.05$.
[c] Sum of head, organs, bone, and hide potassium (as derived from hide mass); used as one independent variable.

components indicate inconsistency from steer to steer in the contribution of potassium in each component to whole-body potassium. Much of this inconsistency may be due to experimental errors other than nuclear decay in measuring potassium in the components.

From the grouping of component parts into carcass and noncarcass potassium, it was found that both variables accounted for a significant amount of variation in whole-body potassium (Table 9). Regression coefficients of 1.54 and 1.21 were found for carcass potassium of phases I and II, respectively. The regression coefficients for noncarcass potassium were less than unity. In phase I, noncarcass potassium as an independent variable increased the variation accounted for by 4.4% when used with intact carcass potassium (Table 9). In phase II, the noncarcass potassium was considerably smaller because of the lower GI radioactivity levels, and an additional 2.0% of the variation in whole-body potassium was accounted for.

A final grouping of steer component potassiums was carried out according to the model of von Döbeln[18] into STL potassium, sum of HO, CB, and hide potassium (defined as lean muscle-free tissue potassium), and GI potassium. The increase in variance accounted for by all three variables over STL potassium alone was 5.1% and 4.1% for phases I and II, respectively. Significant regression coefficients were found for all three groups in both phases in addition to some significant breed-type comparisons (Table 9), these findings indicate that differences among steers in amount of muscle-free lean tissue as well as GI potassium are additional sources of variation in whole-body potassium, as von Döbeln had suggested to be the case with humans.

The SE of body mass as predicted from the component parts, each used as an independent variable, was about 1% as compared with 3.3% when intact carcass potassium, noncarcass potassium, and the breed-type comparisons were used to predict whole-body potassium. These results indicate the present limitations in precision of estimating whole-body potassium from gamma-ray counting of the component parts as compared with the precision of estimating body mass from mass measurements of the components. While all body component masses account for rather sizable amounts of variation in body mass, the STL potassium, CB potassium, and GI potassium account for most of the variation in body potassium.

Regression of CLMM on Whole-Body Potassium

When whole-body potassium was used to estimate the carcass lean muscle mass, 91.6% of the variation was accounted for with a SE of 4.9%. Accounting for the effect of breed type did not significantly decrease the SE, in contrast to the whole-body potassium versus sum-of-parts analysis, and in contrast to carcass potassium versus CLMM analysis.

The whole-body potassium, sum-of-parts potassium, and carcass potassium means for each breed type adjusted by the method of least squares for differences in CLMM are given in Table 10. In contrast to the decrease in adjusted

TABLE 10 Association of Body Potassium with Breed Type Adjusted for Differences in CLMM (44 Phase II Steers)

Item	Adjusted Means for CLMM				Variation Accounted for[a]
	Holstein	Angus x Holstein	Charolais x Angus	Angus	$R^2 \times 100^b$
Whole-body potassium (g)[c]					
Detector position *A*	923	896	890	886	92.4
Detector position *B*	901	881	874	877	92.5
Sum-of-parts potassium (g)	798	799	841	861	91.7
Intact carcass potassium (g)	596	604	623	636	95.6

[a] CLMM and the three breed-type comparisons were used as the independent variables, and the various measures of body potassium were used as the dependent variables.
[b] All coefficients of determination significant, $P < 0.05$.
[c] Based on mean of counts for 2 days.

whole-body potassiums as beef breeding increased, the adjusted sum-of-parts potassium increased. One half to two thirds of this increase was due to greater amounts of potassium in the carcass per unit of CLMM, and the remainder to larger amounts of noncarcass potassium. Thus, although there was less sum-of-parts potassium per unit of CLMM in cattle of dairy breeding, it was distributed so that it was counted with considerably higher efficiency in the whole-body count than for cattle of beef breeding and favored the Holsteins in terms of a higher ratio of whole-body potassium to CLMM, with little difference between the three other breeds. These results agree with those of Miller and Remenchik,[14] showing the limitation of calibrations based on uniformly distributed sources of radioactivity. Although calibrations of this study were carried out *in vivo*, the use of only one breed limited the accuracy when applying the calibration results to other breeds.

The use of nine body measurements in addition to whole-body potassium increased the variation accounted for in CLMM by 3.2%. Of these measurements, only the partial regression on heart girth was significant and only when body potassium was based on the whole-body count from detector position *B*. The use of body mass, length, width, and heart girth accounted for nearly as much of the variation in CLMM as that accounted for by all nine body measurements together with whole-body potassium (Table 11). Only regressions on body length and heart girth were significant when used in addition to whole-body potassium based on position *B* count. Regression on body length was

TABLE 11 Relation of CLMM to Whole-Body and Body Component Measurements (44 Phase II Steers)

Independent Variable	Dependent Variable (CLMM)[a]					
	b	Sb	b	Sb	b	Sb
Whole-body potassium (g)[b]	0.104*	0.019	–	–	0.099*	0.023
Whole-body potassium (g)[c]	–	–	0.101*	0.018	–	–
Body mass (kg)	−0.032	0.057	−0.054	0.058	0.081	0.089
GI potassium (g)	–	–	–	–	−0.116*	0.054
Muscle-free lean potassium (g)[d]	–	–	–	–	−0.026	0.040
Body adipose mass (kg)	–	–	–	–	−0.068	0.067
Body length (cm)[e]	0.34	0.18	0.42*	0.17	0.15	0.20
Body width (cm)[f]	−0.39	0.32	−0.32	0.31	−0.49	0.32
Body circumference (cm)[g]	0.53*	0.21	0.55*	0.21	0.37	0.21
Constant term	−72.8*		−76.8*		−37.3	
R^2 x 100	94.5		94.6		95.2	
SE (kg)	5.4		5.3		5.2	

[a] Asterisk indicates $P < 0.05$.
[b] Based on the mean of counts on 2 days with detector position *A*.
[c] Based on the mean of counts on 2 days with detector position *B*.
[d] Sum of head, organs, bone, and hide potassium.
[e] Shoulder top to pins.
[f] Width of shoulders.
[g] Heart girth.

not significant when used with whole-body potassium based on the position *A* count. The significant effect of heart girth on the relation between whole-body potassium and carcass composition is in contrast to its nonsignificant effect on the relation of sum-of-parts to whole-body potassium.

The addition of GI potassium and bone, organ, and hide potassium as independent variables increased the variation in CLMM accounted for by less than 1% (Table 11). Although the effect of GI potassium was significant, the SE was reduced to only 5.2 kg, 4.0% of the mean CLMM. The muscle-free lean potassium mass, as von Döbeln[18] defined it, did not significantly affect the relation between whole-body potassium and CLMM, but in the same steers it did significantly affect the relation between whole-body potassium and both STL potassium and carcass potassium. Body adipose mass was also used as an independent variable, but no significant effect was observed, and the sign of the regression coefficient was negative in contrast to that when sum-of-parts potassium was the dependent variable.

SUMMARY

Counter-induced and sample-induced variables were studied to determine what factors influence ^{40}K as measured by liquid scintillation whole-body counters.

A comparison of day-to-day variation in detection efficiency by using a ^{137}Cs standard in two different whole-body counters indicated that fairly stable performances can be achieved, although observed variability probably due to electronic-instrument drift and photomultiplier-tube instability was significantly greater ($P < 0.05$) than nuclear decay variability. Consecutive counts over a 10-day period on a human subject yielded coefficients of variation of 2.7 and 1.8% for the two whole-body counters.

Cattle were used to study the major sample-induced variables found to affect ^{40}K measurement, including diet as it influences the GI radioactivity, variability in positioning animals in the whole-body counter, body conformation, and breed type. By feeding a low-radioactivity diet for 7 days before ^{40}K counting and accounting for differences in body conformation, whole-body potassium predicted sum-of-parts potassium, as determined by ^{40}K counts on separate body components, with a SE of 3.4% and carcass lean muscle mass (CLMM) with a SE of 4.2%.

Breed type affected the relation between whole-body potassium and sum-of-parts potassium significantly ($P < 0.05$) as well as that between carcass potassium and CLMM, but not that between whole-body potassium and CLMM. The effect of potassium found outside the lean tissue on the relation between whole-body potassium and carcass lean potassium and CLMM was also studied.

Measurement of gamma radiation from intact carcasses was a more precise indication of whole-body potassium and of CLMM than measurement of radiation from separate carcass components. Intact carcass ^{40}K count, carcass mass, and three breed-type comparisons together predicted CLMM with a SE of 3.2%.

This work was supported in part by U.S. Atomic Energy Commission Grant No. AT(11-1) 1339, and by grants from the Illinois Department of Agriculture, and the National Livestock and Meat Board.

Portions of this paper are part of a thesis submitted by the senior author to the Graduate College, University of Illinois, in partial fulfillment of the requirements for the Ph.D. degree.

REFERENCES

1. Anderson, E. C. 1959. Data reduction for the Los Alamos human counter, 23 p. Los Alamos Scientific Laboratory, Los Alamos, N. M.
2. Anderson, E. C. 1963. Three component body composition analysis based on potassium and water determinations. Ann. N. Y. Acad. Sci. 110:189.
3. Anderson, E. C. 1965. Determination of body potassium by 4 pi gamma counting, p. 211. *In* G. R. Meneely and S. M. Linde [ed.] Radioactivity in Man. 2nd Symposium.
4. Bryant, E. C. 1960. Statistical Analysis, p. 198. McGraw-Hill, New York.
5. Gillett, T. A., A. M. Pearson and A. H. Kirton. 1965. Variation in potassium and sodium in muscles of the pig. J. Anim. Sci. 24:177.
6. Gillett, T. A., A. M. Pearson, D. M. Allen, and R. A. Merkel. 1967. Variation in potassium and sodium content of bovine muscles. J. Anim. Sci. 26:46.
7. Johnson, J. E., and G. M. Ward. 1966. Body composition of live animals as determined by ^{40}K. I. A crystal-type, whole-body counter for determining body composition of live animals. J. Dairy Sci. 49:1163.
8. Karzmark, C. J. 1965. Photomultiplier "fatigue" effects. Health Phys. 11:54.
9. Kirton, A. H. 1964. Some relations between the potassium and sodium contents of animals and their composition, p. 3. Carcass Composition and Appraisal of Meat Animals, Tech. Conf. Proc., Queens College, University of Melbourne.
10. Kirton, A. H., A. M. Pearson, R. H. Nelson, E. C. Anderson, and R. L. Schuch. 1961. Use of naturally occurring potassium-40 to determine the carcass composition of live sheep. J. Anim. Sci. 20:635.
11. Kulwich, R., L. Feinstein, C. Golumbic, W. R. Kauffman, and R. L. Hiner. 1961. Relation of gamma-ray emission to the lean content of beef rounds. Food Tech. 15:411.
12. Lohman, T. G., B. C. Breidenstein, A. R. Twardock, G. S. Smith, and H. W. Norton. 1966. Symposium on atomic energy in animal science II. Estimation of carcass lean muscle mass in steers by ^{40}K measurement. J. Anim. Sci. 25:1218.
13. Madamba, J. C. 1965. Effects of breed-type, dietary energy level, stilbestrol, and slaughter weight on performance and carcass composition of steers. Ph.D. Thesis. Univ. Illinois, Urbana.

14. Miller, C. E., and A. P. Remenchik. 1963. Problems involved in accurately measuring the K content of the human body. Ann. N. Y. Acad. Sci. 110:175.
15. Remenchik, A. P., and C. E. Miller. 1961. The measurement of total body potassium in man and its relation to gross body composition, p. 311. *In* Whole-body counting. International Atomic Energy Agency, Vienna.
16. Twardock, A. R., T. G. Lohman, G. S. Smith, and B. C. Breidenstein. 1966. Symposium on atomic energy I. The Illinois Animal Science Counter: Performance characteristics and animal radioactivity measurement procedures. J. Anim. Sci. 25:1209.
17. Van Dilla, M. A., G. R. Farmer, and V. R. Bohman. 1961. Fallout radioactivity in cattle and its effects. Science 133:1075.
18. von Döbeln, R. J. 1961. Discussion V6, p. 351. *In* Whole-body counting. International Atomic Energy Agency, Vienna.

J. E. Johnson
G. M. Ward
and L. B. Sasser
Colorado State University

CALIBRATION TECHNIQUES FOR A CRYSTAL-TYPE WHOLE-BODY COUNTER

The ability to measure total body potassium by the gamma-ray emission of its radioactive isotope ^{40}K was reported over a decade ago.[4] Although the application of this technique to the determination of gross body composition seemed promising, it has been used with success rather infrequently. This can be attributed largely to difficulties in calibration and relatively few attempts to assess first the precision and accuracy of ^{40}K whole-body counting data.

These two problems would seem amenable to solution with animals because radioactive isotopes may be given and because the animal may be slaughtered and the total potassium content determined accurately by chemical methods.

This paper is concerned with K calibration techniques used at Colorado State University to determine total body K in swine and cattle and to analyze the whole-body counting data in terms of expected precision and accuracy.

EXPERIMENTAL METHODS

The Colorado State University whole-body counter has been described in detail previously.[2] The detector is a single large high-resolution NaI(Tl)

scintillation crystal (4 by 9 in.) used in a low-background steel room (8 by 8 by 8 ft). All animals are carefully washed before counting, and the count time is 15 to 30 min. The counting positions for swine and cattle are shown in Figures 1 and 2. A constant crystal-to-skin distance of 10 cm is used for all animals, regardless of size. The counting geometry for humans is presently the Argonne standard chair.

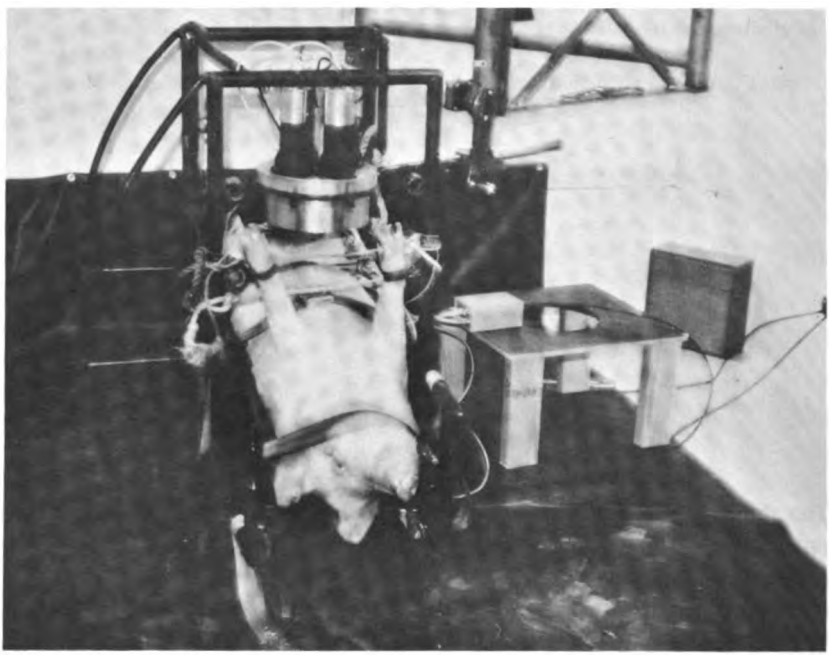


FIGURE 1 Counting position for swine in the Colorado State University whole-body counter.

The counting calibration constant for total body K in animals (counts/30 min-gram K) is determined by values from both ^{42}K-dilution experiments and chemical analysis of the total body K after slaughter. The K concentration in all animal tissues is determined both by ^{40}K counting[5] and by atomic absorption spectrometry. Fat is determined in tissue samples by pentane extraction.[1]

The effect of rumen potassium content on the total body ^{40}K counting rate was estimated by placing polyethylene bags of KCl in solution at various positions in the rumen of a rumen-fistulated cow.

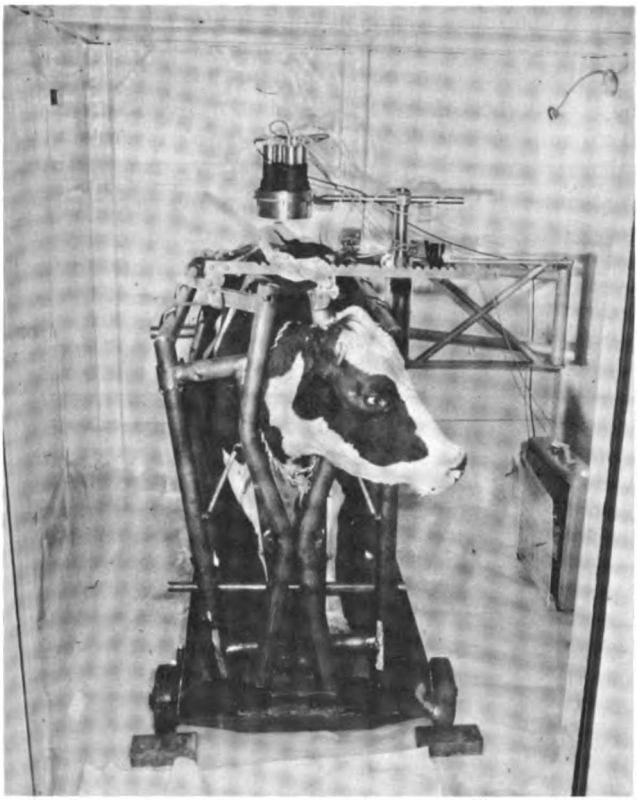


FIGURE 2 Counting position for cattle in the Colorado State University whole-body counter.

RESULTS AND DISCUSSION

The calibration constants or counting efficiencies for potassium in swine are shown in Table 1. These values were derived from both ^{42}K dilution and slaughter analysis. Since the crystal is kept at a fixed distance from the animal, the calibration constant is a function of the size or body weight of the animal. In Figure 3 the calibration constants determined for swine are plotted on log-log coordinates. The equation for this relationship is:

$$\text{counts/30 min-gram K} = 494\ W^{-0.73},$$

where W = the live body weight in kilograms.

TABLE 1 ^{40}K Counting Calibration Constants for Swine

Body Weight (kg)	Method of Measuring Total Body K	Whole-Body Counter K Calibration Constant (counts/30 min-gram K)
24.5	^{42}K	47.5
38.0	^{42}K	38.1
56.8	Slaughter, ^{40}K	27.5
104.0	Slaughter, ^{40}K	20.7
111.0	Slaughter, ^{40}K	18.9
111.0	Slaughter, ^{40}K	20.6
112.0	Slaughter, ^{40}K	17.7
123.0	Slaughter, ^{40}K	16.0

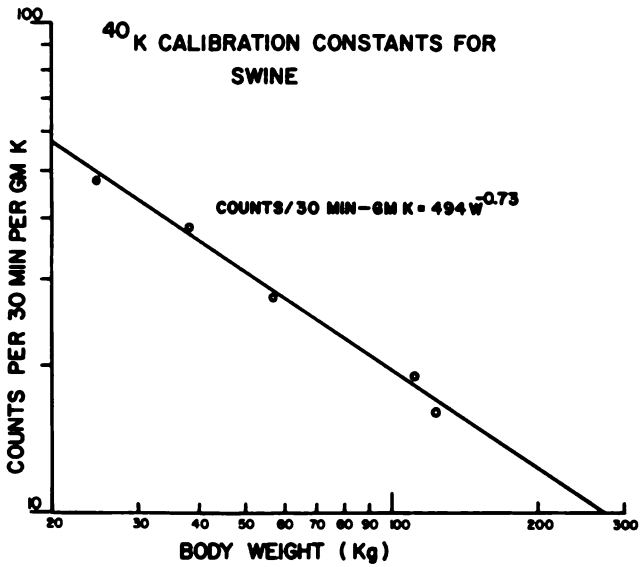


FIGURE 3 The whole-body counter ^{40}K calibration constant for swine as a function of body weight.

The accuracy of any measurement of total body K in a pig will depend on the counting time, the net K count to background ratio, and the body weight. For a 50-kg pig and a counting time of 20 min, the expected coefficient of variation of the counting estimate of total body K is approximately 4%. This would be expected from counting statistics alone. The observed coefficient of

variation of a pig counted repeatedly and repositioned between counts was 4.4%. Swine are completely immobile and docile when on their backs in the counting position used, and the counting time could easily be increased to 30 min. If the total body K is used to estimate lean body mass (LBM), the calculation for a 50-kg pig would give a coefficient of variation for the estimate of approximately 4.4%. If a two-compartment model is used and total body fat is estimated by difference, then variability of its estimate will, of course, increase as the percentage of LBM increases.

The calibration constants observed for cattle are listed in Table 2. These are plotted as a function of body weight in Figure 4. Since the weight range was not as great as for the swine, the values are plotted on linear coordinates. For comparison, the calibration constant for humans in the standard chair

TABLE 2 ^{40}K Counting Calibration Constants for Cattle

Body Weight (kg)	Method of Measuring Total Body K	Whole-Body Counter K Calibration Constant (counts/30 min-gram K)
292	^{42}K	5.07
456	Slaughter, ^{40}K	4.41
485	^{42}K	4.26
510	Slaughter, ^{40}K	4.51
553	Slaughter, ^{40}K	4.22
614	Slaughter, ^{40}K	3.71

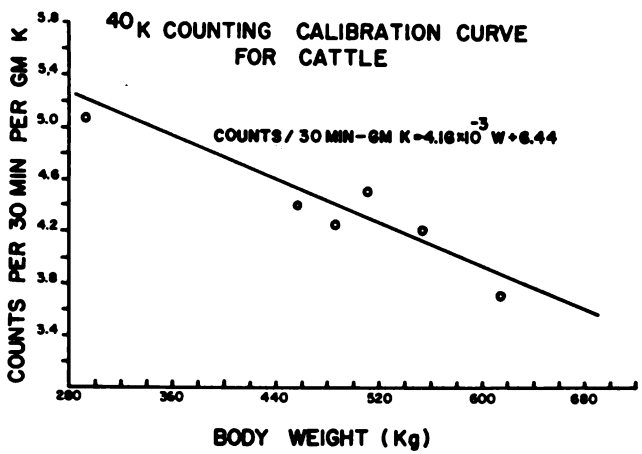


FIGURE 4 The whole-body counter ^{40}K calibration constant for cattle as a function of body weight.

positions was 19.8 counts/30 min-gram K as compared with 4 to 5 counts/30 min-gram K for cattle.

For a 500-kg cow, the net count rate under the ^{40}K total absorption peak (110 KeV) was approximately 3 times the background counting rate. One 545-kg Holstein cow was counted weekly for 12 consecutive weeks, and the coefficient of variation of the total body K estimate was 2.8%.

Although the ^{42}K-dilution technique is an easier calibration method, the ultimate value for total body K must come from slaughter analysis, and the values from the two methods must agree. It is relatively easy to obtain the K concentration in muscle or in the edible portion of the carcass; however, the fraction in bone, hide, and the nonedible portion cannot be neglected and presents the largest experimental difficulty. Phantoms to represent animals seem to be of value only to check reproducibility of counting. Phantoms offer nothing for calibration that cannot be obtained from slaughter and analysis.

With a single-crystal detector, the effect of ^{40}K and other gamma-ray emitters present in the body or the rumen is not as serious as with the liquid scintillation type of detector. One reason is that with a high-resolution crystal there is no contribution from such fallout emitters as ^{137}Cs and ^{54}Mn to the total absorption peak of ^{40}K. If short-lived fission products such as $^{95}Zr/^{95}Nb$, $^{103,106}Ru/^{103,106}Rh$, and $^{140}Ba/^{140}La$ are present in the rumen they may be quickly identified, and their contribution may be subtracted by standard spectrum-stripping techniques.[3]

When bags of water and KCl were placed at various positions in the empty rumen of a 450-kg Holstein cow with a fistulated rumen, the contribution to the net counting rate ranged from 3.0 counts/30 min-gram K at the bottom of the rumen to 3.8 counts/30 min-gram K at the top. Thus the sensitivity of the detector to K in the rumen is less than the average sensitivity for K in the whole body. In another trial the difference in net ^{40}K counting rate from a cow with a normally full (hay ration) to a completely empty rumen was less than 10%. Thus it would seem reasonable that there would be less than 4% error for normal variations in rumen and GI tract contents.

Calibration constants are, however, calculated on the basis of total body K, not including rumen K. To correct the observed counting rate for that due to K in the rumen, the rumen contents are calculated from data of rumen contents as a function of time after feeding and also as a function of body weight. Knowing the hay-to-grain ratio in the ration, the rumen K concentration, and hence the K content, may be calculated. Applying the calibration constants observed in the experiment described above then gives the counting rate due to rumen K, and this is subtracted from the observed counting rate of the animal for calculation of total body K.

The K concentration in all animal tissues is determined either by atomic absorption spectrometry or by ^{40}K counting. As observed in Table 3, the atomic absorption spectrometer method is more sensitive, but both methods predicted the same mean K concentration in tissue fat-free dry solids (FFDS).

TABLE 3 Comparison of K Analysis in Muscle Samples by ^{40}K Gamma-Ray Spectrometry and Atomic Absorption Spectrometry[a]

Analysis	Mean ^{40}K	Mean ^{39}K	Coefficient of Variation[b] (%)
Wet basis	2.86	–	17.8
FFDS	13.63	–	16.6
FFDS	–	13.70	10.2

[a] 35 samples containing 56.48% of moisture and 22.50% of fat.
[b] The coefficient of variation of the K estimate was 2.0% for 14 subsamples of the same sample determined by atomic absorption spectrometry and 4.0% for a meat sample counted 15 times by gamma-ray spectrometry.

The validity of ^{40}K estimates to predict body cell mass (BCM) or lean body mass (LBM) depends on the constancy of K in these compartments. The K concentration in individual skeletal and nonskeletal muscles from a single animal is presented in Table 4. The coefficient of variation of the K concentration in FFDS in muscle is considerably less than the coefficient of variation of the K concentration in nonskeletal muscle.

There is, however, increasing evidence in the Colorado State University laboratory that K concentration in tissue fat-free dry solids is not a constant, but that it decreases as a function of the fat percentage. Although the magnitude of this effect is not great, the LBM of the animal may not be perfectly predicted by total K alone. For this reason, we are presently performing total body water measurements by H_2^3O dilution on all animals in calibration experiments. Bromine-82 has also been used in a number of animals to estimate extracellular water volume since the total body water in the ruminant includes a large fraction of gut water.

SUMMARY

The technique of estimating *in vivo* body K by ^{40}K gamma-ray spectrometry has been shown to be feasible and sufficiently accurate in both swine and

TABLE 4 K Concentration in Individual Skeletal and Nonskeletal Muscles of a Holstein Cow

Muscle	Moisture (%)	Fat (Wet Basis) (%)	g K/kg FFDS	Coefficient of Variation (%)
Skeletal Muscles				
Longissimus dorsi	66.76	9.64	17.5	
Supraspinatus	70.57	5.98	17.5	
Biceps brachii	76.59	7.32	18.3	
Infraspinatus	67.94	14.61	16.2	
Psoas major	66.42	10.20	17.6	
Biceps femoris	67.50	7.50	16.6	
Gluteus medias	66.47	12.60	16.6	
Semitendenosis	70.09	5.06	18.2	
Quadriceps femoris	74.38	3.00	18.5	
Semimembranosis	69.86	7.51	16.7	
Semimembranosis	67.69	8.43	18.2	
Mean	69.48	8.35	17.4	4.6
Nonskeletal Muscles				
Lung	78.04	1.12	16.4	
Tongue	75.62	1.50	15.8	
Liver	70.00	0.66	11.3	
Spleen	74.15	2.32	17.9	
Rumen tissue	78.20	1.28	12.9	
Small intestine	78.50	5.72	17.1	
Large intestine	79.48	4.10	14.6	
Mean	76.28	2.39	15.1	13.0

cattle. This technique has the advantage of measuring a body-composition component directly without the administration of radioactive isotope.

The counter is calibrated from the counting rate of the animal, and the value of total body K is determined by atomic absorption spectrometry and ^{40}K gamma-ray spectrometry of all animal tissues after slaughter. The calibration procedure of ^{42}K dilution is also used. Once the counting calibration constant perfectly predicts total body K determined after slaughter, the instrument has been calibrated and can be used in studies relating total body K to body composition.

This work was partially supported under contract AT(11-1)-1171 with the U.S. Atomic Energy Commission and published as COO-1171-57. It is published with the approval of the Director of the Colorado Agricultural Experiment Station as Scientific Series Paper No. 1213.

REFERENCES

1. Association of Official Agricultural Chemists. 1955. Official Methods of Analysis. J. Ass. Offic. Agr. Chem. Washington, D.C. 8th ed. 386 p.
2. Johnson, J. E., and G. M. Ward. 1966. Body composition of live animals as determined by ^{40}K. I. A crystal-type, whole-body counter for determining body composition of live animals. J. Dairy Sci. 49:1163.
3. Johnson, J. E., G. M. Ward, and H. F. Stewart. 1966. Interpretation of gamma-ray spectra of environmental forage samples. Health Phys. 12:37.
4. Miller, C. E., and L. D. Marinelli. 1956. Gamma-ray activity of contemporary man. Science 124:122.
5. Ward, G. M., and J. E. Johnson. 1965. The cesium-137 content of beef from dairy and feed-lot cattle. Health Phys. 11:95.

T. G. Martin
R. B. Harrington
and W. V. Kessler
Purdue University

FACTORS AFFECTING ESTIMATES OF POTASSIUM CONTENT BY WHOLE-BODY LIQUID SCINTILLATION COUNTING

Accuracy of estimating potassium content by whole-body liquid scintillation counting depends on calibration of the liquid scintillation counter and use of appropriate correction factors for variation in counter efficiency and for counting efficiency associated with sample weight. In using a whole-body counter to estimate potassium content of biological samples at Purdue University, certain questions have recurred. Inasmuch as the counter (Sinco-P) has a sample chamber 183 cm long and 51 cm in diameter, the effects of weight and length of sample on potassium estimates have been subject to question. A high activity short standard (45 cm long) has been used to correct for day-to-day variation in counter efficiency. This led to questions concerning the appropriateness of the standard and the effects of activity level on estimates of potassium content.

The objectives of this experiment were to estimate the effects of length of sample, weight of sample, and level of ^{40}K activity in the sample on counter estimates of potassium content and to investigate the appropriateness of two standards in correcting for day-to-day variation in counter efficiency.

EXPERIMENTAL METHODS

Twenty-seven cylinders were constructed of plywood, canvas, and the necessary screws and nails. Three lengths (60, 113, and 166 cm) were constructed with appropriate diameters to accommodate 13.6, 40.9, and 68.2 kg of sugar at each length. Potassium chloride was mixed with the sugar in such a manner that, in each length and weight group, there were cylinders containing 100, 150, and 200 g of potassium. Therefore, a 3 length by 3 weight by 3 activity level factorial arrangement of treatments was formed.

Each cylinder was counted before the sugar–KCl mixture was added, and the effects of length and diameter were found to be nonsignificant. Average net count of the empty cylinders was 152 counts per minute, which was approximately equal to the expected background depression. Since correction for activity of the empty cylinder and background depression would be offsetting corrections, these corrections were not made.

Five replicates of the experiment were conducted on 5 days in the summer of 1966, and five additional replicates were conducted the following winter. These two sets of five replicates each are referred to as sets 1 and 2 in the data analyses.

The short standard was 45 cm long and contained enough potassium to count above 90,000 counts per minute. The human dummy standard was a cloth replica of a human body filled with a sugar–KCl mixture so that it counted in excess of 32,000 counts per minute. These two standards provided a drastic contrast in both geometry and level of activity for calculating potassium content. The standard count appropriate for a given sample was an average of the count before and after the count of the sample. The standard count to which all readings were corrected was an average of all counts taken during collection of a given set of data.

Counting procedure in each replicate was as follows: (a) the short standard, (b) the human dummy, (c) nine cylinders, (d) the human dummy, (e) the short standard, (f) nine cylinders, (g) the human dummy, (h) the short standard, (i) nine cylinders, (j) the human dummy, and (k) the short standard.

In set 1, an efficiency value for each cylinder was calculated by means of a ratio of counts per minute to gamma rays per minute emitted by the ^{40}K known to be present. Nine efficiency curves, as a function of weight, were calculated from set 1 data. These nine efficiency curves, each representing one length by activity level class, were used to correct both set 1 and set 2 data for counting efficiency associated with weight.

Total potassium (grams) was calculated by the following formula:

$$\frac{(C - B)\frac{K}{S}}{196\, f_w},$$

TABLE 1 Mean Grams of Potassium Detected in the Cylinders When the Short Standard Was Used

Length of Cylinders by Set (cm)	Weight: 13.6 kg			Weight: 40.9 kg			Weight: 68.2 kg		
	100 g K	150 g K	200 g K	100 g K	150 g K	200 g K	100 g K	150 g K	200 g K
Set 1									
60	100.19	149.95	199.88	100.69	146.87	199.32	102.26	140.36	196.96
113	99.93	150.25	200.24	99.23	151.85	196.39	97.37	155.72	189.19
166	99.97	149.84	199.66	100.24	149.27	195.71	100.96	147.25	187.08
Set 2									
60	100.63	146.16	199.15	99.07	145.85	192.54	104.14	139.37	193.48
113	99.68	145.77	197.60	97.91	148.88	194.21	94.94	154.59	186.01
166	101.78	148.41	198.42	99.18	147.15	191.62	96.88	141.13	179.84

where

C = average gross sample counts per minute,
B = average background counts per minute,
K = average standard count over the entire experiment,
S = standard count associated with the specific sample,
f_w = efficiency as a function of weight, and
196 = gamma rays per minute per gram of potassium.

The K/S ratio is necessary for Sinco-P because the electronics are not adjusted for small changes in counting efficiency. Efficiency as a function of weight (f_w) is generally a declining exponential function. In some cases, however, f_w may be appropriately described as a quadratic or linear function of weight. This function is described in more detail below (see Figures 1 and 4).

The data were analyzed statistically by routine analysis of variance techniques.

RESULTS AND DISCUSSION

Mean grams of potassium detected for each cylinder in each set are shown in Table 1. Analyses of variance of sets 1 and 2 data are shown in Table 2. In these analyses, a $3^3 \times 2$ factorial model with five observations per cell was used. Since time is often part of the experimental error involved in use of a counter, replicates were not included in this model. Set 1 and set 2 results were different with regard to amount of potassium detected, but were not drastically different with regard to the relative importance of the various sources of variance.

In both sets, the main effect of length, weight, and activity level; the two-factor interactions between length and activity, and between weight and activity; and the three-factor interaction involving length, weight, and activity were all significant sources of variance affecting total grams of potassium detected. In set 2, the main effect of standards and the interaction between length and weight were also significant.

When potassium content was converted to grams of K per kilogram of weight, the main effect of length and the effect of the three-factor interaction involving length, weight, and activity became nonsignificant in set 1. In set 2, this conversion resulted in the main effect of length becoming nonsignificant, while the interaction between weight and standard and between the activity level and standard became significant sources of variation.

In spite of these changes regarding significance, the magnitude of the various components of variance did not differ drastically between set 1 and set 2, with the exception of the main effect of standards. This main effect was created

TABLE 2 Analyses of Variance of Sets 1 and 2, with Replicates Ignored

Source of Variance[a]	df[b]	Total K (g)		g of K/kg	
		Set 1	Set 2	Set 1	Set 2
L	2	29.52*	92.61*	0.013	0.006
W	2	312.19*	633.43*	2,030.983*	2,048.048*
A	2	207,183.68*	199,414.18*	305.634*	296.352*
S	1	0.01	606.72*	0.000	0.871*
L x W	4	9.24	162.52*	0.001	0.107*
L x A	4	267.87*	251.35*	0.091*	0.093*
L x S	2	0.01	0.02	0.001	0.001
W x A	4	171.42*	271.72*	77.248*	76.096*
W x S	2	0.01	0.06	0.001	0.211*
A x S	2	0.01	20.67	0.001	0.031†
L x W x A	8	108.12*	160.66*	0.017	0.045*
L x W x S	4	0.07	0.12	0.001	0.001
L x A x S	4	0.00	0.03	0.001	0.001
W x A x S	4	0.00	0.02	0.000	0.008
L x W x A x S	8	0.89	2.28	0.001	0.004
Error	216	6.40	7.56	0.010	0.010

*$P \leq 0.01$.
†$P \leq 0.05$.
[a]L = length; W = weight; A = activity; S = standard.
[b]Degrees of freedom.

largely by an error in computation of potassium content in the set 2 data. In set 2, the average standard value (K) used in calculating potassium content was the same as that used in set 1. Data comparing the two standards in sets 1 and 2 are shown in Table 3. Adjustment of the estimated potassium content for

TABLE 3 Comparison of Standards in Sets 1 and 2

Items Compared	Short Standard	Human Dummy
Count per minute		
Set 1	91,442	33,127
Set 2	90,748	32,227
Difference	694	900
Potassium (g)		
Set 1	148.39	148.40
Set 2	146.09	149.09
Adjusted set 2[a]	145.02	145.06
Grams of K per kilogram of weight		
Set 1	5.607	5.607
Set 2	5.538	5.652
Adjusted set 2[a]	5.494	5.499

[a] Results in set 2 were calculated by using average standard values appropriate for set 1. Adjustment is downward, proportionate to reduction in standard counts.

differences in standard counts between sets 1 and 2 eliminated the effect of standards on potassium content. At the same time, however, it enlarged the difference between sets. This difference between sets may have been the result of using, on set 2 data, the efficiency curves that were derived from set 1 data.

Two conclusions can be drawn from this comparison:

1. Two standards different in geometry and activity level may be equally efficient in correcting for day-to-day variation in counter efficiency over a short time period but not equally efficient over a long time period.
2. Equations describing counting efficiency for weight should be derived from the data most analogous to the experiment in question.

The effects of length, weight, and activity level on amount of potassium detected and on efficiency were investigated; selected analyses of variance are presented in Table 4. The selected variables were efficiency of potassium detection and the two expressions of amount of potassium detected when the short standard was used in set 1. Data from set 2 are not presented because the results were very similar to those from set 1. Data based on the

TABLE 4 Analyses of Variance of Set 1 Data

Source of Variance[a]	df[b]	Short Standard		Efficiency
		Total g of K	g of K/kg	
Replicates	4	36.88*	0.046*	0.502*
L, l	1	11.60	0.006	1.936*
q	1	17.94	0.009	29.867*
W, l	1	298.33*	1,771.428*	926.727*
q	1	13.56	259.518*	18.670*
A, l	1	207,161.29*	305.628*	1.820*
q	1	28.18†	0.009	0.116
L × W, l × l	1	5.85	0.000	16.328*
l × q	1	0.32	0.000	1.861*
q × l	1	9.66	0.000	0.660†
q × q	1	1.33	0.005	0.189
L × A, l × l	1	57.41*	0.017	3.038*
l × q	1	161.14*	0.050†	1.105*
q × l	1	2.53	0.003	0.000
q × q	1	313.93*	0.097*	0.010
W × A, l × l	1	304.74*	135.276*	0.004
l × q	1	22.18†	0.004	1.943*
q × l	1	14.48	19.217*	0.660†
q × q	1	0.84	0.000	0.001
L × W × A, total	8	56.32*	0.011	1.962*
Error (cylinder × replicate)	104	5.39	0.0084	0.102

*$P \leq 0.01$.
†$P \leq 0.05$.
[a]L = length; l = linear; q = quadratic; W = weight; A = activity.
[b]Degrees of freedom.

human dummy standard also agreed closely with data based on the short standard. Martin *et al.*, in their paper, "Association between Chemical and Counter Estimates of Potassium Content" (this volume, page 341), report a correlation of 0.999 between potassium estimates derived by use of the two standards.

The model used in the data analyses was a randomized complete block with a 3^3 factorial arrangement of treatments. Effects of length, weight, and activity level were broken into linear and quadratic components.

The effect of replicates was statistically significant. However, the component of variance associated with replicates was considerably smaller than the error (cylinder x replicate) variance component.

Main effects of length, weight, and activity level on efficiency and amount of potassium detected are shown in Figures 1, 2, and 3. The effect of length is shown most graphically in Figure 1, where it is apparent that efficiency is

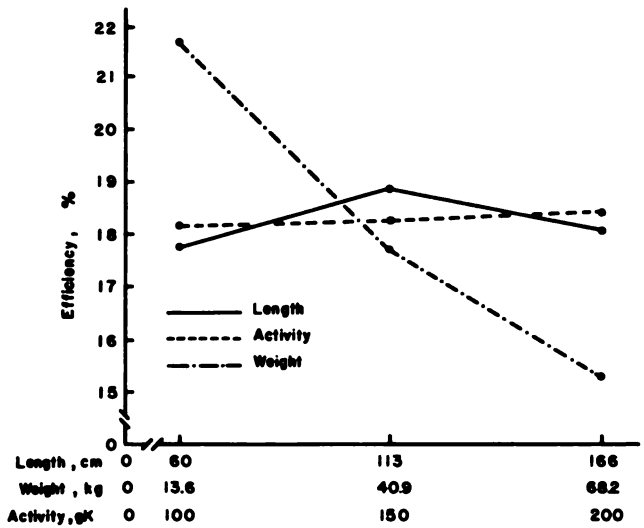


FIGURE 1 Changes in efficiency associated with length, weight, and activity of cylinders.

greatest when the intermediate length (113 cm) cylinders were counted. Since an efficiency curve to correct for variation in counting efficiency associated with sample weight was calculated for each length–activity level class, the effect of length was negligible on total grams of potassium and on grams of K per kilogram of weight detected by the counter (Figures 2 and 3).

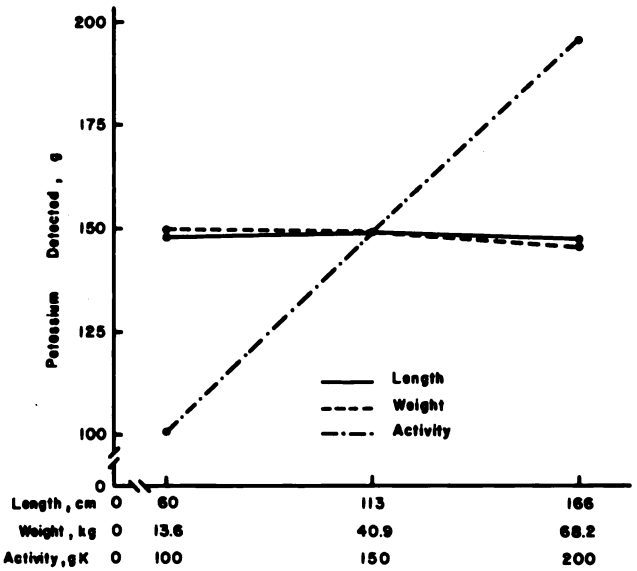


FIGURE 2 Changes in total potassium detected associated with length, weight, and activity of cylinders.

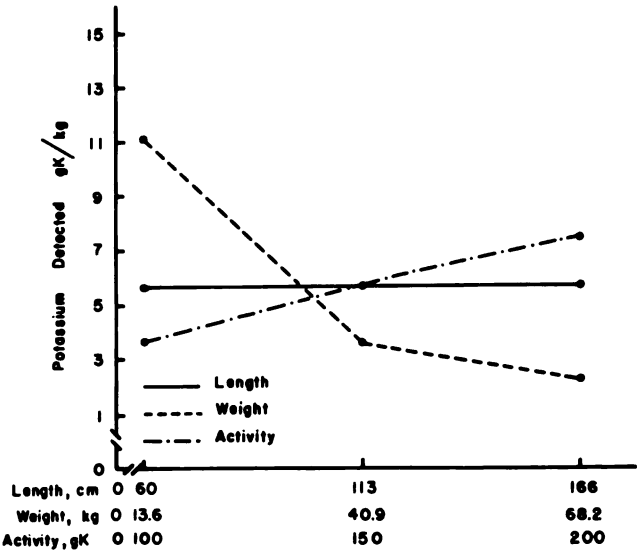


FIGURE 3 Changes in detected grams of K per kilogram of weight associated with length, weight, and activity of cylinders.

Effects of weight on efficiency should theoretically follow an exponential function of weight. The significant linear and quadratic terms are shown graphically in Figure 1. A downward trend with increasing weight in total grams of potassium detected (Figure 2) is indicative of the failure of the efficiency curves to remove completely the variation associated with weight changes. Since grams of K per kilogram of weight is a function of both detected potassium and weight, the linear and quadratic trend in Figure 3 was not unexpected.

Activity level exerted only a minor linear effect on efficiency, as shown in Figure 1, with the efficiency rising with increasing level of ^{40}K activity. An extremely strong linear trend was noted in two variables: total grams of potassium (Figure 2) and grams of K per kilogram of weight (Figure 3). Deviation of these two variables from the known amounts of potassium present was, however, in the opposite direction from that indicated by the efficiency values. This reversal indicates that the efficiency curves derived for higher activity levels underestimated the amount of potassium present and casts some doubt on the advisability of calculating different efficiency curves for each activity level.

The interaction between length and weight was a significant ($P \leqslant 0.01$) source of variation with regard to efficiency (Figure 4). Efficiency changes associated with weight tended to be generally parallel for the two shorter

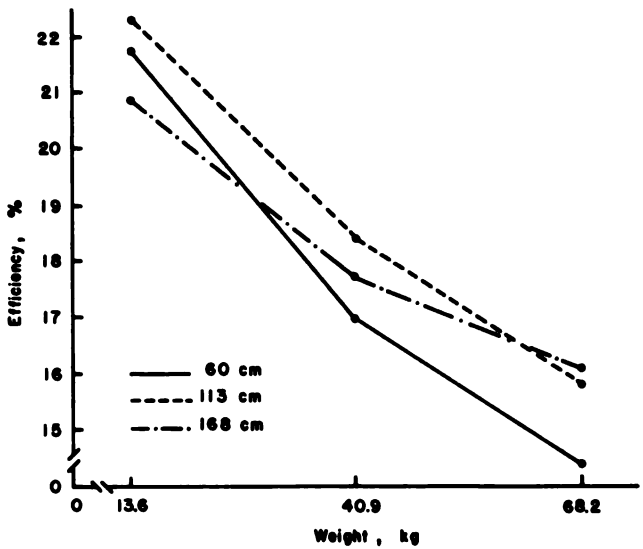


FIGURE 4 Length by weight interaction on efficiency of detection of ^{40}K activity.

cylinders (60 and 113 cm) while the slope of the line associated with the longest cylinders (166 cm) was definitely less pronounced than those for the shorter ones. This interaction indicates that the efficiency curves with regard to weight should be calculated separately for each sample length or that the f_w component in the equation should become a function of weight and length instead of simply a function of weight.

Interaction between length and activity level significantly ($P \leq 0.01$) affected efficiency, as shown in Figure 5. At the higher activity levels (150 and

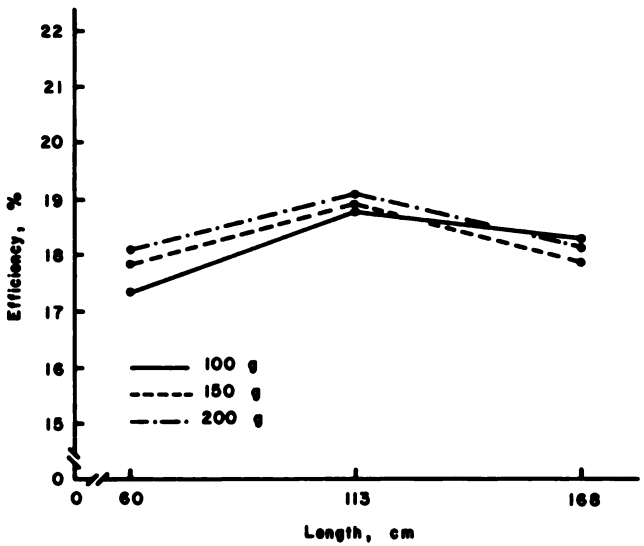


FIGURE 5 Length by activity level interaction on efficiency of detection of ^{40}K activity.

200 g of potassium), the lines were virtually parallel when efficiency was plotted against length. Slopes were different for both segments of the line describing efficiency changes associated with length at 100 g of potassium. Between 113 and 166 cm, a typical linear by linear interaction is depicted. The situation shown in Figure 5 led to use of a separate efficiency curve for weight (f_w) in each activity–length classification. Figure 6 depicts the interaction between length and activity level of total grams of potassium detected. Since a separate efficiency (f_w) curve was calculated for each of the nine length–activity classifications, there is no way of knowing how much this interaction was due to normal variation or to what extent it was created by the method of calculating potassium content. With regard to grams of K per

kilogram of weight, the relationship of the various lines was almost identical to those shown in Figure 6, except that the differences were so small they were difficult to depict graphically.

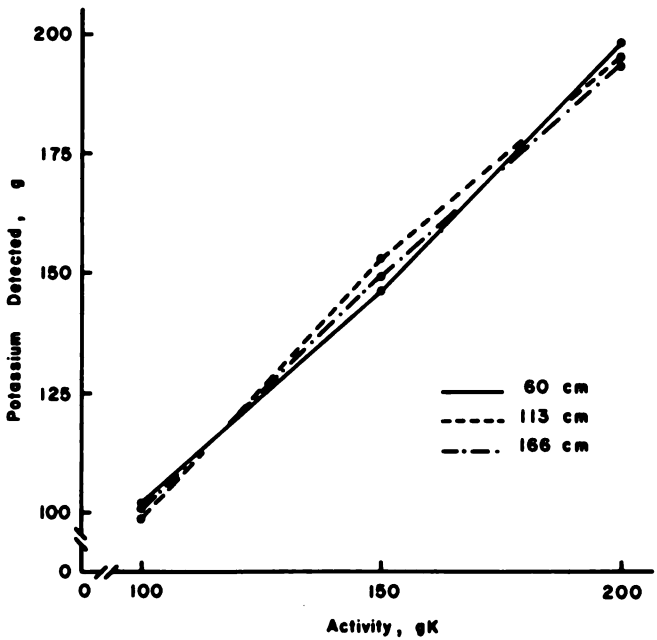


FIGURE 6 Length by activity level interaction on total grams of potassium detected.

Interaction between weight and activity level was a significant ($P \leq 0.01$) source of variation in all three analyses presented in Table 4. This interaction with regard to efficiency is shown in Figure 7. The interaction is almost wholly due to linear by quadratic interaction. When efficiency is plotted against weight for each activity level, each line is linear and quadratic, and the slopes do differ. In Figure 8, the detected potassium is shown in relation to potassium present for each weight group. Each line is essentially linear, and the effect of weight changes the slope of the line.

Division of grams of potassium by weight to obtain grams of K per kilogram changed the scale, so the interaction between weight and activity level of a linear by linear nature would have been created even if there had been no such interaction detected with regard to total grams of potassium. This interaction is shown in Figure 9.

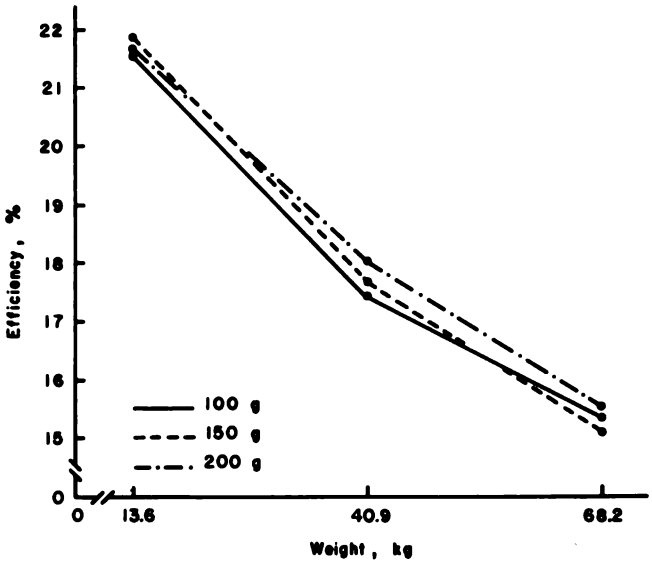


FIGURE 7 Weight by activity level interaction on efficiency of detection of ^{40}K activity.

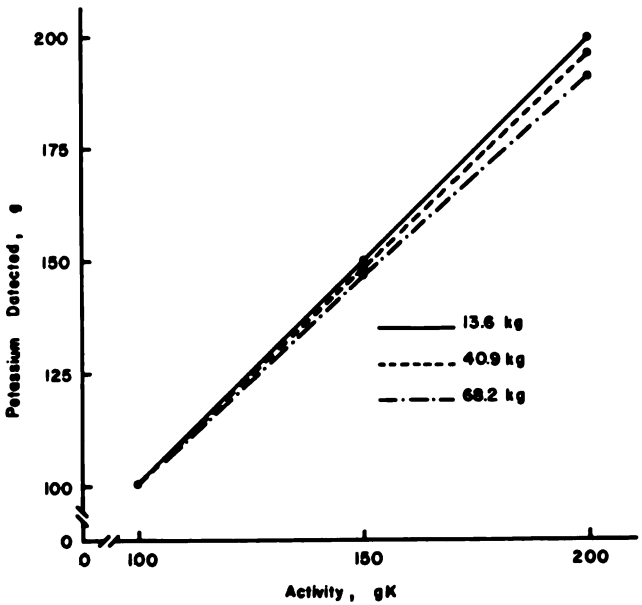


FIGURE 8 Weight by activity level interaction on total potassium detected.

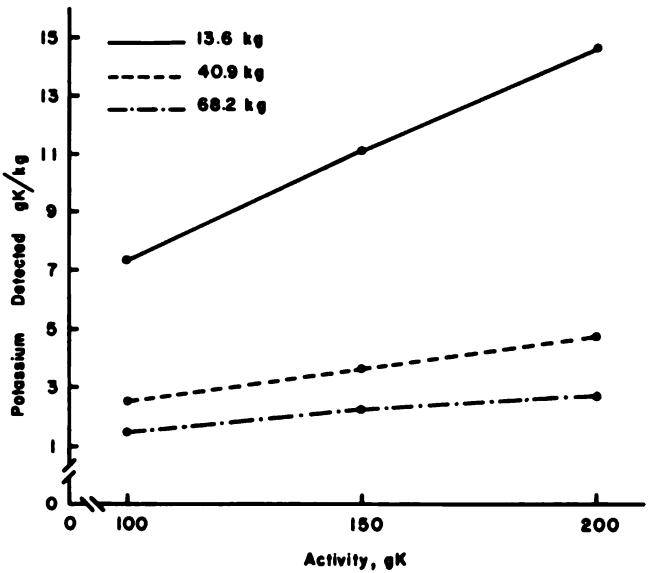


FIGURE 9 Weight by activity level interaction on detected grams of K per kilogram of weight.

SUMMARY

Twenty-seven cylinders containing a sugar–KCl mixture were constructed so that a 3^3 factorial arrangement of treatments was formed with three lengths (60, 113, and 166 cm), three weights (13.6, 40.9, and 68.2 kg), and three activity levels (100, 150, and 200 g of potassium). In addition, two standards were used to correct for day-to-day variation in counter efficiency. Over a short time span, the widely different standards were equally efficient in correcting for time changes in machine efficiency. However, the standards did not maintain this ability over a longer time span.

Length, weight, and activity level all affected efficiency of detection of potassium content. The intermediate length (113 cm) was more efficient than either extreme. The effects of weight were curvilinear and, in all probability, followed the expected exponential curve. Increasing weight decreased efficiency of detection. The effects of activity level, though statistically significant, were of minor importance compared with the effects of weight and length. The interaction between length and weight indicated that the ef-

ficiency curves should correct for effects of both weight and length on efficiency of detection rather than for effects of weight alone.

This work was supported in part by National Institutes of Health Research Grant AM 05551-05.

This paper is issued as Journal Paper No. 3064, Department of Animal Sciences, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

ASSOCIATION BETWEEN CHEMICAL AND COUNTER ESTIMATES OF POTASSIUM CONTENT

T. G. Martin
Purdue University

E. G. Stant, Jr.
Vanderbilt University

W. V. Kessler
Purdue University

H. E. Parker
Purdue University

Liquid scintillation counting and flame photometry have been compared by Kirton and Pearson as methods of measuring potassium content of pork and lamb.[2] Using 10 lambs averaging 40 kg of live weight, they measured grams of potassium per kilogram of separable fat and of separable lean by ^{40}K and flame photometry methods. Separable lean averaged 3.16 and 2.98 g of potassium per kg, respectively, by flame photometry and ^{40}K methods; the corresponding values for separable fat were 0.82 and 0.70 g per kg. Correlations between flame photometry and ^{40}K results were 0.40 and 0.52 in separable fat and separable lean, respectively. They also reported results obtained on 20 ground pork samples and 15 ground lamb samples, each weighing 17.3 kg.[2] Correlations between the methods were 0.98 and 0.94, respectively, for the pork and lamb samples. Potassium content of the pork samples was 0.245 and 0.244%, respectively, by flame photometry and ^{40}K methods; corresponding values for the lamb samples were 0.244 and 0.256%. Relationship between the two estimates was much higher on the more homogeneous and heavier ground samples than on the separable fat and lean samples.

The objective of this experiment was to investigate further the association between chemical and counter estimates of potassium content.

EXPERIMENTAL METHODS

Two sets of samples were used in this investigation. The first set of samples was the carcass, separable lean, separable fat and bone samples of 46 barrow pigs described by Martin *et al.* in their paper (this volume, page 326). There were 12 pigs in each of the 23-, 46-, and 68-kg live weight groups and 10 pigs in the 91-kg group. Two breeds were equally represented in each group. The second set of samples was 27 cylinders containing mixtures of KCl and sugar ranging from 13.6 to 68.2 kg in weight and from 100 to 200 g in total potassium content. There were three lengths in each weight-activity class. The cylinders are described in more detail by Martin *et al.* (this volume, page 326).

All samples were subjected to chemical analysis for potassium by use of a Perkin-Elmer 214 atomic absorption spectrophotometer equipped with a chart recorder. At least three determinations were made on all muscle, fat, bone, and sugar-KCl mixture samples. A value deviating from the sample mean by more than three standard deviations, based on the average within sample variation, was not included in the average. Mean values for each sample were calculated and used in later analyses.

Muscle, fat, and bone samples used for analysis weighed approximately 2.0, 4.0, and 2.0 g, respectively. These samples were dried overnight in a vacuum oven at 70° C., and the dried samples were extracted with petroleum ether on a Goldfisch extraction apparatus for 3 hr to remove most of the ether extractable material present. The extracted sample was digested in 5 ml of concentrated nitric acid until it was clear, and it was then diluted to 100 ml. Three milliliters of this solution was transferred to a 25-ml volumetric flask, and a nitric acid-sodium solution and distilled water were added to bring the volume to 25 ml, the normality to 0.1 N, and the sodium concentration to 1,000 ppm. The sugar-KCl mixtures were prepared by weighing approximately 15 g of the mixture, dissolving this quantity of solid material in water, and bringing the volume to 100 ml. An aliquot of this solution was digested in 5 ml of concentrated nitric acid and subjected to the procedure described above.

Each sample was read for 30 sec on the atomic absorption spectrophotometer at a wavelength of 7,664 Å and a slitwidth of 500 μ. Distilled water was used between samples to establish a base line, and a set of KCl standards containing 1.0 to 10.0 ppm of potassium and 1,000 ppm of sodium in nitric acid (0.1 N) was used to establish a standard curve for each group of samples. The parts per million reading, the sample weight, and the dilution factor were used to calculate grams of potassium per kilogram of sample weight.

The total mass of carcass, separable muscle, separable fat, separable bone, and the sugar-KCl cylinders were each counted two or more times in the large-volume liquid scintillation counter (Sinco-P) described by Christian *et al.*[1]

Sinco-P had been modified to a 4-pi counter prior to use in this study. A count of a sample consisted of four 1-min counts of the sample preceded and followed by two 1-min background counts. Each sample count was corrected for background, background depression, variation in counter efficiency, and counting efficiency for sample weight to obtain disintegrations per minute. Two standards (the short standard and the human dummy) were used to correct for variation in counter efficiency, resulting in two counter estimates of potassium content. The two standards have been described in more detail by Martin *et al.* (this volume, page 326). Disintegrations per minute were converted to total grams of potassium and grams of potassium per kilogram of weight. Mean values for each sample were calculated and used in later analyses.

The efficiency curve for weight of the muscle, fat, and bone samples was derived by counting sugar-KCl mixtures (8.82 g of potassium per kg of sugar) in plastic bags at weights ranging from 2.3 to 45.4 kg. The efficiency curve for carcasses was the same as that used for live pigs and was derived from ^{42}K-dilution studies based on 15 pigs ranging from 21.8 to 92.5 kg of weight. Nine efficiency curves (one for each length and activity level classification) were used for the sugar-KCl cylinders. Since the mixture in each cylinder contained a known amount of potassium (100, 150, or 200 g), the curves derived were much more directly applicable than were those used for carcass, muscle, fat, and bone samples.

The chemical estimate of potassium content of the carcass was obtained by mathematically reconstructing the carcass from potassium contents and weights of muscle, fat, and bone samples. The data were analyzed statistically by routine correlation-regression and analysis of variance techniques.

RESULTS AND DISCUSSION

Mean grams of potassium in the carcass, muscle, fat, and bone for each weight group of pigs, as estimated chemically and by whole-body counting, are shown in Table 1. Only carcass means showed a significant difference between chemically estimated and counter-estimated potassium. The two methods gave highly significant differences in carcass potassium content of the 23- and 46-kg groups, a significant difference at the 68-kg weight, and no significant difference at the 91-kg weight. In all four weight groups, the chemically estimated potassium content of the carcass was lower than the counter-estimated potassium content.

Potassium content of the tibia and femur was determined chemically and used as an estimate of skeletal potassium. Since the leg bones are an early developing part of the skeleton, skeletal potassium may have been underestimated. The decreasing differences between potassium content of carcass

TABLE 1 Mean Grams of Potassium as Estimated by Chemical Analysis and Whole-Body Counting in Pigs of Various Weights

Component and Method of Estimating	Average Weight of Pigs							
	23 kg		46 kg		68 kg		91 kg[a]	
	$\bar{X}$	SE[b]	$\bar{X}$	SE[b]	$\bar{X}$	SE[b]	$\bar{X}$	SE
CARCASS								
Chemical	38.17	0.97*	66.59	1.02*	87.35	1.76†	105.85	2.32
Counter	44.96	0.89	72.66	1.69	94.53	1.69	109.56	5.20
MUSCLE								
Chemical	29.60	0.88	51.67	0.82	68.68	2.04	85.79	1.72
Counter	30.20	1.21	49.58	1.36	66.42	2.09	86.11	1.88
FAT								
Chemical	0.87	0.09	3.51	0.48	6.76	1.16	7.56	0.61
Counter					6.22	1.24	6.33	1.12
BONE								
Chemical	7.70	0.29	11.42	0.50	11.92	0.70	12.50	0.54
Counter	7.66	0.72	10.16	0.81	12.56	0.86	13.81	1.59

[a] Means in 91-kg group are for 10 observations instead of the usual 12.
[b] Symbols denote significant difference between means of chemically estimated and counter-estimated grams of potassium:
*Significant at the 0.01 level.
†Significant at the 0.05 level.

as determined by the two methods noted with weight increase may be because, as the pig increases in weight, the proportion of bone in the carcass decreases and the percentage of carcass potassium in the bones decreases. It may also be due to the increased accuracy of the counting method associated with increased weight.

The general lack of differences between the mean grams of potassium found by the two methods would suggest that whole-body counting can be used to determine accurately the mean potassium content of groups of pigs.

As shown in Table 2, correlations between grams of potassium in carcass and muscle, as estimated by the two methods, were high when calculated over the entire weight range, with the correlations being 0.97 and 0.98 for carcass and muscle, respectively. However, when calculated within breed and weight groups, correlations between methods were lower. The correlations vary widely between weight groups, generally being highest in the 68- and 91-kg groups, especially for muscle and carcass. Muscle samples in particular showed high pooled, within-breed correlations in these heavier (and older) groups, being 0.93 and 0.91 in the 68- and 91-kg groups, respectively. For fat and bone samples, total and pooled correlations were not high enough to be of practical value. This may have been due to the small amounts of potassium present in these components and consequent low count rates and a low degree of counting accuracy.

When grams of potassium per kilogram of component was used, total correlation between the two methods was high (0.92) for the carcass. The pooled correlation for the carcass was 0.58. As estimated by the two methods, grams of potassium per kilogram of component yielded total correlations of 0.42 and 0.55 and pooled correlations of 0.42 and 0.05 for muscle and fat components, respectively. This agreed closely with the correlation of 0.52 between ^{40}K and flame photometry methods for separable lean but not with the correlation of 0.40 for separable fat of 10 lambs reported by Kirton and Pearson.[2] Again, correlations in muscle were highest in the 68- and 91-kg groups, being 0.74 and 0.73, respectively.

It would appear that fair-sized differences in potassium content, such as those occurring between the weight groups, can be detected by whole-body counting, but that small differences, such as those occurring between individual pigs within weight groups, cannot be accurately detected. This is especially pronounced at the lighter weights.

Means and standard deviations of the potassium contents of the sugar-KCl cylinders are shown in Table 3. Two counter estimates were obtained by using two standards. The chemical estimates were, in almost all cases, lower than the counter estimates and averaged 84% recovery of the potassium weighed into the cylinders. The chemical estimates also tended to be considerably more variable than the counter estimates. In the pig data, there was no clear-cut dif-

TABLE 2 Within-Weight-Group Correlations[a] of Chemically Determined with Counter-Estimated Potassium Contents of Component Parts

Component	Within-Weight Groups					
	23 kg	46 kg	68 kg	91 kg[b]	Pooled	Total
GRAMS OF POTASSIUM PER KILOGRAM						
Carcass	0.308	0.358	0.853*	0.517	0.578*	0.916*
Muscle	0.314	0.383	0.736*	0.729†	0.424*	0.417*
Fat	–	–	0.056	0.034	0.053	0.548*
Bone	0.514	0.534	0.514	−0.099	0.332*	0.573*
GRAMS OF POTASSIUM						
Carcass	0.795*	0.451	0.870*	0.765†	0.768*	0.968*
Muscle	0.448	0.617†	0.929*	0.908*	0.836*	0.979*
Fat	–	–	0.362	0.622	0.399	0.662*
Bone	0.534	0.800*	0.899*	0.167	0.466*	0.574*

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Pooled, within-breed correlations.
[b]Correlations in 91-kg group involve 10 observations instead of the usual 12.

TABLE 3 Means and Standard Deviations of Grams of Potassium per Kilogram of Weight in Sugar-KCl Cylinders

Weight and Total Potassium Content	Counter Estimates		Chemical Estimate	Actual Content
	Short Standard	Human Dummy		
68.2 kg				
100 g	1.473[a]	1.473	1.412	1.469[b]
	0.037[c]	0.041	0.082	–
150 g	2.172	2.172	1.831	2.205
	0.113	0.114	0.176	–
200 g	2.809	2.809	2.316	2.940
	0.076	0.069	0.078	–
40.9 kg				
100 g	2.451	2.451	1.791	2.450
	0.018	0.015	0.177	–
150 g	3.659	3.659	3.195	3.674
	0.061	0.052	0.146	–
200 g	4.830	4.830	3.970	4.899
	0.047	0.055	0.411	–
13.6 kg				
100 g	7.352	7.353	6.537	7.349
	0.010	0.024	0.723	–
150 g	11.026	11.026	8.849	11.023
	0.016	0.035	0.650	–
200 g	14.695	14.695	12.826	14.697
	0.022	0.023	1.026	–

[a]Mean.
[b]Three cylinders in each weight-activity level group.
[c]Standard deviation.

ference in variability between the two methods. It is entirely possible that the mean difference between the two methods is a function of sampling accuracy, and it is highly probable that the difference in variability is due to sampling accuracy.

The sampling procedure for the sugar-KCl mixtures was difficult because two types of crystals were in a dry mixture. In the muscle, bone, and fat samples, the potassium was primarily in solution and did not tend to separate out during the process of grinding and sampling.

Extremely close agreement between known potassium content and the counter estimates in the cylinders is probably due to the use of efficiency curves derived from the data collected on the cylinders.

Correlations between the various estimates of potassium content of the cylinders are shown in Table 4. The total correlations, covering a wide range

TABLE 4 Correlations between Various Estimates of Potassium Content in Sugar Standards

Methods of Estimating	Counter Estimates: Short Standard	Counter Estimates: Human Dummy	Chemical Estimate
TOTAL CORRELATIONS[a]			
Human dummy	0.999*	–	–
Chemical estimate	0.977*	0.977*	–
Actual content	0.999*	0.999*	0.976*
POOLED CORRELATIONS[c] WITHIN WEIGHT AND ACTIVITY LEVEL CLASSES			
Human dummy	0.942*	–	–
Chemical estimate	0.130	0.140	–

[a] 25 degrees of freedom.
[b] 17 degrees of freedom.
* Significant at the 0.01 level.

of weights and activity levels, are extremely high (0.976 to 0.999). Much of this high degree of association is due to the automatic relationship created by weight and level of activity.

In the pooled correlations, the association between the two counter estimates involving use of two standards remains high, which tends to indicate that either of the two standards accomplishes the same degree of correction for variation in counter efficiency.

Correlations between chemical and counter estimates of potassium content are seriously reduced when pooled within weight and activity level classes. Actual potassium content in each of the nine classes was a constant.

Variance components and repeatability estimates over the entire weight and activity level ranges are shown in Table 5. The lower repeatability of the

TABLE 5 Variance Components and Repeatabilities of Various Estimates of Potassium Content

Method of Estimating	σ^2 between Cylinders[a]	σ^2 within Cylinders	Repeatability
Counter short standard	19.1623	0.0098[b]	0.999
Counter human dummy	19.1628	0.0100[b]	0.999
Chemical	8.4695	0.6054[c]	0.933

[a] 26 degrees of freedom.
[b] 108 degrees of freedom.
[c] 54 degrees of freedom.

chemical estimates is, in all probability, due to the previously mentioned difficulty in sampling.

Based on the data presented, it appears that accuracy of the chemical method is dependent primarily on collection of a representative sample, whereas accuracy of the ^{40}K counting method is dependent on total amount of potassium present and use of appropriate efficiency curves, background depression curves, and standards for correction of variation in counter efficiency.

It would be difficult to say that one method is superior to the other under all circumstances. In data on animal tissues, Martin *et al.* (this volume, page 428) and Kirton and Pearson[3] have shown that chemical estimates of potassium content are slightly more accurate estimators of body composition than are ^{40}K estimates of potassium content.

SUMMARY

Association between chemical and counter estimates of potassium content were high (r = 0.55 to 0.99) over a wide range of sample weights and total potassium contents. When restricted to narrow weight and total potassium content range, the correlation was drastically reduced. Either method will characterize group differences in potassium content. The two methods cannot always be depended upon to yield the same results in comparing two or more relatively similar samples. Accuracy of the chemical method appears to rely on sampling accuracy, whereas accuracy of the counting method depends on a sizable quantity of potassium and accurate calibration of the counter.

This work was supported in part by National Institutes of Health Research Grant AM 05551-05.

This paper is issued as Journal Paper No. 3062, Department of Animal Sciences, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

REFERENCES

1. Christian, J. E., W. V. Kessler, and P. L. Ziemer. 1962. A 2-pi liquid scintillation counter for determining the radioactivity of large samples including man and animals. Int. J. Appl. Rad. Isotopes 13:557.
2. Kirton, A. H., and A. M. Pearson. 1963. Comparison of methods of measuring potassium in pork and lamb and prediction of their composition from sodium and potassium. J. Anim. Sci. 22:125.
3. Kirton, A. H., and A. M. Pearson. 1963. Relationships between potassium content and body composition. Ann. N. Y. Acad. Sci. 110:221.

PRECISION OF ASSAY OF WHOLE-BODY POTASSIUM IN MAN

Charles E. Miller
Argonne National Laboratory

Alexander P. Remenchik
Loyola University

Wayne V. Kessler
Purdue University

A controversy exists regarding the accuracy of measurement of K in intact, healthy humans. Some groups, principally whole-body counter specialists, maintain that K measurements are intrinsically accurate and that any daily or weekly variations in the measurements reflect changes in the amount of K in the body. Other groups maintain that the amount of K in the body remains constant and that variations of K in the same subject are due to random errors in whole-body counter measurements. As evidenced by the number of papers in the literature, a third group maintains that current whole-body measurements are inaccurate but that a variety of newly developed techniques are more accurate and reliable.

There is no simple, direct method by which the accuracy of a particular whole-body counting system may be assayed since the amount of K in a human cannot be measured directly. One direct approach would be to measure the K content of a cadaver by various whole-body counter techniques and then perform a chemical analysis for K on the complete body. However, in order to make a proper comparative analysis it would be necessary to measure and analyze chemically a large number of cadavers that ranged in weight from approximately 45 to 160 kg, since the calibration factor for a whole-body

counter technique varies as some function of the person's height and weight. Obviously, a project of such scope is impractical because of time, cost, and unavailability of specimens.

Some insight into the accuracy of whole-body counter techniques may be obtained by measuring a large group of subjects for K by various methods and comparing the results. To this end, the K content of each of 44 clinically healthy subjects* whose weights ranged from 44 to 170 kg was measured by either two or three whole-body counter techniques (which employed a sodium iodide crystal as the detector), with a 4-pi liquid scintillation counter, and by an isotope-dilution method. By a careful analysis of the results, some indication of the accuracy of the various techniques for measuring K has been obtained.

MEASUREMENT OF SUBJECTS

The normal gamma-ray spectrum of each subject was obtained first by the tilting-chair technique in the Argonne crystal-type whole-body counter. The subject was seated in the tilting chair (TC) within the whole-body counter, and his spectrum was obtained with each of two sodium iodide crystals located over the chair.

A squat, right cylindrical sodium iodide crystal, 8 in. in diameter by 4 in. thick (called the 8 by 4 crystal), was positioned over the chair with the crystal axis vertical.[3] When a crystal of this shape is used, the gamma rays from the subject's head strike the side of the crystal, those from the torso strike mainly the 8-in. diameter face of the crystal, and those from the legs strike either the side or the face of the crystal. Consequently, the absolute detection efficiency of the system is dependent upon the location of the radioactivity in the body, since the solid angle that is subtended from the radioactive deposit to the crystal is different for the radioactivity in various volumes of the body.

A so-called log crystal (6 in. in diameter and 8 in. long) was located over the chair, with the crystal axis horizontal and parallel to the back and seat of the chair. The cross section of the crystal is the same when viewed from any point in the body. The absolute detection efficiency does depend upon the distance and mass between the radioactive deposit and the detector, but no variation is attributable to the shape of the presenting side of the detector.

Each subject was measured for 40 min with both the 8 by 4 crystal and the log crystal. Measurements, or factors calculated from measurements,

*The term "subject" rather than "patient" is used to emphasize that only healthy, ambulatory persons participated in this study.

made with the 8 by 4 crystal and the log crystal over the tilting chair are designated as 8 x 4 TC and log TC, respectively.

A number of the subjects were also measured in a supine position on a Lucite slab within the whole-body counter. The subject's normal gamma-ray spectrum was recorded with the 8 by 4 crystal located in turn at each of seven equally spaced locations 24.5 cm apart along the body.[3] The crystal was positioned with its axis vertical and the 8-in. diameter face of the crystal located either 30, 35.8, or 37 cm from the surface of the slab. A 30-min measurement was made at each of the seven positions for a total count time of 3.5 hr for each subject.

Unless otherwise stated in the text, the count rates from the seven crystal positions were summed, and one count rate was employed. Measurements or factors calculated from measurements based on this sum count rate are designated 7-crystal sum. This count rate would be equivalent to that obtained by a longitudinal scan of the body that required 3½ hours or to a 30-min count with seven crystals.

The subjects were then measured in the Purdue University 4-pi liquid counter.[1*] Three series of four 1-min measurements were made of the subject's $^{39,40}K$ (normal potassium). Two 1-min background measurements were made before and after measuring the subject and between each series of four 1-min measurements. Background was thus measured for a total of eight 1-min periods, and the subject was measured for a total of twelve 1-min periods.

A carefully measured volume of ^{42}K was then given to each subject, either orally or intravenously, and two equal volumes were pipetted into standard bottles. Each subject was remeasured by each technique 2 to 5 days later, after the ^{42}K had become equilibrated with the subject's $^{39,40}K$. Very short count times were used with the crystal counters for these ^{42}K measurements since the subjects yielded relatively high count rates. All of the urine was collected from each subject between the time of ^{42}K administration and the ^{42}K measurements.

MEASUREMENT OF $^{39,40}K$ AND ^{42}K STANDARDS

In order to avoid the use of the decay scheme of $^{39,40}K$ and ^{42}K, the following procedures were used: For measurement with the crystal whole-body counter, a specified amount of distilled water was added to the ^{42}K standard bottle that was then placed on a tray that had been accurately positioned with a calibrated mechanical fixture 40 cm below the crystal. Repeated measurements made

*Reference article describes 2-pi liquid counter, since converted to 4-pi.

before and during this study demonstrated that the uncertainty in ^{42}K count rates due to pipetting, ^{42}K counting statistics, and positioning of the standard bottles introduced an error of less than 1%. A known amount of KCl was placed in a bottle that was identical in size and shape to that used for the ^{42}K standard, and it was also counted on a tray located 40 cm from the crystal. Although the ^{42}K and the $^{39,40}K$ were counted in different bottles, previous studies had demonstrated that identical spectra (i.e., count rates) were obtained from equal volumes of ^{42}K mixed in the same volumes of distilled water, KOH, or KCl.

In the Purdue 4-pi liquid counter, a known amount of KCl, dissolved in a 2-gallon polyethylene bottle of distilled water, was counted on the same day that a subject's $^{39,40}K$ was measured. The ^{42}K standard, equal in volume to that given to a subject, was then mixed with the contents of this same 2-gallon polyethylene bottle, which was then recounted.

All urine voided between the time of administration and each whole-body measurement was analyzed to determine the amount of ^{42}K eliminated, and each subject's ^{42}K count rate was corrected for excretion and decay to some arbitrary time, usually noon of the day of the measurement. The ^{42}K count rate from the standard bottle was also corrected for decay to this same time.

The ^{42}K:$^{39,40}K$ ratio present in the urine 42 to 50 hr after administration was also computed. From a knowledge of the ^{42}K content of the body and this ratio, the amount of so-called exchangeable potassium in the body could be calculated (isotope-dilution technique).[5]

ANALYSES OF DATA

The data have been analyzed (a) to calculate the calibration factor for each measurement technique employed for each subject in this study; (b) to determine the dependence or lack of dependence of the calibration factor on the weight, height, and other measurements of the subject; (c) to calculate each subject's $^{39,40}K$ content when the calibration constant derived for his own body configuration is used; (d) to evaluate the size of the error that may exist if the average calibration factor corrected for the subject's weight is used to compute the K content of subjects who had not received ^{42}K; (e) to calculate the ratios between the exchangeable K content (found by the isotope-dilution technique) and the total body K (found by each whole-body counter technique); (f) to determine which whole-body counter technique yields more consistent ratios for all subjects; and (g) to evaluate the variability in either isotope-dilution measurements or the exchangeable fraction in humans.

To obtain the necessary factors to perform the various analyses, the

measurements were substituted into the universally used equation for calculating a human's K content:

$$\text{grams K} = A \times \frac{B}{C} \times \frac{D}{E}, \quad (1)$$

where

A is the count rate from the subject's $^{39,40}K$,
B is grams of $^{39,40}K$ in the standard bottle,
C is count rate from $^{39,40}K$ in the bottle,
D is count rate for ^{42}K in the bottle, and
E is count rate for ^{42}K in the subject.

The value of A varies from subject to subject because A is related to the amount of $^{39,40}K$ in the body. The product of the second and third terms is the calibration factor for the whole-body counter, and its value varies for the different subjects if the counter efficiency is dependent on body build. The magnitude of the second term is a constant if both the sensitivity of the counter and the energy calibration of the spectrometer do not change with time. In the Argonne crystal whole-body counter, measurements made with various bottles of KOH, KCl, and KF, all of which contained known amounts of K and were accurately positioned with the aid of a mechanical fixture 40 cm from the face of the crystal, have yielded the same count rate per gram of $^{39,40}K$ during the last 10 years. The magnitude of the last term, D/E, varies from subject to subject and reflects the dependence of the calibration factor upon body build.

Since the possibility exists that the energy calibration may have been slightly different on succeeding days, only the last term, the G (geometry) factor = D/E, is analyzed to evaluate the dependence of the calibration factor on height and weight. Any variation in energy calibration should not affect the magnitude of this term since both D (^{42}K count rate from bottle) and E (^{42}K count rate from subject) were measured on the same day. In the 4-pi liquid counter, the values of A ($^{39,40}K$ count rate from subject) and C ($^{39,40}K$ count rate from standard bottle) were obtained on the same day, so again small changes in counter sensitivity should have introduced equal canceling errors into these two terms and should not have affected the calculated K content of the subject.

The G factors obtained for the subject measured by each technique were fitted with a regression line of the form, $\log(g) = a + b(w)$, with the aid of an electronic computer. The logarithms of the G factors were used as the dependent variables, and the weights of the subjects in kilograms were used as the independent variables. The values and associated standard errors of a and b

for the regression line found to fit the various groups of calibration factors are given in Table 1.

The computer was programmed to calculate the percentage that the subject's actual G factor differed from the value predicted by the regression line for a subject of the same weight.

$$\% \; G \text{ spread} = \frac{G \text{ observed} - G \text{ calculated}}{G \text{ calculated}} \times 100. \qquad (2)$$

To avoid misunderstanding, this percentage difference (Equation 2) is designated % G spread rather than percentage of error although it is the percentage that a subject's K content would be in error if the calculated G factor were used. The % G spread for each subject found when the G factors obtained by each technique were fitted with a regression line is given in Table 2.

The absolute values of these % G spreads (Table 2) were fitted with a linear regression line, % G spread = $a + b(w)$, to determine if the magnitude of the errors was dependent upon the subject's weight. The values and standard errors of a and b of this regression line found to fit the % G spread are given in Table 1 immediately below the parameters of the regression line found to fit the G factors.

FOUR-PI LIQUID SCINTILLATION WHOLE-BODY COUNTER

The G factors found for the 4-pi liquid counter (line 1, Table 1) are very dependent upon the weight of the subject, as demonstrated by the value of B (slope) and standard error of B. The % G spreads, while yielding a positive slope (0.0197), are not statistically dependent (standard error of slope = ±0.0146) upon the weight of the subject ($P > 0.1$). Examination of the % G spreads for the 4-pi liquid counter in column 4 of Table 2 confirms this result in that the five subjects who yielded the largest % G spread (7 to 11.8%) are not grouped at the bottom of Table 2 with the large subjects, but are randomly distributed. In the absence of a statistically significant correlation between error and weight, the mean, the standard deviation of the mean, and the standard error are given at the bottom of Table 2. A mean error of 3.3% with a standard deviation of 3.18% exists for these G factors.

EIGHT BY FOUR CRYSTAL OVER TILTING CHAIR

Since the K content of the subject is routinely calculated with the count rate from two energy bands, two calibration factors are employed. For $^{39,40}K$ measurements the first energy band (Compton band) includes the scattered gamma rays whose energies fall between 0.775 keV and 1.275 MeV, while the

TABLE 1 Parameters of Regression Lines for *G* Factors and for % *G* Spread with Various Whole-Body Counter Techniques

Technique	Energy Band	Number of Subjects		a	SE_a	b	SE_b
4-pi liquid counter	0.8 to 1.8 MeV	42	*G* factor	−0.0293	0.0093	0.001806	0.000093
			% *G* spread	1.4590	1.4471	0.019696	0.014584
8 × 4 tilting chair	Compton	44	*G* factor	0.2012	0.0075	−0.000190	0.000074
			% *G* spread	0.1365	1.1084	0.027403	0.010958
	Photopeak	44	*G* factor	0.2028	0.0083	0.000995	0.000082
			% *G* spread	−0.4735	1.1732	0.036115	0.011599
8 × 4 tilting chair	Compton	29	*G* factor	0.2011	0.0073	−0.000281	0.000093
			% *G* spread	2.4763	1.1034	−0.008218	0.014072
	Photopeak	29	*G* factor	0.2097	0.0064	0.000922	0.000081
			% *G* spread	1.4994	0.8071	0.003655	0.010292
Log tilting chair	Compton	18	*G* factor	0.0431	0.0073	−0.000054	0.000076
			% *G* spread	0.9831	0.9094	0.010925	0.009534
	Photopeak	18	*G* factor	0.2836	0.0099	0.001133	0.000103
			% *G* spread	1.1018	1.1852	0.017324	0.012424
Log tilting chair	Compton	23	*G* factor	0.1073	0.0110	−0.000405	0.000107
			% *G* spread	−1.1599	1.3654	0.041313	0.013206
	Photopeak	23	*G* factor	0.0860	0.0133	0.000834	0.000129
			% *G* spread	−1.2831	1.7824	0.046930	0.017241

TABLE 2 Percentage That Subject's *G* Factor Falls Above or Below Regression Line

Subject	Height (cm)	Weight (kg)	4-pi Liquid	8 x 4 Tilting Chair		Log Tilting Chair			
				Compton	Photopeak	Compton	Photopeak	Compton	Photopeak
1-A1	161.2	43.9	0.07	−1.71	−0.79	−1.00	−0.48	–	–
2-A2	161.2	45.2	−1.30	0.88	2.88	–	–	0.57	1.90
3-B1	154.7	48.5	2.39	0.96	1.24	−0.58	1.06	–	–
4-B2	154.7	49.7	2.51	7.42	1.92	–	–	−2.44	−0.71
5	161.4	52.7	−2.44	−2.68	−2.21	0.84	1.40	–	–
6	178.3	53.7	−0.78	3.66	1.34	–	–	1.98	1.69
7[a]	162.7	55.6	7.93	7.08	9.97	–	–	8.49	6.96
8	163.0	55.9	−0.20	0.64	1.01	1.22	1.79	–	–
9	167.7	56.4	−1.21	0.93	2.41	2.99	3.09	–	–
10	153.5	57.6	7.64	−4.81	−2.66	–	–	−0.37	0.73
11	163.6	57.9	1.88	1.31	1.88	–	–	1.64	3.34
12	170.4	63.3	3.33	−0.81	1.60	–	–	−1.44	−0.03
13	158.8	64.0	5.19	2.76	4.60	–	–	1.54	1.05
14	168.9	70.3	−1.27	−1.41	−1.40	−1.46	−2.33	–	–
15	179.9	71.8	−2.27	−0.50	−1.10	0.70	−1.78	–	–
16	184.2	72.9	−1.01	−0.29	−1.46	–	–	1.51	1.06
17-C3	163.6	73.7	−11.48	−0.49	1.66	0.32	−0.98	–	–
18-C2	163.6	73.7	−5.44	4.00	3.57	4.19	4.54	–	–
19	174.5	74.5	−0.64	−0.86	−2.27	–	–	0.46	−3.67
20	185.2	85.0	0.27	2.22	0.36	–	–	1.31	0.92
21	189.7	87.2	−1.70	1.04	−0.63	–	–	1.98	−1.66
22	178.1	88.4	3.66	3.16	1.40	–	–	1.91	2.81
23-C1	163.6	88.9	−0.87	0.46	2.31	−0.51	0.71	–	–
24	184.1	92.9	−4.97	−2.72	−5.11	−3.77	−7.61	–	–
25-D3	161.2	97.5	1.54	−1.13	−0.87	−2.84	−2.69	–	–
26-D2	161.2	102.3	1.70	0.40	−0.54	−4.30	−3.06	–	–

TABLE 2 Continued

Subject	Height (cm)	Weight (kg)	4-pi Liquid	8 x 4 Tilting Chair		Log Tilting Chair			
				Compton	Photopeak	Compton	Photopeak	Compton	Photopeak
27	165.1	102.5	−2.07	−4.72	−1.67	–	–	–	–
28-E2	166.4	113.8	−8.44	1.57	1.30	2.17	3.41	–	–
29-F2	170.9	118.4	–	0.23	3.27	–	–	1.61	2.31
30-G1	165.7	119.7	1.17	−1.63	−1.47	–	–	–	–
31	160.0	120.1	1.42	−9.17	−9.03	–	–	−9.93	−9.86
32-H1	154.5	121.1	11.30	−3.56	−1.04	–	–	−4.29	−2.94
33-G2	165.7	121.1	4.88	−3.43	−7.04	–	–	−1.31	−3.83
34-H2[a]	154.5	121.8	1.19	4.02	14.37	–	–	7.72	11.23
35	188.1	123.3	−6.72	−8.00	−11.91	–	–	−8.41	−13.24
36-J2	178.5	125.3	2.71	−2.30	−7.38	–	–	–	–
37-D1	161.2	128.0	−1.01	0.13	0.39	−2.91	−3.42	–	–
38	170.0	128.2	8.00	3.44	4.80	–	–	2.14	4.40
39	171.0	128.9	11.76	−0.26	1.43	–	–	2.47	2.24
40-E1	166.4	130.9	−0.81	1.93	2.21	2.09	5.38	–	–
41	172.8	136.6	−1.35	−5.04	−3.12	–	–	−3.88	−0.63
42	160.5	143.1	−3.97	−3.41	−1.38	–	–	2.68	2.18
43-J1	178.5	147.8	−1.80	3.17	−0.34	0.21	−1.06	–	–
44-F1	170.9	149.6	−0.22	9.74	11.02	–	–	8.34	6.34
45	159.2	150.4	–	2.02	7.14	–	–	3.63	8.09
46	183.0	157.0	−5.11	9.94	7.45	3.14	2.96	–	–
47[a]	186.9	167.1	−9.47	−5.60	−15.14	–	–	−3.53	−13.08
NUMBER			42	44	44	18	18	23	23
MEAN			3.30	2.74	2.97	1.96	2.65	2.86	3.29
SD OF MEAN			3.18	2.61	2.86	1.38	1.83	2.60	3.26
SE OF MEAN			0.49	0.39	0.43	0.32	0.43	0.54	0.68

[a]Not included in calculation of mean.

second energy band (photopeak band) includes mainly unscattered gamma rays whose energies fall between 1.325 and 1.575 MeV. In order to calculate the *G* factor for ^{42}K measurements, the same lower energy band is used for the Compton band, but an energy band of 1.375 to 1.625 MeV is used for the photopeak band.

The parameters of the regression line found to fit the *G* factor for the Compton band are given in line 3 of Table 1; those from the photopeak band are given in line 5 of Table 1. The Compton *G* factors support a regression line of negative slope, and the photopeak *G* factors support a regression line of positive slope. Both slopes are significant ($P < 0.01$).

The parameters of the regression line calculated to fit the percent errors by which the observed *G* factors differ from the calculated *G* factors are given in lines 4 and 6, Table 1. The slopes of these regression lines are significant ($P < 0.01$) and demonstrate that the spread in *G* factors along the regression line increases as the weight of the person increases.

Inspection of the percentage by which the observed *G* factors differ from the regression line given in columns 5 and 6 of Table 2 discloses that the large variations exist for subjects who weigh more than 120 kg. To ensure that the *G* factor of the large subjects did not unduly affect the regression line calculated for the *G* factor for the entire series of subjects, new regression lines were calculated for the 29 subjects who weighed between 43.88 and 119.71 kg. The parameters for these regression lines (lines 7 and 9 of Table 1) are not statistically different from those found to fit the entire series of 44 subjects that included 15 subjects who weighed between 120 and 157 kg. Since approximately the same regression line was obtained, the % *G* spread for each of these 29 subjects was very close to those in Table 2. However, while the % *G* spreads for the total series of 44 subjects were related to the weights of the subjects, no correlation exists between the % *G* spread and the weights of these 29 subjects. The % *G* spreads based on the Compton data had a mean of 1.858 (SD of mean = 1.650, SE of mean = 0.306), and those based on the photopeak data yielded a mean of 1.648 (SD of mean = 1.189, SE of mean = 0.221).

LOG CRYSTAL OVER TILTING CHAIR

When the log crystal was placed into operation initially, it was positioned over the chair at an arbitrarily selected reproducible height with the aid of the mechanical fixture used to position the 8 by 4 crystal. After measuring several subjects, it was found necessary to raise the crystal 2.5 cm to accommodate several of the larger subjects. A number of subjects were subsequently measured with the log crystal at this higher position. The *G* factors obtained with the log crystal must therefore be analyzed as two groups.

TABLE 3 Parameters and Standard Errors of the Parameters of the Regression Lines Log $G = a + b$(wt) Found to Fit the G Factors Obtained by Each Technique for One Series of Nine Subjects and Another Series of Ten Subjects

Technique	Energy Band	Number of Subjects	a	SE_a	b	SE_b
4-pi liquid counter	0.8 to 1.8 MeV	9	−0.0036	0.0187	0.00154	0.00023
		10	−0.0621	0.0266	0.00197	0.00023
8 x 4 tilting chair	Compton	9	0.2319	0.0157	−0.00063	0.00019
		10	0.1806	0.0153	0.00007	0.00013
	Photopeak	9	0.2497	0.0124	0.00029	0.00015
		10	0.2012	0.0141	0.00107	0.00012
Log tilting chair[a]	Compton	9	0.1222	0.0125	0.00066	0.00015
		10	0.0392	0.0170	−0.00001	0.00014
	Photopeak	9	0.1217	0.0131	0.00024	0.00016
		10	0.0264	0.0196	0.00117	0.00017
7-crystal sum[b]	Compton	9	−0.6654	0.0095	0.00056	0.00012
		10	−0.5694	0.0127	0.00068	0.00011
	Photopeak	9	−0.6144	0.0058	0.00121	0.00007
		10	−0.5112	0.0118	0.00133	0.00010

[a] Log crystal at closer position over tilting chair for nine subjects, farther position for ten subjects.
[b] Face of 8 by 4 crystal 30 cm from bed for nine subjects, and 35.8 cm for ten subjects.

The parameters of the regression lines that fit the log data for 18 subjects when the crystal was located at the closer distance are given in lines 11 and 13 of Table 1. Neither the *G* factor nor the associated % *G* spread based on the Compton data yields a statistically significant slope. The *G* factors based on the photopeak data support a significant slope ($P < 0.01$), while the % *G* spread does not yield a statistically significant slope.

The parameters of the regression lines found to fit the log crystal *G* factors for the 23 subjects measured with the crystal raised 2.5 cm are given in lines 15 and 17 of Table 1. Interestingly, both the Compton and photopeak *G* factors yield statistically significant slopes and intercepts. The slopes of the % *G* spread are also significant ($P < 0.01$).

SEVEN-CRYSTAL TECHNIQUE

When the study of potassium by the 7-crystal technique was begun, the measurements were made with the face of the crystal 30 cm above the surface of the rigid Lucite slab used as a bed. This spacing had proven satisfactory for all studies of the distribution of ^{226}Ra, ^{137}Cs, ^{154}Eu, and other radioelements in humans. Unfortunately, in the present study it was necessary to reposition the crystal at 35.8 cm and then at 37 cm above the bed to accommodate some of the very large, obese subjects.

The sets of *G* factors obtained for nine measurements with the crystal 30 cm above the bed and for ten measurements with the crystal 35.8 cm above the bed were analyzed. To obtain a valid comparison with the % *G* spreads obtained with other counting techniques, the *G* factors obtained by the other methods for the same subjects were also fitted with regression lines. The parameters and their standard errors for these regression lines are given in Table 3. The % *G* spreads for each subject with each technique for both groups are given in Table 4.

The *G* factors for the 7-crystal sum are highly dependent upon the weight of the subject (lines 11 through 14, Table 3). Also, the individual *G* factors for each subject fall very near the regression lines as evidenced by the small % *G* spread values given in columns 9 and 10 of Table 4.

The slopes of the regression lines found to fit the *G* factors for the groups of nine subjects with the 8 x 4 TC and log TC are much less steep and are completely different from the slopes found for the group of ten subjects or for the entire series of subjects. The slopes for these two techniques were influenced unduly by the inclusion of subjects 33 and 35 in this small series of nine subjects. The slopes of the regression lines found to fit the *G* factors for the two groups with the 4-pi liquid counter also are statistically different from each other but not as much as those obtained with the tilting-chair technique. The slopes of the regression lines found to fit the *G* factors with the 7-crystal

TABLE 4 Percentage That Subject's *G* Factor Falls Above or Below Regression Line

Subject	Height (cm)	Weight (kg)	4-pi Liquid	8 x 4 Tilting Chair		Log Tilting Chair		7-Crystal Sum	
				Compton	Photopeak	Compton	Photopeak	Compton	Photopeak
NINE SUBJECTS WITH CRYSTAL 30 cm ABOVE BED									
2-A2	161.2	45.2	−4.42	−1.54	−0.58	−0.18	−0.11	−1.52	−2.21
4-B2	154.7	49.7	−0.47	5.32	−0.78	−2.90	−2.06	−0.83	0.38
10	153.5	57.6	5.01	−5.91	−4.01	−0.38	0.45	1.27	0.89
12	170.4	63.3	1.14	−1.39	1.13	−1.12	0.48	−1.18	−0.40
13	158.8	64.0	3.01	2.23	4.23	1.92	1.66	2.66	1.83
19	174.5	74.5	−2.09	−0.30	−0.93	1.46	−1.67	0.20	−0.12
21	189.7	87.2	−2.41	2.93	2.84	3.78	2.15	0.32	0.40
33-G2	165.7	121.1	6.26	1.87	1.69	2.48	4.68	−3.59	−1.57
35	188.1	123.3	−5.38	−2.74	−3.29	−4.77	−5.27	2.83	0.87
MEAN			3.36	2.69	2.17	2.11	2.06	1.60	0.96
SD OF MEAN			2.01	1.84	1.44	1.53	1.82	1.18	0.74
SE OF MEAN			0.67	0.61	0.48	0.51	0.61	0.39	0.24

TABLE 4 Continued

Subject	Height (cm)	Weight (kg)	4-pi Liquid	8 x 4 Tilting Chair		Log Tilting Chair		7-Crystal Sum	
				Compton	Photopeak	Compton	Photopeak	Compton	Photopeak
TEN SUBJECTS WITH CRYSTAL 35.8 cm ABOVE BED									
17-C3	163.6	73.7	−7.18	−0.16	0.66	0.54	−1.18	−3.24	−2.55
18-C2	163.6	73.7	−0.84	4.34	2.55	4.42	4.34	2.71	2.04
23-C1	163.6	88.9	3.35	−0.13	1.02	−0.42	0.37	−0.13	−0.74
25-D3	161.2	97.5	5.53	−2.21	−2.27	−2.83	−3.09	−1.13	−1.40
26-D2	161.2	102.3	5.50	−0.98	−2.02	−4.34	−3.50	0.63	1.13
28-E2	166.4	113.8	−5.45	−0.52	−0.44	2.02	2.84	2.80	2.65
37-D1	161.2	128.0	1.68	−2.76	−1.57	−3.18	−4.07	−0.16	0.74
40-E1	166.4	130.9	1.77	−1.20	0.16	1.78	4.64	0.34	0.61
43-J1	178.5	147.8	0.11	−1.00	−2.64	−0.24	−1.89	−3.16	−3.26
46	182.9	157.0	−3.60	4.92	4.80	2.60	2.01	1.54	0.94
MEAN			3.50	1.82	1.81	2.24	2.79	1.58	1.61
SD OF MEAN			2.37	1.70	1.38	1.53	1.41	1.28	0.94
SE OF MEAN			0.74	0.54	0.44	0.48	0.44	0.41	0.30

technique are not particularly different even though the crystal was at different heights from the bed for each series. The values of *A* found with the 7-crystal sum for the two groups are different, a result that would be expected since the height of the crystal above the bed differed.

Appropriate statistical tests demonstrated that no significant correlation exists between the weights of the subjects and the % *G* spreads given in Table 4 for any of the four counting techniques. Consequently, means and SD and SE of means are given at the bottom of each column. The 4-pi liquid counter *G* factors yielded the largest mean error, while the 7-crystal sum yielded the smallest % *G* spread. It should be emphasized at this point that a good fit to the *G* factors from only ten, or even 20, subjects does not demonstrate the superiority of a technique but may reflect accidental subject selection.

DISCUSSION OF ANALYSES OF *G* FACTORS

The parameters and associated standard errors of the regression lines calculated to fit these data denote that the *G* factors are highly dependent upon the subject's weight. Of great importance is the finding that the *G* factors obtained for some subjects are as much as 10 to 12% above or below the regression line found to fit the whole series of measurements.

Significantly, there is very little, if any, correlation between errors in *G* factors found by the 4-pi liquid counter and those found by the tilting-chair technique, although there is a high degree of correlation between the errors found by the two tilting-chair techniques (Table 2). For five subjects (10, 17, 28, 32, and 39) of the seven whose 4-pi liquid counter *G* factors fell more than 6% off the calculated regression line, the *G* factor found with the 8 x 4 TC fell within 2.7% of the regression line for these data. The *G* factor for the sixth subject (38) fell 4.8% off the line, and the *G* factor for the seventh subject (35) was much farther from the regression line, 11.9% versus –6.72%.

On the other hand, while there is a mean difference of 1.84% between the % *G* spreads for the 8 by 4 crystal and the % *G* spreads for the log crystal, the subjects (31 and 35) whose *G* factors fell 9% and 12% below the regression line found with the 8 by 4 crystal also fell 9.86% and 13.2% below the regression line found with the log crystal. Conversely, the subjects (44 and 45) whose *G* factors fell 11% and 7.1% above the 8 by 4 crystal regression line also fell 6.3% and 8.08% above the regression line found for the log crystal *G* factor. This agreement proves conclusively that the count rate obtained with the tilting-chair technique is dependent upon the location of the ^{42}K in the body, and that these variations are not due to instrumentation errors.

About 1.0% of the 1.8% mean difference between the errors of columns 6 and 10, Table 2, is due to counting statistics associated with ^{42}K counts of

the subjects. The addition of 0.8% must be ascribed to movement of the subject and to the varying sensitivities of the two very differently shaped crystals to distribution of ^{42}K in the body.

These data demonstrate the necessity of measuring many subjects before any claim can be made that a particular whole-body counter design yields a count rate that is independent of body build and K distribution. The *G* factors obtained for 18 subjects with the log crystal at the closer of the two positions did not diverge from the regression line at higher body weights as did the *G* factor when the crystal was at the greater distance. Consequently, these data obtained with the log crystal can be used to suggest that the *G* factors will fall much closer to the regression line if the closer crystal spacing is used, and, therefore, the superiority of this crystal spacing is implied. However, these data when compared with the results obtained with the 8 by 4 crystal prove just the opposite. Examination of the % *G* spread values given for the 8 by 4 crystal, wherein all subjects were measured with the same crystal spacing, reveals that the subjects whose *G* factors fell farthest from the regression line for the 8 by 4 crystal *G* factors happened to have been measured with the log crystal when it was located at the greater distance. In fact, the *G* factors found for these 18 subjects with the log crystal at the closer spacing fall farther from the regression line than the *G* factors found for these subjects with the 8 by 4 crystal. As would be expected, placing the crystal closer to the body increases the spread in *G*-factor variation.

EXCHANGEABLE K RATIO

The *G* factor obtained from the ^{42}K measurements on each subject was used to calculate his body content of K. Each subject's body K value, measured by the isotope-dilution method, was divided by the value calculated from the whole-body counter technique; this ratio is given in percent in Table 5. The whole-body counter K values are presented in this form because the results are easier to comprehend since the values are all essentially normalized to the same base line, and because these ratios can be analyzed to determine the variability in the so-called exchangeable K pool of the body from subject to subject.

The SD in counts per minute associated with each $^{39,40}K$ measurement was calculated as follows:

$$SD = \sqrt{\frac{B}{T_1} + \frac{C}{T_2}}, \qquad (3)$$

TABLE 5 Exchangeable K Ratio Calculated with Subject's *G* Factor

Subject	Height (cm)	Weight (kg)	4-pi Liquid		8 x 4 Tilting Chair		7-Crystal Sum		Log Tilting Chair	
			Ratio	C.V.	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.
41	172.8	136.6	82.43	1.28	91.97	2.37	–	–	88.63	1.98
47	186.9	167.1	77.34	1.08	100.03	2.13	–	–	87.03	1.65
42	160.5	143.1	110.17	2.16	106.46	3.05	–	–	109.33	2.69
1-A1	161.2	43.9	90.96	2.00	98.38	3.92	–	–	96.65	3.16
3-B1	154.7	48.5	105.01	2.00	110.88	3.86	–	–	105.17	2.98
5	161.4	52.7	101.07	2.00	98.14	3.61	–	–	93.80	2.88
8	163.0	55.9	108.23	1.84	112.59	3.58	–	–	109.89	2.84
32-H1	154.5	121.1	107.04	1.95	92.71	3.14	–	–	92.60	2.74
27	165.1	102.5	99.09	1.38	103.58	2.64	–	–	–	–
30-G1	165.7	119.7	98.13	1.96	98.21	3.02	–	–	–	–
45	159.2	150.4	–	–	95.36	2.97	–	–	99.23	2.58
24	184.1	92.9	90.67	0.99	92.16	2.10	–	–	93.49	1.70
15	179.9	71.8	90.81	0.98	93.64	2.22	–	–	95.08	1.81
9	167.7	56.4	72.58	1.68	78.97	3.62	–	–	82.06	3.02
14	168.9	70.3	85.86	1.25	90.81	2.68	–	–	87.87	2.12
MEAN			94.24		97.59				95.45	
44-F1	170.9	149.6	82.04	1.24	84.81	2.39	90.63	1.41	89.25	2.03
38	170.0	128.2	93.01	1.86	93.25	2.90	93.33	1.67	100.89	2.60
39	171.0	128.9	85.41	1.86	98.19	3.07	90.40	1.69	90.51	2.45
34-H2	154.5	121.8	90.02	2.09	94.84	3.09	90.12	1.81	98.37	2.66
33-G2	165.7	121.1	92.98	1.98	94.55	3.00	97.75	1.50	96.39	2.60
35	188.1	123.3	90.59	1.26	90.40	2.30	91.38	1.19	94.19	2.00
13	158.8	64.0	87.86	1.56	83.51	3.00	91.49	1.45	91.96	2.70
10	153.5	57.6	88.74	2.09	90.13	3.70	93.03	1.78	93.01	3.40
31	160.0	120.1	87.00	2.36	86.34	3.35	–	–	86.59	2.85

TABLE 5 Continued

Subject	Height (cm)	Weight (kg)	4-pi Liquid		8 x 4 Tilting Chair		7-Crystal Sum		Log Tilting Chair	
			Ratio	C.V.	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.
29-F2	170.9	118.4	–	–	96.84	2.93	93.87	1.66	95.87	2.46
21	189.7	87.2	95.54	0.99	95.77	2.20	96.43	1.04	89.07	1.79
19	174.5	74.5	95.99	1.13	90.58	2.36	88.84	1.08	86.67	1.94
2-A2	161.2	45.2	92.44	2.01	92.47	4.09	–	–	–	–
7	162.7	55.6	92.13	1.81	87.97	3.49	–	–	–	–
4-B2	154.7	49.7	97.60	1.98	98.14	3.89	–	–	–	–
12	170.4	63.3	92.83	1.86	100.80	3.75	–	–	–	–
22	178.1	88.4	94.25	1.31	94.54	2.55	–	–	91.74	2.16
11	163.6	57.9	92.48	1.58	94.47	3.22	–	–	92.66	2.78
20	185.2	85.0	93.88	1.10	96.81	2.36	–	–	94.69	1.99
16	184.2	72.9	98.24	1.12	99.06	2.40	–	–	95.48	2.03
6	178.3	53.7	90.83	1.34	97.52	3.07	–	–	92.83	2.57
NUMBER			20		21		11		17	
MEAN			91.69		93.38		92.47		92.95	
SD OF MEAN			4.04		4.81		2.74		3.88	
SE OF MEAN			0.91		1.05		0.83		0.94	
17-C3	163.6	73.7	108.33	1.66	91.62	3.17	–	–	96.89	2.62
18-C2	163.6	73.7	101.90	1.67	98.43	3.33	92.94	1.77	87.40	2.48
23-C1	163.6	88.9	98.55	1.56	89.03	2.80	89.86	1.55	83.98	2.15
25-D3	161.2	97.5	90.39	2.57	94.50	3.98	94.58	2.36	92.37	3.21
26-D2	161.2	102.3	103.38	2.74	90.61	3.64	87.43	2.07	95.94	3.04
28-E2	166.4	113.8	105.91	1.60	94.77	2.84	92.90	1.59	95.70	2.32
36-J2	178.5	125.3	90.70	1.53	84.26	2.35	89.10	1.49	–	–
37-D1	161.2	128.0	97.16	2.07	95.11	3.16	94.53	1.78	95.31	2.54
40-E1	166.4	130.9	99.82	1.47	92.61	2.50	89.80	1.39	86.70	2.00

TABLE 5 Continued

Subject	Height (cm)	Weight (kg)	4-pi Liquid Ratio	4-pi Liquid C.V.	8 x 4 Tilting Chair Ratio	8 x 4 Tilting Chair C.V.	7-Crystal Sum Ratio	7-Crystal Sum C.V.	Log Tilting Chair Ratio	Log Tilting Chair C.V.
43-J1	178.5	147.8	91.82	1.39	87.90	2.26	87.84	1.28	88.84	1.86
46	182.9	157.0	97.36	1.33	79.23	2.16	89.35	2.04	95.23	1.99
NUMBER			11		11		10		10	
MEAN			98.67		90.73		90.83		91.84	
SD OF MEAN			6.02		5.46		2.67		4.69	
SE OF MEAN			1.82		1.64		0.84		1.48	

where

B = background count rate in counts per minute,

T_1 = length of time in minutes background was counted,

C = gross counts per minute from subject, and

T_2 = length of time in minutes subject was counted.

A modified coefficient of variation (CV) of each K determination, defined as a percentage of the net count rate, $\% = (SD/C - B) \times 100$, is given with each ratio in Table 5. This percentage reflects only the statistical uncertainty introduced into the ratio by the $^{39,40}K$ measurement and does not include the statistical uncertainty present in the ^{42}K measurements. The statistical uncertainty (which is less than 1%) present in the ^{42}K count rates (in G factors) has been ignored in order to emphasize the magnitude of the variability present in K measurements with all these techniques. The SD associated with the isotope-dilution measurement amounted to 3.2% and was ignored since it is common to all ratios given for a particular subject.

In order to simplify Table 5, only the exchangeable K ratios based on the K contents calculated from the photopeak energy bands are given. The subjects are listed in Table 5 in the sequence in which they were measured and not by weight. Data on subjects who were measured more than once are designated by a letter and chronological number.

The ratios for the first 15 subjects listed in Table 5 are included only to permit a comparison of the ratios found for each subject by the three whole-body counter techniques. The absolute magnitude of these 15 ratios is known to be questionable and should not be considered to represent the exchangeable K ratio for these subjects. The counting system purchased expressly to measure the ^{42}K content of the urine yielded slightly erratic background count rates during the period in which the first 15 subjects were measured. Although no individual measurement was grossly incorrect, a statistical analysis of a series of repetitive determinations demonstrated that a larger variation existed than had been predicted. This counter was repaired by the manufacturer, and stable reproducible measurements were obtained thereafter. The errors introduced into the urine measurements did not significantly influence the excretion correction applied to the G factors but did significantly influence the exchangeable ratios.

These ratios obtained for the remaining 32 subjects prove conclusively that any individual K measurement made by any technique can contain a sizable error. In some subjects, three techniques yield the same values, whereas the fourth yields a much different value. Ratios of 93.01, 93.25, and 93.33, respectively, were found for subject 38 with the 4-pi liquid counter, the 8 x 4 TC, and the 7-crystal technique, but a ratio of 100.89 was found with the log TC. Reanalysis of the original data obtained with the log crystal revealed that the

calculations are correct. For subject 44-F1, the 4-pi liquid counter, the 8 x 4 TC, the 7-crystal, and the log TC techniques yielded ratios of 82.04, 84.81, 90.65 and 89.25, respectively. The agreement between the last two techniques plus the preconceived belief that the exchangeable K ratio should be about 90% would lend support to the assumption that the 4-pi liquid counter and the 8 x 4 TC measurements are low; this is an assumption, however, not a proven fact.

The series of 44 measurements was divided into three groups, and the mean, the SD of mean, and the SE of mean for the measurements made by each technique are given in Table 5. The first group of 15 subjects includes measurements made while the urine ^{42}K counter was erratic; the second group of 21 subjects includes healthy individuals who followed a normal life at home; and the third group consists of 11 measurements made of 5 persons who were fasting in a hospital metabolic ward but who followed a prescribed course of exercise and were not at bed rest.

The mean ratios for the first group for the 4-pi liquid counter and log TC agree fairly well, while the mean ratio for the 8 x 4 TC is appreciably higher ($\simeq 2\%$). The mean ratios for all techniques for the second group of 21 subjects agree surprisingly well. The grand mean of the four individual means is 92.62 or within 1 SE of the mean for each of the techniques except the 4-pi liquid counter, where the mean is only 0.02 beyond a single SE of 0.91.

The means of the ratios for the third group of five subjects in Table 5 with the 8 x 4 TC, the 7-crystal, and the log TC agree but are somewhat lower than those found for the second group by the same techniques. The mean value for the 4-pi liquid counter is extremely high and does not agree with those for the other techniques for this group nor with the ratio found with the 4-pi liquid counter for the second group. Undoubtedly, the measurements of the four subjects for whom ratios of over 100% were found are erroneous; however, no reason can be advanced for these discrepancies.

The slightly lower ratios for the 11 measurements of the third group with the 8 x 4 TC technique probably reflect one change in subject position procedure. Except for this series of measurements, each subject was told to sit with his knees resting out against the arm rests of the chair and with his hands clasped in his lap. Measurements of ^{42}K made several years ago showed that the count rate would decrease several percent if a subject placed his arms alongside his body in the chair and that the count rate might increase a few percent if he held his knees together, in which position the knees are slightly closer to the crystal. For the last series of measurements, the subject's knees were taped in the desired position (with easily broken masking tape to avoid subject fatigue), and he wore gloves with fingers that had been sewn together. Consequently, the subject remained in the same position even though he may

have slept during the measurement. This procedure may account for the absence of any ratios as high as 100 such as were seen in the second group.

VARIABILITY OF EXCHANGEABLE K RATIOS

A controversy exists as to the relationship between the K value measured by the isotope-dilution technique and the actual K content of the body. Some investigators maintain that all the body K is readily exchangeable and that the value found by the dilution technique is the true value. However, we found that the mean exchangeable ratios given by each technique were about 0.92.

An analysis of the exchangeable K ratios (Table 5), which ranged from 86 to 95% for the individual subjects, seems to suggest that this ratio is consistent among all subjects. The values for the standard deviation of the mean of the exchangeable ratios determined by the 7-crystal technique for the two groups of subjects (2.74% and 2.67%) is less than the 3.2% standard deviation present in the isotope-dilution measurement alone. The uncertainty in the normal K measurement has been ignored. When the two groups of subjects are pooled, the 21 exchangeable K ratios yield a mean ratio of 91.65%. Only four, instead of the acceptable seven, ratios fall over 1 SD from this mean, and no ratios (one excepted) fall outside 2 SD. For three subjects (35, 21, and 43-J1) of the four whose ratios fell more than 1 SD beyond the mean, practically the same exchangeable K ratios were found with two of the other whole-body counter techniques. Consequently, the variability in the exchangeable ratios found with the 7-crystal sum represents either statistical counting uncertainties in the isotope-dilution measurements or differences in the ratios among subjects. However, the 3.2% statistical uncertainty associated with the isotope-dilution measurement overshadows any differences that may exist among the individual subjects.

The large variability among the exchangeable K ratios found with 4-pi liquid counter, 8 x 4 TC, and log TC measurement techniques must be attributed to errors in counting either the normal body K or the ^{42}K in the body.

EXCHANGEABLE K RATIOS BASED ON REGRESSION LINE *G* VALUES

The data were recalculated to determine if an average calibration factor based on the subject's weight could be employed for K measurement or whether it was really necessary to administer ^{42}K to every subject for accurate results.

The K content of each subject was recalculated with the *G* factor given by the regression line for a person of his weight. The exchangeable K ratios obtained for the 4-pi liquid counter, the 8 x 4 TC, and the log TC based on those K values are given in Table 6. The 7-crystal sum results were not recalculated because of the limited number of measurements included in each series.

The mean ratios obtained for the second group of about 20 subjects were 2.13%, 1.13%, and 0.39% higher for the 4-pi liquid counter, the 8 x 4 TC, and the log TC, respectively, when the regression line *G* value was used than when the subject's own *G* factor was used. The standard deviation of the mean was slightly larger for the 4-pi liquid counter but considerably larger for the two tilting-chair techniques. The acceptable exchangeable K ratios of 93.01 and 90.58 for subjects 38 and 35, respectively, obtained with the 4-pi liquid counter became 100.45 and 84.49, respectively (Table 6). In the 8 x 4 TC and log TC measurements, the exchangeable K ratios of 90.4 and 94.19 found by the two techniques for subject 35 decreased to 79.63 and 81.72, and the already high exchangeable ratios of 94.84 and 98.37 for subject 34-H2 increased to the impossibly high values of 108.47 and 109.41.

The observation that the exchangeable K ratios for these subjects agree with the mean exchangeable K ratio for the group when their own *G* factors were used, but differ greatly from the mean when the regression line value of *G* was used, suggests that the % *G* spread observed for these subjects does, in fact, reflect different counting efficiencies for K in the body and that a very significant error will be introduced for some subjects in 4-pi liquid, 8 x 4 TC, and log TC measurements if an average calibration factor is used.

When the regression line *G* factors were used for the third group of 11 subjects, the results for the 8 x 4 TC and log TC ratios were approximately the same as for the second group. The mean ratios for these two techniques increased 0.80% and 0.47%, respectively, and a greater spread in exchangeable K ratios was noted. However, results were considerably different for the 4-pi liquid data. The mean ratio for the 4-pi liquid counter decreased 2.79%, and the SD of mean decreased significantly from 6.02 to 4.12%. The impossibly high ratios of 108.33 and 101.90 (Table 5) for subject 17-C3 and 17-C2 (same subject at different times) decreased to 96.85 and 96.46, respectively, and the ratios of 105.91 for subject 28-E2 decreased to 97.46. The ratios of 103.38 for subject 26-D2 increased slightly. The exchangeable ratios for these subjects based on the 4-pi liquid counter, which decreased to a physically acceptable value, suggest that the ^{42}K measurements made during this third series must be slightly erratic. Significantly, the mean ratio also decreased to a value nearer the mean ratios found by the other techniques.

In order to obtain comparable data for the 7-crystal sum, the exchangeable K ratios of the last 10 measurements in Table 5 were recalculated with the *G* factors predicted by the regression lines found to fit this limited series of

TABLE 6 Exchangeable K Ratio Calculated with Regression Line *G* Factor

Subject	Height (cm)	Weight (kg)	4-pi Liquid		8 x 4 Tilting Chair		7-Crystal Sum		Log Tilting Chair	
			Ratio	C.V.	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.
41	172.8	136.6	81.31	1.28	89.10	2.37	–	–	88.07	1.98
47	186.9	167.1	70.02	1.08	84.87	2.13	–	–	75.64	1.65
42	160.5	143.1	105.79	2.16	104.98	3.05	–	–	111.71	2.69
1-A1	161.2	43.9	91.02	2.00	97.60	3.92	–	–	96.19	3.16
3-B1	154.7	48.5	107.51	2.00	112.25	3.86	–	–	106.89	2.98
5	161.4	52.7	98.59	2.00	95.97	3.61	–	–	95.12	2.88
8	163.0	55.9	108.01	1.84	113.72	3.58	–	–	111.86	2.84
32-H2	154.5	121.0	119.13	1.95	91.74	3.14	–	–	89.87	2.74
27	165.1	102.5	97.03	1.38	101.84	2.64	–	–	–	–
30-G1	165.7	119.7	99.27	1.96	96.76	3.02	–	–	–	–
45	159.2	150.4	–	–	102.17	2.97	–	–	107.25	2.58
24	184.1	92.9	86.16	0.99	87.44	2.10	–	–	86.34	1.70
15	179.9	71.8	88.75	0.98	92.60	2.22	–	–	93.39	1.81
9	167.7	56.4	71.69	1.68	80.87	3.62	–	–	84.59	3.02
14	168.9	70.3	84.76	1.25	89.53	2.68	–	–	85.82	2.12
MEAN			93.52		96.10				94.83	
44-F1	170.9	149.6	81.85	1.24	94.15	2.39	90.63	1.41	94.90	2.03
38	170.0	128.2	100.45	1.86	97.72	2.90	93.33	1.67	105.33	2.60
39	171.0	128.9	95.45	1.86	99.59	3.07	90.40	1.69	92.53	2.45
34-H2	154.5	121.8	91.09	2.09	108.47	3.09	90.12	1.81	109.41	2.66
33-G2	165.7	121.1	97.52	1.98	87.89	3.00	97.75	1.50	92.70	2.60
35	188.1	123.3	84.49	1.26	79.63	2.30	91.38	1.19	81.72	2.00
13	158.8	64.0	92.42	1.56	87.35	3.00	91.49	1.45	92.92	2.70
10	153.5	57.6	95.52	2.09	87.72	3.70	93.03	1.78	93.69	3.40
31	160.0	120.1	88.23	2.36	78.54	3.35	–	–	78.05	2.85

TABLE 6 Continued

Subject	Height (cm)	Weight (kg)	4-pi Liquid Ratio	4-pi Liquid C.V.	8 x 4 Tilting Chair Ratio	8 x 4 Tilting Chair C.V.	7-Crystal Sum Ratio	7-Crystal Sum C.V.	Log Tilting Chair Ratio	Log Tilting Chair C.V.
29-F2	170.9	118.4	–	–	100.01	2.93	93.87	1.66	98.08	2.46
21	189.7	87.2	93.91	0.99	95.16	2.20	96.43	1.04	87.59	1.79
19	174.5	74.5	95.37	1.13	88.52	2.36	88.84	1.08	83.49	1.94
2-A2	161.2	45.2	91.23	2.01	95.13	4.09	–	–	–	–
7	162.7	55.6	99.43	1.81	96.74	3.49	–	–	–	–
4-B2	154.7	49.7	100.05	1.98	100.02	3.89	–	–	–	–
12	170.4	63.3	95.92	1.86	102.41	3.75	–	–	–	–
22	178.1	88.4	97.69	1.31	95.86	2.55	–	–	94.31	2.16
11	163.6	57.9	94.21	1.58	96.24	3.22	–	–	95.76	2.78
20	185.2	85.0	94.13	1.10	97.15	2.36	–	–	95.55	1.99
16	184.2	72.9	97.25	1.12	97.61	2.40	–	–	96.49	2.03
6	178.3	53.7	90.11	1.34	98.83	3.07	–	–	94.40	2.57
NUMBER			20		21				17	
MEAN			93.82		94.51				93.34	
SD OF MEAN			4.91		7.26				7.71	
SE OF MEAN			1.10		1.58				1.87	

TABLE 6 Continued

Subject	Height (cm)	Weight (kg)	4-pi Liquid Ratio	4-pi Liquid C.V.	8 x 4 Tilting Chair Ratio	8 x 4 Tilting Chair C.V.	7-Crystal Sum Ratio	7-Crystal Sum C.V.	Log Tilting Chair Ratio	Log Tilting Chair C.V.
17-C3	163.6	73.7	95.88	1.66	93.13	3.17	–	–	95.94	2.62
18-C2	163.6	73.7	96.35	1.67	101.94	3.33	92.94	1.77	91.37	2.48
23-C1	163.6	88.9	97.68	1.56	91.08	2.80	89.86	1.55	84.57	2.15
25-D3	161.2	97.5	91.78	2.57	93.67	3.98	94.58	2.36	89.88	3.21
26-D2	161.2	102.3	105.14	2.74	90.12	3.64	87.43	2.07	93.00	3.04
28-E2	166.4	113.8	96.96	1.60	95.99	2.84	92.90	1.59	98.97	2.32
36-J2	178.5	125.3	93.15	1.53	78.04	2.35	89.10	1.49	–	–
37-D1	161.2	128.0	96.17	2.07	95.48	3.16	94.53	1.78	92.04	2.54
40-E1	166.4	130.9	99.00	1.47	94.65	2.50	89.80	1.39	91.37	2.00
43-J1	178.5	147.8	90.16	1.39	87.59	2.26	87.84	1.28	87.90	1.86
46	182.9	157.0	92.38	1.33	85.13	2.16	89.35	2.04	98.04	1.99
NUMBER			11		11				10	
MEAN			95.88		91.53				92.31	
SD OF MEAN			4.12		6.34				4.44	
SE OF MEAN			1.24		1.91				1.98	

TABLE 7 Exchangeable K Ratios Calculated for Ten Subjects with Regression *G* Values

Subject	Height (cm)	Weight (kg)	4-pi Liquid		8 x 4 Tilting Chair		7-Crystal Sum		Log Tilting Chair	
			Ratio	C.V.	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.
17-C3	163.6	73.7	100.40	1.66	92.47	3.17	–	–	95.74	2.62
18-C2	163.6	73.7	100.89	1.67	101.21	3.33	94.96	1.77	91.19	2.48
23-C1	163.6	88.9	101.56	1.56	90.39	2.80	89.40	1.55	84.29	2.15
25-D3	161.2	97.5	95.04	2.57	92.94	3.98	93.54	2.36	89.51	3.21
26-D2	161.2	102.3	108.62	2.74	89.41	3.64	88.71	2.07	92.58	3.04
28-E2	166.4	113.8	99.63	1.60	95.20	2.84	95.73	1.59	98.42	2.32
36-J2	178.5	125.3	95.21	1.53	77.37	2.35	85.63	1.49	–	–
37-D1	161.2	128.0	98.17	2.07	94.66	3.16	95.73	1.78	91.42	2.54
40-E1	166.4	130.9	100.86	1.47	93.83	2.50	91.40	1.39	90.73	2.00
43-J1	178.5	147.8	91.18	1.39	86.79	2.26	85.53	1.28	87.15	1.86
46	183.0	157.0	93.03	1.33	84.34	2.16	90.85	2.04	97.14	1.99
MEAN			98.60		90.74		91.12		91.77	
SD OF MEAN			4.84		6.34		3.84		4.40	
SE OF MEAN			1.46		1.91		1.22		1.39	

10 subjects. It should be emphasized that for the 4-pi liquid counter, 8 x 4 TC, and log TC techniques, the regression line found to fit these 10 subjects, rather than the line found to fit the entire series, was used. The exchangeable K ratios for each subject for each method as well as the mean, the SD of mean, and the SE of mean of the ratios for each technique are given in Table 7.

The mean ratio for the 7-crystal sum increased slightly from 90.83 (Table 5) to 91.12 (Table 7), and the SD of mean increased from 2.67 to 3.84. A slightly greater range in exchangeable ratios exists for the individual subjects—85.53 to 95.73 (Table 7) versus 87.43 to 94.58 (Table 5)—when the regression line *G* values are used.

For the 4-pi liquid counter, the exchangeable K ratios for one subject increased to the impossibly high value of 109%. However, the exchangeable K ratios for subjects 17-C3 and 28-E2 decreased from the impossibly high values of 108.33 and 105.91 to physically possible values of 100.55 and 100.13, respectively. The SD of mean also decreased from 6.02 when the person's own *G* factor was used to 4.85 when the regression line *G* factor was used. These results suggest also that the ^{42}K measurements were in error.

These extremely divergent exchangeable K ratios obtained by the 4-pi liquid counter, 8 x 4 TC, and log TC techniques when the regression line *G* factors are used demonstrate that any isolated single measurement of a subject may contain as much as a 10% error if the person is not given ^{42}K to calibrate the counter for his own unique body build.

COMPARISON OF *G* FACTORS FOUND AT DIFFERENT POSITIONS ALONG THE BODY

Analyses were performed to determine if the variability observed with the tilting-chair technique or the 4-pi liquid counter could be explained with results obtained at the seven positions along the body. The results of one analysis are presented because these are pertinent to other techniques currently being tested by other investigators.

The net ^{42}K count rate obtained when the 8 x 4 crystal was located over the subject at each of seven positions was divided into the count rate obtained from the same amount of ^{42}K in a bottle on the tray at 40 cm. A *G* factor was thus obtained for each of the seven positions for each of nine subjects measured with the 8 x 4 crystal 30 cm above the bed.

The *G* factors for each of the seven positions for nine subjects were fitted with regression lines. The % *G* spread that the observed *G* factors fell above or below the regression line for each position is given in Table 8. In addition, the *G* factors obtained with the 7-crystal sum, the 4-pi liquid counter, the 8 x 4 TC, and the log TC for these nine subjects were also fitted with regres-

TABLE 8 Percent *G* Spread Observed for Seven Crystal Positions and for Each Technique

Subject	Height (cm)	Weight (kg)	Crystal Position 1	2	3	4	5	6	7	7-Crystal Sum	4-pi	8 x 4 TC	Log TC
Compton Energy Band													
2-A2	161.2	45.2	−9.86	−3.42	−1.23	−0.20	1.05	4.74	−1.91	−1.52	−4.43	−1.54	−0.18
4-B2	154.7	49.7	4.19	−1.04	−2.49	−1.32	−0.08	−0.94	−1.84	−0.83	−0.47	5.32	−2.90
10	153.5	57.6	9.43	4.66	−2.34	−2.56	−1.28	6.17	7.05	1.27	5.01	−5.91	−0.38
12	170.4	63.3	−7.36	−3.92	0.62	0.94	1.64	−2.97	−1.20	−1.18	1.14	−1.39	−1.12
13	158.8	64.0	11.06	2.50	0.98	1.84	1.78	0.45	3.57	2.66	3.01	2.23	1.92
19	174.5	74.5	0.80	2.69	5.63	0.38	−5.14	−7.91	−3.23	0.20	−2.09	−0.30	1.46
21	189.7	87.2	−7.14	0.77	2.58	3.22	1.70	−4.16	−3.93	0.32	−2.41	2.93	3.78
33-G2	165.7	121.1	−8.00	−6.48	−7.44	−5.79	1.39	13.17	4.17	−3.59	6.26	1.87	2.48
35	188.1	123.3	9.82	4.88	4.32	3.86	−0.85	−6.70	−2.10	2.83	−5.38	−2.73	−4.77
Photopeak Energy Band													
2-A2	161.2	45.2	−12.60	−3.84	−3.21	−0.76	2.11	5.62	−0.53	−2.21	−4.43	−0.58	−0.11
4-B2	154.7	49.7	6.50	−1.06	−1.44	1.26	0.34	0.71	−0.24	0.38	−0.47	−0.78	−2.06
10	153.5	57.6	6.60	2.71	−0.71	−3.48	−0.92	5.54	7.90	0.89	5.01	−4.01	0.45
12	170.4	63.3	−7.57	−1.94	0.23	0.69	2.81	−0.24	0.19	−0.40	1.14	1.13	0.48
13	158.8	64.0	14.29	2.82	1.30	1.69	−2.00	−2.07	−3.42	1.83	3.01	4.23	1.66
19	174.5	74.5	2.68	1.30	6.11	0.22	−5.71	−10.19	−3.98	−0.12	−2.09	−0.93	−1.67
21	189.7	87.2	−7.09	3.25	1.04	1.63	2.94	5.64	−1.68	0.40	−2.41	2.84	2.15
33-G2	165.7	121.1	−6.23	−7.31	−4.14	−4.23	3.64	19.17	6.47	−1.57	6.26	1.69	4.68
35	188.1	123.3	6.66	4.70	1.16	3.23	−2.81	−9.71	−3.97	0.87	−5.38	−3.29	−5.27

sion lines, and the % *G* spread is given in Table 8. The sign associated with the % *G* spread is opposite to the variation in ^{42}K count rate from the human. That is, if the ^{42}K yielded a higher count rate than predicted, the observed *G* factor, $^{42}K_{bottle}/^{42}K_{man}$, would be less than the regression line and thus would carry a negative sign.

Very large % *G* spreads are observed for each crystal position. Of particular significance is the fact that while one person exhibits a negative value for position 1 over the head and a positive value for position 7 over the feet, a second subject exhibits the opposite result, and other subjects yield either positive or negative values at these two positions. Nevertheless, the *G* factors based on the sum of the count rates from the seven positions exhibit a much smaller *G* factor spread than the 4-pi liquid counter, the 8 x 4 TC, or the log TC techniques. No explanation can be given for the large % *G* spread observed with the 4-pi liquid counter in view of the small spread found for the 7-crystal sum. The two techniques are similar, and equivalent results would be expected.

The same analyses were performed with the data obtained by all four techniques for the 11 subjects included in the third group. In this case the 7-crystal measurements were made with the 8 x 4 crystal 35.8 cm above the surface of the bed. The subjects' weights ranged from 73.7 to 157 kg versus 45.2 to 123.3 kg for the first group. The *G* factors for each of the seven positions exhibited approximately the same spread around the regression line as those for the previous group of subjects. Again, the *G* factors based on the sum count rate of seven crystal positions fell very close to their regression line.

The large variations in % *G* spread values given in Table 8 demonstrate the impracticability of counting a supine subject with one or two stationary crystals. This analysis was included because some whole-body counter groups have proposed the use of an uncollimated scan technique whereby the crystal transverses a distance 60 to 80 cm along the body. If the resultant *G* factors were fitted with a regression line, they would fall above or below the line an amount approximately equal to the average of the three values for % *G* spread (Table 8) that cover that length. A typical 75-cm scan along the torso would be equivalent to positions 2, 3, and 4. The % *G* spread for subject 33-*G*2 for such a scan would be about –5%, while the spread for subject 35 would be about +2%. Consequently, these data suggest that the % *G* spread will be reduced as more crystals are employed.

G FACTOR FOR SAME SUBJECT AT DIFFERENT TOTAL-BODY WEIGHTS

A special study was conducted with a number of obese subjects to determine if the *G* factors obtained for a subject as he lost weight decreased along the

regression line that was derived from many subjects of different weights. After the first series of K measurements was completed, the subject fasted for several weeks in a hospital metabolic ward. During the starvation period, the subject followed a prescribed course of exercise and remained active. The complete series of measurements was repeated approximately 1 week after the subject had terminated his fast. Measurements of the same subject are designated by letter and number in the tables; the number designates the sequence of the measurement. Measurements designated C, D, E, and J were made of subjects who successfully completed this program and lost 15.2, 30.5, 17.1, and 22.5 kg, respectively. Measurements 37, 26, and 25 in Table 2, designated D1, D2, and D3, were made of one subject before starvation, during a period when this subject resumed eating, and then at the end of a second starvation period.

With the 4-pi liquid counter, the *G* factors for subjects C1, D1, E1, and J1 before starvation fell 0.87, 1.01, 0.81, and 1.80% below the regression line (Table 2). After starvation, the *G* factors for two of these subjects, C3 and E2, fell 11.48 and 8.44% below the *G* factors predicted by the regression line for subjects at their new weights. The new *G* factors for subjects D3 and J2 were 1.54 and 2.71% above the regression line. Thus, the ^{42}K in subjects C3 and E2 was counted more efficiently after diet than would have been predicted from measurement of other subjects of the same weight.

With both tilting-chair techniques, the *G* factors based on the photopeak band for subjects C, D, and E changed about as predicted by the regression lines. For example, the *G* factor for the 8 x 4 TC for C1 before starvation was 2.31% above the line, and for C3, after starvation it was 1.66% above the line. However, the *G* factor obtained for J2 after starvation decreased much faster than predicted, from 0.34% below the regression line to 7.38% below the line. Unfortunately, because of a technical error, the log TC measurement was not obtained, so it is not possible to compare 8 x 4 TC and log TC results for this subject.

Only 11 measurements were made with the 7-crystal technique, with the 8 by 4 crystal located 35.8 cm above the bed. Consequently, the results are biased because of the limited number of *G* factors employed and because all measurements were of the same subjects. It is noteworthy that the *G* factors did decrease with weight, approximately as predicted by the regression line found to fit these 11 measurements. Both *G* factors obtained for a subject agreed within about 1%, whether they fell above or below the regression line. For example, the *G* factors for one subject (J1 and J2) were 2.63% and 3.89% below the regression line. Significantly, this was the one subject whose 8 x 4 TC *G* factor decreased much more than expected.

This analysis indicates that the *G* factors changed as the subjects lost weight but not, at least for the 4-pi liquid counter and the 8 x 4 TC, as predicted by

the regression line. The *G* factors obtained for any subject at two different total-body weights with the 7-crystal sum seemed to change as predicted by the slope of the regression line, but not enough subjects were studied to prove it.

WEIGHT/HEIGHT AS THE INDEPENDENT VARIABLE

Various investigators have suggested that the calibration factors would be more closely correlated to the weight/height ratios than to just the total weights.[2] Each time a regression analysis that employed weight was performed, a second regression analysis that employed weight/height was also performed. The results of the latter analyses have been omitted because, for every whole-body counter technique, the mean *G* spreads obtained from these analyses based on weight/height were equal to or larger than those based on weight alone.

When the results of the analyses for individual subjects were compared, it was found that the *G* factors for some subjects fell closer to the regression line based on weight/height than to the regression line based on total weight. However, the opposite effect occurred just as often, and as a result an equal or larger mean *G* spread occurred.

The use of weight/height might reduce the spread in *G* factors around the regression line if the subject's weight were more or less uniformly distributed along his body. Actually, the spread in *G* factors results because the weight is not uniformly distributed. Since the absorption of gamma rays is an exponential function of mass, the use of a linear average of the subject's weight does not correct for the nonlinear absorption.

DISCUSSION OF PREVIOUSLY PUBLISHED DIVERGENT RESULTS

Some of the conclusions reached in this paper are contradictory to results published previously.[4] In the previous study we published the following observations: (a) The *G* factors found with the 8 x 4 TC for 24 patients who weighed between 50.3 and 95.3 kg fell within 3% of the mean *G* factor and did not depend upon the weight of the patients; (b) the *G* factors for four very obese patients who weighed between 142.1 and 164.8 kg were much larger than those for the lighter patients; (c) the *G* factors based on the count rate of any combination of, or of all the same, crystal positions had a much larger spread than those obtained with the 8 x 4 TC technique; and

(d) the exchangeable K ratios found for the 29 patients were dependent upon the weight of the patients. The exchangeable K ratios varied from about 89% for the lighter patients to slightly over 100% for the very obese patients.

In the previous analyses, the *G* factors for the series of 24 patients had been correlated with weight, weight/height, and exponential relationships of weight/height. Recently, the entire series of 28 *G* factors was fitted with the regression line, $\log_{10}(G) = A + B(w_{kg})$. As found for the series of 44 subjects discussed above, the *G* factors based on the Compton energy band do not support a regression line that has a significant slope. However, approximately the same parameters were found for the regression line based on the photopeak *G* factors for this earlier series of 28 patients as for the current series of 44 subjects. The *G* factors for some of the larger patients fell 10% above or below the regression line, and the *G* factor obtained for the smallest patients (50.3 kg) fell 10% above the regression line. Notably, none of the *G* factors obtained for the smaller subjects included in the present study deviated more than 5% from the regression line.

One explanation can be advanced for the high *G* values found for the small patients, the divergent *G* factors found with the 7-crystal technique, and the dependence of exchangeable K ratios on weight. The 29 persons included in the previous study were all patients who were at bed rest in a hospital. Consequently, the divergent *G* factor probably reflects a nonnormal distribution of ^{42}K in their bodies.

The seemingly divergent results obtained for the patients in the earlier study cannot be attributed to any errors in measurement for several reasons: (a) Practically the same equipment and counting techniques were used for both studies, (b) approximately the same parameters were found for the regression line, and (c) the same count rate per gram of $^{39,40}K$ was found during both studies. Consequently, the exchangeable K ratios are probably correct.

CONCLUSIONS

The results of these analyses have demonstrated that (a) the calibration factors for all whole-body counter techniques are dependent upon the weight of the subject; (b) the single mathematical expression, $\log_{10}(G) = A + B(w_{kg})$ will fit the curve of calibration factors obtained for subjects whose weights range from 43.88 kg to 157 kg; (c) the actual calibration factor found for a particular subject may differ by as much as 10% from the value predicted by the regression line; (d) the exchangeable K content of the body as measured by the isotope-dilution technique is very close to 92% of the total body K for all active subjects, including those who have starved for several weeks and lost

considerable weight; (e) the calibration factors and exchangeable K ratios obtained with the 7-crystal technique did not exhibit the variability observed with the other techniques; and (f) every subject should be given ^{42}K in order to obtain the correct calibration factor for his body build, if very accurate K measurements are desired.

This work was supported in part by the U.S. Atomic Energy Commission and by U.S. Public Health Service Research Grant RH 00283, Division of Radiological Health, USPHS General Research Support Grant 1S01-FR-5368, and a grant from the American Medical Association Education and Research Foundation Grants-in-Aid for Research Project AMA-ERF No. 149.

REFERENCES

1. Christian, J. E., W. V. Kessler, and P. L. Ziemer. 1962. A 2-pi liquid scintillation counter for determining the radioactivity of large samples including man and animals. Int. J. Appl. Radiat. Isotop. 13:557.
2. Marinelli, L. D. 1966. Geometrical and physical parameters in whole-body gamma-ray spectrometry measurements. p. 31. ANL-7220, Argonne National Laboratory, Argonne, Ill.
3. Miller, C. E. 1962. An experimental evaluation of multiple-crystal arrays and single-crystal techniques. p. 81. *In* Whole-body counting. International Atomic Energy Agency, Vienna.
4. Miller, C. E., and A. P. Remenchik. 1963. Problems involved in accurately measuring the K content of the human body. Ann. N. Y. Acad. Sci. 110:175.
5. Remenchik, A. P., and C. E. Miller. 1962. The measurement of total body potassium in man and its relation to gross body composition. p. 331. *In* Whole-body counting. International Atomic Energy Agency, Vienna.

G. M. Ward, Presiding

GENERAL DISCUSSION

G. M. WARD In this session we have heard something about the kinds of instrumentation we would need to tackle the problem of whole-body counting.

The last three papers dealt with the use of whole-body counters for large animals. Because my name is on the first paper, I might be accused of not being particularly objective in this area, so I will skim over this. The one point that I think we should consider is that in all these studies, the animals employed were slaughtered animals, and in most cases chemical analyses were made of them. I would hope that we have some data to present to the people working in the medical field and some answers to some of the questions that were raised yesterday about variability in animals and the biological meaning of differences in water and potassium content. I might ask some of the speakers whether they do have anything definite to provide you. Ultimately, I would hope that the people working with animals will have many answers to contribute to this problem, as suggested by Dr. Brožek.

Another crucial point that Dr. Panaretto made earlier is that we should decide what we want to measure and what precision we feel we must have. This topic was not specifically covered in the preceding papers. Maybe the speakers are not prepared to tackle this problem now, but it is one that cer-

tainly should be given consideration. A point that was made a couple of times is that when one uses animals, particularly large ones, the animal is the potassium source, and this is what is being measured. On the other hand, the interest in the animal husbandry field, more commonly, is in the composition of the carcass, of some meat quantity such as edible meat, trimmed lean, or some other fraction that is described in terms of meat. So it is difficult to make these transitions sometimes.

In his paper, Dr. Lohman made the point that he made his ^{42}K dilution calibrations only on Angus cattle which would be, I presume, the smallest of the animals he studied as well as the most compact, differing considerably in geometry from the others. His four breeds of animals representing the range from Angus to Holsteins have much difference in ultimate body size, and considerable differences in geometry. If it is not an unfair question, perhaps I might ask him whether ^{42}K dilution calibrations with the other breeds might solve some of these problems.

Dr. Johnson pointed out some features of the crystal-type detector, and particularly, that this detector would pick up spurious radioactivity which may be introduced in the diet. Potassium in the feed, of course, is a considerable factor in ruminant diets because the potassium intake is quite large, and has to be considered. Fission products are something that we have had in variable quantities in the last decade. At present, they are almost nonexistent. No one can say what the situation might be at any moment, but currently it is not a serious problem.

Dr. Martin concluded these papers by showing with his swine data that potassium could be estimated nearly as accurately with his liquid scintillation counter as it could be by chemical analysis, which one has to accept as a standard method. If we cannot do it accurately chemically, there is not much hope. He concluded by showing the interactions he found with the standard weight activity and geometry interaction. Before entertaining questions from the audience, I wish to give the speakers the opportunity to reply to some of the questions I have raised. Dr. Miller is the first volunteer.

CHARLES E. MILLER We analyzed the calibration factors both as a function of the patients' weight and as a function of weight/height. The use of weight/height rather than weight does not reduce the amount that the calibration factors fall off the regression line. A very large spread in calibration factors for patients who weighed over 260 pounds was observed for the very simple reason that their weight was not uniformly distributed along the body. These patients usually had a very large fat pad around the buttocks. Since the absorption of gamma rays varies as an exponential of the thickness and not as a linear function of thickness, the use of the linear function of

weight/height does not correct for the nonlinear exponential term. As would be expected, the calibration factors for some patients will fall closer to the associated regression line when plotted against weight/height rather than against weight, but the calibration factors for other patients will fall farther away from the regression line. The patients were counted with the crystal located, in turn, at seven positions along the body and calibration factors calculated for each of the seven positions. These analyses disclosed that the distribution of ^{42}K within the body varied from patient to patient. Some patients contained as much as 10% more ^{42}K in the head, the feet, or both the head and feet than the average patient; other patients contained up to 10% less ^{42}K in these volumes. Naturally, patients who contained a higher than expected amount in the head and feet contained less than the average amount in their trunks. Calibration factors derived for a whole-body counting technique that employed a scan along a patient's torso, but did not include his legs, would vary significantly for patients of the same weight. Consequently, the legs must be scanned even though the K signal to background is very poor over the legs.

In rebuttal to Dr. Anderson's presentation: He discussed the decrease in efficiency obtained with a single crystal as he moved the source through an object 20 cm thick. This variation in efficiency would have been reduced considerably if he had used a detector on both sides. A thicker phantom should be used since an average person is about 24 cm thick, and the heaviest ones included in our study were 35 to 37.5 cm thick. The variation in calibration factors obtained for the 250-, 300-, or 375-lb people included in our study may be more typical of the results expected when large animals are studied than the results obtained for people who weigh less than 200 lb.

I did not stress the fact that the logarithm of the calibration factor (not the linear value) was plotted on the abscissa, and weight was plotted on the ordinate. When plotted in this manner, the data for all the patients whose weights ranged from 100 to 360 lb, fell along a straight line. Since the efficiency factor used by Dr. Anderson is the reciprocal of the calibration factor, I question whether his curved plot, which diverged for persons who weighed over 200 lb, would become a straight line if he replotted his data on a semilog plot.

I was very much interested to hear a speaker say that 10% of a cow's K is located in the skeleton. We found that only 92% of the human's total-body K was exchangeable. I would not expect the ^{42}K tracer given to the human to exchange to any significant degree with the normal K present in the skeleton; therefore, I wonder if the 10% you find in the cow's skeleton doesn't substantiate the 8% nonexchangeable we find for humans. We do have plots of the $^{42}K/^{40}K$ ratio along the body. In some instances the ratio varies con-

siderably along the length of the body, and the ^{42}K simply is not in equilibrium with the ^{40}K. We made our ^{42}K measurements in these patients 36 hours after they received it. On different occasions we measured at 24 hours, 36 hours, and 48 hours to see if we could see any difference.

E. C. ANDERSON To answer Dr. Miller's questions, I would certainly agree that when one has to deal with a subject whose weight is 350 lb, he is definitely outside the range that our calculations were intended to cover, and, indeed, I would expect that, at that weight, one would begin to have serious problems. I think my intention in presenting the data for this computational method was twofold: First, I was trying to confirm the hypothesis that we adopted sometime in the past, mainly that for the 4-pi system and people of the normal weight range, total mass was an adequate predictor of counting efficiency. But I also hope that the size of the numbers that I arrived at for the nonuniformity effects will be a warning that the upper limit of this range is very likely in the region of 200 lb; also, I presented it in the hope that these calculations might be usefully extended into the higher regions for the benefit of people who find it necessary to study subjects of such large masses. With respect to the log plot's straightening out the curve, I think probably it would, but if our theoretical model means anything, what it is telling us is that the curve is not expressible by any simple analytic function. The functional relationships involved are based primarily on an exponential integral which has to be computed by numerical analysis, and any straightening effect would be a rather superficial one. It might be useful, but it would not define anything. With respect to the problem of contamination of the environment and its improvement, the fallout situation is far better now than it has ever been. Early in 1967, the Cesium-137 body burden in people was down by something like the factor 3 from the peak it reached back in 1964. It is down to the lowest level it has had at any time since then. Actually, the situation with respect to fallout debris is even better than what one might assume. The large atmospheric tests ceased several years ago. The point is that the cesium appearing now is cesium that is coming back from stratospheric fallout of debris, whereas the cesium that was present several years ago was derived from tropospheric debris injected in Nevada; and back in the 1950's and the early 1960's, the Cesium was accompanied by large amounts of the shorter-life fission products—zirconium, niobium, barium, lanthanum, and so on. So, I would expect that the situation for fallout debris is quite a bit better. Of course, if the testing resumes, things will get messy again. I was quite interested to see that the barium and lanthanum were evident from the Chinese tests. I have one question in this connection. I would like to ask Dr. Lohman the date of measurement of the reconstructed steer in his Figure 2.

T. G. LOHMAN That steer was measured in 1963.

E. C. ANDERSON And it did show considerable, significant quantities of the zirconium? Well, we are not out of the woods yet, but perhaps things are getting better.

R. L. PRESTON I would like to ask a question of Dr. Lohman. It was not quite clear to me from the data you presented whether you actually did any chemical potassium analyses on the samples you counted. Were all your correlations derived from counts only?

T. G. LOHMAN That's right. There were no chemical determinations of potassium done in any of these studies. The components were counted again separately for ^{40}K, and our coefficients of determination were computed from the components.

W. B. WEIL This is not a question; it is a statement. I am glad I do not have to make a decision about whether to buy a liquid scintillation counter or a whole-body counter that employs crystals, because you have the same problem today that we had 10 years ago. I believe both Dr. Anderson and Dr. Miller will agree. I was a neophyte in this several years ago, and I still am, compared with these two gentlemen. My interest in the liquids and crystals was proportional to how often I met these fellows. So when I tried to get the money to build a whole-body counter at Purdue, I talked with Dr. Anderson more often than I did with Dr. Miller. The AEC was leaning toward liquids, so I got the liquid scintillation whole-body counter for Purdue, and you have seen some of the results. What I am saying is that when you really get down to specifics on this, they are compatible. The ideal situation would be to have both. When I went to Florida, I built a hybrid. Neither counter worked as well as both of them, but I want Dr. Miller and Dr. Anderson to know that one counter—the hybrid—is doing both things; and I think both gentlemen would agree with the results.

T. G. MARTIN In our attempts to reconstruct the total body or the total carcass from its component parts, our experience has been in contrast with Dr. Lohman's experience. We have almost invariably obtained a higher total potassium content of the parts than we have obtained in the total sample. I can see several reasons why this could be, but our results are in direct contrast with those reported by Dr. Lohman.

One additional comment: The paper from our group which will be in the proceedings, but which was not reported here today, dealing with the effects of length, weight, and level of activity on counter performance, does have a main-effect graph in it on the effects of length on efficiency. Since we held

the weight constant at all lengths, the diameter changed, of course, as was discussed by Dr. Anderson, and at the shortest length, which was 24 in. we did have a lower efficiency than we did at the intermediate length, and then it immediately dropped from the intermediate length to the 66-in. length (which was virtually the length of the counter) back to about the same level it was at 24 in. Now, Dr. Anderson's presentation would explain this beautifully. The lower efficiency with the shorter length and the larger diameter was a reflection of self-absorption; the lower efficiency at the greater length was the result of activity escaping at the ends of the counter. What we concluded on the basis of our study was that if we have a set of samples that encompasses a drastic variation in length of samples, with our particular Sinco-P counter, we must do one of two things. We must either include length in our efficiency calibration curve as a second factor and possibly even as an interaction factor of length and weight, or for each distinct set of samples of different lengths we must compute a separate efficiency weight curve in order to use it in our computer program for calculating potassium content from the results of the scintillation counter work.

T. G. LOHMAN I would like to reply to Dr. Ward's initial question about our ^{42}K calibrations and the possibility of using different breeds with ^{42}K instead of just the Angus breed, and to see if we would get different counting efficiencies with the different breeds. I think that if we did this study, it would probably verify the results we observed. It needs to be done because body conformation does affect the counting efficiency in cattle. However, for the dairy-type conformation, our evidence shows that the potassium distributed in dairy animals counted more efficiently than that in the beef animal. This would thus influence our calibrations.

In response to Dr. Martin's comment, I would like to say that when we sum up the parts for an animal of the Angus breed (which is the one we have used to calibrate our counter for ^{42}K) versus the whole-body potassium, our sum of parts agrees well with the total in the whole body, by the whole-body count. It is when we add the other breeds, which are counted more efficiently, that the whole-body potassium in general overestimates the potassium as determined from the sum of parts.

COMPARATIVE EVALUATION OF METHODS

B. C. Breidenstein
T. G. Lohman
and H. W. Norton
University of Illinois

COMPARISON OF THE POTASSIUM-40 METHOD WITH OTHER METHODS OF DETERMINING CARCASS LEAN MUSCLE MASS IN STEERS

Recognition of the need to define composition of the body more adequately than can be done by live weight within breed or type is indicated by the frequency of use of carcass measurements in current beef cattle research literature. While retail cutout is one of the most useful carcass measurements, the various cutout methods are subject to varying interpretations. The most definable measure of meat production is a chemically derived fat-free or fat-constant boneless muscle mass. Moreover, such a criterion of performance represents a measurement of the primary purpose of meat animal production, namely, the conversion of ingested feed into lean meat. Chemically derived components are often impractical either because of economic limitations or because of the need to retain the animal intact for such purposes as breeding. It is apparent, however, that nondestructive indicators of body composition can be tested most suitably by using a chemically derived entity as a dependent variable. This paper compares several alternative indirect methods of determining body composition with a chemically derived estimate of muscle mass.

PROCEDURE

PRESLAUGHTER TREATMENT

The steers used consisted of four breed types slaughtered at one of four pre-established slaughter weights. In addition, the experimental design included high-energy and medium-energy diets as well as stilbestrol treatment. The design was a 4 x 4 x 2 x 2 factorial conducted in 2 successive years. The distribution within slaughter-weight and breed-type groups for all criteria is shown in Table 1.

TABLE 1 Number of Steers in Each Slaughter Weight-Type Group[a]

Slaughter Weight (kg)	D	B x D	B x B	B	Total
305	5 (3)	7 (4)	6 (3)	8 (3)	26 (13)
385	7 (3)	8 (4)	6 (3)	8 (3)	29 (13)
465	6 (4)	7 (4)	7 (3)	5 (4)	25 (15)
544	7 (4)	6 (4)	4 (2)	6 (3)	23 (13)
TOTALS	25 (14)	28 (16)	23 (11)	27 (13)	103 (54)

[a]D = Dairy type (Holsteins); B x D = Beef x dairy cross (Angus bulls on Holstein cows); B x B = Beef cross (Charolais bulls on Angus cows); B = Beef (Angus). Numbers in parentheses indicate the number of steers in each group that were subjected to whole-body ^{40}K counting.

The animals subjected to whole-body counting were fed a crimped oat diet for 1 week prior to counting so as to minimize the contribution of the contents of the gastrointestinal tract to the whole-body ^{40}K count. Physical measurements including body length, measured from shoulder top to pin, and body circumference, measured at the heart girth, were also taken.

Off-test weight was determined to the nearest 0.45 kg prior to feeding the animal on the weekly weigh date at which the steer was nearest to its pre-established slaughter weight. The steers were also weighed after whole-body ^{40}K counting, and this weight was used in the analysis of the live-animal data.

SLAUGHTER AND DRESSING

Dressing procedure was according to a modified "packer style," the only deviation being the removal of the "heart fat." The carcasses were split down the midline, "shrouded" in a conventional manner, and chilled for 48 hours at 1°C. Chilled side weights were then recorded to the nearest 0.23 kg.

CARCASS MEASUREMENTS

Carcass length was measured to the nearest 0.25 cm on the right side between the anterior edge of the split ischium (aitch bone) and the anterior edge of the first rib. The right side of each carcass was then "ribbed" between the twelfth and thirteenth ribs in the manner prescribed in the USDA Beef Grading Standards[7] for determining fat thickness and *longissimus* area.

Fat thickness was measured at three locations established by first determining the long axis of the *longissimus* cross section (medial to lateral tip) exposed in ribbing at the twelfth rib. All fat thicknesses were measured perpendicular to the outer periphery of the subcutaneous fat cover over the *longissimus*, and at a point established perpendicular to the long axis of the *longissimus*. Fat thicknesses 1, 2, and 3 were measured at points one fourth, one half, and three fourths of the distance from the medial to the lateral tip of the *longissimus*. Tracings of the perimeter of the *longissimus* were made on acetate film for subsequent measurement of cross-sectional *longissimus* area by means of a polar planimeter. Fat thicknesses were measured to the nearest 0.025 cm, and *longissimus* area was measured to the nearest 0.064 sq cm.

Carcass specific gravity was determined between 48 and 54 hr after slaughter by separately suspending the right forequarter and the right hindquarter in water at 1°C and determining the underwater weight to the nearest 5 g. Specific gravity was calculated as follows:

$$\text{sp gr} = \frac{\text{48-hr side weight in air}}{\text{48-hr side weight in air–side weight under water}}.$$

CUTTING AND TRIMMING PROCEDURES

Kidney and pelvic fat includes all body cavity fat from the hindquarter removed to the underlying musculature or skeletal structure. Trimmed hindquarter had the kidney and pelvic fat and the flank removed from the conventional hindquarter. With the hindquarter lying skin side down on a table, the flank including all cod fat was removed by separating on a straight line from a point immediately adjacent to the round muscles and perpendicular to the table to a point on the thirteenth rib lateral to the lateral tip of the *longissimus* by a distance equal to one eighth of the long axis measurement as used in determining fat thickness location.

The standard trimmed lean was derived by removing all bones, trimming the subcutaneous fat to approximately 0.75 cm on the round roasts, rump roast, loin roasts, rib roast, and chuck roasts, and removing as much intermuscular fat as possible, still retaining the shape of the cut. All lean trim from

these major cuts and the boneless mass from the remainder of the carcass was "knife separated" into lean and fat. The sum of the weights of the roasts and lean trim were expressed as a percentage of carcass weight at cutting time (7 to 9 days after slaughter).

Samples for proximal analysis were then taken from the round roasts, rump roast, loin roasts, rib roast, chuck roasts, lean trim from the four major cuts, and lean trim from the remainder of the carcass. The weight of ether-extractable fat[1] was subtracted from the boneless mass weight within each of these components. The weight of the fat-free lean from each of the parts was summed and doubled to derive total carcass lean muscle mass.

POTASSIUM DETERMINATION

All potassium values were determined by means of the liquid scintillation counter described by Twardock *et al.*[6] and Lohman *et al.*[5] Immediately before live ^{40}K counting, steers were washed and then restrained in a cart that was designed specifically for whole-body counting and that afforded standardized positioning in the counter. The data reported here were obtained with the detector tanks closed at the top and open 56 cm at the bottom. Each steer was counted for two consecutive 2-min periods, and the count was adjusted for background by measuring the radiation of the empty counter for 2 to 4 min before and after each body count. Two to 3 days after slaughter, each carcass quarter was counted in the 4-pi geometry counter for two consecutive 2-min periods with 2-min background counts immediately before and after each sample count.

The potassium content from the count was calculated as described by Lohman.[4] The data were analyzed by the method of least squares.

RESULTS AND DISCUSSION

The general characteristics of the animals used in these studies are shown in Tables 2 and 3. The dependent variable used in each regression equation was carcass lean muscle mass (CLMM) expressed as a weight or as a percentage of the carcass. The other variables were used in various combinations to estimate CLMM. In this discussion, CLMM is expressed as a percentage unless otherwise stated. Since the inclusion of fitted constants for breed type generally improved the regressions, they have been included in each of the models with the exception of equations based on data obtained on the live animals where the effect of breed type was negligible (see Tables 8 and 9).

Davis *et al.*,[2] Hedrick *et al.*,[3] and others have demonstrated that skilled operators using ultrasonic devices can measure fat thickness and *longissimus*

TABLE 2 Population Characteristics for 103 Steers Used in Carcass Studies Only

Characteristic	Mean	SD	Coefficient of Variation
Off-test weight (kg)	423.0	85.0	20.1
Carcass weight (kg)	241.0	56.0	23.3
Fat thickness 1 (cm)	1.22	0.75	61.6
Fat thickness 3 (cm)	1.00	0.74	74.2
Fat thickness 3 (cm)	0.73	0.55	74.6
Longissimus area (sq cm)	60.4	8.7	14.4
Kidney and pelvic fat (%)[a]	3.42	1.19	34.6
Carcass length (cm)	118.8	8.0	6.8
Carcass specific gravity	1.0687	0.0142	13.2
Trimmed hindquarter (kg)	45.19	8.62	19.1
Trimmed hindquarter (%)[a]	37.98	2.29	6.2
Side standard trimmed lean (kg)	74.7	14.5	19.4
Side standard trimmed lean (%)[a]	62.77	4.22	6.7
Carcass potassium (g)	629.0	107.0	17.0
Carcass lean muscle mass (kg)	132.8	23.3	17.5
Carcass lean muscle mass (%)[a]	56.2	5.8	10.4

[a] As percentage of carcass.

TABLE 3 Population Characteristics for 54 Steers Subjected to Whole-Body ^{40}K Counting

Characteristic	Mean	SD	Coefficient of Variation
Counter weight (kg)	407.0	80.0	19.7
Fat thickness 2 (cm)	1.00	0.77	7.0
Fat thickness 3 (cm)	0.78	0.62	80.0
Longissimus area (sq cm)	58.6	8.7	14.9
Body length (cm)	117.0	9.6	8.2
Body circumference (cm)	80.0	14.8	8.2
Whole-body potassium (g)	919.0	156.0	17.0
Carcass lean muscle mass (kg)	130.0	22.3	17.1
Carcass lean muscle mass (% of carcass)	54.8	5.9	10.8

area in live cattle. The usefulness of each of three sites of fat-thickness measurement in various combinations with each other and with carcass weight as indicators of CLMM have been studied. The coefficient of variation of CLMM as a percentage of carcass weight was 10.4%. Equation 1 of Table 4 includes only breed-type constants, reducing the coefficient of variation to

TABLE 4 Regression Equations for Predicting Carcass Lean Muscle Mass as a Percentage of Carcass

Independent Variable	Equation Number 1	2	3	4	5	6	7	8	9
Off-test weight (kg)	–	−0.049*	−0.016*	−0.027*	−0.020*	−0.032*	−0.042*	−0.037*	−0.065*
Fat thickness 1 (cm)	–	–	−1.185†	−1.006	–	–	–	–	–
Fat thickness 2 (cm)	–	–	−2.208*	−2.253*	−2.257*	−2.299*	−4.003*	–	–
Fat thickness 3 (cm)	–	–	−4.127*	−3.793*	−4.857*	−4.367*	–	−6.201*	–
Longissimus area (sq cm)	–	–	–	0.120†	–	0.132*	0.175*	0.128†	0.210*
Additive constant	56.25	76.77	69.68	66.81	70.34	67.06	67.24	68.49	71.03
R^2	0.186	0.681	0.833	0.843	0.825	0.837	0.802	0.817	0.714
SE of estimate	5.34	3.36	2.47	2.41	2.51	2.44	2.68	2.57	3.20
Coefficient of Variation	9.5	6.0	4.4	4.3	4.5	4.3	4.8	4.6	5.7

*$P < 0.01$.
†$P < 0.05$.

9.5%. The further inclusion of off-test weight (Equation 2, Table 4) results in a coefficient of variation of 6.0%.

The use of constants for breed type and for fat thicknesses 1, 2, and 3 results in a coefficient of variation of 4.6% (Equation 1, Table 5; compare Table 6), with each fat thickness measurement significant ($P < 0.01$). Addition of carcass weight results in a coefficient of variation of 4.5% (Equation 2, Table 5), with carcass weight and fat thicknesses 2 and 3 highly significant ($P < 0.01$) and fat thickness 1 significant ($P < 0.05$). The remaining equations of Table 5 comprise all possible combinations of the three fat-thickness measurements added to a basic model including breed type and carcass weight. Omission of any one fat thickness results in little increase in the coefficient of variation, fat thickness 3 causing the greatest change. Because of the widespread use of fat thickness 3 in various beef carcass appraisal schemes, Equation 5 of Table 5 is of interest. In predicting weight or percentage of CLMM, fat thickness 3 is equal or superior to either or both fat-thickness measurements 1 and 2, when added to breed type and carcass weight.

Equations 2 through 9 of Table 4 use, as a basic model, constants fitted for breed type and off-test weight. Equation 3 of Table 4, including all three fat-thickness measurements, has a coefficient of variation of 4.4%. Substituting carcass weight for off-test weight (Equation 2, Table 5), results in a coefficient of variation of 4.5% and indicates that live weight and carcass weight are equally effective in predicting percentage of CLMM when combined with the three fat thicknesses and breed type. In Equation 3 of Table 4, fat thicknesses 2 and 3 are highly significant ($P < 0.01$), and fat thickness 1 is significant ($P < 0.05$). The addition of *longissimus* area (Equation 4, Table 4) reduces the coefficient of variation from 4.4 to 4.3%. Fat thickness 1 is not significant, whereas all other criteria are highly significant ($P < 0.01$) except *longissimus* area, which is significant ($P < 0.05$). Omitting fat thickness 1 (Equation 5, Table 4) results in a minor increase in coefficient of variation. When fat thicknesses 2 and 3 and *longissimus* area are included (Equation 6, Table 4), all are significant ($P < 0.01$). Again, because of the widespread use of fat thickness 3 and *longissimus* area in beef evaluation schemes, Equation 8 of Table 4, with a coefficient of variation of 4.6%, is of interest.

Either live weight or carcass weight, and fat thicknesses 2 and 3 and *longissimus* area, have been shown to have merit as predictors of CLMM. Fat thickness 1 may be rejected because (a) virtually no increase in coefficient of variation results from its omission, (b) locating the correct site of measurement is difficult, and (c) minor differences in the medial-lateral location of the site may make appreciable differences in the measured fat thickness. A model including breed type, off-test weight, fat thicknesses 2 and 3, and *longissimus* area (Equation 6, Table 4) has a coefficient of variation of 4.3%

TABLE 5 Regression Equations for Predicting Carcass Lean Muscle Mass as a Percentage of Carcass

Independent Variable	Equation Number							
	1	2	3	4	5	6	7	8
Carcass weight (kg)	–	−0.023*	−0.029*	−0.032*	−0.038*	−0.028*	−0.041*	−0.052*
Fat thickness 1 (cm)	−1.876*	−1.259†	–	−1.291†	–	−1.987*	–	−2.669*
Fat thickness 2 (cm)	−2.999*	−2.204*	−2.233*	–	–	−3.586*	−4.086*	–
Fat thickness 3 (cm)	−4.708*	−4.116*	−4.873*	−5.723*	−6.522*	–	–	–
Additive constant	64.90	68.44	68.94	69.54	70.08	69.04	70.12	71.93
R^2	0.812	0.829	0.820	0.811	0.801	0.800	0.775	0.738
SE of estimate	2.61	2.50	2.55	2.61	2.66	2.69	2.84	3.06
Coefficient of variation	4.6	4.5	4.5	4.7	4.7	4.8	5.1	5.5

*$P < 0.01$.
†$P \leq 0.05$.

TABLE 6 Regression Equations for Predicting Carcass Lean Muscle Mass (kg)

Independent Variable	Equation Number 1	2	3	4	5	6	7	8
Carcass weight (kg)	–	0.511*	0.497*	0.487*	0.473*	0.495*	0.463*	0.430*
Fat thickness 1 (cm)	10.862*	−2.928†	–	−3.015†	–	−5.051*	–	−6.940*
Fat thickness 2 (cm)	11.853†	−5.907*	−5.974*	–	–	−9.935*	−11.205*	–
Fat thickness 3 (cm)	1.224	−12.000*	−13.760*	−16.307*	−18.170*	–	–	–
Additive constant	107.26	28.17	29.34	31.14	32.38	29.92	32.68	37.93
R^2	0.400	0.931	0.928	0.923	0.920	0.916	0.906	0.886
SE of estimate	18.58	6.34	6.44	6.66	6.76	6.96	7.34	8.07
Coefficient of variation	14.0	4.8	4.9	5.0	5.1	5.2	5.5	6.1

*$P < 0.01$.
†$P < 0.05$.

and a standard error of 2.4%, and accounts for 83.7% of the variation. Equation 6 of Table 7, for estimating weight of CLMM, has a coefficient of variation of 5.5% and a standard error of 7.3 kg, and accounts for 90.9% of the variation. Of the variables used which can be measured in the live animal, this model combines precision and expediency appropriately, but it should be remembered that some of the measurements used in this analysis were actually determined on the carcass.

Tables 8 and 9 include only the 54 animals subjected to whole-body ^{40}K counting and various live physical measurements; they received a low-counting diet for 1 week. Lohman[4] found that body length and body circumference added to whole-body count and total body mass resulted in a lower coefficient of variation than when replaced by constants fitted for breed type. The use of a model including whole-body potassium determined by ^{40}K counting, total body mass and body measurements (Equation 5, Table 8) resulted in a coefficient of variation of 3.6% and a standard error of 2.0%, and accounted for 89.3% of the variation. Omitting whole-body potassium from the analysis (Equation 2, Table 8) increases the standard error to 2.9% and the coefficient of variation to 5.2%; thus, addition of whole-body potassium to other easily obtained live measurements decreases the deviation mean square by more than one half. Incorporation of fat thickness and loin eye area measurements into the model (Equations 6 and 7, Table 8) were of little use in further decreasing the standard error.

Tables 10 and 11 use as a basic model the breed-type constants and carcass weight. The lowest error results from inclusion of standard trimmed lean (Equation 7, Table 10) with a coefficient of variation of 1.4% and a standard error of 0.8%, and accounted for 98.2% of the variation. Equation 8 of Table 10, including carcass potassium determined by carcass ^{40}K count, results in a coefficient of variation of 3.7% and a standard error of 2.07%, and accounts for 88.0% of the variation. Carcass specific gravity (Equation 5, Table 10) results in a coefficient of variation of 4.0% and a standard error of 2.23% and accounts for 86.2% of the variation. Equation 9 of Table 10 is worthy of note because it employs a combination of variables widely used in beef carcass evaluation schemes. Carcass weight, fat thickness 3, and percentage of kidney and pelvic fat were all significant ($P < 0.01$), but *longissimus* area was not significant. This combination resulted in a coefficient of variation of 4.0% and a standard error of 2.27%, and accounted for 85.9% of the variation.

Carcass weight and fat thicknesses 2 and 3 (Equation 1, Table 12; compare Table 13) has a coefficient of variation of 4.5%, a standard error of 2.55%, and accounts for 82.0% of the variation, and utilizes only expedient, inexpensive measurements that are totally nondestructive of carcass value. The addition

TABLE 7 Regression Equations for Predicting Carcass Lean Muscle Mass (kg)

Independent Variable	Equation Number 1	2	3	4	5	6	7	8	9
Off-test weight (kg)	–	0.244*	0.310*	0.249*	0.302*	0.243*	0.222*	0.235*	0.181*
Fat thickness 1 (cm)	–	–	−2.398	−1.426	–	–	–	–	–
Fat thickness 2 (cm)	–	–	−3.327	−3.571	−3.427	−3.636	−7.124*	–	–
Fat thickness 3 (cm)	–	–	−9.931*	−8.124*	−11.410*	−8.939*	–	−11.839*	–
Longissimus area (sq cm)	–	–	–	0.648*	–	0.665*	0.753*	0.659*	0.816*
Additive constant	133.00	29.62	15.40	−0.14	16.73	0.21	0.57	2.47	7.31
R^2	0.064	0.837	0.892	0.910	0.890	0.909	0.899	0.906	0.882
SE of estimate	22.86	9.15	7.94	7.28	7.97	7.27	7.59	7.36	8.19
Coefficient of Variation	17.2	6.9	6.0	5.5	6.0	5.5	5.7	5.5	6.2

*$P < 0.01$.

TABLE 8 Regression Equations for Predicting Carcass Lean Muscle Mass as a Percentage of Carcass

Independent Variable	Equation Number 1	2	3	4	5	6	7
Counter weight (kg)	−0.053*	−0.042†	−0.037†	−0.046*	−0.115*	−0.103*	−0.105*
Fat thickness 2 (cm)	–	–	−1.614	–	–	−1.390	−1.54
Fat thickness 3 (cm)	–	–	−1.737	–	–	−0.637	−0.564
Longissimus area (sq cm)	–	–	–	0.090	–	–	0.074
Body length (cm)	–	0.314*	0.182*	0.336*	0.139*	.071	.088
Body circumference (cm)	–	−0.206*	−0.089	−0.236*	−0.092	−0.033	−0.057
Whole-body potassium (g)	–	–	–	–	0.038*	0.034*	0.033*
Additive constant	76.24	72.32	67.77	71.55	66.93	64.91	64.32
R^2	0.510	0.766	0.820	0.772	0.893	0.913	0.917
SE of estimate	4.16	2.94	2.63	2.93	2.01	1.85	1.83
Coefficient of variation	7.4	5.2	4.7	5.2	3.6	3.3	3.3

*$P < 0.01$.
†$P < 0.05$.

TABLE 9 Regression Equations for Predicting Carcass Lean Muscle Mass (kg)

Independent Variable	Equation Number 1	2	3	4	5	6	7
Off-test weight (kg)	0.256*	0.193*	0.198*	0.173*	0.012	0.019	0.012
Fat thickness 2 (cm)	–	–	−0.861	–	–	−0.259	−1.079
Fat thickness 3 (cm)	–	–	−4.148	–	–	−1.176	−0.788
Longissimus area (sq cm)	–	–	–	0.472†	–	–	0.394*
Body length (cm)	–	0.711*	0.531*	0.825*	0.270†	0.232	0.322†
Body circumference (cm)	–	0.042	0.212	−0.118	0.324†	0.364†	0.235
Whole-body potassium (g)	–	–	–	–	0.096*	0.093*	0.088*
Additive constant	25.95	−39.20	−46.58	−43.21	−52.60	−54.30	−57.43
R^2	0.843	0.895	0.903	0.906	0.950	0.951	0.958
SE of estimate	8.89	7.43	7.29	7.11	5.18	5.26	4.90
Coefficient of variation	6.7	5.6	5.5	5.4	3.9	4.0	3.7

*$P < 0.01$.
†$P < 0.05$.

TABLE 10 Regression Equations for Predicting Carcass Lean Muscle Mass as a Percentage of Carcass

Independent Variable	Equation Number 1	2	3	4	5	6	7	8	9
Carcass weight (kg)	−0.038*	−0.110*	−0.052*	−0.085*	−0.014*	−0.022†	−0.016*	−0.238*	−0.032*
Fat thickness 3 (cm)	−6.522*	–	–	–	–	–	–	–	−6.022*
Longissimus area (sq cm)	–	0.297*	–	–	–	–	–	–	0.095
Kidney and pelvic fat (%)	–	–	−1.564*	–	–	–	–	–	−1.427*
Body length (cm)	–	–	–	0.097	–	–	–	–	–
Carcass specific gravity	–	–	–	–	375.134*	–	–	–	–
Trimmed hindquarter (%)	–	–	–	–	–	1.676*	–	–	–
Side standard trimmed lean (%)	–	–	–	–	–	–	1.150*	–	–
Carcass potassium (g)	–	–	–	–	–	–	–	0.090*	–
Additive constant	70.08	64.85	74.09	65.18	−341.32	−2.33	−12.14	56.71	67.49
R^2	0.801	0.746	0.742	0.691	0.862	0.792	0.982	0.880	0.859
SE of estimate	2.67	3.01	3.03	3.32	2.23	2.73	.80	2.07	2.27
Coefficient of variation	4.7	5.4	5.4	5.9	4.0	4.9	1.4	3.7	4.0

*$P \leq 0.01$.
†$P \leq 0.05$.

TABLE 11 Regression Equations for Predicting Carcass Lean Muscle Mass (kg)

Independent Variable	Equation Number 1	2	3	4	5	6	7	8	9
Carcass weight (kg)	0.473*	0.293*	0.420*	0.299*	0.520*	−0.209*	−0.211*	−0.093*	−0.501*
Fat thickness 3 (cm)	−18.170*	–	–	–	–	–	–	–	−17.575
Longissimus area (sq cm)	–	0.654*	–	–	–	–	–	–	0.114
Kidney and pelvic fat (%)	–	–	−3.345*	–	–	–	–	–	−3.185*
Body length (cm)	–	–	–	0.648†	–	–	–	–	–
Carcass specific gravity	–	–	–	–	925.999*	–	–	–	–
Trimmed hindquarter (kg)	–	–	–	–	–	3.856*	–	–	–
Side standard trimmed lean (kg)	–	–	–	–	–	–	2.343*	–	–
Carcass potassium (g)	–	–	–	–	–	–	–	0.256*	–
Additive constant	32.38	23.15	43.52	−16.04	−981.86	8.70	8.46	−5.81	29.32
R^2	0.920	0.883	0.881	0.871	0.931	0.922	0.992	0.962	0.935
SE of estimate	6.76	8.18	8.25	8.58	6.27	6.71	2.08	4.66	6.13
Coefficient of Variation	5.1	6.2	6.2	6.5	4.7	5.1	1.6	3.5	4.6

*$P < 0.01$.
†$P < 0.05$.

TABLE 12 Regression Equations for Predicting Carcass Lean Muscle Mass as a Percentage of Carcass

Independent Variable	Equation Number 1	2	3	4	5	6	7	8	9
Carcass weight (kg)	−0.029*	−0.020	−0.054*	−0.012	−0.011	0.001	−0.005	−0.014*	−0.186*
Fat thickness 2 (cm)	−2.233*	−1.528†	−2.112*	−1.420†	−2.433*	−1.217†	−1.568†	−0.342	−0.571
Fat thickness 3 (cm)	−4.873*	−5.047*	−4.088*	−5.479*	−5.012*	−3.383*	−4.162*	−0.348	−2.491*
Longissimus area (sq cm)	–	0.090	0.165*	–	–	–	–	–	–
Kidney and pelvic fat (%)	–	−1.276*	–	−1.429*	–	–	–	–	–
Body length (cm)	–	−0.062	–	–	−0.142	–	–	–	–
Carcass specific gravity	–	–	–	–	–	291.730*	–	–	–
Trimmed hindquarter (%)	–	–	–	–	–	–	1.206*	–	–
Side standard trimmed lean (%)	–	–	–	–	–	–	–	1.102*	–
Carcass potassium (g)	–	–	–	–	–	–	–	–	0.072*
Additive constant	68.94	72.44	64.43	69.40	81.67	−252.19	15.04	−9.08	58.21
R^2	0.820	0.867	0.835	0.861	0.823	0.906	0.867	0.982	0.895
SE of estimate	2.55	2.23	2.45	2.25	2.54	1.85	2.20	.79	1.95
Coefficient of variation	4.5	4.0	4.4	4.0	4.5	3.3	3.9	1.4	3.5

*$P \leq 0.01$.
†$P < 0.05$.

TABLE 13 Regression Equations for Predicting Carcass Lean Muscle Mass (kg)

Independent Variable	Equation Number 1	2	3	4	5	6	7	8	9
Carcass weight (kg)	0.497*	0.458*	0.456*	0.532*	0.494*	0.566*	0.059*	−0.161*	0.039
Fat thickness 2 (cm)	−5.974*	−4.023†	−5.779*	−4.302†	−5.935*	−3.628†	−3.764†	−0.962	−1.129
Fat thickness 3 (cm)	−13.760*	−14.120*	−12.499*	−15.005*	−13.734*	−10.321*	−11.082*	−1.501	−6.815*
Longissimus area (sq cm)	–	0.136	0.265	–	–	–	–	–	–
Kidney and pelvic fat (%)	–	−2.800*	–	−2.937*	–	–	–	–	–
Body length (cm)	–	0.182	–	–	0.028	–	–	–	–
Carcass specific gravity	–	–	–	–	–	673.479	–	–	–
Trimmed hind-quarter (kg)	–	–	–	–	–	–	2.688*	–	–
Standard trimmed lean (kg)	–	–	–	–	–	–	–	2.207*	–
Carcass potassium (g)	–	–	–	–	–	–	–	–	0.209*
Additive constant	29.34	10.15	22.10	30.29	26.87	−712.00	8.89	8.65	−1.95
R^2	0.928	0.939	0.929	0.939	0.927	0.956	0.951	0.994	0.968
SE of estimate	6.44	5.98	6.36	5.96	6.47	5.00	6.35	2.01	4.28
Coefficient of variation	4.9	4.5	4.8	4.5	4.9	3.8	4.8	1.5	3.2

*$P < 0.01$.
†$P < 0.05$.

of *longissimus* area (Equation 3, Table 12) causes a further small reduction in error.

Another simply obtained criterion under laboratory conditions is specific gravity. Its addition to the basic model (Equation 6, Table 12) resulted in a coefficient of variation of 3.3% and a standard error of 1.85% and accounted for 90.6% of the variation. In the absence of fat thickness measurements (Equation 5, Table 10), a coefficient of variation of 4.0% and a standard error of 2.23% were observed, with 86.2% of the variation accounted for. Thus, it appears that there is merit in combining fat-thickness measurements and specific gravity in estimating CLMM. Carcass potassium combined with fat-thickness measurements (Equation 9, Table 12) results in a coefficient of variation of 3.5% and a standard error of 1.95% and accounts for 89.5% of the variation. Equation 8 of Table 10, which excludes the fat-thickness measurements, results in a coefficient of variation of 3.7% and a standard error of 2.07% and accounts for 88.0% of the variation.

It may sometimes be desirable to proceed to some level of dissection of carcasses or carcass parts as a means of estimating CLMM. The addition of percentage of kidney and pelvic fat to a model including breed type, carcass weight, and fat thicknesses 2 and 3 (Equation 4, Table 12) results in a coefficient of variation of 4.0% and a standard error of 2.25% and accounts for 86.1% of the variation. The exclusion of kidney and pelvic fat (Equation 1, Table 12) results in a coefficient of variation of 4.5% and a standard error of 2.55% and accounts for 82.0% of the variation. Another possibility involving minimal time, effort, and destruction of carcass value is to use trimmed hindquarter. A model including breed type, carcass weight, and percentage of trimmed hindquarter (Equation 6, Table 10) results in a coefficient of variation of 4.9% and a standard error of 2.73% and accounts for 79.2% of the variation. A similar model, but including fat-thickness measurements (Equation 7, Table 12), results in a coefficient of variation of 3.9% and a standard error of 2.20% and accounts for 86.7% of the variation. A comparison of Equations 4 and 7 of Table 12 indicates that adding percentage of kidney and pelvic fat to the basic model was about as effective as adding percentage of trimmed hindquarter. As percentage of kidney and pelvic fat is more easily obtained, it should be preferred to trimmed hindquarter.

It is sometimes possible to do a careful job of semidissection, represented in this study by standard trimmed lean. Equation 7 of Table 10, including breed type, carcass weight, and standard trimmed lean, resulted in a coefficient of variation of 1.4% and a standard error of 0.80% and accounted for 98.2% of the variation. Addition of fat-thickness measurements 2 and 3 (Equation 8, Table 12) did not reduce the error.

SUMMARY

A total of 103 steers representing four breed types and slaughtered at four different weights were used in a study to compare several alternative methods of determining carcass lean muscle mass (CLMM). Fifty-four steers were subjected to whole-body counting after consuming a low-counting diet for a week. Constants for breed type were included in all regressions except those using whole-body potassium and physical measurements of the live animal. The dependent variable was weight or percentage of CLMM.

The inclusion of either live weight or carcass weight in a regression model results in appreciable decrease in the coefficient of variation. Fat thicknesses measured at one half and three fourths of the medial to lateral axis of the *longissimus* were useful criteria, but little or no reduction in the coefficient of variation resulted from including fat thickness measured at one fourth of the medial to lateral axis. Inclusion of *longissimus* area generally reduced the coefficient of variation. Carcass specific gravity was a useful indicator of CLMM, and the coefficient of variation was further reduced by including carcass weight and fat thickness. Trimmed hindquarter was not better than other more easily acquired measures. Whole-body ^{40}K counting resulted in an appreciable reduction in coefficient of variation over other easily obtained measurements of the live animal, and carcass ^{40}K counting resulted in the lowest coefficient of variation of any regression model except that including standard trimmed lean. The model including standard trimmed lean had the lowest coefficient of variation of any model tested, 1.4%, and this was not reduced by including the fat thicknesses.

REFERENCES

1. Association of Official Agricultural Chemists. 1955. Official methods of analysis. 8th ed. Association of Official Agricultural Chemists. Washington, D. C.
2. Davis, J. K., R. S. Temple, W. C. McCormick. 1966. A comparison of ultrasonic estimates of rib-eye area and fat thickness in cattle. J. Anim. Sci. 25:1087.
3. Hedrick, H. B., W. E. Meyer, M. A. Alexander, S. E. Zobrisky, and H. D. Naumann. 1962. Estimation of rib-eye area and fat thickness of beef cattle with ultrasonics. J. Anim. Sci. 21:362.
4. Lohman, T. G. 1967. Distribution of potassium, as determined by whole-body ^{40}K counting, and its relation to carcass composition in steers. Ph.D. Thesis. Univ. Illinois, Urbana.
5. Lohman, T. G., B. C. Breidenstein, A. R. Twardock, G. S. Smith, and H. W. Norton. 1966. Symposium on atomic energy in animal science. II. Estimation of carcass lean muscle mass in steers by ^{40}K measurement. J. Anim. Sci. 25:1218.

6. Twardock, A. R., T. G. Lohman, G. S. Smith, and B. C. Breidenstein. 1966. Symposium on atomic energy. I. The Illinois animal science counter: performance characteristics and animal radioactivity measurement procedures. J. Anim. Sci. 25:1209.
7. U. S. Department of Agriculture, Consumer and Marketing Service, Official United States Standards For Grades of Carcass Beef. 1965. Service and Regulatory Announcement. Consumer and Marketing Service. 99.

R. L. Preston
D. P. Hutcheson
H. B. Hedrick
L. E. Jeremiah
S. E. Zobrisky
and G. F. Krause
University of Missouri

INDICES OF CHEMICAL COMPOSITION IN LIVE OVINES AND THEIR CARCASSES

The major chemical constituents of the body are water, protein, and fat. The strong relationship that exists between these three chemical constituents is due primarily to variations in fat content. This is a well-known relationship and requires little discussion. The content of water and protein tends to be rather constant on a fat-free basis once animals have reached "chemical maturity" as described by Moulton.[7] Thus, if one can estimate the percentage of one of these constituents, estimation of the other two can be made rather accurately.

Research requiring information on body composition would be greatly advanced if techniques could be developed for estimating water, protein, or fat composition without destruction of the body or carcass for chemical analysis. Several techniques have been studied and employed for this purpose. They include potassium as an indicator of body protein composition, specific gravity as an indicator of fat composition, and various empirical measures such as fat trim, fat thickness, weight, and several others.

The work reported in this paper relates several of these direct and empirical measures to actual chemical composition of lamb carcasses.

METHODS

Fifty-six lambs (28 ewes and 28 wethers) of Texas origin near 6 to 7 months of age and with an initial weight ranging from 26.75 to 39.75 kg were used in this study. The lambs were divided into two outcome groups on the basis of initial relative "condition." Two ewes and two wethers from each outcome group were slaughtered initially for carcass composition measurements. The remaining lambs in each outcome group were divided equally on the basis of sex and assigned to three lots, giving a total of six lots with four ewes and four wethers each. All lambs were shorn immediately prior to the start of this experiment.

The lambs were fed *ad libitum* a "finishing ration" containing cottonseed hulls, steam-crimped corn, soybean meal, cane molasses, minerals, and vitamins. The ration was calculated to contain 105 g of crude protein and 2,670 kcal of digestible energy per kilogram of ration. One of three minor dietary variables was fed to one lot from each outcome group.

Following the initial slaughter of four ewes and four wethers, the remaining 48 lambs were slaughtered in groups of 12 every third week. At each of these subsequent slaughter times, one ewe and one wether were slaughtered from each lot; therefore, there was one lamb representing each treatment, sex, and outcome group combination at each slaughter period. Feed was removed 20 hr and water was removed 12 hr prior to slaughter.

On each slaughter date, the wether lambs were thoroughly washed once with water, and ^{40}K emission was determined with the whole-body counter. All lambs were then slaughtered, and various parts from each lamb were weighed, including the full and empty gastrointestinal tract, pelt, and wool. These weights were subsequently used to calculate the wool-free empty-body weight of each lamb. The warm carcass was weighed prior to washing, then washed and reweighed. After a 24-hr chilling period near 3° C, the carcasses were weighed again. At this time, ^{40}K emission was determined on all carcasses. Specific gravity was determined by water displacement. Approximately 24 hr later (48 hr after slaughter), the carcasses were split on the band saw. The right side was used to determine carcass cutout, namely, fat trim and retail yield, according to the methods of Kemp[4] and Ringkob *et al.*[9] The left side was physically dissected into soft tissue (muscle and fat) and bone; these samples were individually ground twice through a meat grinder that had plates with 3-mm openings, thoroughly mixed, and sampled for chemical analysis.

COUNTING PROCEDURE FOR ^{40}K EMISSION

On each slaughter date, all wethers were thoroughly washed once* and counted alive in the University of Missouri 2-pi, 6-cell, low-level, liquid scintillation counter as described by Lohman *et al.* in the article on "Factors Affecting ^{40}K Measurement in the Whole Body and Body Components" (this volume, page 291). Twenty-four hours later, the chilled carcasses from all lambs (wethers and ewes) were similarly counted.

Counts were made for 5 min with the samples placed approximately 1.2 cm from the surface of the detectors; periodic 5-min background counts were made throughout the counting period.

Calculation of potassium concentration was based on factors including the ^{40}K emission scheme, ^{40}K content in potassium, counter efficiency, and background damping due to the mass of the counting sample. Estimated K in the live animal was recorded as percentage of the wool-free shrunk body weight, and the estimated K in the carcass was recorded as percentage of the warm carcass weight.

SPECIFIC GRAVITY

Specific gravity was determined on all carcasses after a 24-hr chill at near 3°C by displacement of water at the same temperature as the carcasses. Special care was taken to free any entrapped air in the carcasses.

OMENTAL FAT

At slaughter, the omental fat associated with the gastrointestinal tract was weighed and the amount was expressed as a percentage of the wool-free empty-body weight and as a percentage of the empty gastrointestinal tract.

KIDNEY FAT

At the time the carcasses were being separated for retail yield measurement and sampled for chemical analysis, the fat that could be pulled easily from the carcass in association with each kidney was weighed and expressed as a percentage of the warm carcass weight.

*Preliminary work indicated that further washing did not significantly reduce the counting rate of the animal.

FAT TRIM AND RETAIL YIELD

The wholesale cuts from the right side were processed into retail cuts; the subcutaneous and internal fat were trimmed to a maximum thickness of 0.6 cm. The amount of fat trim was expressed as a percentage of the 48-hr carcass weight; the trimmed wholesale cuts along with any lean trim were expressed as a percentage of the 48-hr carcass weight and called retail yield of the carcass.

FAT THICKNESS MEASUREMENTS

Fat thickness was measured on the right side of the carcass at the time the carcasses were being cut and separated. Measurements taken are shown in Figure 1.

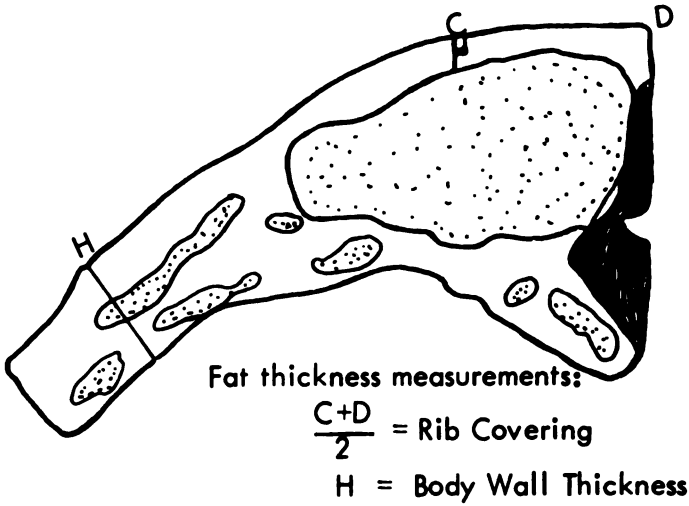


FIGURE 1 Cross section of lamb loin at the last rib.

CARCASS GRADE

Carcass grade was determined on the carcasses after a 24-hr chill; specifications in the current USDA grading system[11] were employed.

CHEMICAL PROCEDURES

Moisture, fat, nitrogen, sodium, and potassium were chemically determined* on the soft tissue sample (including the kidney and kidney fat) and bone

*Analyses performed by Missouri Agricultural Experiment Station chemical laboratories.

sample from the left side of each carcass according to the procedures outlined by AOAC.[1] The actual chemical composition of the warm carcass was reconstructed on the basis of the amount of each chemical constituent found in the soft tissue and bone sample. Any change in weight from the warm carcass weight was assumed to be moisture.

STATISTICAL ANALYSES

Correlation-regression analyses were performed on the data from all lambs; moisture, fat, and nitrogen content of the warm carcass were used as dependent variables. Similarly, correlation-regression analyses were performed on the estimated live animal potassium content (^{40}K) and the carcass potassium content (^{40}K) on the 28 wether lambs. Regression analyses were also performed on each subgroup to determine if there were any significant differences between the regression coefficients and adjusted means of the two outcome groups, two sex groups, and three treatment groups. No differences were found; therefore, the data were pooled. Procedures were those outlined by Snedecor.[10] Arc-sin transformation of the data expressed as a percentage did not materially change the correlation coefficients.

RESULTS

INDICES OF FAT CONCENTRATION IN OVINE CARCASSES

Table 1 shows the relative value of various indices of fat concentration in the lamb carcasses observed in this study. A high relationship was found between fat concentration and water concentration, as has been reported by a number of other workers. There was also a strong relationship between nitrogen concentration and fat concentration. The relation between potassium concentration and fat concentration was not as high as expected; only 62% of the variation in fat concentration was associated with variations in potassium concentration. The calculated potassium content on a fat-free basis from the regression equation yields a value of 0.318% or 81 mEq/K/kg of fat-free tissue. Of the total potassium in the carcass, 11% (range, 8 to 14%) was present in the bone. A much higher relationship was found between sodium concentration and fat concentration. This probably reflects a strong negative correlation between the proportion of bone and the proportion of fat, since 49% (range, 45 to 57%) of the total carcass sodium was present in the bone. The relationship between potassium as estimated by ^{40}K emission, and fat concentration was rather poor; thus, only 25% of the variation in fat concentration was associated with variations in potassium as estimated by ^{40}K. Specific gravity rather effectively estimated fat concentration; over 75% of

TABLE 1 Indices of Fat Concentration (%) in Ovine Carcasses[a]

Independent Variable	Correlation Coefficient	b	c	$S_{y \cdot x}$
DIRECT MEASURES				
Water (%)	−0.991	-1.29	9.56×10^{1}	0.856
Nitrogen (%)	−0.931	-2.95×10^{1}	1.01×10^{2}	2.30
Potassium (%)	−0.789	-2.50×10^{2}	7.96×10^{1}	3.88
Potassium (%) (^{40}K)	−0.495	-1.37×10^{2}	5.46×10^{1}	5.49
Sodium (%)	−0.896	-4.66×10^{2}	7.62×10^{1}	2.81
Specific gravity	−0.871	-4.33×10^{2}	4.82×10^{2}	3.10
EMPIRICAL MEASURES				
Fat trim (%)	0.945	1.55	9.55	2.07
Retail yield (%)	−0.887	−1.56	1.57×10^{2}	2.92
Omental fat (%)[b]	0.816	4.89	1.41×10^{1}	3.66
Kidney fat (%)[c]	0.792	7.19	1.48×10^{1}	3.85
Rib covering (cm)	0.870	1.76×10^{1}	1.62×10^{1}	3.11
Body wall (cm)	0.892	8.45	1.08×10^{1}	2.86
Wool-free empty-body weight (kg)	0.750	7.48×10^{-1}	−1.07	4.18
Warm carcass weight (kg)	0.792	1.10	8.49×10^{-1}	3.86
24-hr cold carcass weight (kg)	0.797	1.12	6.56×10^{-1}	3.81
Carcass grade[d]	0.683	3.52	−2.26	4.61

[a] Average fat concentration = 25.88% (S = 6.26%).
[b] Percentage of wool-free empty-body weight.
[c] Percentage of warm carcass weight.
[d] Prime (+) = 12; prime = 11; prime (-) = 10; choice (+) = 9; choice = 8; choice (-) = 7; good (+) = 6; good = 5; good (-) = 4.

the variation in fat concentration was associated with differences in specific gravity.

Several empirical measures were rather effective estimators of fat concentration. Thus, percentage of fat trim, percentage of retail yield, and body wall thickness were all highly associated with fat concentration. Also, various body and carcass weights were rather highly associated with fat concentration.

INDICES OF NITROGEN CONCENTRATION IN OVINE CARCASSES

The statistical summary concerning nitrogen concentration in ovine carcasses is shown in Table 2. In general, the relationships were of magnitudes similar to those observed for fat concentration. The indicated Y-intercept for the concentration of nitrogen on a fat-free basis, 3.30%, is equivalent to 20.6% of crude protein, which compares favorably with a range of 18.9% to 21.2% reported by Kirton *et al.*[6]

INDICES OF WATER CONCENTRATION IN OVINE CARCASSES

The statistical summary concerning water concentration is shown in Table 3. The magnitude of the statistical data is similar to that observed for fat and nitrogen concentration. The indicated Y-intercept for the concentration of water on a fat-free basis is 73.7%, which compares favorably with a range of 73.2 to 75.0% reported by Kirton *et al.*[6]

RELATION BETWEEN POTASSIUM-40 CONCENTRATION IN THE LIVE ANIMAL AND THE CONCENTRATION OF FAT, NITROGEN, WATER, AND POTASSIUM IN THE SUBSEQUENT CARCASS

Table 4 shows the statistical data relating carcass composition as estimated in the live animal by ^{40}K emission. The correlation coefficients are quite low; the standard errors of estimate are almost as high as the standard deviations observed for the dependent variables themselves.

POTASSIUM-40 CONCENTRATION IN THE CARCASS AS AN ESTIMATOR OF ACTUAL POTASSIUM CONCENTRATION IN THE CARCASS

Table 5 shows the pertinent statistical data for comparing ^{40}K in the carcass as an estimator of actual potassium concentration in the carcass. The actual data relating these two variables are plotted in Figure 2. The correlation coefficient is quite low, indicating that at best only 26% of the variation in actual

TABLE 2 Indices of Nitrogen Concentration (%) in Ovine Carcasses[a]

Independent Variable	Correlation Coefficient	b	c	$S_{y \cdot x}$
DIRECT MEASURES				
Water (%)	0.889	3.66×10^{-2}	5.64×10^{-1}	0.0913
Fat (%)	−0.931	-2.94×10^{-2}	3.30	0.073
Potassium (%)	0.787	7.89	8.48×10^{-1}	0.123
Potassium (%) (^{40}K)	0.496	4.32	1.63	0.173
Sodium (%)	0.829	1.36×10^{1}	1.06	0.112
Specific gravity	0.821	1.29×10^{1}	-1.10×10^{1}	0.114
EMPIRICAL MEASURES				
Fat trim (%)	−0.894	-4.63×10^{-2}	3.03	0.090
Retail yield (%)	0.834	4.65×10^{-2}	−1.34	0.110
Omental fat (%)[b]	−0.786	-1.49×10^{-1}	2.90	0.123
Kidney fat (%)[c]	−0.714	-2.05×10^{-1}	2.86	0.140
Rib covering (cm)	−0.805	-5.15×10^{-1}	2.82	0.118
Body wall (cm)	−0.865	-2.59×10^{-1}	3.00	0.100
Wool-free empty-body weight (kg)	−0.746	-2.35×10^{-2}	3.39	0.133
Warm carcass weight (kg)	−0.780	-3.44×10^{-2}	3.32	0.125
24-hr cold carcass weight (kg)	−0.783	-3.48×10^{-2}	3.32	0.124
Carcass grade[d]	−0.635	-1.04×10^{-1}	3.37	0.154

[a] Average nitrogen concentration = 2.541% (S = 0.198%).
[b] Percentage of wool-free empty-body weight.
[c] Percentage of warm carcass weight.
[d] Prime (+) = 12; prime = 11; prime (-) = 10; choice (+) = 9; choice = 8; choice (-) = 7; good (+) = 6; good = 5; good (-) = 4.

TABLE 3 Indices of Water Concentration (%) in Ovine Carcasses[a]

Independent Variable	Correlation Coefficient	b	c	$S_{y \cdot x}$
DIRECT MEASURES				
Nitrogen (%)	0.889	2.16×10^{1}	-8.98×10^{-1}	2.22
Fat (%)	−0.991	-7.61×10^{-1}	7.37×10^{1}	0.657
Potassium (%)	0.761	1.85×10^{2}	1.42×10^{1}	3.15
Potassium (%) (^{40}K)	0.511	1.08×10^{2}	3.12×10^{1}	4.17
Sodium (%)	0.875	3.50×10^{2}	1.62×10^{1}	2.34
Specific gravity	0.861	3.28×10^{2}	-2.92×10^{2}	2.47
EMPIRICAL MEASURES				
Fat trim (%)	−0.924	−1.16	6.63×10^{1}	1.86
Retail yield (%)	0.885	1.20	-4.61×10^{1}	2.26
Omental fat (%)[b]	−0.803	−3.70	6.29×10^{1}	2.89
Kidney fat (%)[c]	−0.786	−5.48	6.25×10^{1}	3.00
Rib covering (cm)	−0.859	-1.34×10^{1}	6.13×10^{1}	2.48
Body wall (cm)	−0.880	−6.40	6.54×10^{1}	2.30
Wool-free empty-body weight (kg)	−0.722	-5.52×10^{-1}	7.39×10^{1}	3.36
Warm carcass wt. (kg)	−0.765	-8.18×10^{-1}	7.26×10^{1}	3.13
24-hr. cold carcass wt. (kg)	−0.771	-8.33×10^{-1}	7.27×10^{1}	3.09
Carcass grade[d]	−0.655	−2.59	7.47×10^{1}	3.67

[a] Average water concentration = 54.01% (S = 4.81%).
[b] Percentage of wool-free empty-body weight.
[c] Percentage of warm carcass weight.
[d] Prime (+) = 12; prime = 11; prime (-) = 10; choice (+) = 9; choice = 8; choice (-) = 7; good (+) = 6; good = 5; good (-) = 4.

TABLE 4 Relation between Potassium (^{40}K) Concentration (%) in the Live Ovine and Concentration (%) of Fat, Nitrogen, Water, and Potassium in the Warm Carcass

Dependent Variable	Correlation Coefficient	b	c	$S_{y \cdot x}$
Carcass fat (%)[a]	−0.417	-2.35×10^{2}	6.23×10^{1}	5.91
Carcass N (%)[b]	0.389	6.65	1.49	0.182
Carcass H_2O (%)[c]	0.397	1.75×10^{2}	2.68×10^{1}	4.69
Carcass K (%)[d]	0.518	9.89×10^{-1}	5.64×10^{-2}	0.0189

[a] Average fat concentration = 25.88% (S = 6.26%).
[b] Average nitrogen concentration = 2.541% (S = 0.198%).
[c] Average water concentration = 54.01% (S = 4.81%).
[d] Average potassium concentration = 0.2146% (S = 0.0197%).

TABLE 5 Warm Carcass Potassium (^{40}K) Concentration as an Estimator of Actual Potassium Concentration (%) in the Warm Carcass

Item	Statistic
Average K concentration in warm carcass (%)	0.2146 (S = 0.0197%)
Correlation coefficient (r)	0.510
Regression coefficient (b)[a]	4.44×10^{-1}
Standard error of b	1.02×10^{-1}
Y-intercept (c)	1.21×10^{-1}
$S_{y \cdot x}$	1.71×10^{-2}

[a]Significantly different from $\beta = 1.0$, $P < 0.01$.

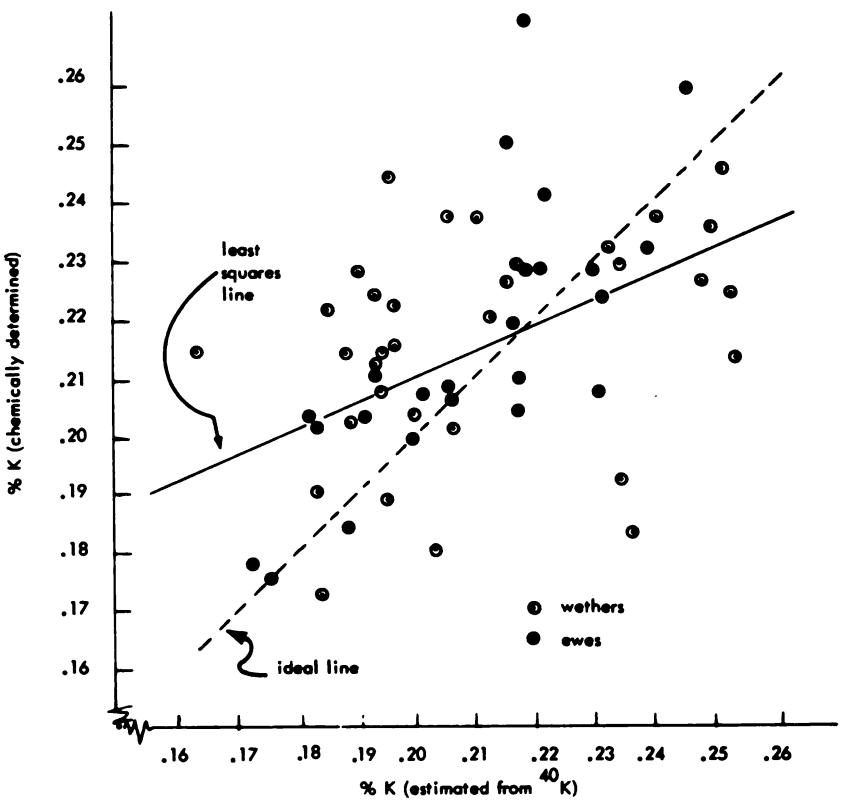


FIGURE 2 Relation between potassium concentration in ovine carcasses as determined chemically and estimated from ^{40}K emission.

potassium concentration of the carcass was associated with ^{40}K emission. Furthermore, the actual regression coefficient (0.444) differs significantly ($P < 0.01$) from the theoretical ideal. These are probably the major reasons that ^{40}K emission was not related to a higher degree with fat, nitrogen, and water concentration in the carcass (see Tables 1, 2, and 3). The mean potassium concentration in these carcasses (0.2146%) was somewhat higher than the mean potassium concentration (0.2104%) as estimated from ^{40}K emission.

DISCUSSION

RELATIVE VALUE OF VARIOUS INDICES OF CARCASS COMPOSITION

All the indices reported in this paper were related to the various carcass components to varying degrees. Therefore, evaluating these indices in terms of their real potential as estimators of carcass composition becomes a problem. One method would be to calculate the coefficient of variation by using the standard error of estimate and the average value observed for the particular carcass component of interest. However, this method does not give a true picture of relative merit because certain carcass components do not vary over a wide range (e.g., nitrogen concentration). A preferred method would be to evaluate the amount of variation in the dependent variable that is associated with variation in the independent variable (R^2). It would seem that at least 60% of the variation in the dependent variable should be associated with the independent variable before it will serve effectively as an estimator of body composition.

With this method of evaluating the various indices, all of the indices listed in Table 1 are valid estimators of fat concentration except for ^{40}K emission, wool-free empty-body weight, and carcass grade. Similarly in Table 2, all indices listed are acceptable estimators of nitrogen concentration except for ^{40}K emission, kidney fat percentage, wool-free empty-body weight, and carcass grade. Of the indices listed for water concentration, percentage of potassium, ^{40}K emission, wool-free empty-body weight, warm carcass weight, 24-hr cold carcass weight, and carcass grade (Table 3) do not meet the grade criteria given in Tables 1 and 2. When one compares the correlation coefficients shown in Table 4 with the grade criteria, none of the carcass components listed can be effectively estimated by ^{40}K emission in the live animal. Similarly in Table 5, only 26% of the variation in potassium concentration in the warm carcass was associated with ^{40}K emission, which indicates it is a rather poor estimator of actual potassium concentration in the same carcass. This is about

the same reliability with which concentration of K in the live animal (^{40}K) predicted concentration of K in the warm carcass.

NONDESTRUCTIVE ESTIMATORS OF CARCASS COMPOSITION

Of the indices that do not require grinding and chemical analysis of the carcass, the most valuable estimators of fat, nitrogen, and water concentration in the ovine were, in decreasing order of reliability, percentage of fat trim, body wall thickness, retail yield, specific gravity of the 24-hr cold carcass, rib covering, omental fat as a percentage of wool-free empty-body weight, 24-hr cold carcass weight, kidney fat as a percentage of warm carcass weight, warm carcass weight, wool-free empty-body weight, carcass grade, and ^{40}K emission. Some of these indices, however, are possible artifacts and would apply only under the conditions in which they were determined. This would be especially true for wool-free empty-body weight, warm carcass weight, and 24-hr cold carcass weight.

The equation relating fat content to specific gravity gives an apparent specific gravity of lamb fat near 0.882, and 1.11 for the specific gravity of the fat-free body mass. These compare with 0.900 for subcutaneous lamb fat[2] and 1.10 for lean body mass.[5] The equation found in our work is slightly different from that found by Garrett *et al.*[3] for lambs; however, when the mean specific gravity observed in this paper is used, the estimated fat concentration would be 24.4% and 25.7%, respectively, for the equation of Garrett *et al.*[3] and for the one presented in this paper.

Pitts and Hollifield[8] have published data indicating that fatness in the total body of obese guinea pigs deviates from that estimated from the eviscerated carcass when the carcass contains more than 28% of fat. When omental fat (percentage of the empty gastrointestinal tract) is plotted as a function of carcass fat (Figure 3), there is no indication of a change in trend as carcass fat concentration increases. If the relationship shown here is a valid measure, it can be assumed that fatness of the carcass and fatness of the viscera in lambs tend to parallel each other over the range in fatness observed in this study.

SUMMARY

A total of 56 lambs (28 ewes and 28 wethers) were used to study several indices of ovine carcass composition. These indices included ^{40}K emission from the live animal as well as the carcass and several other direct and empirical measures of carcass composition. ^{40}K emission did not effectively predict

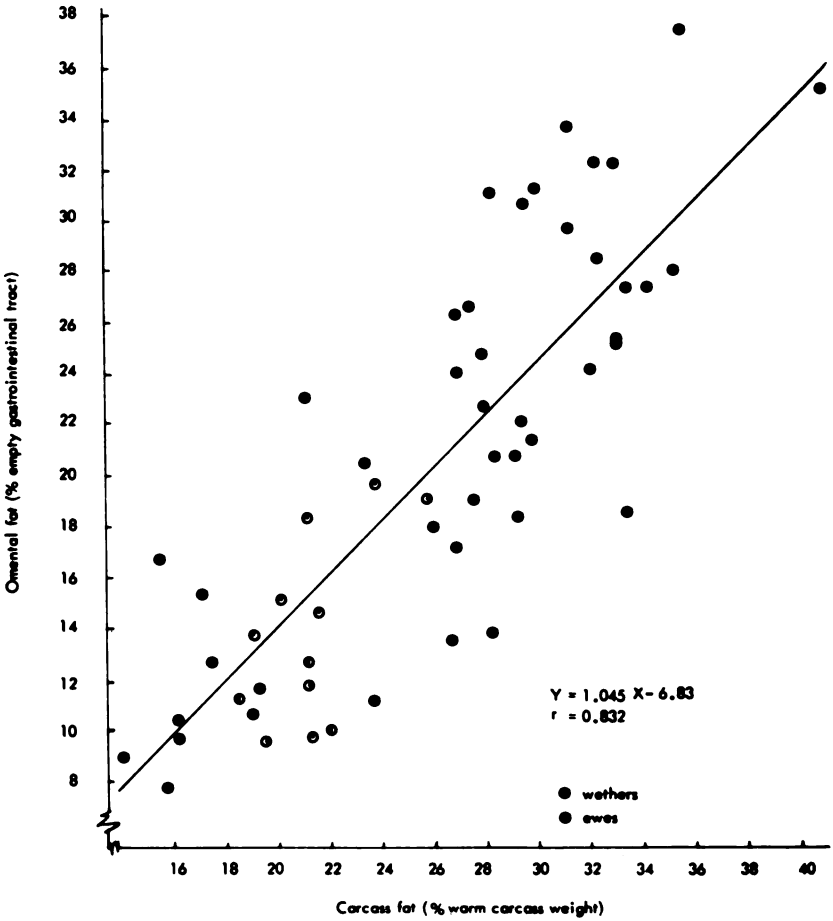


FIGURE 3 Relation between omental fat content of the viscera and carcass fat concentration.

carcass composition in either the live animal or the carcass. Several other carcass indices estimated carcass composition. Those with the highest reliability include percentage of fat trim, body wall thickness, retail yield, specific gravity of the 24-hr cold carcass, rib covering, omental fat as a percentage of wool-free empty-body weight, 24-hr cold carcass weight, kidney fat as a percentage of warm carcass weight, and warm carcass weight. The statistical parameters for each of these indices are included in this paper.

This work was supported in part by Public Health Service Research Grant 03292, from the National Institutes of Health.

This paper is a contribution from the Missouri Agricultural Experiment Station, Journal Series Number 5137, and its use is approved by the Director.

Assistance rendered by the following persons is gratefully acknowledged: O. Lewis, S. Grebing, P. Telle, C. Kinsey, J. Looney, G. Poe, D. Woodson, M. Alexander, W. Coffman, K. Flack, B. Schofield, and M. Sagebiel.

REFERENCES

1. Association of Official Agricultural Chemists. 1965. Methods of analysis. 10th ed. Association of Official Agricultural Chemists, Washington, D. C.
2. Fidanza, F., A. Keys, and J. F. Anderson. 1953–1954. Density of fat in man and other mammals. J. Appl. Physiol. 6:352.
3. Garrett, W. N., J. H. Meyer, and G. P. Lofgreen. 1959. The comparative energy requirements of sheep and cattle for maintenance and gain. J. Anim. Sci. 18:528.
4. Kemp, J. D. 1952. Methods of cutting lamb carcasses. Fifth Ann. Recip. Meat Conf., Proc. p. 89.
5. Keys, A., and J. Brožek. 1953. Body fat in adult man. Physiol. Rev. 33:245.
6. Kirton, A. H., M. J. Ulyatt, and R. A. Barton. 1959. Composition of some fat-free carcasses and bodies of New Zealand sheep. Nature 184:1724.
7. Moulton, C. R. 1923. Age and chemical development in mammals. J. Biol. Chem. 57:79.
8. Pitts, G. C., and G. Hollifield. 1963. Fatness of the total body as estimated from measurements on the eviscerated carcass. Science 141:718.
9. Ringkob, T. P., S. E. Zobrisky, C. V. Ross, and H. D. Naumann. 1964. Measurements of muscle and retail cuts of lamb. Mo. Agr. Exp. Sta. Res. Bull. 876.
10. Snedecor, G. H. 1956. Statistical methods applied to experiments in agriculture and biology. 5th ed. Iowa State College Press, Ames.
11. U. S. Department of Agriculture. 1960. Official U.S. standards for grades of lamb, yearling mutton, and mutton carcasses. Agr. Marketing Service.

T. G. Martin
E. G. Stant, Jr.
W. V. Kessler
M. D. Judge
and J. E. Christian
Purdue University

ASSOCIATIONS OF BODY POTASSIUM, CHEMICAL COMPOSITION, AND CARCASS MEASUREMENTS WITH FAT-LEAN COMPOSITION DETERMINED BY PHYSICAL SEPARATION

Research workers have investigated several indirect estimators of total body and carcass composition. Complete chemical analysis of the body or complete physical separation into fat and lean components, or both, are costly and time consuming. Furthermore, such destructive techniques cannot be readily used on human subjects or on animals intended for use in a breeding herd.

Exceptionally high associations have been reported between potassium content and lean body mass in humans by Muldowney *et al.*[17] and in humans and rats by Talso *et al.*[20] Kirton *et al.*[13] reported correlations of percentage of potassium in the empty body of pigs (82 to 100 kg) with percentage of water, 0.86 to 0.87; with percentage of ether extract, –0.87 to –0.89; and with percentage of protein, 0.76 to 0.78. Kirton and Pearson[14] found the potassium content of the carcasses of lambs to be correlated with composition data on the dressed carcass as follows: 0.81 with percentage of water, –0.87 with percentage of ether extract, 0.94 with percentage of protein, 0.81 with percentage of separable lean, –0.92 with percentage of separable fat, and 0.81 with percentage of separable bone.

Use of potassium, as estimated by liquid scintillation counting of the naturally occurring potassium-40 isotope, to estimate body composition has

had variable degrees of success. Anderson[1] and Zobrisky *et al.*[23] first suggested the possible use of potassium-40 in estimating fat–lean composition of animals. Martin *et al.*[16] reported grams of potassium per kilogram of live pig to be significantly correlated (–0.79) with back-fat thickness, a commonly used estimator of body composition. Breidenstein *et al.*[5] reported that a model including breed, sex, live weight, carcass weight, live animal ^{40}K count, and carcass ^{40}K count accounted for 91.3% of the variation in carcass lean muscle mass and yielded a standard error of estimate of 1.12 kg of carcass lean muscle mass in pigs averaging 95 kg live weight. Judge *et al.*[12] reported ^{40}K counts per minute per lb of live weight to be significantly negatively correlated with excess fat expressed as percentage of live weight in three of four groups of lambs. Breidenstein *et al.*[4] reported that a model including sex, age, live weight, carcass weight, potassium content of the live animal, and potassium content of the carcass accounted for 87.0% of the variation in carcass lean muscle mass and yielded a standard error of estimate of 0.28 kg of carcass lean muscle mass in lambs averaging 33.4 kg of live weight.

Relatively simple carcass measurements have been shown to be of value in predicting muscle proportions and retail yield or retail value, or both, of lamb carcasses by Hoke,[10] Zinn,[22] Hiner and Thornton,[9] Field *et al.*,[8] and Carpenter *et al.*[6] However, the time required to obtain measurements by carcass separation may discourage extensive use of these prediction equations.

Two experiments are reported here. The objective of the first was to investigate the associations between potassium content of the live animal, potassium content of the carcass, chemical composition of the carcass, and fat–lean composition of the carcass as determined by physical separation in pigs ranging from 23 to 91 kg of live weight. The objective of the second was to compare the accuracy of carcass measurements with the accuracy of potassium content as estimators of the edible portion content of lamb carcasses.

EXPERIMENTAL METHODS

EXPERIMENT ONE

Two groups of 24 cross-bred barrow pigs were used. The first group consisted of 24 Yorkshire x Chester White barrows reared in the fall and winter of 1962, and the second group consisted of 24 Yorkshire x Duroc barrows reared in the spring and summer of 1963. Each breed group consisted of four randomly selected barrows from each of six litters. One of the four members of each litter was randomly assigned to each of four slaughter-weight groups. Slaughter-weight groups consisted of pigs to be sacrificed at 23, 46, 68 and 91 kg of live weight.

All pigs were weighed weekly, and each pig was subjected to ^{40}K counting (4-min counts) in the large-volume, liquid scintillation counter at the time it reached its assigned slaughter weight. After ^{40}K counting, the pigs were sacrificed, and each was divided into carcass and noncarcass components. The noncarcass component consisted of blood, head, and all internal organs. Carcass and noncarcass components were weighed, and ^{40}K emissions were counted in the large-volume, liquid scintillation counter at 24 hr post-mortem.

Each carcass was physically separated into muscle, fat, and bone components. These components were weighed, and ^{40}K emissions were counted in the large volume, liquid scintillation counter. The muscle component was ground and mixed thoroughly, and two 237-ml sample jars were filled for use in subsequent chemical analyses. The fat component was ground and mixed thoroughly, and one 237-ml sample jar was filled for subsequent chemical analyses. In the absence of an adequate method of grinding the entire skeleton, the femurs and tibias were used as a sample of the skeleton. The shafts of these bones were cut into discs 0.15 to 0.30 cm thick, and the discs were stored in sample jars. All samples were frozen and held at -18°C.

Analyses of all muscle, fat, and bone samples for protein (Kjeldahl), ether extract, and moisture were performed as described by AOAC[2] and Benne *et al.*[3] Potassium content was determined by a modification of the method described by Willis.[21] Protein, ether extract, and moisture were expressed as percentages, and potassium was expressed as grams per kilogram of tissue. Total kilograms of protein, ether extract, and moisture, and total grams of potassium were calculated for each sample by using the above-described proportions and sample weights. On the basis of the chemical analyses and sample weights, additional variables, such as fat-free muscle, were generated.

EXPERIMENT TWO

Fifty-one purebred and cross-bred lambs of Cheviot, Hampshire, Rambouillet, Shropshire, and Southdown breeding, both ewes and wethers, were available for this study. Live weight and age at slaughter were known. Each lamb was subjected to ^{40}K counting in the large-volume, liquid scintillation counter prior to slaughter, and the results were used to calculate grams of potassium per kilogram of weight and total grams of potassium. Prior to counting, the lambs were shorn and washed thoroughly.

At 48-hr post-mortem, each carcass was weighed and counted in the large-volume, liquid scintillation counter. Carcass measurements taken have been described by Judge *et al.*[11] and consist of (a) a mean of three fat-thickness measurements over the *longissimus dorsi* muscle, (b) a single (one of the above three) fat-thickness measurement over the *longissimus dorsi* muscle, (c) a single fat-thickness measurement over the lower rib, (d) *longissimus dorsi*

area, (e) chilled carcass weight, (f) kidney and kidney fat weight, and (g) leg and loin weight. The carcasses were then separated into edible portion, excess fat, and bone.

STATISTICAL ANALYSIS

Data in Experiment 1 were subjected to the analysis of variance to determine the magnitude of the effects of weight group and of the breed or season, or both. Linear regression (both simple and multiple) and correlation techniques were used in both experiments to estimate correlation and regression coefficients and standard errors of estimate.

RESULTS AND DISCUSSION

EXPERIMENT ONE

Means and standard errors of the ages and weights of the live pigs and of the weights of the various component parts are shown in Table 1. Because of the loss of one pig in each of the two 91-kg breed groups, these groups involve

TABLE 1 Means and Standard Errors of Age at Slaughter, Weight of Live Pigs, and Weights of Component Parts

Variable	Breed[a]	Weight Group Means				SE[b]
		23 kg	46 kg	68 kg	91 kg	
Age (days)	Y-C	57.3	89.8	116.2	155.0	2.3
	Y-D	68.7	94.3	121.2	152.6	
Live pig (kg)	Y-C	25.2	47.2	71.7	91.0	0.9
	Y-D	24.9	45.2	68.0	90.8	
Noncarcass (kg)	Y-C	9.0	12.7	18.3	22.6	0.5
	Y-D	9.0	12.5	17.9	21.5	
Carcass (kg)	Y-C	16.2	33.2	52.1	67.6	0.7
	Y-D	16.8	31.0	48.9	67.6	
Carcass muscle (kg)	Y-C	9.7	17.5	23.8	31.9	0.5
	Y-D	10.3	17.9	25.7	32.1	
Carcass fat (kg)	Y-C	2.0	7.7	16.2	21.9	0.6
	Y-D	1.4	5.3	12.6	22.2	
Carcass bone (kg)	Y-C	4.2	7.0	9.8	12.6	0.3
	Y-D	4.6	6.8	9.9	12.9	

[a] Y-C = Yorkshire x Chester White; Y-D = Yorkshire x Duroc.
[b] Standard error of mean of 6 observations.

only five animals instead of six. Means and standard errors of means of selected variables are presented in Table 2.

TABLE 2 Means and Standard Errors of Means of Selected Variables

Variable	Breed[a]	Weight Group 23 kg	46 kg	68 kg	91 kg	SE[b]
LIVE PIG						
Counter potassium (g K/kg)	Y-C	2.17	1.98	1.61	1.48	0.15
	Y-D	2.25	1.85	1.56	1.28	
CARCASS						
Counter potassium (g K/kg)	Y-C	2.78	2.13	1.76	1.75	0.06
	Y-D	2.67	2.41	2.03	1.44	
Protein (%)	Y-C	15.81	14.56	12.97	12.88	0.20
	Y-D	16.38	14.69	13.02	11.81	
Ether extract (%)	Y-C	19.28	29.71	36.02	39.16	0.67
	Y-D	17.07	27.60	36.59	43.71	
Moisture (%)	Y-C	49.17	42.48	36.13	35.64	0.87
	Y-D	50.59	43.38	37.68	33.50	
MUSCLE						
Chemical potassium (g K/kg)	Y-C	2.90	2.97	2.87	2.82	0.05
	Y-D	3.03	2.88	2.69	2.54	
Protein (%)	Y-C	16.47	17.92	18.09	17.76	0.28
	Y-D	15.59	16.92	16.22	15.86	
Ether extract (%)	Y-C	15.68	17.73	19.76	21.58	0.65
	Y-D	14.53	18.64	22.57	25.16	
Moisture (%)	Y-C	66.73	63.46	61.04	60.04	0.60
	Y-D	67.28	63.25	59.30	57.64	
Fat-free muscle (%)	Y-C	50.35	43.65	36.61	37.12	1.14
	Y-D	52.46	46.87	40.77	35.55	
FAT						
Chemical potassium (g K/kg)	Y-C	0.57	0.58	0.54	0.42	0.03
	Y-D	0.46	0.46	0.35	0.27	
Protein (%)	Y-C	4.27	3.89	3.56	3.29	0.15
	Y-D	4.50	3.86	2.68	2.50	

TABLE 2 Continued

Variable	Breed[a]	Weight Group 23 kg	46 kg	68 kg	91 kg	SE[b]
Ether extract (%)	Y-C	73.93	78.76	80.30	82.16	0.85
	Y-D	77.24	80.36	83.89	86.12	
Moisture (%)	Y-C	21.16	16.97	15.70	13.96	0.69
	Y-D	18.18	15.69	12.72	10.85	
BONE						
Chemical potassium (g K/kg)	Y-C	1.78	1.72	1.32	0.97	0.07
	Y-D	1.75	1.59	1.10	0.99	
Protein (%)	Y-C	21.43	19.91	19.11	18.26	0.41
	Y-D	21.41	19.68	18.76	18.04	
Ether extract (%)	Y-C	4.38	9.80	10.59	12.90	0.84
	Y-D	6.42	14.39	15.62	18.09	
Moisture (%)	Y-C	27.29	23.10	17.49	14.46	0.90
	Y-D	28.93	19.48	15.80	13.18	

[a]Y-C = Yorkshire x Chester White; Y-D = Yorkshire x Duroc.
[b]Standard error of mean of 6 observations.

Total correlations, over all live weight groups, between grams of potassium per kilogram of component and percentages of protein, ether-extractable fat, and water in the same component are shown in Table 3. Correlations involving counter-estimated potassium are listed beside the corresponding ones involving chemically determined potassium. In all cases, correlations involving counter-estimated potassium were lower than those involving chemically determined potassium. Correlations of potassium with chemical constituents in the carcass component were probably the only ones large enough to be of practical value. These total correlations ranged from 0.92 to 0.94 for counter-estimated potassium and from 0.96 to 0.97 for chemically determined potassium. Correlations involving percentage of ether extract were negative.

Pooled correlations within breed and weight group were lower than the corresponding total correlations, especially for counter-estimated potassium in the carcass. Pooled correlations of chemically determined potassium concentrations with percentages of chemical constituents in the carcass ranged from 0.66 to 0.76. This indicates a lower relationship between potassium concentration and percentage of chemical composition within breed and weight groups. The total correlations are high because of high correlations between weight group means. The general developmental pattern is characterized by

TABLE 3 Correlations between Grams of Potassium per Kilogram and Percentage of Chemical Constituents within Components

Component and Constituent (%)	Total Correlations[a]		Pooled Correlations[b]	
	Chemical K	Counter K	Chemical K	Counter K
CARCASS[c]				
Protein	0.959*	0.923*	0.661*	0.566*
Ether extract	−0.966*	−0.940*	−0.685*	−0.581*
Water	0.963*	0.924*	0.756*	0.534*
MUSCLE				
Protein	0.566*	0.132	0.675*	0.390*
Ether extract	−0.826*	−0.478*	−0.658*	−0.319†
Water	0.748*	0.511*	0.570*	0.329*
FAT[d]				
Protein	0.708*	0.678*	0.615*	0.383
Ether extract	−0.686*	−0.363	−0.425*	0.237
Water	0.698*	0.266	0.458*	−0.442
BONE[c]				
Protein	0.646*	0.427*	0.049	0.017
Ether extract	−0.634*	−0.548*	0.080	0.148
Water	0.799*	0.523*	0.085	0.017

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Total correlations, ignoring weight groups.
[b]Pooled correlations within breed and weight group.
[c]Correlations involving counter estimates of potassium in the carcass and bone involve 44 rather than the usual 46 observations.
[d]Correlations involving counter estimates of potassium in the fat involve only 68- and 91-kilogram groups.

increasing fat content and decreasing potassium content. The predictive value of the relationship was much lower for animals of the same weight than for those differing in weight.

Correlations in the 91-kg group were generally higher than the correlations in the lighter weight groups. In the carcass, correlations of chemically determined potassium concentration with percentages of protein, ether-extractable fat, and water ranged from 0.73 to 0.78 in the 91-kg group. These values were slightly lower than the values reported by Kirton *et al.*[13] for frozen carcasses of 24 pigs (181 to 220 lb live weight) and by Kirton and Pearson[14] for carcasses of 10 lambs. They reported correlations of percentage of potassium with percentage of protein, 0.78 and 0.94, with percentage of ether extract, 0.88 and 0.87, and with percentage of water, 0.87 and 0.81 for pigs and lambs, respectively.

Table 4 lists the total correlations, over all weight groups, of counter-estimated grams of potassium per kilogram of live pig and carcass and of chemically determined grams of potassium per kilogram of carcass with percentages of protein, ether-extractable fat, and water in various components.

TABLE 4 Correlations[a] of Grams of Potassium per Kilogram of Live Pig and Carcass with Percentage of Chemical Constituents of Various Components[b]

Component and Constituent (%)	Live Pig	Carcass[c]	
	Counter K	Counter K	Chemical K
CARCASS			
Protein	0.909	0.923	0.959
Ether extract	−0.930	−0.940	−0.966
Water	0.908	0.924	0.963
MUSCLE			
Protein	0.071	−0.146	0.042
Ether extract	−0.886	−0.788	−0.906
Water	0.905	0.849	0.937
FAT			
Protein	0.781	0.656	0.722
Ether extract	−0.782	−0.702	−0.754
Water	0.778	0.704	0.749
BONE			
Protein	0.715	0.730	0.722
Ether extract	−0.769	−0.639	−0.700
Water	0.883	0.803	0.886

[a] Total correlations, ignoring weight groups.
[b] All correlations significant at the 0.01 level, except those involving percentage of protein in muscle.
[c] Correlations involving counter estimates of potassium in the carcass involve 44 rather than the usual 46 observations.

There was no significant correlation between counter-estimated potassium concentration in the live pig or carcass with percentage of protein in the muscle. This was also the case for newborn to 4-month-old calves.[16] However, counter-estimated potassium concentrations in the live pig and carcass were highly significantly correlated (r = 0.79 to 0.94) with percentages of ether extract and water in the muscle of the pig. This contrasts with the calf results of Martin *et al.*,[16] where the only significant correlation was between potassium concentration in the carcass and percentage of fat in separable muscle.

Similar to the results reported in Table 3, prediction of chemical composition of the carcass (r = 0.96 to 0.97) was more accurate than prediction of chemical composition of the other components. Correlations involving chemically determined potassium concentration in the carcass were higher in most cases than the corresponding ones involving counter-estimated potassium.

When the within-weight-group correlations were examined, it was found that a particular correlation varied widely among weight groups, usually having the largest value in the 91-kg weight group. In several cases the correlation was smallest in the 23-kg group and increased with increasing live pig weight. However, there were exceptions where the correlation was lowest in the 46- or 68-kg groups. In an investigation of the association between potassium content and body composition of newborn pigs, Curtis *et al.*[7] found a positive association between potassium and ether-extract contents and a negative association between potassium and moisture contents. These results strongly suggest that the prediction equation that might be applicable to a given set of animals is dependent on the age of the animals.

The pooled correlations within breed and weight group shown in Table 5 correspond with the total correlations in Table 4. In nearly every case, they are much lower than the corresponding total correlations. Pooled correlations of percentages of chemical constituents in the carcass with counter-estimated potassium concentrations in the live pig and carcass ranged from 0.34 to 0.36, while those with chemically determined potassium concentrations in the carcass ranged from 0.66 to 0.76.

Table 6 lists the same correlations as Tables 4 and 5, but only for the 91-kg group. The correlations of counter-estimated potassium concentration in the live pig with percentage of chemical composition in most components were higher in the 91-kg group than the corresponding pooled correlations. However, those involving counter-estimated potassium in the carcass were generally as low as, or lower than, the corresponding pooled correlations. Correlations involving chemically determined potassium were either of the same magnitude or slightly higher than the corresponding pooled correlations.

In the 91-kg group, correlations of counter-estimated potassium concentration in live pigs with percentage of protein and ether extract in the carcasses were 0.82 and –0.76, respectively. These correlations were in the same range as correlations of percentage of potassium in empty body with percentage of protein (0.77) and percentage of ether extract (–0.89) in frozen carcasses of pigs with an average live weight of 90 kg reported by Kirton *et al.*[13]

Since potassium concentration of various components was most highly correlated with percentages of chemical constituents in the carcass, equations to predict percentages of chemical composition of the carcass from potassium concentrations were calculated for the 91-kg group. The Y-intercepts (a), re-

TABLE 5 Pooled Correlations[a] of Grams of Potassium per Kilogram of Live Pig and Carcass with Percentage of Chemical Constituents of Various Components

Component and Constituent (%)	Live Pig	Carcass[b]	
	Counter K	Counter K	Chemical K
CARCASS			
Protein	0.348†	0.566*	0.661*
Ether extract	−0.364†	−0.581*	−0.685*
Water	0.336†	0.534*	0.756*
MUSCLE			
Protein	0.273	−0.242	0.314
Ether extract	−0.432*	−0.070	−0.633*
Water	0.391†	0.085	0.655*
FAT			
Protein	0.182	−0.172	−0.264
Ether extract	−0.169	0.050	−0.137
Water	0.121	−0.071	0.064
BONE			
Protein	0.082	0.178	0.142
Ether extract	−0.217	0.306	−0.062
Water	0.138	0.124	0.244

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Pooled correlations within breed and weight group.
[b]Correlations involving counter estimates of potassium in the carcass involve 44 rather than the usual 46 observations.

gression coefficients (b), and standard errors of estimate ($S_{y \cdot x}$) of the simple regressions of percentages of protein, ether-extractable fat, and water in the carcass on grams of potassium per kilogram of live pig and carcass are listed in Table 7.

Correlations between grams of potassium per kilogram of live pig and carcass with physical-separation data are shown in Table 8. Over all weight groups, grams of potassium per kilogram of live pig was less highly correlated with the various percentages of component parts than was carcass potassium concentration. Percentages of separable fat in the carcass and in the live pig and percentage of carcass in the live pig were negatively correlated with potassium concentrations. This is because these variables increased with age while potassium concentration decreased with age. These increases in percentages of components can be attributed to fat increasing at a faster relative rate than live weight.

TABLE 6 Correlations of Grams of Potassium per Kilogram of Live Pig and Carcass with Percentage of Chemical Constituents of Various Components, in the 91-kg Group Only[a]

Component and Constituent (%)	Live Pig	Carcass[b]	
	Counter K	Counter K	Chemical K
CARCASS			
Protein	0.819*	0.284	0.755†
Ether extract	−0.759†	−0.552	−0.765†
Water	0.590	0.473	0.743†
MUSCLE			
Protein	0.616	−0.331	0.335
Ether extract	−0.656	−0.249	−0.682†
Water	0.567	0.050	0.826*
FAT			
Protein	0.342	0.130	−0.078
Ether extract	−0.454	−0.071	−0.147
Water	0.393	0.021	0.087
BONE			
Protein	0.159	−0.254	0.212
Ether extract	−0.260	0.211	−0.159
Water	−0.057	0.602	0.366

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Pooled within-breed correlations in 91 kg group.
[b]Correlations involving counter estimates of potassium in the carcass involve 9 rather than the usual 10 observations.

When pooled correlations within breed and weight group were calculated, they were found to be much lower than the corresponding total correlations. The correlations within the 91-kg group (Table 8), were higher than the pooled correlations, although, with one exception, they were not as high as the total correlations. Correlations involving counter-estimated potassium in the live pig and carcass were all below 0.66 and were nonsignificant in the 91-kg group.

Percentage of edible portion in hams and loins of 200-lb pigs had been found by Martin *et al.*[16] not to be significantly correlated with counter-estimated grams of potassium per kilogram of live pig. These correlations were lower than corresponding correlations reported by Kirton *et al.*[15] between grams of counter-estimated potassium per kilogram of live lamb and percentages of separable fat, lean, and bone ranging from 0.57 to 0.86. Those between lamb carcass potassium concentration and percentages of fat, lean, and bone were nonsignificant, as was found here for pigs.

TABLE 7 Simple Regressions of Percentages of Chemical Constituents on Grams of Potassium per Kilogram of Component in the 91-kg Group Only[a,b] ($Y = a + bX$)

Y Carcass	X (g K/kg)		a	b	R^2	$S_{y.x}$
Protein (%)	Live pig	(counter K)	5.973	4.662	0.671	0.448
	Carcass	(chemical K)	3.266	5.795	0.569	0.513
Ether extract (%)	Live pig	(counter K)	58.243	−12.304	0.576	1.449
	Carcass	(chemical K)	67.634	−16.727	0.585	1.434
Water (%)	Live pig	(counter K)	21.971	9.337	0.348	1.754
	Carcass	(chemical K)	9.866	15.867	0.552	1.454

[a] Regressions calculated on a pooled within-breed basis.
[b] Regressions involving counter estimates of potassium in the carcass involve 9 rather than the usual 10 observations.

In both live pig and carcass, grams of chemical potassium per kilogram of carcass was more highly correlated with percentages of component parts than was counter-estimated potassium. Grams of chemical potassium per kilogram of carcass was correlated 0.80 with percentage of fat-free muscle in carcass, –0.75 with carcass fat as a percentage of live weight, and –0.93 with carcass as a percentage of live weight. In order to determine chemically the potassium content of the carcass, it was necessary to physically separate carcass. Therefore, percentage of carcass in the live pig was known, and a high correlation of –0.93 between chemical potassium concentration in the carcass and percentage of the live pig as carcass was of no practical value.

Over all live weight groups, total correlations between grams of potassium in component and kilograms of chemical constituents in the same component are shown in Table 9. Because the total amount of all chemical constituents and potassium increased as live weight increased, all correlations were positive. Correlations involving chemically determined potassium content were higher than the respective ones involving counter-estimated potassium. In addition to the correlations within carcass, those within the muscle component and those involving chemically determined potassium content in the fat were also high. In each component, the correlation of potassium content with kilograms of ether extract had the smallest value. This was not the case when percentages of chemical constituents and potassium concentration were used (Table 3).

When calculated over a large weight span, chemically determined potassium was shown to be highly correlated with kilograms of chemical constituents present in muscle and carcass components. This was especially true for protein, water, and fat-free muscle where the correlations were 0.99. Similar high

TABLE 8 Correlations of Grams of Potassium per Kilogram of Live Pig and Carcass with Their Percentage Component Parts

Trait	Live Pig	Carcass	
	Counter K	Counter K	Chemical K
Total correlations[a]			
PERCENTAGE OF CARCASS AS			
Fat-free muscle	0.844*	0.913*	0.931*
Muscle	0.741*	0.868*	0.843*
Fat	−0.854*	−0.942*	−0.924*
Bone	0.763*	0.814*	0.778*
PERCENTAGE OF LIVE PIG AS			
Fat-free carcass muscle	0.757*	0.819*	0.866*
Carcass muscle	0.540*	0.677*	0.668*
Carcass fat	−0.864*	−0.950*	−0.931*
Carcass bone	0.643*	0.681*	0.653*
Carcass	−0.785*	−0.849*	−0.808*
Correlations within 91-kg group[b]			
PERCENTAGE OF CARCASS AS			
Fat-free muscle	0.662	0.573	0.797†
Muscle	0.485	0.298	0.633
Fat	−0.380	−0.326	−0.530
Bone	0.520	0.400	0.644
PERCENTAGE OF LIVE PIG AS			
Fat-free carcass muscle	0.503	0.228	0.332
Carcass muscle	0.074	−0.334	−0.107
Carcass fat	−0.493	−0.502	−0.750†
Carcass bone	0.323	0.394	0.254
Carcass	−0.551	−0.546	−0.932*

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Total correlations, ignoring weight groups.
[b]Pooled within-breed correlations in 91-kg group.

correlations between total amounts of potassium and chemical constituents have been reported for rat carcasses and humans by Talso *et al.*[20] and for pig muscle by Pfau and Kallistratos.[18]

Pooled correlations within breed and weight group between kilograms of ether extract and grams of potassium were greatly reduced in the carcass and muscle components but were still significant in the fat component. The pooled correlations of kilograms of protein and water with grams of counter-estimated potassium were considerably lower than the corresponding total correlations, while those involving chemically determined potassium were reduced much less.

TABLE 9 Correlations between Grams of Potassium and Kilograms of Chemical Constituents within Components

Component and Constituent (kg)	Total Correlations[a]		Pooled Correlations[b]	
	Chemical K	Counter K	Chemical K	Counter K
CARCASS[c]				
Protein	0.992*	0.963*	0.880*	0.740*
Ether extract	0.936*	0.890*	0.225	0.467*
Water	0.990*	0.961*	0.854*	0.744*
MUSCLE				
Protein	0.995*	0.978*	0.927*	0.845*
Ether extract	0.925*	0.929*	0.140	0.169
Water	0.993*	0.979*	0.911*	0.777*
Fat-free muscle	0.994*	0.980*	0.921*	0.795*
FAT[d]				
Protein	0.939*	0.604*	0.846*	0.563*
Ether extract	0.795*	0.162	0.652*	0.466†
Water	0.931*	0.408	0.820*	0.206
BONE[c]				
Protein	0.720*	0.622*	0.591*	0.389†
Ether extract	0.555*	0.401*	0.049	−0.041
Water	0.756*	0.570*	0.566*	0.262

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Total correlations, ignoring weight groups.
[b]Pooled correlations within breed and weight group.
[c]Correlations involving counter estimates of potassium in the carcass and bone involve 44 rather than the usual 46 observations.
[d]Correlations involving counter estimates of potassium in the fat involve only 68- and 91-kg groups.

Total correlations, calculated over all weight groups, between grams of potassium in live pig and carcass and kilograms of chemical constituents in various components are listed in Table 10. All correlations were positive, and in almost all cases, they were higher than the respective correlations between percentages of chemical constituents and potassium concentrations (Table 4). Total protein content of muscle was highly correlated (r = 0.96 to 0.99) with the various potassium contents. This was in contrast to the lack of significant correlations between percentage of protein in muscle and potassium concentrations as given in Table 4. Kilograms of ether extract in the carcass and muscle were less highly correlated (r = 0.84 to 0.94).

In practically every case, chemically determined potassium content of the carcass was more highly correlated with kilograms of chemical constituents in the various components than was counter-estimated potassium content.

Correlations of counter-estimated potassium content of live pig with kilograms of constituents in the muscle were: protein, 0.96; ether extract, 0.84;

TABLE 10 Correlations[a] of Grams of Potassium in Live Pig and Carcass with Kilograms of Chemical Constituents in Various Components[b]

Component and Constituent (kg)	Live pig	Carcass[c]	
	Counter K	Counter K	Chemical K
CARCASS			
Protein	0.964	0.963	0.992
Ether extract	0.877	0.890	0.936
Water	0.953	0.960	0.990
MUSCLE			
Protein	0.956	0.965	0.990
Ether extract	0.836	0.888	0.905
Water	0.931	0.958	0.977
Fat-free muscle	0.938	0.961	0.980
FAT			
Protein	0.884	0.839	0.887
Ether extract	0.877	0.874	0.931
Water	0.892	0.840	0.896
BONE			
Protein	0.928	0.934	0.951
Ether extract	0.755	0.825	0.817
Water	0.730	0.694	0.728

[a] Total correlations, ignoring weight groups.
[b] All correlations significant at the 0.01 level.
[c] Correlations involving counter estimates of potassium in the carcass involve 44 rather than the usual 46 observations.

water, 0.93; and fat-free muscle, 0.94. Using the same whole-body counter, Stant[19] found counter-estimated potassium content of live calves from birth to 4 months of age to be correlated (over all age groups) with kilograms of constituents in lean muscle as follows: protein, 0.90; ether-extractable fat, 0.84; moisture, 0.93; and fat-free lean, 0.92. The total correlations of grams of counter-estimated potassium in pig carcasses with kilograms of chemical constituents of 0.89 to 0.96 were slightly lower than the respective correlations of 0.97 to 0.98 in calves reported by Stant.[19]

Pooled correlations within breed and weight group between grams of potassium and kilograms of ether extract were generally very low and non-significant, as shown in Table 11. This is in contrast to previous work on calves where kilograms of fat (ether extract) in the meat was found by Stant[19] to be the only chemical constituent in the meat significantly correlated within age groups (r = 0.72 to 0.75) with counter-estimated grams of potassium in the live calf and carcass. All correlations involving chemical constituents in fat and bone were low (below 0.60). As noted in previous cases, correlations involving chemically determined grams of potassium were

TABLE 11 Pooled Correlations[a] of Grams of Potassium in Live Pig and Carcass with Kilograms of Chemical Constituents in Various Components

Component and Constituent (kg)	Live Pig	Carcass[b]	
	Counter K	Counter K	Chemical K
CARCASS			
Protein	0.793*	0.740*	0.880*
Ether extract	0.041	0.467*	0.225
Water	0.643*	0.744*	0.854*
MUSCLE			
Protein	0.625*	0.705*	0.836*
Ether extract	−0.211	0.279	−0.050
Water	0.474*	0.688*	0.706*
Fat-free muscle	0.532*	0.691*	0.736*
FAT			
Protein	0.339†	0.332†	0.357†
Ether extract	0.143	0.434*	0.315
Water	0.355†	0.409†	0.304
BONE			
Protein	0.545*	0.597*	0.356†
Ether extract	−0.254	0.078	−0.462
Water	0.300	0.501*	0.355†

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Pooled correlations within breed and weight group.
[b]Correlations involving counter estimates of potassium in the carcass involve 44 rather than the usual 46 observations.

higher than those involving counter-estimated potassium content. Pooled correlations of kilograms of protein and water in carcass and muscle with grams of counter-estimated potassium in live pig and carcass ranged from 0.47 to 0.79, while those involving grams of chemically determined potassium in carcass ranged from 0.71 to 0.88.

Correlations within the 91-kg group (Table 12) exhibited the same trends as the pooled correlations. In general, the correlations in the 91-kg group were slightly larger than the corresponding pooled correlations. Exceptions were those involving grams of counter-estimated potassium in the carcass. Correlations of grams of counter-estimated potassium in live pig and grams of chemically determined potassium in carcass with kilograms of protein and water in carcass ranged from 0.81 to 0.92.

The Y-intercepts (a), regression coefficients (b), and standard errors of estimate ($S_{y.x}$) of the simple regressions of kilograms of chemical constituents in carcass and muscle on grams of potassium in various components are given

TABLE 12 Correlations of Grams of Potassium in Live Pig and Carcass with Kilograms of Chemical Constituents in Various Components[a]

Component and Constituent (kg)	Live Pig	Carcass[b]	
	Counter K	Counter K	Chemical K
CARCASS			
Protein	0.870*	0.697	0.886*
Ether extract	0.068	0.469	0.438
Water	0.806*	0.728†	0.885*
MUSCLE			
Protein	0.872*	0.655	0.879*
Ether extract	−0.085	0.192	0.116
Water	0.877*	0.726†	0.915*
Fat-free muscle	0.892*	0.724†	0.914*
FAT			
Protein	0.446	0.545	0.684†
Ether extract	0.134	0.493	0.513
Water	0.433	0.530	0.565
WATER			
Protein	0.719†	0.743†	0.581
Ether extract	−0.373	−0.082	−0.609
Water	0.259	0.608	0.324

*Significant at the 0.01 level.
†Significant at the 0.05 level.
[a]Pooled within-breed correlations in 91-kg group only.
[b]Correlations involving counter estimates of potassium in the carcass involve 9 rather than the usual 10 observations.

in Table 13 for the 91-kg group. The variation (R^2) in kilograms of chemical constituents accounted for by potassium content of various components was greater than when grams of potassium per kilogram was used to estimate percentage of chemical constituents (Table 7). The coefficients of determination (R^2) were larger for chemically determined potassium than for counter-estimated potassium.

The much lower pooled correlations are indicative of a high correlation between cell means and would indicate that potassium and body composition estimates obtained at an early age might be good estimators of potassium content and body composition of groups of pigs at a later standard age.

EXPERIMENT TWO

Means of the variables observed on 51 lambs are shown in Table 14. Means of carcass measurement variables observed on these lambs were reported by Judge *et al.*[11]

The 14 measurements were used to predict percentage of edible portion in the carcass. The multiple correlation, when all 14 measurements were used,

TABLE 13 Simple Regressions of Kilograms of Chemical Constituents on Grams of Potassium in Components, in the 91-kg Group Only[a] ($Y = a + bX$)

Y	X (grams of K)		a	b	R^2	$S_{y.x}$
CARCASS						
Protein (kg)	Live pig	(counter K)	3.631	0.037	0.757	0.373
	Carcass	(counter K)	2.892	0.048	0.486	0.735
	Carcass	(chemical K)	1.079	0.068	0.785	0.351
Water (kg)	Live pig	(counter K)	12.895	0.082	0.649	1.075
	Carcass	(counter K)	8.662	0.132	0.530	1.848
	Carcass	(chemical K)	6.005	0.164	0.782	0.847
MUSCLE						
Protein (kg)	Live pig	(counter K)	2.309	0.024	0.760	0.242
	Carcass	(counter K)	1.784	0.032	0.429	0.545
	Carcass	(chemical K)	0.686	0.044	0.773	0.236
Water (kg)	Live pig	(counter K)	10.736	0.064	0.770	0.623
	Carcass	(counter K)	8.100	0.096	0.527	1.349
	Carcass	(chemical K)	6.027	0.121	0.838	0.523
Fat-free muscle (kg)	Live pig	(counter K)	13.066	0.091	0.796	0.815
	Carcass	(counter K)	12.041	0.111	0.525	1.572
	Carcass	(chemical K)	6.740	0.168	0.835	0.733

[a]Regressions calculated on a pooled within breed basis. Regressions involving counter estimates of potassium in the carcass involve 9 rather than the usual 10 observations.

TABLE 14 Means and Standard Deviations of Variables Observed on 51 Lambs

Variable	Mean	SD
Age at slaughter (days)	288	54.7
Slaughter weight (kg)	39.0	3.06
Carcass weight (kg)	22.77	2.32
Live total potassium (g)	65.7	9.46
Live potassium content (g/kg)	1.641	0.238
Carcass total potassium (g)	50.9	5.57
Carcass potassium content (g/kg)	2.258	0.301
Edible portion (% of carcass)	63.04	4.17
Excess fat (% of carcass)	21.88	5.52
Bone (% of carcass)	14.23	2.11
Edible portion weight (kg)	14.3	1.36
Excess fat weight (kg)	5.01	1.44
Edible portion (% of live weight)	36.57	1.98
Excess fat (% of live weight)	12.79	3.57

TABLE 15 Prediction Equations to Estimate Percentage of Edible Portion in Lamb Carcasses Using All Counter Data, Age, and Weights[a]

Parameter	Equation Number 1	2	3	4	5	6	7
Intercept, b_0	44.63[b]	46.02	39.34	38.87	39.89	35.54	39.95
	31.57[c]	11.67	7.71	7.57	7.58	4.42	3.04
g K/kg carcass weight, b_1	9.67	8.95	8.86	9.19	10.27	10.85	10.23
	15.33	2.00	1.98	1.81	1.63	1.40	1.33
Age (days), b_2	0.016	0.017	0.015	0.015	0.012	0.010	–
	0.009	0.009	0.009	0.008	0.008	0.008	
Carcass weight (kg), b_3	−0.838	−0.911	−0.744	−0.739	−0.158	–	–
	1.613	0.546	0.497	0.493	0.222		
Slaughter weight (kg), b_4	0.299	0.304	0.376	0.411	–	–	–
	0.354	0.339	0.326	0.312			
Total g of K (live), b_5	0.133	0.130	0.023	–	–	–	–
	0.166	0.150	0.053				
g K/kg (live weight), b_6	−4.78	−4.64	–	–	–	–	–
	6.78	6.07					
Total g K (carcass), b_7	−0.033	–	–	–	–	–	–
	0.695						
Multiple correlation	0.76	0.76	0.76	0.76	0.75	0.75	0.74
SE of estimate	2.90	2.86	2.85	2.83	2.85	2.84	2.86

[a] Order of variables determined by order of deletion of least useful variable in stepwise regression analysis.
[b] Regression coefficient.
[c] Standard error of regression coefficient.

was 0.84. Since Judge *et al.*[11] reported a multiple correlation of 0.78 using only the seven carcass measurements and a multiple correlation of 0.77 using only three measurements which were easily obtained, it was felt that the accuracy of prediction of carcass composition from ^{40}K counting should be compared with that obtained by carcass measurements.

Multiple regression techniques were used to estimate predictive accuracy and multiple correlations. The predictive accuracy (r = 0.76) of all carcass and live animal counter variables plus age and weights was only slightly less than that obtained when carcass measurements were used to predict percentage of edible portion in the carcass (Table 15). A single variable, grams of potassium per kilogram of carcass weight, was virtually as accurate (r = 0.74) as the multiple regression. In a similar analysis, only variables measured on the live animal were used (Table 16). The multiple correlation of 0.46 is considerably lower than that observed for carcass measurements. Grams of potassium per kilogram of live weight predicted percentage of edible portion in the carcass almost as accurately (r = 0.44) as the multiple regression did. These relationships tend to be slightly lower than those reported by Kirton and Pearson.[14]

Predictive accuracies of various combinations of carcass measurements, age, live weight, and counter variables on seven parameters of body composition are shown in Table 17. Edible portion weight was predicted quite accurately (r = 0.84) by data on live animals, largely because of the part–whole correlation between live weight and edible-portion weight (r = 0.80). When all counter data were used, 81.0% (r = 0.90) of the variation in edible portion weight was accounted for by the model. This was only slightly less than the 87.0% reported by Breidenstein *et al.*[4] Grams of potassium per kilogram of carcass or live weight, or both, accounted for much of the accuracy in predicting percentage of edible portion in the carcass and in the live animal.

Excess fat weight, excess fat as a percentage of carcass weight, and excess fat as a percentage of live weight were, in general, predicted accurately. When all counter- and carcass-measurement data were used in the prediction, fat thickness was the best single indicator of excess fat content. When all counter data were used, a combination of carcass weight (r = 0.54) and total grams of potassium in the carcass (r = –0.47) predicted excess fat weight very well (r = 0.83); while excess fat as a percentage of carcass and of live weight was predicted quite accurately (r = 0.75 and r = 0.77) by grams of potassium per kilogram of carcass weight. When only data on the live animals were used, the accuracy of prediction dropped considerably. The best single estimator of fat content was grams of potassium per kilogram of live weight (r = –0.53, with excess fat weight; r = –0.47, with excess fat as a percentage of carcass weight; and r = –0.49, with excess fat as a percentage of live weight).

In general, potassium content used in conjunction with weight and age variables will predict composition of lamb carcasses with virtually the same

TABLE 16 Prediction Equations to Estimate Percentage of Edible Portion in Lamb Carcasses Using Only Counter Data, Age, and Weight of the Live Animal[a]

Parameter	Equation Number 1	2	3	4
Intercept, b_0	52.74[b]	50.16	51.89	50.49
	13.47[c]	5.69	3.89	3.69
g of K/kg live weight, b_1	10.42	11.64	11.09	7.65
	7.07	4.03	3.78	2.23
Total g of K (live), b_2	−0.081	−0.114	−0.107	–
	0.185	0.098	0.095	
Age (days), b_3	0.005	0.004	–	–
	0.011	0.011		
Slaughter weight (kg), b_4	−0.075	–	–	–
	0.350			
Multiple correlation	0.46	0.46	0.46	0.44
SE of estimate	3.85	3.81	3.78	3.79

[a] Order of variables determined by order of deletion of least useful variable in stepwise regression analysis.
[b] Regression coefficient.
[c] Standard error of regression coefficient.

TABLE 17 Multiple Correlations and Standard Errors of Estimate for Predicting Composition of Lamb Carcasses

Dependent Variable	All Counter Data plus Carcass Measurements[a]		Age, Weight, and All Counter Data[b]		Age, Weight, and Counter Data on Live Animals[c]	
	R	SE	R	SE	R	SE
EDIBLE PORTION						
Weight (kg)	0.93	0.62	0.90	0.65	0.84	0.78
As % of carcass weight	0.84	2.67	0.76	2.90	0.46	3.85
As % of live weight	0.66	1.80	0.59	1.73	0.44	1.90
EXCESS FAT						
Weight (kg)	0.95	0.56	0.87	0.76	0.56	1.25
As % of carcass weight	0.93	2.45	0.83	3.33	0.50	4.99
As % of live weight	0.95	1.37	0.86	1.94	0.52	3.17
BONE AS % OF CARCASS WEIGHT	0.87	1.26	0.71	1.59	0.43	1.98

[a] Variables used in Table 15 plus those used by Judge *et al.*, reference 11.
[b] Variables used in Table 15.
[c] Variables used in Table 16.

accuracy obtained by use of carcass measurements. Prediction based only on live animal variables is considerably less accurate than prediction based on both live animal and carcass variables.

SUMMARY

In the swine data, correlations of grams of potassium per kilogram of sample with percentage of chemical constituents of various components were almost always higher when chemically determined potassium content was used than when counter-estimated potassium content was used. All correlations involving percentage of ether-extractable fat were negative. Potassium content of the total carcass was more highly correlated with the percentages of chemical constituents of the carcass than was potassium content of the muscle, bone, or fat with the percentages of chemical constituents in the respective component parts.

The correlation between grams of potassium per kilogram of sample and percentage of chemical constituents was lower when pooled within breed and weight group than when calculated as total correlations ignoring breed and weight group classifications. In general, these correlations were higher in the 91-kg weight group than in lighter groups. Simple regressions of percentage of chemical constituents in the carcass on grams of potassium per kilogram of carcass and live pig were calculated.

Grams of potassium per kilogram of live pig was less highly correlated with physical separation estimates of composition than was grams of potassium per kilogram of carcass. Percentage of fat-free muscle in the carcass and percentage of carcass fat in the live pig were highly correlated with potassium concentration in the carcass.

Correlations between total grams of potassium and kilograms of chemical constituents were higher than those between grams of potassium per kilogram and percentage of chemical constituents. In this case also, correlations involving chemically determined potassium were higher than those involving counter-estimated potassium. All correlations between grams of potassium and kilograms of chemical constituents, including kilograms of separable fat, were positive. Pooled correlations were lower than total correlations, and correlations in the 91-kg weight group were generally higher than those in the lighter weight groups. Correlations involving grams of potassium in the live pig were generally lower than those involving grams of potassium in the carcass. Simple regressions of kilograms of chemical constituents in the carcass on total grams of potassium in the carcass and live pig were calculated.

In the lamb data, multiple regression based on live and carcass weights, age, grams of potassium per kilograms of carcass and live animal, and total

grams of potassium in the carcass and live lamb were found to yield virtually the same accuracy of prediction of percentage of edible portion in the carcass as did a multiple regression based on carcass measurements described by Judge *et al.*[11] Grams of potassium per kilogram of carcass was the primary predictor of percentage of edible portion in both the carcass and the live animal. Live weight, carcass weight, and total grams of potassium were primary predictors of edible-portion weight and excess-fat weight. Primary predictors of excess fat as a percentage of both the carcass and the live animal were fat-thickness measurements, and grams of potassium per kilogram of carcass and per kilogram of live animal. Predictions of carcass composition were considerably less accurate from data obtained on the live animal than from data obtained on the carcass.

This work was supported in part by National Institutes of Health Research Grant 05551-05.

This paper is issued as Journal Paper Number 3059, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

REFERENCES

1. Anderson, E. C. 1959. Applications of natural gamma measurements to meat. Food Res. 24:605.
2. Association of Official Agricultural Chemists. 1960. Official methods of analysis. 8th ed. Association of Official Agricultural Chemists, Washington, D. C.
3. Benne, E. J., N. H. Van Hall, and A. M. Pearson. 1956. Analysis of fresh meat. J. Ass. Offic. Agr. Chem. 39:937.
4. Breidenstein, B. C., T. G. Lohman, E. E. Hatfield, G. S. Smith, and H. W. Norton. 1965. Comparison of K^{40} measurement with other methods for determining lean muscle mass in sheep. J. Anim. Sci. 24:860.
5. Breidenstein, B. C., T. G. Lohman, G. S. Smith, and H. W. Norton. 1965. K^{40} and other indices of carcass lean muscle mass in pigs. J. Anim. Sci. 24:860.
6. Carpenter, Z. L., G. T. King, F. A. Orts, and N. L. Cunningham. 1964. Factors influencing retail carcass value of lambs. J. Anim. Sci. 23:741.
7. Curtis, S. E., C. J. Heidenreich, and T. G. Martin. 1967. Relationship between body weight and chemical composition of pigs at birth. J. Anim. Sci. 26:749.
8. Field, R. A., J. D. Kemp, and W. Y. Varney. 1963. Indices for lamb carcass composition. J. Anim. Sci. 22:218.
9. Hiner, R. L., and J. W. Thornton. 1962. Study of certain lamb and carcass quality factors. J. Anim. Sci. 21:511.
10. Hoke, K. E. 1961. Factors affecting yield of cuts in lamb carcasses. Recip. Meat Conf. Proc. 14:163.
11. Judge, M. D., T. G. Martin, and J. B. Outhouse. 1966. Prediction of carcass composition of ewe and wether lambs from carcass weights and measurements. J. Anim. Sci. 25:92.

12. Judge, M. D., M. Stob, W. V. Kessler, and J. E. Christian. 1963. Lamb carcasses and live lamb evaluations by potassium-40 and carcass measurements. J. Anim. Sci. 22:418.
13. Kirton, A. H., R. H. Gnaedinger, and A. M. Pearson. 1963. Relationship of potassium and sodium content to the composition of pigs. J. Anim. Sci. 22:904.
14. Kirton, A. H., and A. M. Pearson. 1963. Comparison of methods of measuring potassium in pork and lamb and prediction of their composition from sodium and potassium. J. Anim. Sci. 22:125.
15. Kirton, A. H., A. M. Pearson, R. H. Nelson, E. C. Anderson, and R. L. Schuch. 1961. Use of naturally occurring potassium-40 to determine the carcass composition of live sheep. J. Anim. Sci. 20:635.
16. Martin, T. G., W. V. Kessler, E. G. Stant, Jr., J. E. Christian, and F. N. Andrews. 1963. Body composition of calves and pigs measured by large volume liquid scintillation counting and conventional chemical analyses. Ann. N. Y. Acad. Sci. 110:213.
17. Muldowney, F. P., J. Crooks, and M. M. Bluhm. 1957. Relationship of total exchangeable potassium and chloride to lean body mass, red cell mass and creatinine excretion in man. J. Clin. Invest. 362:1375.
18. Pfau, Von A., and G. Kallistratos. 1963. Untersuchungen zur Bestimmung von korperbestandteilen lebender Schweine uber ^{40}K-Gamma-Radioaktivitatsmessungen. II. Der Kaliumgehalt der Muskulatur eines 110 kg. schweren Schweines. Z. Tierz. Zuchtungsbiol. 79:249.
19. Stant, E. G., Jr. 1963. Body composition of calves measured by large volume liquid scintillation counting and chemical analyses. M. S. Thesis, Purdue University. (Library, Purdue Univ., Lafayette, Ind.)
20. Talso, P. J., C. E. Miller, A. J. Carballo, and I. Vasquez. 1960. Exchangeable potassium as a parameter of body composition. Metabolism 9:456.
21. Willis, J. B. 1960. The determination of metals in blood serum by atomic absorption spectroscopy. III. Sodium and potassium. Spectrochem. Acta 16:555.
22. Zinn, D. W. 1961. Cutting methods as related to lamb carcass evaluation. Recip. Meat Conf. Proc. 14:177.
23. Zobrisky, S. E., H. D. Nauman, A. J. Dyer, and E. C. Anderson. 1959. The relationship between the potassium isotope, K^{40} and meatiness of live hogs. J. Anim. Sci. 18:1480.

A. Bensadoun
J. T. Reid
L. D. Van Vleck
O. L. Paladines
and B. D. H. Van Niekerk
Cornell University

COMPARISON OF VARIOUS INDIRECT METHODS WITH DIRECT METHODS OF DETERMINING BODY COMPOSITION IN RUMINANTS

Most available indirect methods of determining body composition are not precise enough to justify their use in indirect calorimetry or as a tool of genetic selection. The problem of determining body composition *in vivo* in the ruminant animal is further complicated by the presence of a large mass of ingesta which constitutes a significant portion of the body weight, even when animals are fasted for 15 to 20 hours. It becomes mandatory then to devise means of predicting empty-body weight (ingesta-free body) as a step in resolving the gross chemical composition of the sheep body.

In this report, the following aspects of indirectly estimating body composition are discussed: (a) relationship between shrunk live weight (body weight taken after 20 hours of fast) and directly measured empty-body weight; (b) usefulness of antipyrine, *N*-acetyl-4-amino-antipyrine, and daily creatinine excretion as predictors of body composition; and (c) relationship between directly measured body protein and potassium.

EXPERIMENTAL PROCEDURE

Body-composition data on 125 male castrated sheep were available for this study. One hundred twenty-three sheep were part of two tissue-analysis experiments designed to determine the effect of grinding and pelleting on the utilization of sheep rations. Two additional sheep were included because of their extreme body composition: a very lean 4-month-old Dorset Horn lamb and a 27-month-old Shropshire x Hampshire wether.

The first experiment included 63 sheep. Thirty-six older sheep and 27 younger sheep (20 and 8 months old, respectively, at the beginning of the experiment) were studied. Nine 20-month-old sheep and nine 8-month-old sheep were than slaughtered to assess the initial body composition. The remaining 45 sheep were assigned at random to the treatments of a factorial design with three levels of intake and three diets superimposed on two age groups. The three rations fed were: chopped timothy hay; pelleted, ground timothy hay; and a pelleted mixture of corn meal (45%) and ground timothy hay (55%). The low level of intake was adjusted so that the animals would be in a slight positive weight balance. The sheep on the medium level were fed on an equalized-feed-intake basis, irrespective of diet, whereas the high level of intake represented *ad libitum* feeding.

The second experiment was carried out with 60 sheep that were 8 months old at the beginning of the experiment. Twelve of these were slaughtered to establish the base line body composition for the remaining 48 sheep, which were assigned to the treatments of a factorial design consisting of the three levels of intake and two diets. The two rations fed were: chopped, second-cutting alfalfa hay, and the same kind of hay in the ground, pelleted form. The three levels of intake were established as described for the first experiment.

At the completion of the feeding periods, which lasted 196 and 156 days, respectively, for the first and second experiments, the sheep were slaughtered. The day before slaughter the sheep were shorn very closely. The chemical components of the wool were not included in the computation of the empty-body composition. The sheep received water but were deprived of feed during the 18- to 20-hour period prior to slaughter. A shrunk body weight measurement was made just before slaughter. The empty (i.e., ingesta-free) bodies were sampled and analyzed for their gross chemical composition and energy content by a procedure already described.[1] The contents from the rumen-reticulum and those from the rest of the digestive tract were collected in two separate samples.

The sheep available for the experiments were crossbred. The origin of the animals, their ages, and the ration they received for at least 4 months prior to slaughter are given in Table 1.

TABLE 1 Origin, Age, and Nutritional Treatment of 125 Experimental Sheep

Origin and Number of Sheep	Age at Slaughter (months)	Treatment
DORSET HORN		
1	4	Supplementary
HAMPSHIRE × SUFFOLK × SHROPSHIRE		
9	8	Chopped timothy[a]
12	8	Alfalfa pellets[b]
6	15	Chopped timothy[a]
24	15	Chopped alfalfa[c]
6	15	Timothy pellets[b]
6	15	Corn–timothy pellets[d]
24	15	Alfalfa pellets[b]
RAMBOUILLET × COLUMBIA		
9	20	Chopped timothy[a]
9	27	Chopped timothy[a]
9	27	Timothy pellets[b]
9	27	Corn–timothy pellets[d]
SHROPSHIRE × HAMPSHIRE		
1	27	Corn–timothy pellets[d]

[a] Timothy hay harvested on June 4, 1959.
[b] Timothy or alfalfa hay ground through a 1/16-in. screen prior to pelleting.
[c] Second cutting alfalfa hay.
[d] Pelleted mixture of 45% corn meal and 55% ground timothy hay.

RESULTS AND DISCUSSION

BODY COMPOSITION

The population of the sheep studied represents a wide range in age and nutritional history. It is no surprise that a very wide range in body composition was obtained. The average of, and the range in, the weights of the major chemical constituents are presented in Table 2. The same chemical constituents expressed as percentages of the ingesta-free body are recorded in Table 3.

RELATION BETWEEN SHRUNK LIVE WEIGHT AND EMPTY-BODY WEIGHT

The relationship between shrunk live weight and empty-body weight and all the others presented in this report were studied by calculating regression

TABLE 2 Weights of Constituents of 125 Sheep Bodies

Constituent	Average (kg)	Range (kg)
Shrunk body weight[a]	39.92	17.10–75.75
Gut contents	5.99	3.21–12.13
Fat[b]	7.74	0.64–29.24
Protein[b]	5.27	2.08–9.45
Water[b]	19.52	9.72–35.94
Ash[b]	1.27	0.58–2.41

[a]Sheep received water but were deprived of feed 18 to 20 hours prior to weighing.
[b]Chemical components in the ingesta-free bodies.

TABLE 3 Percentage of Constituents in the Ingesta-Free Bodies of 125 Sheep

Constituent	Average (%)	Range (%)
Water	60.02	39.58–73.86
Fat	19.79	4.12–46.61
Protein	16.020	11.23–19.20
Ash	3.983	1.72–5.79

equations for each of the subsamples included in this study. The 123 sheep for which the ration was known were divided into the following subgroups according to ration fed: chopped timothy, chopped alfalfa, pelleted timothy, pelleted alfalfa, and corn–timothy pellets. The various regression coefficients were examined for homogeneity, and the data were pooled accordingly.

Since the empty-body weight represents the largest proportion of the shrunk live weight, it is to be expected that these two variables would be highly correlated, especially if one considers the great variability of the empty-body weight (Table 4).

The regression analysis revealed that for both the pelleted and the chopped rations the species of forage, *Phleum protense* and *Medicago sativa* (timothy versus alfalfa), did not significantly affect the regression. On that basis, the data were regrouped into the following categories: chopped hay, pelleted hay, and corn–hay pellets. A test of homogeneity of the regression coefficients obtained for each of these groups showed that the linear equation computed for the chopped-hay group was significantly different at the 2% level of probability from that computed for either the pelleted hay or the corn–hay pellet

TABLE 4 Relations between the Shrunk Live Weight and the Empty-Body Weight of Sheep Ingesting Various Diets

Diet	Prediction Equation[a]	Obser-vations	Empty-Body Weight (kg)	r^b	$S_{y \cdot x}{}^c$ (kg)
All diets	$Y = -3.280 + 0.931X$*	125	33.9 ± 13.1	0.994	1.5
	$Y = 20.102 + 1.152X - 20.446 \log X$†	125	33.9 ± 13.1	0.995	1.4
Chopped hay	$Y = -2.230 + 0.876X$*	57	29.9 + 9.3	0.994	1.0
Pelleted hay	$Y = -2.217 + 0.919X$*	51	34.5 ± 13.6	0.997	1.1
	$Y = 17.191 + 1.096X - 16.834 \log X$†	51	34.5 ± 13.6	0.997	1.0
Corn–hay pellets	$Y = -1.933 + 0.928X$*	15	46.6 ± 14.7	0.996	1.4
Pelleted feeds	$Y = -2.415 + 0.928X$*	66	37.3 ± 14.6	0.997	1.2
	$Y = 13.299 + 1.066X - 13.500 \log X$†	66	37.3 ± 14.6	0.997	1.1

*Linear equation.
†Curvilinear equation. A curvilinear equation is presented when it significantly ($P < 0.05$) improved the predictive value of the linear model.
[a] Y = Empty-body weight (kg); X = Shrunk live weight (kg).
[b] Correlation coefficients.
[c] Standard error of estimate.

group. For these last-mentioned groups, a common equation describes the relationship as accurately as the corresponding two equations.

The relationship between empty-body weight and shrunk live weight was significantly ($P < 0.01$) curvilinear only when sheep were fed pelleted hay. As a result, the curvilinear equation calculated for the pooled data improved significantly ($P < 0.01$) the accuracy of the prediction by the linear model.

At a given shrunk live weight, the empty body is significantly larger for the sheep fed pelleted feeds than for those receiving chopped hay. At a shrunk body weight of 45.36 kg (100 lb) it was computed that the ingestal fill contributes 17% of the body weight of a sheep fed chopped hay, whereas it would contribute only 13% of the body weight if the sheep had received a pelleted ration.

For 93 sheep included in this study, daily dry matter intake prior to a 20-hr fast and various physical dimensions (height at withers, length from shoulders to pin bones, and heart girth) were measured. When these measurements were considered in a multiple regression equation in addition to shrunk body weight, no statistical improvement was obtained over the equation that included as independent variables shrunk live weight and the logarithm of shrunk live weight.

A corollary to the previous finding that pelleting modifies the relationship between shrunk live weight and empty-body weight is the fact that pelleting of the diet decreases the amount of fill in the "shrunk sheep" (Table 5). To

TABLE 5 Gut Contents of 123 Sheep on Various Experimental Diets

Diet	Observations	Gut Contents: Weight, Actual (kg)	Gut Contents: Weight, Adjusted[b] (kg)	Gut Contents: Percentage of Shrunk Body Weight[a], Actual (%)	Gut Contents: Percentage of Shrunk Body Weight[a], Adjusted[b] (%)
Chopped hay	57	6.67	7.08	18.2	17.7
Pelleted hay	51	5.42	5.41	10.0	13.6
Corn–hay pellets	15	5.60	4.75	10.7	11.9

[a] Shrunk body weight taken after 18 to 20 hours of fast, sheep having access to water.
[b] Adjusted to mean shrunk live weight of all sheep, 39.9 kg.

gain more insight into this problem, the gut water and the gut dry matter were separately regressed on shrunk live weight. The average gut water and gut dry matter for each of the main types of feeds are recorded in Table 6. A test of homogeneity of the regression coefficients (Table 7) revealed that at a given body weight there were no significant differences in gut dry matter between

TABLE 6 Gut Water and Gut Dry Matter of 123 Sheep on Various Experimental Diets

Diet	Observations	Gut Water		Gut Dry Matter	
		Actual (kg)	Adjusted[a] (kg)	Actual (kg)	Adjusted[a] (kg)
Chopped hay	57	5.93	6.28	0.74	0.79
Pelleted hay	51	4.70	4.69	0.72	0.72
Corn–hay pellets	15	4.81	4.12	0.79	0.61

[a] Adjusted to mean shrunk live weight of all sheep, 39.9 kg.

TABLE 7 Relation between the Shrunk Live Weight and Gut Dry Matter or Gut Water

Item	Regression Equation[a]	Observations	r[b]	$S_{y \cdot x}$[c] (g)	Gut Dry Matter or Water (g)
GUT DRY MATTER					
Chopped hay	$Y_1 = 138 + 0.164X$	57	0.76	146	739 ± 225
Pelleted rations	$Y_1 = 209 + 0.124X$	66	0.78	159	738 ± 250
GUT WATER					
Chopped hay	$Y_2 = 1970 + 1.081X$	57	0.77	935	5932 ± 1470
Pelleted rations	$Y_2 = 2234 + 0.582X$	66	0.66	956	4723 ± 1381

[a] Y_1 = Gut dry matter (g); Y_2 = Gut water (g); and X = shrunk body weight (kg).
[b] r = correlation coefficient.
[c] $S_{y \cdot x}$ = standard error of estimate.

the three types of feed considered. On the other hand, gut water was significantly larger ($P < 0.01$) for the sheep fed chopped hay than for those receiving the pelleted rations. At a mean shrunk body weight of 39.9 kg, this difference in gut water was equal to 1.6 liters.

The problem of deriving an estimate of the empty-body weight of ruminants has long been recognized. Makela[4] studied the relationships between the dry matter intake and various measurements of digestive tract contents of cows: the contents of the reticulum–rumen, the contents in the intestines, the percentage of dry matter in the alimentary tract contents, and the percentage of dry matter in the reticulum–rumen. The strongest relationship was that between the dry matter intake and the dry matter in the gut.

Stroud[7] established a multiple regression equation for data obtained with cattle in which 75% of the variation in reticulum–rumen contents was ac-

counted for by three factors: dry-matter intake, percentage of dry matter fed as hay, body weight, and curvilinear functions of the preceding variables. Also, in that study the steers were deprived of feed and water for 12 to 17 hr prior to measurements of the alimentary tract contents.

In both of these studies with cattle,[4,7] the gut contents were strongly correlated with the dry-matter intake. In the present study, the correlation between these two measurements was not significant (Table 8). This difference

TABLE 8 Correlation Coefficients between Various Measurements and the Weight of the Ingesta of 93 Sheep

Independent Variable	X^a	r^b
Shrunk live weight (kg)	43.2 ± 13.6	0.46*
Height (inches)	27 ± 2.1	0.41*
Length (inches)	28 ± 2.8	0.34*
Girth (inches)	34 ± 4.2	0.38*
Dry matter intake (kg)	1.2 ± .7	0.10

[a] Mean and standard deviation of the independent variable considered.
[b] Simple correlation coefficient. *Significant at the 1% level of probability. The mean digestive tract content was equal to 1.80 kg ± 0.55.

might reflect the fact that in the present experiment, although the sheep were deprived of feed for 20 hr prior to slaughter, they had access to water.

PROPORTION OF THE TOTAL GUT CONTENTS LOCATED IN THE RUMEN

In the present study, the rumen and reticulum contents represented 71.3% ± 5.66% of the total gut contents. The nature of the diet (Table 9) did not sta-

TABLE 9 Proportion of the Total Gut Content Weight Located in the Rumen and Reticulum of 123 Sheep

Diet	Rumen and Reticulum Contents (% of Total Gut Content)
All diets	71.3 ± 5.7
Chopped timothy	72.5 ± 4.0
Pelleted timothy	73.0 ± 5.0
Corn-timothy pellets	74.5 ± 5.0
Chopped alfalfa	72.3 ± 5.0
Pelleted alfalfa	67.3 ± 6.0

tistically affect the proportion of the total gut contents found in these two compartments. This value is very similar to the 72% obtained by Mitchell *et al.*[5] for the "first three stomachs" of sheep. Makela[4] reported a value of 72.8% for cattle. In studies in which sheep are fitted with a rumen cannula large enough to empty the rumen and reticulum, the total gut content weight could be derived from the knowledge of the rumen and reticulum content weight.

USE OF ANTIPYRINE (AP), N-ACETYL-4-AMINO-ANTIPYRINE (NAAP), AND CREATININE EXCRETION AS PREDICTORS OF BODY WATER

An earlier study concerned with the accuracy with which AP, NAAP, and urinary output of creatinine could be used as predictors of body composition in 65 living sheep has been published.[1,8] The experimental protocol for the use of these indices has also been published.[1,8] In this report the pooled results obtained with 125 sheep (including the first 65 animals studied), described in the experimental section, are presented.

To evaluate the merit of AP and NAAP as predictors of body water, no attempt was made to equate the water spaces of these two drugs with empty-body water or total body water. Instead, the empty-body water determined by desiccation was regressed on the dilution spaces of these two drugs, and the relationships were evaluated statistically. The results of such regression analyses for the various parameters considered are presented in Table 10.

TABLE 10 Prediction of Empty-Body Water of Sheep by Various Means[a]

Independent Variable	r[b]	$S_{y \cdot x}$[c]	CV[d]
Live weight	0.969	1.367	7.0
AP[e]	0.940	1.876	9.6
NAAP[f]	0.953	1.664	8.5
Creatinine	0.965	1.446	7.4
AP, NAAP	0.964	1.469	7.5
AP, NAAP, live weight	0.980	1.300	6.6
AP, NAAP, creatinine, live weight	0.986	0.930	4.8

[a] 125 sheep were used in these studies.
[b] r = correlation coefficient.
[c] $S_{y \cdot x}$ = standard error of estimate.
[d] CV = coefficient of variation, that is, standard error of estimate expressed as a percentage of the mean of the dependent variable.
[e] Antipyrine.
[f] N-acetyl-4-amino-antipyrine.

One means of evaluating an indirect method of estimating body composition (in this particular case, body water) is to assess statistically the additional information it provides beyond that supplied by body weight alone. By using this criterion it was shown that the combined use of AP, NAAP, and live weight in a multiple regression equation (Table 10) decreases significantly ($P < 0.05$) the standard error of estimate of the equation in which live weight is the sole predictor of empty-body water. The standard error of estimate is further decreased when all four independent variables are considered (AP, NAAP, creatinine, and live weight). It is not possible to generalize on the practical value of the 31% improvement in the standard error of estimate achieved with that equation. Reid *et al.*, in their paper on "Some Peculiarities in the Body Composition of Animals" (this volume, page 19), report that when sheep are fed under normal conditions, and especially when their body weight is less than 45 kg, the variation in live weight is associated with over 96% of the variability in body composition. Under these conditions, it would be difficult to justify the use of the predictors discussed. However, when sheep are starved and refed, Reid *et al.* (this volume, page 19) have shown that the body composition at a given body weight is markedly different from that of sheep kept in continuous, positive energy balance. Under these conditions, AP, NAAP, and creatinine, either singly or in combination, would be useful predictors of body composition.

RELATIONSHIP BETWEEN DIRECTLY MEASURED EMPTY-BODY PROTEIN AND POTASSIUM

The failure of AP, NAAP, creatinine excretion, density, and other indirect indices (except the hydrogen isotopes) to provide satisfactory accuracy in resolving the composition of the live animal stimulated interest in the estimation of body potassium by whole-body counting of the naturally occurring potassium-40 isotope. In this procedure, the underlying principle is the assumption that potassium and body protein are highly correlated. Most of the early evidence was not evaluated statistically over a wide range of body composition.[2] Recently Kirton *et al.*[3] investigated the relationships between the potassium analyzed by flame photometry and the composition of pig empty bodies. These authors suggested, "potassium is of questionable value for predicting the chemical composition of pig bodies or carcasses, unless methods are found for reducing some of the errors in these relationships."

In this report, the standard errors of chemical determination of potassium and protein in the sheep empty body were estimated. The minimum standard error of estimate associated with the prediction of body protein from body potassium was calculated after the effect of analytical errors had been nullified.

This minimum standard error of estimate provides a theoretical limit of accuracy in the prediction of body protein from body potassium, when body potassium is determined by an indirect method such as whole-body counting.

Forty-six sheep were used. These sheep received either chopped hay or the same hay in the pelleted, ground form for 4 months prior to slaughter. The whole empty bodies were ground in a high capacity grinder. The ground material was homogenized by passing it through the grinder six times. Four subsamples (15 to 20% of the original weight) were freeze-dried and subsequently ground with dry ice to a fine powder. Chemical analyses were performed on this final sample. For each sheep, four replicates were analyzed for protein by the Kjeldahl method, and eight replicates were analyzed for potassium by atomic absorption spectrometry. For potassium analyses, digestion of the tissue samples was carried out over low heat first with nitric acid and then, after most of the organic matter had been oxidized, with hydrogen peroxide.

The mean composition of the sheep is presented in Table 11. The potassium content of the fat-free mass (Table 12) for various body weight ranges is of the same order of magnitude as that reported by Spray and Widdowson[6] for other species. The wide range in potassium and protein content accounts in part for

TABLE 11 Percentage Composition of the Empty Bodies of 46 Sheep Used in the Potassium Experiment

Component	Mean	SD	Range
Fat (%)	18.51	5.02	4.10–36.83
Water (%)	60.77	6.41	47.21–72.58
Protein (%)	16.75	1.51	13.58–19.18
Ash (%)	4.11	0.81	2.65–5.80
K (mg/g of dry matter)	6.33	1.64	3.59–9.60

TABLE 12 Potassium Content of the Empty Bodies of 46 Sheep

		Potassium per 100 g	
Body Weight Range	Observations	Fat-Free Mass (mg)	Protein (mg)
20–30 kg	9	292	1453
30–40 kg	16	293	1425
40–50 kg	10	289	1414
50–60 kg	7	285	1373
60–70 kg	3	315	1460
73.2 kg	1	276	1399

TABLE 13 Prediction of Empty-Body Protein (kg) from Empty-Body Potassium (g) and Other Parameters

Independent Variable	Regression Coefficients b_1	b_2	b_3	a[a]	R[b]	$S_{y \cdot x}$[c] (kg)	CV[d] (%)
Potassium (g)	0.0686	–	–	0.1518	0.973	0.339	5.9
Live weight (kg)	0.1172	–	–	1.6210	0.976	0.316	5.5
Empty-body weight (kg)	0.1116	–	–	1.1309	0.975	0.327	5.7
Potassium, live weight	0.0308	0.0646	–	0.5630	0.989	0.219	3.8
Potassium, empty-body weight	0.0342	0.0623	–	0.7664	0.988	0.229	4.0
Potassium, empty-body weight, log empty-body weight	0.0313	0.0143	4.5158	4.1866	0.991	0.219	3.5

[a] Constant term of regression equation.
[b] Total or multiple correlation coefficient.
[c] Standard error from regression.
[d] Coefficient of variation, that is, standard error of estimate expressed as a percentage of the mean empty-body protein.

the high correlation coefficients observed in the relationships between empty-body protein and body potassium (Table 13). Consideration of body potassium, empty-body weight, and the logarithm of empty-body weight provided ($P < 0.01$) more refinement in the prediction of empty-body protein than shrunk live weight alone. More information concerning this particular multiple regression equation is given in Table 14. The standard partial regression

TABLE 14 Analysis of a Multiple Regression Equation Predicting Body Protein (Y) from Potassium (X_1), Body Weight (X_2), and the Logarithm of Body Weight (X_3)

Item	Coefficient
TOTAL CORRELATION	
$r_{y}{}^{1}$	0.973
$r_{y}{}^{2}$	0.976
$r_{y}{}^{3}$	0.978
STANDARD PARTIAL REGRESSION COEFFICIENTS	
b_1	0.443
b_2	0.119
b_3	0.444
MULTIPLE CORRELATION	0.991

TABLE 15 Analysis of Variance Components of Potassium and Protein Determinations in 46 Sheep Empty Bodies[a]

Source of Variation	Potassium (8) df	Potassium (8) σ^2	Potassium (4) df	Potassium (4) σ^2	Protein df	Protein σ^2	Covariate (4) df	Covariate (4) σ^2
Among sheep	45	398	45	369	45	2,072,061	45	27,002
Among determinations within sheep	316	32	138	36	138	9,201	138	–25
TOTAL	361	430	183	405	183	2,081,262	183	26,977

[a] An average of eight subsamples were analyzed for estimating the empty-body potassium, and four subsamples were analyzed for measuring empty-body protein. For potassium determinations, the variance components (σ^2) and degrees of freedom (df) have been calculated for eight replicates and for the first four replicates. To estimate the covariance components, the first four potassium subsamples were considered.

coefficients show that the logarithm of the empty-body weight provides as much information as body potassium.

The variance in body protein and potassium was partitioned by analysis of variance into components associated with variation among sheep and analytical errors of determination (Table 15). The empty-body protein was determined with greater accuracy than was empty-body potassium. This is also illustrated in Table 16. The coefficient of variation associated with the determination of body protein when one analysis is performed is 1.6%, whereas the corresponding value for body potassium is 7.0%. This underlines the fact that one cannot ignore errors in measuring body chemical components when deriving regression equations relating these components. Neither the dependent variable nor the independent variable is determined without error. By using the variance components presented in Table 15, the correlation coefficient between body protein and body potassium was expressed as a function of the number of replicates used for potassium (N_k) and protein (N_p) analyses.

$$r = \frac{27{,}002.27}{\sqrt{\left(368.96 + \frac{36.49}{N_k}\right)\left(2{,}072{,}061 + \frac{9201.156}{N_p}\right)}}.$$

The residual covariance term corresponding to the protein–potassium interaction among determinations within sheep was ignored because it was not significant. This function is presented in Figure 1, for $N_p = 4$. The standard errors of estimate corresponding to each value of the correlation coefficient are plotted on the same figure. Had duplicate analyses been used for each carcass analysis, as is often done, the standard error of estimate would have been 440 g and would have been 30% higher than that observed in this study. When the number of replicate analyses increases, the asymptotic value for the standard error of estimate is 310 g of protein for a mean empty-body protein of 5.75 kg. This figure provides us with an estimate of the *limit of accuracy* that could be achieved when potassium is determined by an indirect procedure.

These investigations were supported by Public Health Service Research Grant Number AM-02889 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

The authors express their thanks to Professor R. M. Forbes, of the University of Illinois at Urbana, for his generous cooperation in the analyses of potassium in carcass samples.

TABLE 16 Analytical Errors Associated with the Direct Body Protein and Potassium Determinations

Item	Replicate	Standard Error (g)	CV[a] (%)
Protein	1	96	1.6
	4	48	0.8
Potassium	1	6	7.0
	8	2	2.5

[a]Coefficient of variation, that is, standard deviation expressed as a percentage of the mean empty-body protein or mean empty-body potassium.

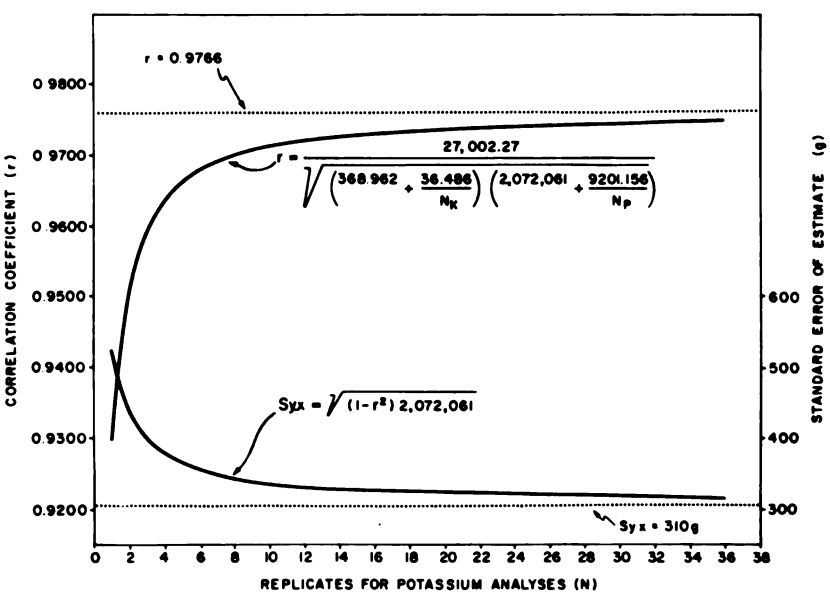


FIGURE 1 Effect of the number of potassium analyses (N_k) on the correlation coefficient and the standard error of estimate of the relationship between body protein and body potassium. [The number of protein analyses (N_p) was set at four.]

REFERENCES

1. Bensadoun, A., B. D. H. Van Niekerk, O. L. Paladines, and J. T. Reid. 1963. Evaluation of antipyrine, N-Acetyl, 4-Amino-Antipyrine and shrunk body weight in predicting the chemical composition and energy value of the sheep body. J. Anim. Sci. 22:604.
2. Ciba Foundation colloquia on aging. 1958. IV. Water and electrolyte metabolism in relation to age and sex. Churchill, London.
3. Kirton, A. H., R. H. Graedinger, and A. M. Pearson. 1963. Relationship of potassium and sodium content to the composition of pigs. J. Anim. Sci. 22:904.
4. Makela, A. 1956. Studies on the question of bulk in the nutrition of farm animals with special reference to cattle. Acta Agr. Fenn. 85:1.
5. Mitchell, H. H., W. G. Kammlade, and T. S. Hamilton. 1928. Relative energy value of alfalfa, clover and timothy hay for the maintenance of sheep. Agr. Exp. Sta. Bull. 317.
6. Spray, Christine M., and Elsie M. Widdowson. 1949. The effect of growth and development on the composition of mammals. Brit. J. Nutr. 3:332.
7. Stroud, J. W. 1961. The development of indirect methods for the estimation of the chemical composition and energy value of the living cattle. Ph.D. Thesis, Cornell University, Ithaca, New York.
8. Van Niekerk, B. D. H., J. T. Reid, A. Bensadoun, and O. L. Paladines. 1963. Urinary creatinine as an index of body composition. J. Nutr. 79:463.

G. F. Fries
and G. P. Lynch
U.S. Department of Agriculture

EXCHANGEABLE-POTASSIUM AND BODY-COMPOSITION MEASUREMENTS IN SHEEP

The quantitative prediction of the various body components of living animals has been of concern for many years. A workable method of prediction must have several important characteristics. The method must be nondestructive, reproducible, and accurate, as well as relatively simple and economical to apply.

It has been recognized for many years that the three body components, water, fat and fat-free tissue, can be predicted from the determination of either body water or body fat.[3] In recent years a considerable amount of work has been carried out with humans, relating exchangeable sodium (Na_e) and exchangeable potassium (K_e) to total body water and other body components.

Moore *et al.*[6] report that the sum of Na_e and K_e accounts for about 95% of the osmotically active cations of the body. They cite work indicating that the sum of Na_e and K_e, as determined by isotope dilution, is closely related to total body water. Using improved methods, Boling and Lipkind[1] found that the sum of Na_e and K_e correlated exceedingly well with total body water as determined by tritium dilution (r = 0.991).

These methods of determining Na_e and K_e have been well described and can be readily adapted to farm animals by use of commonly available equipment. The short half-lives of ^{24}Na and ^{42}K permit K_e and Na_e to be estimated with minimal problems of radioactive contamination. Holding the animals and waste products for a week would allow the total administered activity to decay to a level that could be ignored.

As a result of these considerations, we carried out studies to determine the relationships between Na_e and K_e in lambs and the composition of their carcasses. The results indicated that exchangeable potassium alone shows the most promise as a predictor of carcass composition. Therefore, additional work has been carried out to determine some of the factors that may affect the accuracy of the exchangeable-potassium measurement.

MATERIALS AND METHODS

Thirty-two animals were used in the various phases of the studies. In general, the measurements made included Na_e, K_e, carcass water, carcass fat, and carcass fat-free tissue. In some of the later work, where the interest was only in certain aspects of the problem, not all measurements were made. On three occasions there was a question as to whether the animals received the appropriate isotope dose, so the results from these animals were excluded. For these reasons the number of comparisons reported is less than the total number of animals used.

Cross-bred ram lambs weighing from 25 to 40 kg were used. Three days prior to the administration of the isotopes, the lambs were placed in metabolism crates. The lambs were fed twice daily. The diet consisted of approximately 500 g/day of a concentrate mixture and alfalfa hay *ad libitum*.

The lambs were given an oral dose of 50 μc of ^{24}Na and 100 μc of ^{42}K. Total collections of urine were made for the three periods from 0 to 24 hr, 24 to 48 hr, and, except in the earliest observations, 48 to 72 hr. The activity of ^{24}Na and ^{42}K was determined by differential gamma counting,[4] and the stable sodium and potassium were determined by flame spectrophotometry.[5]

The exchangeable sodium and exchangeable potassium were calculated by using the equation:

$$Na_e \text{ or } K_e = \frac{100 - \left(A + \frac{B}{2}\right)}{B/C}, \qquad (1)$$

TABLE 1 Correlation Coefficients and Regression Equations Relating the Various Measures of Body Composition and Body Pools of Exchangeable Sodium, Potassium, and Sodium + Potassium in the Live Sheep

Parameters	Number	r	Regression Equation	S_b
LIVE WEIGHT VERSUS				
Carcass water	24	0.85*	$CW = 0.2406(LW) + 0.892$	0.0317
Carcass fat-free tissue	24	0.84*	$CF = 0.3681(LW) + 0.048$	0.0512
Na_e	18	0.46	$Na_e = 25.48(LW) + 1265$	12.43
K_e	21	0.80*	$K_e = 51.03(LW) - 282.8$	8.68
$Na_e + K_e$	17	0.83*	$Na_e + K_e = 77.60(LW) + 940.8$	13.26
CARCASS WEIGHT VERSUS				
Na_e	18	0.13	$Na_e = 39.09(CWT) + 1557$	21.98
K_e	21	0.80*	$K_e = 93.71(CWT) + 28.85$	15.89
$Na_e + K_e$	17	0.84*	$Na_e + K_e = 137.7(CWT) + 1528$	23.23
CARCASS WATER VERSUS				
Na_e	18	0.34	$CW = 0.00173(Na_e) + 5.255$	0.00121
K_e	21	0.89*	$CW = 0.00369(K_e) + 3.757$	0.00047
$Na_e + K_e$	17	0.84*	$CW = 0.00252(Na_e + K_e) - 0.007$	00.00040
CARCASS FAT-FREE TISSUE VERSUS				
Na_e	18	0.36	$CF = 0.00288(Na_e) + 6.155$	0.00188
K_e	21	0.86*	$CF = 0.00522(K_e) + 5.037$	0.00070
$Na_e + K_e$	17	0.87*	$CF = 0.00371(Na_e + K_e) - 0.812$	0.00053

*Correlation coefficient at significant level.

where

Na_e = exchangeable sodium (mEq),
K_e = exchangeable potassium (mEq),
A = ^{24}Na or ^{42}K excreted in urine from 0 to 24 hr (% dose),
B = ^{24}Na or ^{42}K excreted in urine from 24 to 48 hr (% dose), and
C = Na or K excreted in urine from 24 to 48 hr (mEq).

When the excretion of ^{24}Na and ^{42}K was followed through 72 hr, second values for Na_e and K_e were calculated by Equation 1 with the time intervals for A, B, and C changed appropriately.

One week after dosing with the isotopes, the lambs were slaughtered. The warm carcasses were weighed immediately after slaughter, placed in plastic bags, and frozen. Prior to analysis, the frozen carcasses were sliced into thin strips, ground, and mixed. Samples of ground carcass were freeze-dried to constant weight for removal of moisture. Portions of the dried samples were then used for fat extraction with diethyl ether.

RESULTS AND DISCUSSION

The regression equations and correlation coefficients relating some of the more important variables studied are presented in Table 1. The values of Na_e, K_e, and their sum were based on the estimates from the 24- to 48-hr collection period, because this was the only collection period common to all animals. All correlations involving the various parameters were significant ($P < 0.01$), except those involving Na_e. Some of the more interesting relationships, involving K_e or $Na_e + K_e$ and live weight, carcass water, and carcass fat-free tissue are illustrated in Figures 1–6.

As would generally be expected, there were high correlations between live weight and the carcass components, water and fat-free tissue. In order to be useful, any proposed method must be an improvement over live weight as a predictor of the components in question. The correlations between K_e and the carcass components, water and fat-free tissue, were both higher than corresponding correlations with live weight. The correlation between K_e and carcass water was the highest correlation between any of the variables examined in this study.

The use of the sum, $Na_e + K_e$, did not improve the prediction of carcass composition. In the case of carcass water, the correlation was somewhat lower than the correlation with K_e alone. These results may appear to be in disagreement with the work in humans based on tritium dilution,[1,6] in which the correlations between Na_e and K_e individually with body water are low, while the

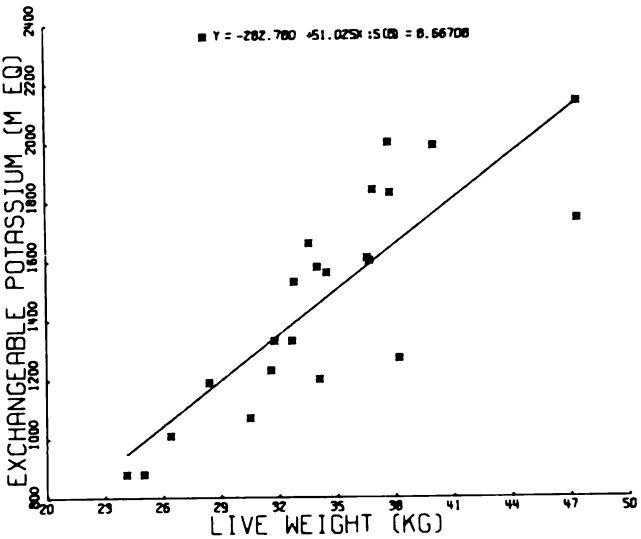


FIGURE 1 Total exchangeable potessium and live weight in sheep.

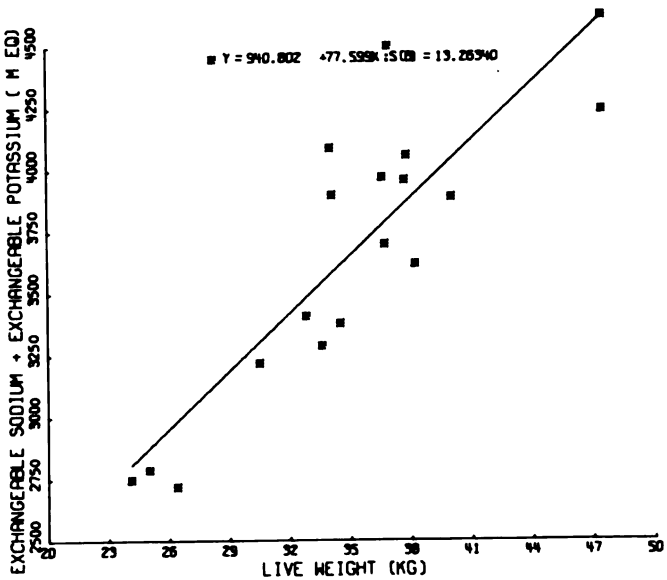


FIGURE 2 Total exchangeable sodium plus total exchangeable potessium and live weight in sheep.

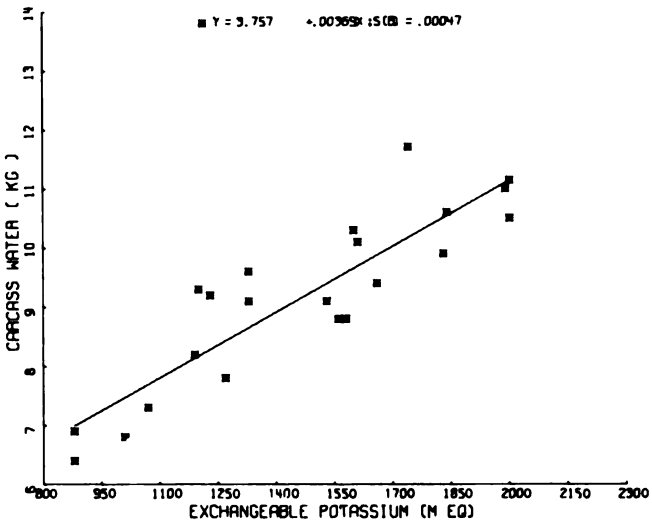


FIGURE 3 Total exchangeable potassium and carcass water in sheep.

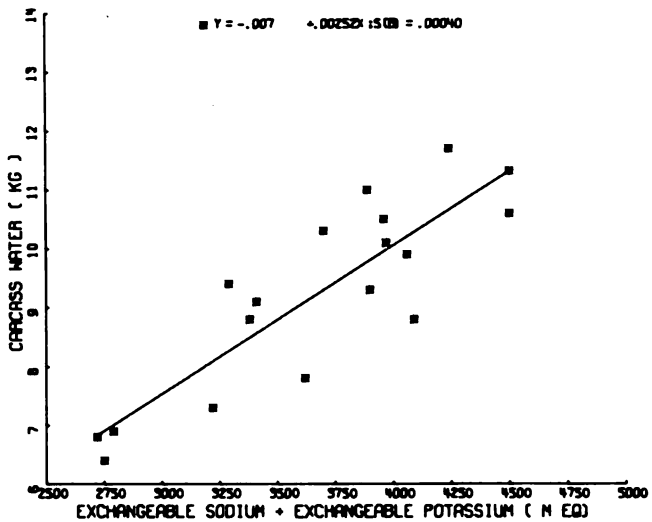


FIGURE 4 Total exchangeable sodium plus total exchangeable potassium and carcass water in sheep.

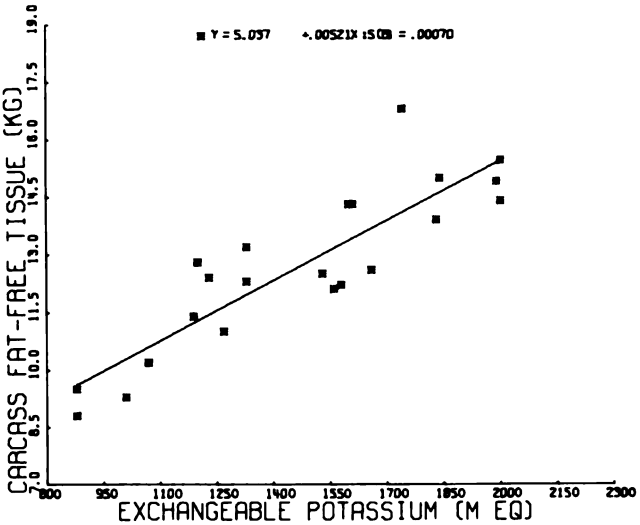


FIGURE 5 Total exchangeable potassium and carcass fat-free tissue in sheep.

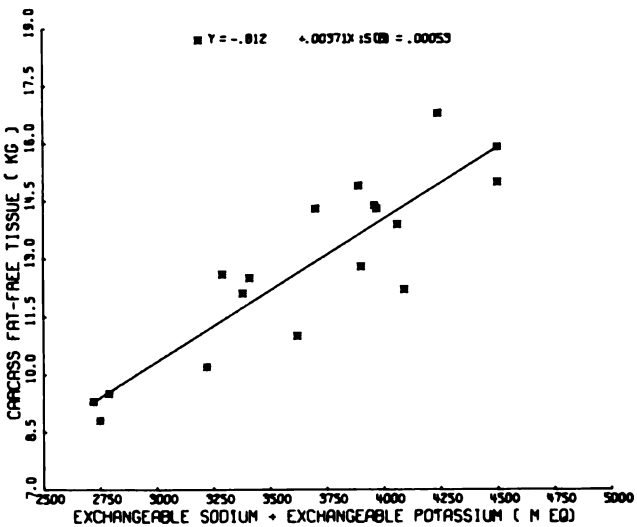


FIGURE 6 Total exchangeable sodium plus total exchangeable potassium and carcass fat-free tissue in sheep.

correlation of the sum with body water is high. However, this work relates the measurements to carcass water, while the human work relates them to whole-body water, so the difference may be more apparent than real.

The only measurements in our study that may be directly compared with the work with humans are the relations of Na_e and K_e to live weight. In spite of the low correlation, the equation relating Na_e to live weight in grams (Table 1) is in very close agreement with the equation

$$Na_e = 21.01 \text{ (body weight)} + 1414, \quad (2)$$

which Moore[6] found with male humans. In contrast, the equation relating K_e to live weight in rams (Figure 1) had greater slope and lower intercept than the corresponding equations for different age groups in the male human. Whether the difference is due to the lack of similarity in physiological age or to a true species difference cannot be stated at this point.

The correlations between Na_e, K_e, or $Na_e + K_e$ and the various measures of body composition were generally lower than analogous correlations reported for humans.[1,6] While one cannot rule out possible species or technique differences, examination of both sets of data suggests that the primary reason for the difference was that the human work covered a much wider range of body weight than our sheep work.

The results of our studies indicate that measurement of K_e shows some promise as a predictor of carcass composition, especially carcass water. Whether it would be better than the sum of Na_e and K_e for whole-body composition cannot be stated on the basis of our work. In either case, the usefulness of the method will depend upon improved precision of the K_e determination. The Na_e measurements were much less variable, and their determination posed no apparent problems.

Some of the factors that may have influenced our K_e measurements are problems associated with counting, failure to reach equilibrium, fecal potassium excretion, and variation in the gastrointestinal contents.

In this work ^{42}K was assayed by counting its gamma radiation. This is a convenient but somewhat inefficient method when routine equipment is used. In addition, when ^{24}Na is used, it is not possible to count the ^{42}K without the contribution of ^{24}Na. The correction for the ^{24}Na will be an additional source of error in the ^{42}K determination. The use of a large-volume detector such as described by Boling,[1] for counting the beta radiation of ^{42}K, would probably be a much more satisfactory method. If the measurement of Na_e is not necessary, the counting problems are greatly simplified.

All relationships between K_e and composition reported here were based on K_e measurements calculated from the urine excretion of ^{42}K during the 24- to 48-hr period. It has been reported[2] that among humans individuals may require different lengths of time to come to equilibrium and that K_e values calculated from single observations may not have much value. In our later work, we made a second estimate of K_e based on the activity of the urine

collected from 48 to 72 hr. The relationship between the two estimates is presented in Figure 7. The mean values for the two times were identical, and the correlation was high (0.96). However, the variation would suggest that using an average of the two estimates would be a better practice than using either estimate alone.

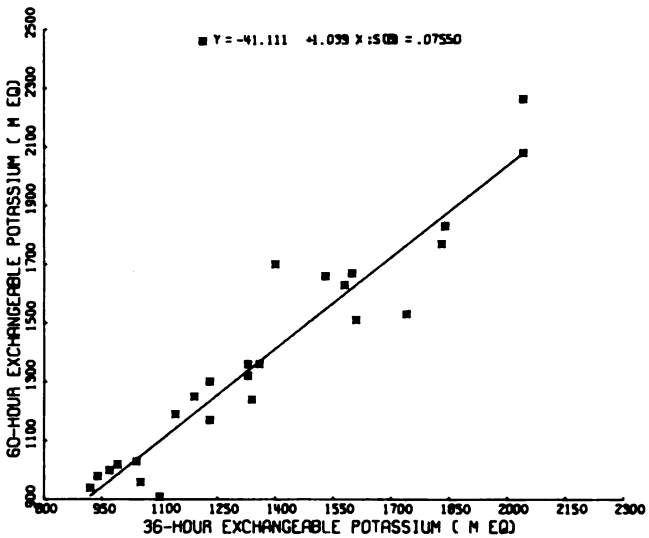


FIGURE 7 Total exchangeable potassium estimated from urine collected 24 to 48 hr after dosing (36 hr) and 48 to 72 hr after dosing (60 hr).

The length of time for the tracer to mix with the K_e space is only one aspect of the equilibrium problem. A second aspect, which is much more important in this work than in the human work, arises from the relatively large and discontinuous intake of stable potassium from the diet. It can be readily estimated that an average hay and concentrate diet fed at maintenance may contain from 20 to 30% as much potassium as the estimated K_e. This would make it questionable whether equilibrium could ever be attained except by feeding at very frequent intervals.

Early in our work we found that successive K_e measurements based on urine collection periods of less than 24 hr could be very erratic, apparently because of the irregularity of potassium intake. However, recalculation of the data on a 24-hr collection basis removed most of this variation. A typical but not extreme example of this is shown in Table 2. For this reason, the 24-hr collection period for the urine samples was adopted for all our work.

TABLE 2 Estimates of Exchangeable Potassium Calculated from 8- or 16-hr Collection Periods and 24-hr Collection Periods

8 or 16 hr		24 hr	
Time (hr)	K_e (mEq)	Time (hr)	K_e (mEq)
24–32	1,078	–	–
32–48	1,149	24–48	1,139
48–56	1,126	32–56	1,144
56–72	1,210	48–72	1,191
72–80	1,041	56–80	1,154

The large intake of potassium relative to the body content of potassium suggested that variations in the potassium content of the gastrointestinal tract might have an important influence on estimates of K_e. In addition, unlike in the human, feces may be an important route of potassium excretion in the ruminant. If the feces is an important or variable route of excretion, failure to measure this source of potassium loss would contribute to errors in the measurement of K_e. An experiment[4] was carried out to examine the importance of these questions.

Four lambs were used in a 4 x 4 Latin square design. The four dietary treatments ranged from all hay to all concentrate. Timothy hay was used in place of alfalfa in order to reduce the potassium intake. Total collection sodium and potassium balances were carried out with a K_e measurement during the last 3 days of the balance trial. At the end of the experiment, the lambs were slaughtered and a whole-body analysis was carried out.

Some of the more important results of this study are summarized in Table 3. Two points are of particular concern for the measurement of K_e.

The animals receiving only hay excreted significantly ($P < 0.01$) more of the ^{42}K dose by fecal route than the animals receiving the other diets. This

TABLE 3 Effect of Diet on Routes of Potassium Excretion and Exchangeable Potassium of Four Sheep

Diet		Potassium	72-hr ^{42}K Excretion		Potassium	Exchangeable
Hay (parts)	Concentrate (parts)	Intake (mEq/day)	Urine (% dose)	Feces (% dose)	Balance (mEq/day)	Potassium (mEq)
3	0	295	33.5	10.5	33.7	1,282
2	1	235	33.6	4.6	37.1	1,228
1	2	197	30.5	3.2	29.1	1,215
0	3	146	18.1	4.5	37.7	1,258

difference is large enough that it would affect the K_e calculation if the fecal excretion of ^{42}K were ignored. With the diets other than all hay, the fecal excretion of ^{42}K was fairly small and relatively constant over a wide range of intake. The intermediate diets typify the diets used in our work. While ignoring fecal ^{42}K may have overestimated K_e, it probably did not greatly affect the conclusions drawn.

The second point of interest is the significant ($P < 0.05$) differences in K_e resulting from the dietary treatments. In contrast to the other K_e values we determined, the fecal excretion of ^{42}K was included in calculating these values. The values for K_e were higher at both dietary extremes. The elevated fecal excretion of potassium and the higher levels of potassium found in the rumen support the possibility that increased potassium in the gastrointestinal tract is the cause of the higher K_e with the hay diet. An explanation for the higher K_e with the concentrate diet is not readily apparent.

The demonstration of significant dietary effects on the K_e values and the fecal excretion of ^{42}K indicate that studies of this type should be carried out under defined conditions. While some flexibility under the intermediate conditions may eventually be possible, present knowledge of these conditions would not warrant it.

The relationship of the average exchangeable potassium to the whole-body and carcass composition is presented in Table 4. The small numbers and the

TABLE 4 Relationship of Exchangeable Potassium to Whole-Body and Carcass Composition in Four Sheep

		Whole Body			Carcass		
Live Weight (kg)	K_e (mEq)	Water (kg)	Fat (kg)	Fat-Free Tissue (kg)	Water (kg)	Fat (kg)	Fat-Free Tissue (kg)
31.6	1,274	16.7	4.42	22.2	9.2	3.06	12.4
31.8	1,286	18.3	3.32	24.1	9.6	2.14	13.2
28.4	1,140	15.1	3.97	20.7	8.2	2.53	11.4
32.7	1,282	16,5	3.40	22.2	9.1	2.38	12.3

lack of great variation in the animals preclude an extensive analysis of the data. In general, the trends in K_e and the water and fat-free tissue contents of both the whole body and carcass were similar. The various components of the carcass were closely related to the corresponding components of the whole body. This suggests that most of the conclusion drawn about carcass composition would also be applicable to whole-body composition.

SUMMARY

Studies were carried out on sheep to determine the relation of exchangeable sodium, exchangeable potassium, and their sum to carcass composition of sheep. There was a high correlation between K_e and the carcass components, water and fat-free tissue. The sum $Na_e + K_e$ did not improve the prediction of these components. Secondary studies indicated that the estimate of K_e can be affected by diet.

On the basis of this work, K_e shows promise as a predictor of carcass composition in living sheep. Because of the factors that may affect K_e, these measurements should be carried out under defined feeding conditions. Further work would be required to improve the accuracy of this method.

REFERENCES

1. Boling, E. A., and J. B. Lipkind. 1963. Body composition and serum electrolyte concentrations. J. Appl. Physiol. 18:943.
2. Flear, C. T. G., W. T. Cooke, A. Sivyer, and J. Domenet. 1963. Measurement of total exchangeable potassium. Clin. Chim. Acta 8:768.
3. Lawes, J. B., and J. H. Gilbert. 1859. Experimental inquiry into the composition of some of the animals fed and slaughtered as human food. Phil. Trans., Pt. II, p. 494.
4. Lynch, G. P., G. F. Fries, and R. L. Hiner. 1968. Nutritional effects on potassium space estimates in sheep. J. Anim. Sci. 27:370.
5. Lynch, G. P., J. W. Thornton, R. L. Hiner, H. F. Travis, and G. H. Wellington. 1965. Variation in the sodium and potassium levels of lambs subjected to pre-slaughter carbon dioxide immobilization. J. Anim. Sci. 24:235.
6. Moore, F. D., K. H. Olesen, J. D. McMurrey, V. H. Parker, M. R. Ball, and C. M. Boyden. 1963. The body cell mass and its supporting environment. W. B. Saunders Co., Philadelphia.

Margaret A. Flynn
Fikri Hanna
Charles H. Long
R. Y. Asfour
Ruth N. Lutz
and S. E. Zobrisky
University of Missouri

DEUTERIUM-OXIDE DILUTION AS A PREDICTOR OF BODY COMPOSITION IN CHILDREN AND PIGS

Little is known of changes in body composition of animals in the early growth period. Data from direct chemical analyses of entire bodies and tissues of domestic animals at wide age intervals from conception to cell maturity indicate a decrease in percentage of total body water (TBW) and an increase in percentage of fat and protein.[1,6,11,16,23,24]

Direct chemical analysis of the living human or of the same animal during the growth period is not possible, so investigators have looked for accurate and precise indirect methods of assessing body composition.[5] This investigation is an attempt to compare dilution techniques *in vivo* with direct chemical analyses of whole bodies of piglets for TBW, fat, nitrogen, and potassium. The same methods were used to measure total body water and extracellular water (ECW) *in vivo* in normal and undernourished preschool children, to supplement the meager data on body compartments in the literature for this age group.

MATERIALS AND METHODS

The subjects used to test the techniques were eight newborn cross-bred Hampshire x Duroc piglets, four of each sex, from the same litter and seven Poland China piglets, five males and two females. At 2, 9, 16, 23, 30, 37, 70, and 102 days after birth, TBW and ECW were determined indirectly in one of the piglets selected at random. The piglet was then killed for direct chemical analysis of the whole body. Two Poland China piglets were also subjected to direct and indirect analyses at 28 days, three at 38 days, and two at 45 days of age. They were housed in confinement on the University of Missouri swine farm and had access to a standard creep ration until weaned at 4 weeks of age, when they received another ration designed to meet their nutritive needs according to the National Research Council recommendations.[17]

The subjects used for the study of normal children were 20 preschool children from 1 to 6 years of age who were examined by a pediatrician before each determination and found free of disease. They were within two standard deviations of normal weight and height according to the Iowa growth charts.[14] Results of serum protein and hematocrit determinations were within the normal range. Thirteen of these children were studied twice or more over a period of at least 2 years. Values for body water compartments of seven children under 1 year of age were available from Hanna.[12]

Calculations of nutrients from consecutive 5-day food intake records kept by the mothers, and from diet histories, revealed that the children consumed at least two thirds of the Recommended Dietary Allowances of the National Research Council.

The subjects used for the study of undernourished children were eight American and two Lebanese preschool children hospitalized with simple undernutrition. The children were 1 to 3 years of age. They were more than 2 SD below the mean length for age and over 2.5 SD below the mean weight for age by the Iowa growth charts.[14] They were free from chronic, inborn, endocrine, or metabolic diseases. Total body water and ECW were measured at the beginning of the study and at the end of at least 10 weeks of nutritional rehabilitation. The adequacy of their repletion diets was documented by the fact that all the children gained weight and some increased in height. Both of the Lebanese children ate a diet in which 50% of the calories came from Laubina (a 15% vegetable protein mixture), 40% came from full cream powdered milk, and 10% from sugar.[2]

The tracer used in this study to assess indirectly the amount of TBW was deuterium oxide. It is nonradioactive, and it distributes rapidly and uniformly

throughout the body without being metabolized or causing toxicity in the amounts used. Deuterium equilibrates with hydrogen in molecules other than water and overestimates TBW by 1%.[20] Sodium thiocyanate was selected as a tracer for ECW because it is administered by injection, has the same equilibration time in children as deuterium oxide, 2.5 hr,[7] and includes transcellular water and red-blood-cell water.[10] Total body water measured by deuterium oxide also includes transcellular water and red-blood-cell water. The difference between TBW and ECW is intracellular water (ICW). Because both TBW and ECW methods measure transcellular water, the estimate of ICW can be expected to be precise. Since ICW is in the fat-free part of the cell, it is assumed to represent a measure of body-cell mass. Nitrogen and potassium also measure body-cell mass.

A sterilized, injectable solution containing 1.5 mg of thiocyanate and 0.995 g of deuterium oxide per milliliter of solution was used for the indirect estimation of TBW and ECW. The solution was weighed in a sterilized syringe to the nearest 0.01 mg. One and a half milliliters of solution per kilogram of body weight was given to each animal intraperitoneally after a 5-ml blood sample was drawn from the jugular vein, followed at once by 2 ml of isotonic saline from another syringe to wash in any deuterium oxide in the needle. The empty syringe that had contained the deuterium oxide was reweighed to determine the exact amount injected. After 2.5 hr, 5 ml of blood was drawn. The two blood samples were allowed to clot. The serum was drawn off and sealed in ampules and frozen for later analyses. Deuterium oxide was determined by the falling-drop technique,[21] and thiocyanate was analyzed by spectrophotometry.[7] In the *in vivo* determinations, urine losses and equilibration of deuterium with hydrogen in molecules other than water were not considered. These two errors were estimated by Schloerb[20] to add up to 1 to 2% of TBW. The tracers were given to the children by intravenous infusion.

For direct analysis, each piglet was killed by intracardiac injection of Nembutal.® The abdominal cavity was opened. The intestines were removed and stripped of their contents. The empty body, which included the intestines, was weighed, packaged in a polyethylene bag, and frozen at -25°C. Subsequently, the frozen empty body was cut on a band saw into slices approximately 0.5 cm thick. In a -25°C walk-in freezer, these slices were passed first through a 0.5-cm plate of a heavy duty meat grinder and then twice through a 0.25-cm plate. An aliquot of the mixed ground whole body was put in glass jars and stored at -25°C. The intestinal contents were thoroughly mixed, and an aliquot sample was put into glass jars and stored at -25°C. Each sample was thawed and thoroughly mixed before subsamples were removed for water, fat, nitrogen, ash, and potassium determinations.[3]

RESULTS AND DISCUSSION

PIGS

The total body water values, as determined in young piglets by direct and indirect analysis and expressed as percentage of body weight, are given in Table 1. By necessity the indirect method included intestinal contents, therefore, the values for the corrected body water (values for empty body and intestinal contents) were used for comparison. The mean difference between direct, corrected total body analysis and *in vivo* analysis was +0.4% (S=0.239), whereas that between direct empty-body analysis and *in vivo* analysis was +0.1% (S=0.201). The statistical hypothesis that there is no difference between the two techniques of measurement was not disproved. The two-tail "t" was 1.54, whereas the "t" value required for significant difference at the 0.05% level is 2.14 with 14 degrees of freedom. In only 2 of the 14 animals for which intestinal contents were analyzed did correction for the contents change the percentage of TBW by as much as 1 percent. Judging from the limited number of animals used, the error introduced by the necessary inclusion of intestinal contents in the indirect analysis appears to be insignificant in swine of this age. Direct analyses of the whole animal involve losses of volatile substances other than water when vacuum desiccation is carried out at 100°C. There is also a possibility that water that would not normally be freely exchangeable in the living body could be released by such vigorous desiccation, and vice versa. These results show that the *in vivo* estimations of TBW in healthy, young piglets measures the same volume as desiccation. In their study of pigs, Groves and Wood[11] found *in vivo* body values slightly below those obtained by desiccation (r = 0.81) on the same animals.

In the piglets studied, the percentage of TBW in the whole body or fat-free body decreased with age (Table 1), similar to the findings of Wood and Groves[24] on piglets of about the same age and those reported by Hansard[13] for older pigs that weighed 39 to 42 kg. The average TBW of the piglets reported here was 79% of fat-free weight during their growth phase. Pace and Rathbun[18] reported average TBW in adult mammals to be 73.2% of fat-free weight. Apparently, TBW in adult and in growing animals differs, and the adult formula for estimating body fat on the basis of TBW was not applicable to this study. All the pigs in this study were lean, with a range of 4% to 13% fat, and did not have the extreme variations of fat reported in many other studies.[1]

The percentage of ECW in the piglets (ranging from 45 to 55%) was much higher than values (ranging from 28 to 45%) for children aged 3 days to 3 months studied by Hanna.[12] Ratios of the weights of potassium to nitrogen

TABLE 1 Direct and Indirect Methods for Estimating Total Body Water, Expressed as Percentage of Body Weight

Pig			Body Water (%)					
			Indirect Analysis	Direct Analysis			Difference[a]	
Sex	Age (days)	Weight (kg)	D_2O Dilution	Empty Body	Intestinal Contents	Corrected Total Body	Corrected Total Body D_2O	Empty Body D_2O
F	2	2.08	77.6	78.3	[b]	78.2	+0.7	+0.7
M	9	2.48	73.3	72.3	71.2	72.3	−1.0	−1.0
F	16	3.46	72.3	71.4	72.2	71.4	−0.9	−0.7
F	23	5.80	70.0	69.4	82.1	69.7	−0.3	−0.6
M	30	7.70	68.6	67.8	76.5	69.3	+0.7	−0.8
F	37	7.12	67.6	68.3	82.6	69.3	+1.7	+0.7
M	79	21.74	68.5	67.9	85.2	67.9	−0.6	−0.6
M	102	31.60	68.2	69.0	75.3	69.3	+1.1	+0.8
F	28	4.30	76.0	77.1	83.7	77.2	+1.2	+1.1
M	28	5.90	72.1	73.3	81.5	73.3	+1.2	+1.2
M	38	10.50	72.3	72.6	78.9	73.1	+0.8	+0.3
F	38	8.44	73.5	72.4	79.4	72.8	−0.7	−1.1
M	38	6.54	73.5	74.7	82.2	75.0	+1.5	+1.2
M	45	10.50	72.1	72.8	82.1	72.8	+0.7	+0.7
M	45	8.48	73.8	73.7	82.5	73.7	−0.1	−0.1

[a] Mean difference +0.4 ± 0.239 +0.1 ± 0.201.
[b] Insufficient weight for analysis.

vary from 92 to 110 (Table 2) and compare well with analyses of skeletal muscle in piglets: 101 to 111 reported by Pfau.[19] The calculated concentration of potassium in ICW was higher than the 150 to 160 mEq per liter usually accepted as normal. Extracellular water appears to be overestimated by thiocyanate in pigs, so the data for ECW and ICW are considered unreliable. Another investigator has found that thiocyanate does not measure ECW in pigs reliably and has suggested that the pig excretes or metabolizes thiocyanate in a manner different from the human function (E. Widdowson, personal communication).

TABLE 2 Body Composition of Piglets on Fat-Free Weight Basis by Direct Analysis

Age of Pig (days)	Fat-Free Weight (kg)	N (g)	K (g)	$\frac{K}{N} \times 1{,}000$
2	1.99	44	5.0	113
9	2.24	61	6.7	110
16	3.15	88	8.7	99
23	5.16	143	15.1	106
30	6.76	190	19.4	102
37	6.33	191	18.5	96
79	19.15	578	58.7	101
102	28.38	859	91.6	107
28	4.15	103	9.8	95
28	5.49	145	13.5	93
38	9.71	257	24.2	94
38	7.75	203	19.4	96
38	6.11	159	15.0	94
45	9.79	267	24.2	91
45	7.95	214	20.4	95

In both litters of pigs the percentages of nitrogen and total nitrogen increased with weight and age, as would be expected in growing animals building tissue. Wood and Groves' equation[24] for predicting nitrogen from TBW gave results that agree within 20% of analyzed values for the animals weighing from 2 to 10 kg but greatly underestimated nitrogen in the two heavier animals.

NORMAL CHILDREN

Results of the *in vivo* studies of TBW of preschool children are given in Figure 1. Shortly after birth, the percentage of TBW of the children was high.

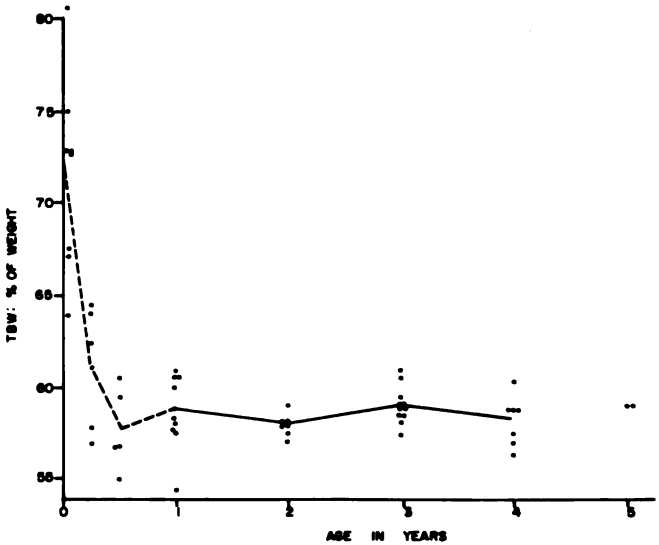


FIGURE 1 Total body water as percentage of body weight and age in normal children.

Then it decreased, approximating values of young adults from other studies by the first year, averaging 58.7% of body weight; and it changed little as the children grew to school age. No increase in percentage of TBW was noted at 2 and 3 years of age over that at 1 year of age, as reported by Friis-Hansen.[10]

Figure 2 reveals that there is a rapid decrease in percentage of ECW in the first year of life, with an average of 28.5% of body weight at 1 year and about 26.7% at 2 and 3 years, which compares with ECW in nine preschool children studied by Friis-Hansen.[10]

Percentage of ICW was variable from birth to 1 year (Figure 3). At 1 year, the average percentage of ICW was 30% of body weight, at 2 to 3 years, 31.4%; and at 3 to 4 years, 32%.

While TBW approximated adult values of about 59% of body weight by the end of the first year, ICW stabilization took longer, reaching a plateau between the second and third years. Longitudinal values for each of 13 preschool children are reported elsewhere.[8]

When the values for normal children were plotted against total body weight, there was a linear relationship between absolute amounts of TBW and weight, excluding the newborn period (r = 0.99) (Figure 4). Absolute amounts of ECW also showed a linear correlation with weight. The presentation of the data in this manner minimizes the differences in body water compartments as shown in Figures 1 and 2.

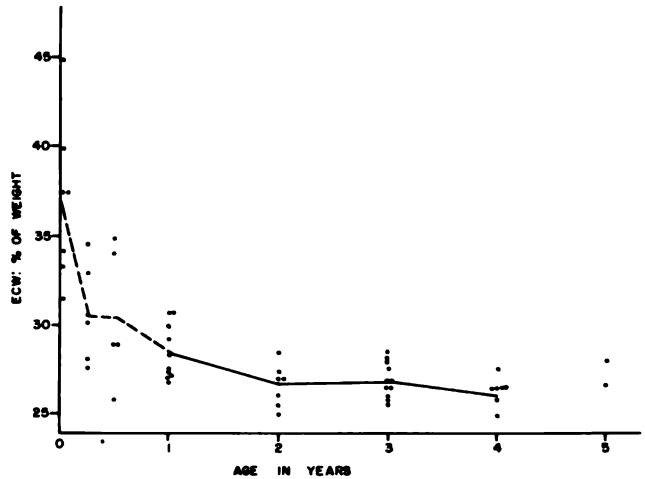


FIGURE 2 Extracellular water as a percentage of body weight and age in normal children.

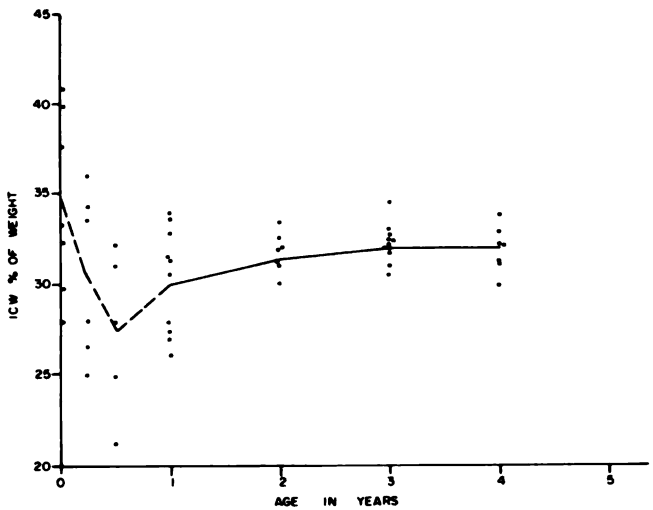


FIGURE 3 Intracellular water as a percentage of weight and age in normal children.

UNDERNOURISHED CHILDREN

The percentage of TBW of the undernourished children was significantly higher than that of the normal children.[9] The values for percentage of TBW

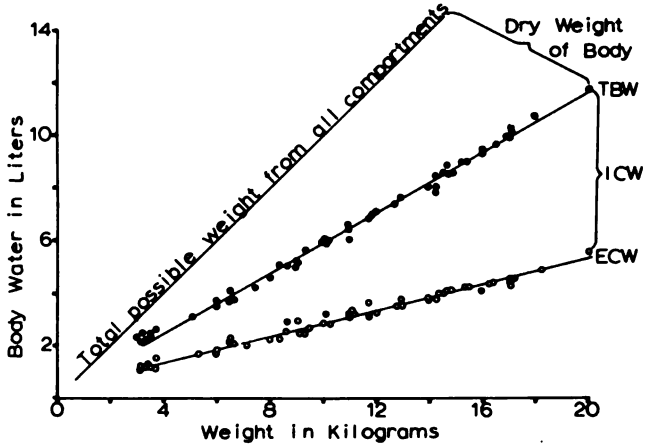


FIGURE 4 Body composition of normal children end weight.

decreased in all but one child over a period of 10 weeks to 4 months of adequate food intake.

The percentage of ECW of the undernourished children was above normal in every instance, with a range of 31 to 43%. During rehabilitation, the percentage of ECW returned to normal in all but one child (Figure 5). The exceptional child had severe undernutrition and had not had sufficient time to replete completely.

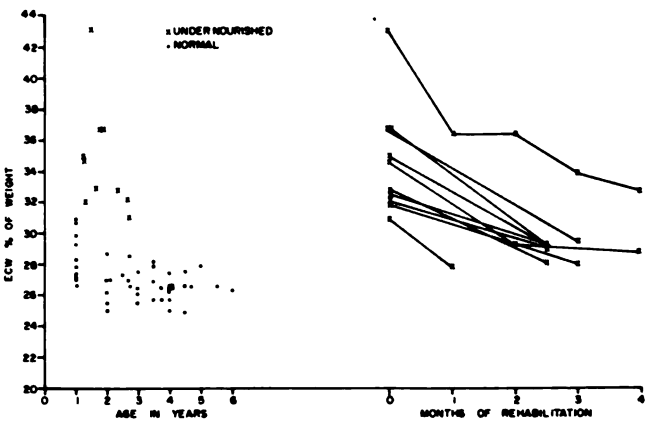


FIGURE 5 Percentage of extracellular water in normal and undernourished children. Decrease in percentage of extracellular water during repletion of undernourished children.

Percentage of ICW in the undernourished children was lower than in normal children and increased to within normal values above 31% of body weight with 10 weeks or more of adequate feeding. When energy and protein intakes are increased, the body builds new cell mass which contains greater amounts of ICW. Body height and weight increased in all the children, which suggests protein deposition. During nutritional rehabilitation, increases in absolute amounts of ICW in the undernourished children were greater than in the normal children of the same age. Expressed as gain in liters per month, the average increase in ICW in the undernourished children was four times that of the normal children (Table 3).

TABLE 3 Increase in ICW in Normal and Undernourished Preschool Children of Comparable Ages During Growth (Liters per Month)

Normals		Undernourished
0.08		0.07
0.08		0.23
0.12		0.18
0.07		0.37
0.04		0.28
0.09		0.20
0.06		0.40
0.09		0.29
0.04		0.80
0.07		0.28
0.05		
AVERAGE	0.07	0.31
MEDIAN	0.07	0.28

In one of the Lebanese children hospitalized for 4 months, percentage of ICW fluctuated during repletion while percentages of TBW and ECW were decreasing. Perhaps water-poor fat, rather than lean body mass, was being laid down at various stages. Keys *et al.* found that fat formation took precedence over lean mass formation during repletion of adults until the point when exercise was initiated.[15]

In studies of extreme cases of undernutrition reported in the literature, the percentage of TBW was higher than in the subjects reported here.[4,22] Many of those children had clinical edema. In the present study only two children had clinical edema. On the basis of the present study, water compartments are significantly altered without clinical edema. These data indicate

that measurement of ECW may be a good tool for diagnosis of early undernutrition.

SUMMARY

In vivo estimations of TBW by deuterium-oxide-dilution technique measure the same volumes that desiccation measures in young pigs.

Total body water in 20 normal preschool children between 1 and 6 years of age was relatively constant at about 59% of body weight.

Extracellular water did not stabilize to young adult levels of 26 to 27% of body weight until the second or third year of age.

Intracellular water also took longer to stabilize, reaching a plateau at 32% of body weight by 3 years of age.

Values for TBW and ECW for eight American and two Lebanese preschool children with simple undernutrition were significantly elevated upon hospital admission. When the children were fed an adequate diet for 10 weeks or longer, the values for body water compartments reached those for normal. On the basis of this study, measurement of thiocyanate space may be a valuable tool for early diagnosis of simple undernutrition.

REFERENCES

1. Armsby, H. P., and C. R. Moulton. 1925. The animal as a converter of matter and energy. Chemical Catalog Co. Inc., New York.
2. Asfour, R. Y., R. I. Tannous, Z. I. Sabry, and J. W. Cowan. 1965. Protein-rich food mixtures for feeding infants and young children in the Middle East. Amer. J. Clin. Nutr. 17:148.
3. Association of Official Agricultural Chemists. 1965. Official methods of analysis. 10th ed. Washington, D. C.
4. Brinkman, G. L., M. Bowie, B. Friis-Hansen, and J. D. Hansen. 1965. Body water in kwashiorkor during and after edema. Pediatrics 36:94.
5. Brožek, J. 1961. Techniques for measuring body composition. National Academy of Sciences, Washington, D. C.
6. Callow, E. H. 1948. Comparative studies of meat. II. The changes in the carcass during growth and fattening, and their relation to the chemical composition of the fatty and muscular tissues. J. Agr. Sci. 38:174.
7. Doxiadis, S. A., and D. Gairdner. 1948. Estimation of extracellular fluid volume by thiocyanate method in children and adults. Clin. Sci. 6:259.
8. Flynn, M. A., F. M. Hanna, and R. N. Lutz. 1967. Estimation of body water compartments of pre-school children. I. Normal children. Amer. J. Clin. Nutr. 20:1125.
9. Flynn, M. A., F. M. Hanna, R. Y. Asfour, and R. N. Lutz. 1967. Estimation of body water compartments of pre-school children. II. Undernourished children. Amer. J. Clin. Nutr. 20:1129.

10. Friis-Hansen, B. J. 1957. Changes in body water compartments during growth. Acta Paediat. 46 (Suppl.):110.
11. Groves, T. D., and A. J. Wood. 1965. Body composition studies on the suckling pig. Can. J. Anim. Sci. 45:14.
12. Hanna, F. M. 1963. Changes in body composition of normal infants in relation to diet. Ann. N. Y. Acad. Sci. 110:840.
13. Hansard, S. 1963. Radiochemical procedure for estimating body composition in animals. Ann. N. Y. Acad. Sci. 110:229.
14. Jackson, R. L., and H. G. Kelly. 1945. Growth charts for use in pediatric practice. J. Pediat. 27:215.
15. Keys, A., J. Brožek, A. Henshel, O. Mickelsen, and H. L. Taylor. 1950. *In* Biology of human starvation. Vol. I. U. Minnesota Press, Minneapolis.
16. Lawes, J. B., and J. H. Gilbert. 1959. Experimental enquiry into the composition of animals fed and slaughtered for human food. Roy. Soc. (London), Trans. 149:493.
17. National Academy of Sciences-National Research Council. 1964. Nutrient requirements of swine. NAS-NRC Pub. 1192. National Academy of Sciences-National Research Council, Washington, D. C.
18. Pace, N., and E. N. Rathbun. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158:677.
19. Pfau, A. 1965. Whole body potassium measurements. *In* J. Brožek [ed.] Symposia of the society for study of human biology. Vol. VII. Human body composition. Pergamon Press.
20. Schloerb, P. R., B. J. Friis-Hansen, I. S. Edelman, A. K. Solomon, and F. D. Moore. 1950. Measurement of total body water in the human subject by deuterium oxide dilution with consideration of deuterium distribution. J. Clin. Invest. 29:1296.
21. Schloerb, P. R., B. J. Friis-Hansen, I. S. Edelman, D. Sheldon, and F. D. Moore. 1951. The measurement of deuterium oxide in body fluids by the falling drop method. J. Lab. Clin. Med. 37:653.
22. Smith, R. 1960. Total body water in malnourished infants. Clin. Sci. 19:275.
23. Widdowson, E., and R. McCance. 1957. Effect of low protein diet on the chemical composition of the bodies and tissues of young rats. Brit. J. Nutr. 11:198.
24. Wood, A. J., and T. D. Groves. 1965. Body composition studies on the suckling pig. Can. J. Anim. Sci. 45:8.

BODY-COMPOSITION METHODOLOGY IN MILITARY NUTRITION SURVEYS

Harry J. Krzywicki
and C. Frank Consolazio
U.S. Army Medical Research
and Nutrition Laboratory
Fitzsimons General Hospital

It is the responsibility of the Office of The Surgeon General to evaluate periodically the nutritional adequacy of the military ration. As a result, the U.S. Army Medical Research and Nutrition Laboratory has, for many years, been directed to conduct nutrition surveys in military camps (see references 20, 31, 32, 35, 38, 40) to enable the commanding officer of the laboratory to fulfill his responsibilities to the Surgeon General.

Some studies have been conducted to relate the daily energy expenditure to daily minimal allowances, and in others the nutritional status of troops has been evaluated by biochemical and anthropometric measurements (see references 12–14, 21, 22, 39). Unfortunately, these studies have not always permitted a direct correlation between the nutrient intake with the biochemical evaluation, body composition, anthropometric measurements, and work performance.

The evaluation of the nutritional adequacy of the ration, although important, cannot be accepted as the ultimate evaluation of nutritional status of the individual, because of the many unknown factors that are present. There is no question, as has been emphasized repeatedly by the leading nutritional and medical authorities, that overnutrition or obesity is the major nutritional

disease in the United States. Obesity in terms of total body composition is still relatively unstudied with respect to morbidity and work performance. Little is known of the influence that the fat/muscle mass ratio of the individual has on his body composition and work performance or energy expenditure. It is particularly important to the military to evaluate that degree of variation in the ratio of fat to muscle mass, which will not significantly impair work performance.

As a result, this laboratory initiated a series of nutrition surveys on troops living in military camps throughout the United States, under varied conditions of temperature and environment (heat, cold, and altitude), and on troops performing light, moderate, and heavy physical activities.

These data included information on food intake, body composition, anthropometric measurements, maximal work performance, and biochemical evaluation. Data are now being compiled, by age groups, at each military camp to determine the interrelationships and intrarelationships existing between nutritional status, work performance, and body composition.

METHODS

Teams composed of members from four divisions of this laboratory were utilized in the first nutrition survey of approximately 500 soldiers at Fort Carson, Colorado, in November 1963 (Table 1). Later surveys were conducted primarily by members of the Bioenergetics Division with the assistance of medical and nursing personnel from the laboratory and from the installations studied. In addition to the body-composition aspects of the Fort Carson survey, special emphasis was placed on the physiological, biochemical, and clinical evaluation of the group of 500 subjects plus that randomly selected population of 112 soldiers in eight age groups (14 men per group) ranging from 17 to 50+ years of age (17–19, 20–24, and so on). In some instances, the 112 quota was not available because of the limited number of men in the older age groups. Additional information has also been obtained on obese individuals of all ages as well as a group of WAC's (Women's Army Corps). Body composition was to be estimated in terms of quantities of water, fat, protein, mineral, and total body density, with the aim of selecting a combination of methods that would serve to define the active metabolizing tissue mass, or allow for ranking of individuals into anthropometrically similar groups. Additional surveys were then conducted at Fort Huachuca, Arizona, in April 1966 and at Fort Campbell, Kentucky, in March 1967. In all of these surveys, soldiers were randomly selected from units where mess hall surveys were being conducted so that correlations could be studied between nutriture, work performance,

TABLE 1 Body-Composition Observed Measurements, Fort Carson, 1963

Minimal Measurements on 500 Subjects

1. Mass, age, height
2. Skinfolds
   Triceps, scapula
3. Circumferences
   Xiphisternal, waist, buttocks, calf

Comprehensive Measurements on 112 Subjects

1. Mass, age, height
2. Bony diameters
   Wrist, elbow, biacromial, knee, ankle, bi-iliac
3. Body diameters
   P.A. Chest, bihumeral, lateral chest, bideltoid
4. Circumferences
   Max. forearm, waist, biceps, buttocks, max. calf, Xiphisternal
5. Skinfolds
   Triceps, scapular, iliac crest
6. Body volume by water displacement
7. Residual lung volume by calculation of N_2 dilution
8. Total body water by calculation of HDO or HTO dilution
9. Potassium-40 counting

and body composition. Although planned from the start of the first survey, utilization of the Automatic Data Processing Center (USAMRNL) to determine further correlations between the various parameters studied is expected to begin during the summer of 1967.

Body density was computed from body volume as measured by the direct water-displacement technique of Allen *et al.*,[3] corrected for the residual lung volume. Residual lung volume was estimated by a modified Rahn *et al.*[34] nitrogen washout technique in which a Nitralyzer* was used. Gastrointestinal gas was omitted in the calculation of gas-free body volume. The human body volumeter, which has been reported elsewhere,[12] is shown in Figure 1.

Krzywicki and Chinn reported[23] on calibration of the body volumeter by repeatedly drawing off 2-liter aliquots of water and noting the manometer scale changes. This resulted in a factor of 2.100 ± 0.014 liters/cm.[23] The water-level manometer is backed by a machine-engraved centimeter rule (0.05-cm graduations) and could be interpolated to 0.01 cm with the aid of an enlarging lens. Each 0.01-cm change represented 0.021 liter of volume. Error propagation based on two manometer scale readings and the subject's ability to effect a forced maximal expiration reproducible to 100 ml permitted fat to

*Custom Engineering and Development Co., St. Louis, Missouri.

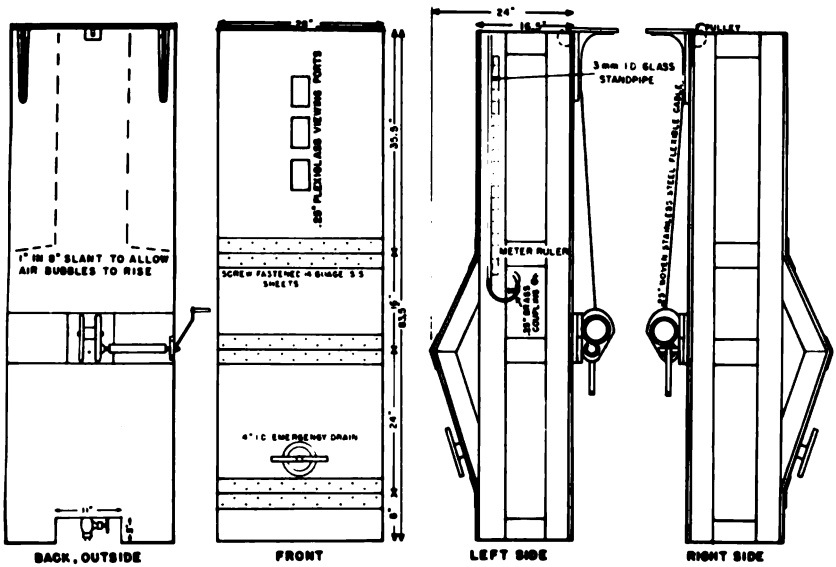


FIGURE 1 Human body volumeter.

be estimated with ± 0.488 kg, if the observed body volume is corrected for the measured residual lung volume. However, this precision is decreased to ± 1.52 kg when a mean residual volume of 1.250 liters is accepted to correct the measured body volume.

A newer commercially fabricated model,* made of a cardboard hollow honeycomb core with overlying fiberglass matting, reinforced and waterproofed with polyester resins, has proved to be very durable. It weighs about 250 lb and has survived over 5,000 miles of shipment by truck. A most recent addition was a filtering system utilizing a Jabsco self-priming pump† capable of delivering 21 gallons per minute, to remove such debris as is carried in mechanically or mucous that can be expectorated. The system is energized about every fourth subject for a period of 4 to 5 min and leaves the water crystal clear. Wescodyne‡ is added to the water for its bactericidal qualities.

Total body water was estimated at Fort Carson by the method of Liebman *et al.*,[25] wherein the dilution of 600 μc of orally ingested tritiated water, after a 4-hr period of equilibration of tritium with body fluids, was measured in the urine by means of a Packard Tri-Carb‡ liquid scintillation counter. An alter-

*Plasticrafts, Inc., Denver, Colorado.
†Jabsco Pump Co., Costa Mesa, California.
‡ Wescodyne West Chemical Prod., Inc., Long Island City, New York.
‡ Packard Instrument Co., LaGrange, Illinois.

nate method for estimating total body water in soldiers under 20 years of age was performed by the dilution technique after ingestion of 50 ml of deuterium oxide (99.5%) as suggested by von Hevesy and Hofer[42] and modified by Edelman and Moore.[17] The concentration of D_2O was determined in the urine after a 4-hr equilibration period by use of a MicroTek Gas Chromatograph.*

Total body potassium of soldiers at Fort Carson was determined by measurement of potassium-40 in the Colorado State Public Health Department's whole-body NaI crystal counter,[15] after the method of Marinelli.[27] Body counting was of 45-min duration per individual. The body potassium counts at Fort Huachuca and Fort Campbell were determined in the Hanford mobile shadow shield whole-body counter (NaI crystal) reported by Palmer *et al.*[29] and shown in Figure 2.

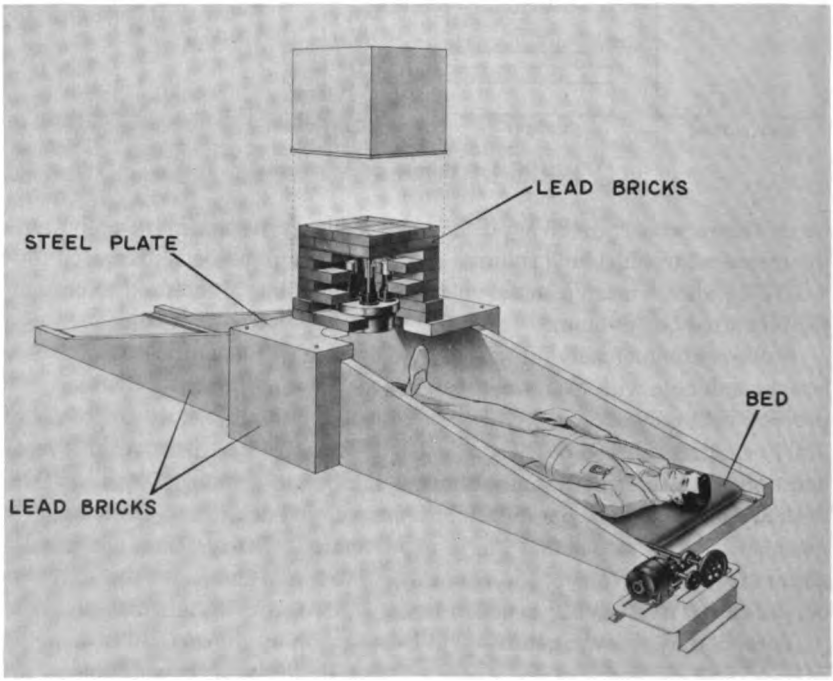


FIGURE 2 Shadow shield whole-body counter.

A series of selected anthropometric measurements included the arm and scapula skinfolds as designated by the Committee on Nutritional Anthropometry,[11] four bony diameters as suggested by Trotter,[41] two bony diame-

*MicroTek Instrument Co., Baton Rouge, Louisiana.

ters as well as four soft tissue diameters as reported by Maresh,[26] and six body and extremity circumferences as reported by Behnke *et al.*[6] Body weight was estimated to the nearest 0.05 kg on a Fairbanks-Morse body scale equipped with a Spinks Paddle Dash Pot,* while body height was recorded to the nearest centimeter on an engine-engraved steel centimeter rule. At Fort Huachuca and Fort Campbell, a Homs Full Capacity Beam Scale† was used to observe body weight.

Only deuterium oxide dilution for total body water was used in later surveys because AEC limitations preclude normal utilization of tritiated water (sewage disposal, consent of subjects, and so on). Evans blue dye for estimating blood volume and predicting adiposity as reported by Allen *et al.*[2] was added during the Fort Huachuca and Fort Campbell surveys.

RESULTS

Only the minimal anthropometric observations listed in Table 1 were made on the 500 soldiers studied clinically and biochemically at Fort Carson. It was anticipated that a correlation would exist from the comprehensive body-composition estimate observed in the 112 soldiers that could be utilized in some predictive way.

The data in Table 2 show the mean and standard deviation in body weight and density in two (lightest and heaviest) of 14 subjects measured at seven inter-

TABLE 2 Changes in Body Weight and Density in Two (Lightest and Heaviest) of 14 Subjects Measured at 4-hr Intervals

Body Weight (kg)	Density (g/ml)
46.7 ± 0.2	1.088 ± 0.002
79.2 ± 0.4	1.043 ± 0.002

Standard deviation of individual observation (14 subjects) = 0.0021 density unit.

vals over a 24-hr period. An analysis of variance for the body density unit change of all 14 subjects over the 24-hr period shows the standard deviation of a single observation to be 0.002 density unit.

Table 3 depicts data previously reported[23] on 173 males randomly selected from all walks of life at Fitzsimons General Hospital and this laboratory

*Fairbanks-Morse Co., Chicago, Ill.; Spinks Co., Atlanta, Georgia.
† Model 150, KTHM, Homs Scale Co., Burlingame, California.

TABLE 3 Body Density and Percentage of Fat in Adult Males, Fitzsimons General Hospital

Age Group	*n*	Body Weight (kg)	Density (g/ml)	Fat (%)
17–19	9	71.9 ± 14.4	1.060 ± 0.016	19.6 ± 7.0
20–24	35	73.6 ± 7.5	1.060 ± 0.013	19.5 ± 5.5
25–29	29	76.8 ± 14.0	1.053 ± 0.017	22.6 ± 7.3
30–34	15	85.8 ± 17.6	1.044 ± 0.013	26.3 ± 6.1
35–39	13	76.2 ± 10.6	1.043 ± 0.012	26.9 ± 3.6
40–44	25	75.4 ± 11.1	1.042 ± 0.012	27.1 ± 5.5
45–49	24	76.2 ± 10.0	1.038 ± 0.010	29.3 ± 4.5
50–54	12	75.5 ± 10.1	1.032 ± 0.026	32.8 ± 9.1
55–59	4	79.0 ± 10.3	1.031 ± 0.021	32.5 ± 4.8
60–64	5	69.7 ± 7.5	1.026 ± 0.010	34.7 ± 4.5
65–69	2	68.6 ± 2.1	1.017 ± 0.001	38.7 ± 0.6
TOTAL	173			

(USAMRNL). In each instance, residual lung volumes were computed from Chinn and Allen's formula[9] which incorporates body weight, age, and the average of the bilateral arm and scapula skinfolds. Gastrointestinal gas was not considered in the gas-free body volume. Body fat was calculated from the formula of Allen *et al.*[3] wherein percentage of body fat = [4.834/density – 4.366] · 100.

The mean body weight, density, and percentage of body fat was subgrouped into 5-year age increments for the 173 subjects studied. The data show a progressive decline in mean body density with age (from 1.060 g/ml at ages 17 to 19, to 1.017 g/ml at ages 65 to 69) as well as a gradual increase in body fat (from 19.6% at ages 17 to 19, to 38.7% for the oldest age group).

Of the 112 subjects screened for the complete body-composition study at Fort Carson only 97 had fully completed all the measurements scheduled. As a matter of interest, 8 obese volunteers were included as part of the survey.

The data in Table 4 group the 97 subjects measured at Fort Carson into 5-year age increments plus the small group of 8 obese subjects and includes the mean body mass (weight), the calculated body density, and the estimated percentage of body fat with standard deviations for each group. In these selected groups, body mass increased to age 34 (64.9 to 80.2 kg), and then levelled off by age 44, to be followed by a sharp decrease of 7.2 kg by age 49 (73.0 kg). The small number of subjects in the 45- to 49-year-old group and those aged 50 and over made these values unreliable for comparison.

TABLE 4 Body Density and Percentage of Fat in Adult Males, Fort Carson, 1963

Age Group	*n*	Body Weight (kg)	Density (g/ml)	Fat (%)
17–19	15	64.9 ± 8.9	1.073 ± 0.007	14.4 ± 2.9
20–24	19	68.3 ± 9.5	1.066 ± 0.012	16.6 ± 4.8
25–29	15	75.4 ± 10.6	1.062 ± 0.014	18.7 ± 6.3
30–34	14	80.2 ± 17.3	1.061 ± 0.021	19.0 ± 9.6
35–39	15	77.4 ± 13.4	1.050 ± 0.016	23.9 ± 7.2
40–44	9	78.3 ± 10.2	1.049 ± 0.013	24.2 ± 5.6
45–49	8	73.0 ± 13.5	1.041 ± 0.009	28.0 ± 4.2
50+	2	77.3 ± 3.1	1.048 ± 0.008	24.4 ± 1.3
TOTAL	97			
Obese 22–45	8	110.5 ± 12.9	1.020 ± 0.011	37.1 ± 5.1

In general, body density, as shown in Table 4, demonstrated a progressive decline with age for all groups (from 1.073 to 1.041 g/ml from ages 17 to 49). Since body fat as percentage of body weight was calculated from a formula involving density, percentage of body fat varied inversely with density (Table 4). Note also, the progressive increase in body fat with age and particularly that this increase occurred independently of any body weight change and appears to be age dependent (from 14.4% fat at ages 17 to 19, to 28.0% fat at ages 45 to 49). The additional group of 8 obese subjects (Table 4) showed a mean body mass of 110.5 kg, which constituted the lowest body density (1.020 g/ml). This group had an estimated mean body fat burden of 37.1%.

The data in Table 5 group 125 subjects studied at Fort Huachuca into 5-year age increments, plus a group of 21 obese subjects, in a manner similar to that depicted in Table 4. Again, body density showed a progressive decline with age for all groups except those aged 50+ years (from 1.077 to 1.042). Subjects beyond 50 years of age were notably leaner than all the other subjects, except for the groups aged 17 to 19 and 30 to 34 years. Similarly, as with the Fort Carson subjects, the Huachuca soldiers showed an increase in body fat with age except for the previously mentioned groups aged 50+ years. This increase in body fat again appears age dependent and is not related to body weight. The 21 obese subjects as a group were leaner than the 8 obese soldiers studied at Fort Carson; however, they, too, exhibit marked body fat burdens ranging from 28.0 to 36.8% (mean = 32.4%).

TABLE 5 Body Density and Percentage of Fat in Adult Males, Fort Huachuca, 1966

Age Group	*n*	Body Weight (kg)	Density (g/ml)	Fat (%)
17–19	16	65.5 ± 7.0	1.077 ± 0.004	12.4 ± 1.9
20–24	21	75.8 ± 12.8	1.064 ± 0.022	18.0 ± 8.8
25–29	17	74.9 ± 10.2	1.059 ± 0.023	20.0 ± 8.1
30–34	14	69.8 ± 7.5	1.058 ± 0.018	20.9 ± 8.0
35–39	18	73.0 ± 13.6	1.048 ± 0.022	25.0 ± 9.6
40–44	17	77.0 ± 13.2	1.047 ± 0.020	24.4 ± 9.5
45–49	16	80.4 ± 12.5	1.042 ± 0.017	27.4 ± 7.3
50+	6	70.0 ± 12.0	1.045 ± 0.010	26.0 ± 4.8
TOTAL	125			
Obese 23–52	21	93.1 ± 5.7	1.031 ± 0.010	32.4 ± 4.4

A group of adult females of the Women's Army Corps were studied at Fort Huachuca. Their mean body weight, density, and percentage of fat is shown in Table 6. Only the groups aged 17 to 19 and 20 to 24 years constituted

TABLE 6 Body Density and Percentage of Fat in Adult Females, Fort Huachuca, 1966

Age Group	*n*	Body Weight (kg)	Density (g/ml)	Fat (%)
17–19	14	60.8 ± 7.4	1.045 ± 0.018	26.3 ± 8.8
20–24	19	62.5 ± 8.4	1.038 ± 0.021	30.4 ± 9.5
25–29	3	59.4 ± 4.5	1.032 ± 0.023	31.9 ± 1.9
30–34	0			
35–39	4	60.9 ± 1.2	1.034 ± 0.009	31.2 ± 3.3
40–44	2	56.0 ± 14.0	1.041 ± 0.015	28.1 ± 21.0
45–49	2	68.1 ± 10.4	1.039 ± 0.017	28.6 ± 7.6
50+	1	71.7	1.039	28.5
TOTAL	45			

sizable populations. A decrease in density was noted in the 20- to 24-year-old group (0.007 unit), which represented a 4.1% increase in percentage of body fat over the 17- to 19-year-olds. The small number of subjects in the remaining groups made these groups unrealistic for comparisons.

Most recent data in body density were collected at Fort Campbell, Kentucky, and are shown in Table 7. Similar changes in body density and percentage of fat were observed as in the previous surveys. The entire normal Fort

TABLE 7 Body Density and Percentage of Fat in Adult Males, Fort Campbell, 1967

Age Group	*n*	Body Weight (kg)	Density (g/ml)	Fat (%)
17–19	29	74.8 ± 9.2	1.065 ± 0.027	17.4 ± 6.6
20–24	37	77.9 ± 8.1	1.061 ± 0.019	19.2 ± 7.2
25–29	30	79.8 ± 11.0	1.048 ± 0.017	24.7 ± 4.8
30–34	23	76.3 ± 12.0	1.055 ± 0.018	21.0 ± 6.7
35–39	22	76.4 ± 3.6	1.048 ± 0.021	24.6 ± 9.0
40–44	20	83.2 ± 25.4	1.042 ± 0.015	27.3 ± 6.6
45–49	9	83.6 ± . 5.5	1.040 ± 0.017	28.1 ± 7.6
50+	0			
TOTAL	170			
Special Training Group				
17–24	24	79.0 ± 10.5	1.047 ± 0.015	25.3 ± 6.2
Obese				
19–49	26	88.9 ± 9.1	1.033 ± 0.023	31.2 ± 3.4

Campbell population was markedly heavier than counterparts studied at Fort Carson and Fort Huachuca, except for subjects 30 to 34 and 35 to 39 years of age observed at Fort Carson. The same trends in decreased body density with age and increased percentage of body fat were noted, except for subjects 30 to 34 and 35 to 39 years of age.

In addition, 24 subjects 17 to 24 years of age in a special training group were measured. The group was composed of soldiers who had failed to pass Army physical fitness tests after 8 weeks of basic training and were kept on a rigorous athletic program until able to pass these tests. Their body density approximated that of the 25- to 29-year-old subjects (1.047 g/ml) and represented 25.3% of body fat.

The obese individuals at Fort Campbell (Table 7), although spanning a wide range of ages (19 to 49 years), had a mean body density of 1.033 g/ml and 31.2% of body fat.

Table 8 shows the mean body density, its range, and the fat-free weight of subjects aged 20 to 40 years as reported by various investigators and assembled by Behnke[5]; data reported elsewhere by Kryzwicki and Chinn[23,24]; and, for

TABLE 8 Estimated Fat-Free Weights on Groups of Adults 20 to 40 Years of Age from Body Density Determinations

Investigator	*n*	Density (g/ml)	Range	Fat-Free Body Weight (kg)
UNDERWATER WEIGHING				
Behnke (1942)	99	1.064	1.016–1.092	61.3
Osserman (1949)	81	1.063	1.016–1.095	63.5
Brožek (1952)	25	1.063		60.2
von Döbeln (1956)	35	1.072	1.020–1.099	61.2
Pascale (1956)	88	1.068	1.020–1.089	59.1
GAS DISPLACEMENT				
Siri-Behnke (1957)	31	1.051	1.014–1.081	61.9
DIRECT WATER DISPLACEMENT				
USAMRNL (1960)[a]	93	1.052	1.010–1.094	59.1
USAMRNL (1963)[b]	67	1.059	1.015–1.094	59.5
USAMRNL (1966)	70	1.057	1.020–1.078	57.7
USAMRNL (1967)	112	1.053	1.012–1.088	59.5

[a]Data of Krzywicki and Chinn, reference 23.
[b]Data of Krzywicki and Chinn, reference 24.

comparison, consolidated data on 70 and 112 subjects aged 20 to 40 years from Fort Huachuca and Fort Campbell. The data show these groups of subjects have a relatively constant fat-free body weight with the exception of the 1966 Fort Huachuca group (57.7 kg fat-free mass). Data on adult males 17 to 25 years of age are reported in Table 9. Mean body weights, density, and estimated body fat percentages reported by Krzywicki and Chinn[23,24] are compared with current data from Fort Huachuca and Fort Campbell surveys (1966 and 1967) and the early data of Pascale *et al.*[33] Body density for these groups ranges from 1.059 (1960) to 1.069 (1963 and 1966) and estimated body fat ranges from 19.9 to 15.7%.

Body potassium, as estimated from potassium-40 counting, is shown only for the various age groups studied at Fort Carson in 1963 (Table 10).

The mean total body potassium described by Krzywicki and Chinn[24] for all age groups studied is presented and expressed in grams of K per kilogram of body weight. The mean total body potassium increased from 141.1 g (age 17 to 19) to a peak of 153.2 g (age 30 to 34) and gradually decreased to 130.5 g (age 45 to 49). The obese subjects resembled the 35- to 39-year age group in terms of total body potassium. However, the concentration of potassium in the body exhibited a progressive decline from 2.10 g (age 17 to

TABLE 9 Body Weight, Density, and Percentage of Fat in Adult Males 17 to 25 Years of Age

Investigator	*n*	Body Weight (kg)	Density (g/ml)	Fat (%)
Pascale (1956)	88	68.3 ± 11.1	1.068 ± 0.012	16.0
USAMRNL (1960)[a]	60	73.1 ± 10.3	1.059 ± 0.013	19.9
USAMRNL (1963)[b]	36	66.7 ± 9.1	1.069 ± 0.032	15.7
USAMRNL (1966)	37	71.4 ± 11.8	1.069 ± 0.018	15.6
USAMRNL (1967)	66	76.3 ± 8.7	1.063 ± 0.022	18.8

[a]Data of Krzywicki and Chinn, reference 23.
[b]Data of Krzywicki and Chinn, reference 24.

TABLE 10 Body Weight and Potassium in Males, Fort Carson, 1963

Age Group	*n*	Body Weight (kg)	Total K (g)	Total K (g/kg Body Weight)
17–19	15	64.9 ± 8.9	141.1 ± 12.7	2.19 ± 0.17
20–24	19	68.3 ± 9.5	145.5 ± 18.9	2.17 ± 0.24
25–29	15	75.4 ± 10.6	147.5 ± 11.5	2.02 ± 0.81
30–34	14	80.2 ± 17.3	153.2 ± 20.8	1.97 ± 0.38
35–39	15	77.4 ± 13.4	143.3 ± 21.1	1.89 ± 0.31
40–44	9	78.3 ± 10.2	141.4 ± 15.7	1.82 ± 0.33
45–49	8	73.0 ± 13.5	130.5 ± 14.6	1.83 ± 0.20
50+	2	77.3 ± 3.1	138.6 ± 6.6	1.84 ± 0.06
TOTAL	97			
Obese 22–45	8	110.5 ± 12.9	143.6 ± 14.8	1.39 ± 0.28

19) to 1.83 g (age 45 to 49), when expressed as grams per kilogram of body weight. The obese subjects demonstrated the lowest values (1.39 g) of all groups observed.

Figure 3 shows the mean total potassium content in relation to age and the mean body weight in relation to age for the eight age groups. The total potassium content was highest (153.2 g) in the 30- to 34-year age group (mean age 32) and then decreased to 130.5 g in the 45- to 49-year age group (mean age 46.80). Body weight also increased until 30 to 34 years of age and tended to decrease with age except for the 50+ group.

Figure 4 shows the relation of body density to body potassium (grams per kilogram of body weight) for all subjects studied. The correlation coefficient for the eight age groups totaling 97 soldiers was 0.731.

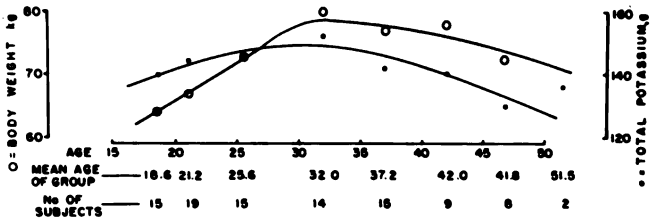


FIGURE 3 Total body weight and body potassium in relation to age.

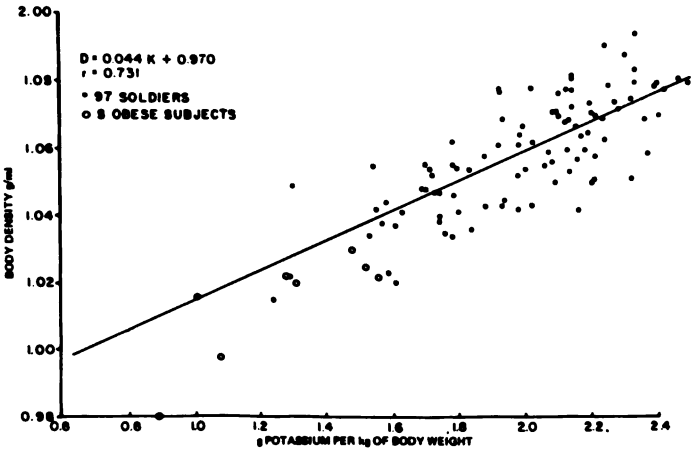


FIGURE 4 Relationship of body density and potassium.

Table 11 shows the results of estimating the dry protein mass of the body from (a) body density by water displacement; (b) ^{40}K counting, by using a conversion factor of 68.1 mEq/kg of lean body mass[18]; and (c) Chinn's estimating equation[8] that incorporated ^{40}K estimates with 24-hr urinary creatinine excretion in the Fort Huachuca subjects. The subjects in this Table reflect valid 24-hr urinary creatinine excretion in those subjects who cooperated and collected 24-hr urines. In other instances, urinary creatinine determinations were unreasonably low and were noted as false, unacceptable 24-hr urine collections.

DISCUSSION

The human body volumeter had been further evaluated by Krzywicki and Chinn[23] to test the effect of *ad libitum* food intake on diurnal variations of

TABLE 11 Comparison of Three Methods of Estimating the Dry Protein Mass of the Body

Age	*n*	Mass (kg)	Density (Allen) Water Displacement	^{40}K Counting (Forbes) 68.1 mEq K/kg LBM	^{40}K and 24-hr Creatinine (Chinn)
17–19	12	65.9	11.1	10.9	12.4
20–24	7	73.2	11.9	11.5	12.4
25–29	5	73.4	11.3	11.9	13.1
30–34	9	71.2	10.8	11.2	12.2
35–39	5	80.3	11.1	12.0	13.6
40–44	7	81.7	11.6	11.5	12.6
45–49	13	81.0	11.1	11.4	12.4
50+	3	76.4	10.5	11.2	11.5
TOTAL	61				

body weight and volume with respect to body density. Any large variation in volume not attributable to food and liquids consumed may be assumed to be gastrointestinal gas. One liter of GI gas could be equivalent to 4.8 kg of body fat in our fat-predicting equation. No diurnal trends in GI gas production were observed in the subjects tested. The propagation of error for the water-displacement technique showed it to fall within the limits (± 0.005 density unit) as prescribed by Siri[37] for this technique. The volumeter showed the standard deviation of an individual observation to be ± 0.0021 density unit. This means that estimates of body fat in the heaviest (79.2 kg) of 14 subjects measured could vary by ± 0.8%, since a 1-unit density change is equivalent to 0.4% of body fat by weight, as suggested by Durnin and Taylor.[16]

For the present, gastrointestinal gas in the body is disregarded. Conflicting reports cite 125 ml of GI gas observed by Bedell *et al.*[4] versus quantities of up to 2,600 ml reported by Blair *et al.*[7] as being present in the GI tract at any one time. Calloway recently suggested (personal communication) that perhaps no more than 200 ml of GI gas is present in the tract at any one time.

Krzywicki and Chinn[23] showed a progressive increase in the mean body weight to age 34 (Fitzsimons group) with a decline in body density that reflects increased body fat. Body density continued to decline although body weight has decreased by approximately 10 kg at age 49. In the older age groups, body density is further decreased, demonstrating an increase in body fat. Fryer[19] reported on 60 males aged 60 years or older and cited a mean body density of 1.0296 g/ml, which indicated 31.7% of body fat and is comparable to our 60- to 64-year age group.

The Fort Carson group follows much the same pattern as indicated above; similar trends of decreased body density with age and the concomitant increased body fat, independent of body weight, were observed. Although similar observations were noted, only trends can be compared here, because residual lung volume was estimated by a prediction equation to correct body volume measured by water displacement in the 173 subjects of the Fitzsimons group.

The residual lung volumes were measured in the subjects at Fort Carson and in all subsequent studies, because predicting residual lung volume presents problems in the older age groups, particularly in individuals with significant pulmonary emphysema. Some of the respiratory gases are entrapped or encapsulated and cannot be measured with accuracy even by nitrogen-washout techniques. Attempts to predict such volumes only add to the error of estimating body density. Then, too, the soldier subjects at Fort Carson, although fewer, were leaner by 1.4 to 7.3 kg, depending on age group, except for those in the 35- to 44-year-old group, which exceeded the body weight of Fitzsimons subjects by 2.4 to 4.8 kg. The data in both studies tend to describe the body fat burdens of populations in terms of the methods used to obtain gas-free body volume. The higher incidence of body fat in the 173 subjects of the Fitzsimons mixed population could be partially due to inaccuracies in obtaining the gas-free volumes when predicting the residual lung volume; however, many other factors were also involved (diet, physical activity, and the like).

The populations studied at Fort Huachuca and Fort Campbell again showed increased body density with age and demonstrated the diversity of randomly selected groups. Of the obese soldiers, the Fort Huachuca group were less obese than the Fort Carson group, and those at Fort Campbell were the least obese.

The body densities of the adult females studied at Fort Huachuca are comparable with data reported by Young *et al.*,[43] who showed that young females 17.2 to 27.2 years of age have a body density ranging from 1.015 to 1.059 g ml^{-1} (with a mean density of 1.034). Allen *et al.*[2] reported similar data for 26 Chinese university women—mean density of 1.040 g ml^{-1} (mean age 22.6 years, mean weight 49.3 kg).

The special training group observed at Fort Campbell reflects the effects of physical training on young men who once tended towards obesity. This group is comparable in fatness to 25- to 29-year-olds. The eventual correlations of their energy expenditures (treadmill walking) with their exhaustive physical training will be of greater interest.

The fat-free body weight of 20- to 40-year-old males is of special interest. As populations were added to Behnke's collation of data,[5] it became increas-

ingly evident that when fat is removed from the human body, a rather stable fat-free body weight is observed. However, the most valid comparisons of the USAMRNL data can be made only with the Siri-Behnke data since the fat predicting equation of Allen *et al.*,[3] like that of Siri,[37] took hydration of the body into account. It appears that as the numbers of subjects or populations studied increases, the fat-free weight of reference man could approximate 60 kg.

Males in the 17- to 25-year-old group showed variations in the percentage of body fat ranging from 15.6 to 19.9%. Novak[28] recently reported the density of seventeen 16.5- to 18.5-year-olds to be 1.0743 g ml^{-1}, but cited no SD for his group. Our 17- to 19-year-olds at Fort Huachuca had a mean density of 1.044 ± 0.077 g ml^{-1}, yet the Fort Campbell group had a lower density of 1.065 ± 0.027, but with a greater spread of body weight. The body densities discussed in this report show that our populations seem mainly to reflect the diversity to be expected when going into survey situations. One cannot assume increased body mass to be age-related. In Tables 3, 4, 5, and 7 the age groupings show great variation in body weight. Another type of ranking soon to be explored will attempt to relate density to mass and stature. This is particularly important with respect to potassium-40 counting.

Krzywicki and Chinn[24] reported that total body potassium in the Fort Carson 1963 subjects showed a downward trend with age, as has been reported by Sievert[36] for the younger age group and also by Allen *et al.*[1] with respect to aging.

The trends in loss of body potassium with age and its relation to body weight agree with those reported by Oberhausen and Onstead[29]; however, the more definitive pattern they expressed reflects the greater number of subjects measured (10,000) in a 2-pi liquid scintillation counter. Our subjects at Fort Carson showed the highest potassium level at the mean age of 32, while theirs peaked out at age 22.

The relationship exhibited between body density and body potassium (grams of K per kilogram of body weight) is expected. Lean adults of high body density would reflect a low fat burden and consequently a greater percentage of cell mass, while obese subjects would show the contrary effect of body fat burdens on proximate composition. The correlation (r = 0.731) reflects mainly the variation in subjects. The very low values of potassium in grams of K per kilogram of body weight as demonstrated by the obese subjects represent the further diluting of body potassium by the large body fat burden they carry.

Attempts to define the loss in body potassium with aging as the change in proportion of muscle protein to collagenous and connective tissues in the active metabolizing tissue mass will require a more comprehensive study of

this mass in terms of its muscle and nonmuscle components. Chinn and Plough[10] reported that the potassium-to-protein ratio in muscle of rats remained constant throughout life, while that of the nonmuscle component decreased steadily from maturity to old age; however, applicability of the rat data to humans has not yet been established.

The percentage of body fat as estimated from body density by direct water displacement was 21.0% and only 19.5% when Forbes' factor[18] was used to estimate lean body weight from total body potassium as measured by ^{40}K counting. Comparisons of the two methods for each subject showed a correlation of $r = 0.815$. One cannot cite either method of calculating as accurate. However, the differences in body fat estimates (1.5%) indicate that these estimates are approaching actual values.

Surveys at Fort Huachuca and Fort Campbell included ^{40}K counting by a recent technique described by Palmer *et al.*[30] and employed a shadow shield whole-body counter (Figure 2). This counter has the advantage of portability even though its gross weight is 6 tons. The shield is made of lead brick. Two men can set up the counter in 4 hours.

The dry protein mass of the individual Fort Huachuca subjects was estimated by three methods: (a) body volume densitometry, (b) ^{40}K shadow-shield counting using Forbes' conversion factor,[18] and (c) ^{40}K counting and 24-hour creatinine excretion incorporating Chinn's equation.[8] A comparison of values from the three methods of calculation showed that Chinn's equation yielded the highest values for this mass. Again, further evaluation of the data is required before any comments can be made as to the validity of Chinn's estimating equation. It is felt that a similar correlation of body fat can be expected between body density by water displacement and ^{40}K counting, as was shown in the Fort Carson group.

SUMMARY

Information has been presented on body density collected at three military installations. Potassium-40 counting has been presented only on personnel at Fort Carson. Data for total body water and anthropometric measurements are now undergoing automatic data processing. It is anticipated that regression or prediction equations that can utilize the data as predictors of body fat will be devised.

Body density decreases with age, which represents an increase of body fat with age but is not related to body weight. The direct water-displacement volumeter is an excellent and simple device for measuring body density in the field and compares favorably with potassium-40 counting for estimating per-

centage of body fat. The fat-free estimates of 20- to 40-year-old males approximates 60.0 kg.

REFERENCES

1. Allen, T. H., E. C. Anderson, and W. H. Langham. 1960. Total body potassium and gross body composition in relation to age. J. Gerontol. 15:348.
2. Allen, T. H., M. T. Peng, K. P. Chen, T. F. Huang, C. Chang, and H. S. Fang. 1956. Prediction of blood volume and adiposity in man from body weight and cube of height. Metabolism 5:328.
3. Allen, T. H., H. J. Krzywicki, W. S. Worth, and R. M. Nims. 1960. Human body volumeter based on water displacement. USAMRNL Report 250.
4. Bedell, G. N., R. Marshall, A. B. DuBois, and J. N. Harris. 1956. Measurement of the volumes of gas in the gastrointestinal tract. Values in normal subjects and ambulatory patients. J. Clin. Invest. 35:336.
5. Behnke, A. R. 1961. Comment on the determination of whole body density and a resume of body composition data. Techniques for measuring body composition. Nat. Acad. Sci., Nat. Res. Counc., p. 118. Washington, D.C.
6. Behnke, A. R., O. E. Guttentag, and C. Brodsky. 1959. Quantification of body weight and configuration from anthropometric measurements. Hum. Biol. 31:213.
7. Blair, H. A., R. J. Dern, and P. L. Bates. 1947. The measurement of gas in the digestive tract. Amer. J. Physiol. 149:688.
8. Chinn, K. S. K. 1966. Potassium and creatinine as indices of muscle and nonmuscle protein in rats. J. Nutr. 90:323.
9. Chinn, K. S. K., and T. H. Allen. 1960. Prediction of residual lung volume for purposes of determining total body tissue volume. USAMRNL Report 252.
10. Chinn, K. S. K., and I. C. Plough. 1965. Estimation of fat-free mass and its 4 components from creatinine excretion. Fed. Proc. 24:315.
11. Committee on Nutritional Anthropometry, Food and Nutrition Board, National Research Council. 1956. Recommendation concerning body measurements for the characterization of nutritional status. Hum. Biol. 28:115.
12. Consolazio, C. F., J. M. Hawkins, F. M. Berger, O. C. Johnson, B. Katzanek, and J. H. Skala. 1955. Nutrition surveys of two consecutive training cycles of the Airborne Training Branch, Co. G., Fort Benning, Ga., Oct–Nov 1953. USAMRNL Report 166.
13. Consolazio, C. F., J. M. Hawkins, O. C. Johnson, R. Ryer, III, J. E. Farley, F. Sauer, and T. E. Friedemann. 1956. Nutrition surveys at five Army camps in various areas of the United States. USAMRNL Report 187.
14. Consolazio, C. F., R. Shapiro, G. J. Isaac, and L. M. Hursh. 1961. Nutritional evaluation of a normal military population. USAMRNL Report 260.
15. Directory of whole body radioactivity monitors. 1964. International Atomic Energy Agency, Vienna, Austria.
16. Durnin, J. G. V. A., and A. Taylor. 1960. Replicability of measurements of density of the human body as determined by underwater weighing. J. Appl. Physiol. 15:142.
17. Edelman, I. S., and F. D. Moore. 1951. Body water, water distribution, and water kinetics as revealed by the use of deuterium oxide. J. Clin. Invest. 30:628.

18. Forbes, G. B., J. Galley, and J. B. Hursh. 1961. Estimation of total body fat from potassium-40 content. Science 133:101.
19. Fryer, J. H. 1962. Studies on body composition in man aged 60 and over, p. 59. *In* N. Q. Shock [ed.] Biological aspects of aging. Columbia University Press.
20. Johnson, R. E., L. V. Crowley, F. Toth, C. J. Koehn, E. P. Monahan, G. G. Lalanne, E. M. Parrott, H. J. Krzywicki, and A. Mancilla. 1949. Nutrition surveys on troops, Alaska winter 1948–49. USAMRNL Report 59.
21. Konishi, F., C. F. Consolazio, J. M. Hawkins, R. Ryer, III, and O. C. Johnson. 1956. Dietary survey of U. S. Army hospitals. Adequacy of the soldier-patient's diet. USAMRNL Report 191.
22. Konishi, F., J. M. Hawkins, M. I. Grossman, F. M. Berger, G. J. Isaac, and T. E. Friedemann. 1956. The dietary composition and adequacy of the food consumed by soldiers under an *ad libitum* regimen. USAMRNL Report 184.
23. Krzywicki, H. J., and K. S. K. Chinn. 1967. Human body density and fat of an adult male population as measured by water displacement. Amer. J. Clin. Nutr. 20:305.
24. Krzywicki, H. J., and K. S. K. Chinn. In press. Body composition of a military population, Fort Carson, 1963. Amer. J. Clin. Nutr.
25. Leibman, J., F. A. Gotch, and I. S. Edelman. 1960. Tritium assay by liquid scintillation spectrometry. Comparison of tritium and deuterium oxide as tracers for body water. Circn. Res. 8:907.
26. Maresh, M. M. 1963. Tissue changes in the individual during growth from x-rays of the extremities. Ann. N. Y. Acad. Sci. 110:465.
27. Marinelli, L. D. 1957. The use of NaI-TI crystal spectrometer in the study of gamma-ray activity *in vivo*. A summary of developments of the Argonne National Laboratory. Brit. J. Radiol. Suppl. 7:38.
28. Novak, L. P. 1967. Body composition and clinical estimation of desirable body weight. Proc. 7th Nat. Conf. Medical Aspects of Sports, p. 58, 1967, Amer. Med. Ass., November 1965.
29. Oberhausen, E., and C. O. Onstead. 1965. Relationship of potassium content with age and sex. Second Symposium, p. 179. *In* G. R. Meneely [ed.] Radioactivity in man. Charles C Thomas. Springfield, Ill.
30. Palmer, H. E., W. C. Hanson, B. I. Griffin, and W. C. Roesch. 1965. Radioactivity in Alaskan Eskimos. Second Symposium, p. 527. *In* G. R. Meneely [ed.] Radioactivity in man. Charles C Thomas. Springfield, Ill.
31. Parrott, E. M., T. E. Friedemann, C. F. Consolazio, W. J. Kuhl, W. R. Best, H. J. Krzywicki, R. W. Powell, J. E. Farley, C. M. Gates, L. J. Caranna, and W. M. DeCrease. 1952. Nutrition survey of a company mess, Fort Sheridan, Illinois, 9–13 and 23–27 June 1952. USAMRNL Report 100.
32. Pascale, L. R., T. Frankel, M. I. Grossman, S. Freeman, I. L. Faller, E. E. Bond, R. Ryan, and L. Bernstein. 1955. Changes in body composition of soldiers during paratrooper training. USAMRNL Report 156.
33. Pascale, L. E., M. I. Grossman, H. S. Sloane, and T. Frankel. 1956. Correlations between thickness of skinfold and body density of 88 soldiers. Hum. Biol. 28:165.
34. Rahn, H., W. O. Fenn, and A. B. Otis. 1949. Daily variations of vital capacity, residual air, and expiratory reserve including a study of the residual air method. J. Appl. Physiol. 1:725.

35. Ryer, R., III, C. F. Consolazio, and F. M. Berger. 1954. Nutrition survey of two company messes, 10th Infantry Division, Fort Riley, Kansas. USAMRNL Report 128.
36. Sievert, R. M. 1951. Measurements of gamma radiation from the human body. Ark. Fys. 3:337.
37. Siri, W. E. 1960. Body composition from fluid space and density: Analysis of methods. Semi-Annual Report, Biol. and Med. Donner Laboratory, Los Angeles, Calif.
38. Staff of MNL. 1953. The effect of vitamin supplementation on physical performance of soldiers residing in a cold environment. USAMRNL Report 115.
39. Staff of USAMRNL. 1957. (Prepared by Indik, B. P., Brophy, E. M., and Levy, L. M.) The relation between the *ad libitum* food intake, body composition, physical performance, and biochemical changes in 100 soldiers in a training company at Fort Carson, Colorado, 1955. USAMRNL Report 214.
40. Swain, H. L., F. M. Toth, C. F. Consolazio, W. H. Fitzpatrick, D. I. Allen and C. J. Koehn. 1948. Three nutrition surveys at Fort Churchill, Manitoba, Canada, 1947–48. USAMRNL Report 40.
41. Trotter, M. 1954. A preliminary study of estimation of weight of the skeleton. Amer. J. Phys. Anthropol. 12:537.
42. von Hevesy, G., and E. Hofer. 1934. Die Verweitzert des Wassers in menschlichen Korper, untersucht mit Hilfe von "schwerem" Wasser als Indicator. Klin. Wochenschr. 13:1524.
43. Young, C. M., M. E. K. Martin, M. Chihan, M. McCarsky, M. J. Manniello, E. H. Harmush, and J. H. Fryer. 1961. Body composition of young women. J. Amer. Diet. Ass. 38:332.

C. F. Sierk, Presiding

GENERAL DISCUSSION

C. F. SIERK Although the program does not call for a discussant, I think this has been a most interesting session, and for those of us who are concerned mainly with the applied aspects, these papers give us much to think about.

B. A. PANARETTO Time and again at this Symposium, people have reported results obtained by the use of expensive, tedious, technically difficult, indirect methods that were no better than those obtained by merely weighing the animals. Now, while this is fair enough and while I think you are entitled to find out whether you are adding anything to your knowledge by the use of these sometimes gigantic pieces of equipment, nobody has commented on a situation in which body weights let you down and lead you astray, and where, possibly, your indirect methods might provide the only clue that the situation was not that which body weight indicated. The best example I can offer to illustrate this point is an experiment done in Australia in 1962 (I think it was by Morris, Howard, and McFarland) in which two groups of sheep in different nutritional states, were shorn at a time when the weather was cold, and studied during the succeeding 14 days when the environmental temperatures were

quite low. During this period both groups lost almost identical amounts of body weight, so if one were relying on the scale to tell one what had happened, the answer would have been that there was no difference, for the animals had responded in an almost identical fashion to the environmental conditions. However, because the authors had been wise enough to make a few simple measurements, it turned out that the group that was most poorly nourished had lost something like 30% of body solids, I believe, during this 14-day period. Because of readjustment in body compartments, retention of water, and so forth, the over-all loss in body weight was exactly the same as with the other group that had lost only 7% of body solids during the period. So, on an energetic basis, the loss was 56,000 kcal in one group and only about 16,000 to 19,000 kcal in the other group. (I cannot be certain that these figures are absolutely accurate, for I am speaking now from memory.) However, I feel that it might be more meaningful sometimes to think of these techniques in the biological-research situation rather than in the technological situation that most authors have used in assessing the methods.

G. LESSER I would like to underline what Dr. Panaretto has just said. One of the papers this morning presented some data on sheep, I think, in which the body weight was better correlated with various other parameters than were, let's say, body water, potassium, and so forth. I think this can probably be true because we can measure weight with a greater accuracy than we can most of the other parameters. However, although I did not see the range of weights, or ages, and so forth, my guess is that these were relatively homogeneous in terms of age, sex, and weight, within that range. In any homogeneous group, one will find about the same proportion of fat within a very narrow range. Therefore, using weight, we can predict almost everything for the entire group. On the other hand, if as is more common (certainly among humans, and probably among many animal populations), they are much less homogeneous in terms of age, sex, weight, conditions of care, and so on, the use of weight for projecting many other body measurements will be, obviously, a very poor one. There I think the indirect methods (even though really one cannot defend them in our present state of knowledge) certainly will give us much better information in terms of prediction than will total body weight.

I. BUNDING I was very interested in many of the discussions at this session. Nowhere have I seen anyone make any reference to the season of the year in which these measurements were taken. Now, we certainly know that in a variety of animals, there are seasonal changes in body composition, and I would be curious to know whether the military people—if they could keep in contact with their subjects over a full year, or over a variety of environmental

conditions—might somehow associate some of these changes, which we know occur in many wild animals, with man, himself.

H. DOORNENBAL Mr. Chairman, I want to make a statement. It is very similar to that made by Dr. Lesser. Unfortunately, I did not hear Dr. Norton's talk, and he may have covered this. The animal scientist often works with market-weight animals, which are physiologically immature and, consequently, are still growing. Because of this, in most cases, relationships between parameters estimating body composition are highly affected by body weight itself. Now this has been clearly illustrated by several speakers who have reported correlation coefficients of 0.9 and up that were usually of equal or even greater magnitude than those for the additional parameters used to estimate body composition. Now, in evaluating the usefulness of additional criteria, would it not be correct to include these with body weight in a multiple regression analysis to determine what percentage of the total variation is explained by body weight itself, and how much an additional parameter adds to the improvement of the prediction equation? I am, of course, not suggesting that body weight alone is the answer. As we know, within a group of animals of similar body weight, there is a considerable variation in body composition. This is one of the problems that animal husbandmen want to solve: to differentiate between fat and lean within a narrow live-weight range. I am referring here to the immediate application of selection for leaner animals, and I am not referring to the biological implications of additional knowledge.

C. F. SIERK Dr. Norton, can you answer that yes or no?

H. W. NORTON I suppose that Dr. Breidenstein's presentation this morning was exactly in line with that comment. Weight was commonly included, and other things were being measured to evaluate what was accomplished over and above weight.

I want to make another distinct comment. Dr. Preston showed us a graph relating potassium by chemical analysis to potassium by ^{40}K measurement, and he indicated that the regression coefficient, which on theoretical grounds ought to be 1, was significantly less than 1. I think that, in most of the uses that we are concerned with, the regression is the right way to look at such things. But, in this particular case, it occurs to me that the reason that the regression appears significantly different from 1 may be because of appreciable errors in the variable that was considered independent. Probably it would be desirable to have a look at those data on the basis that both variables are subject to error. In that case, I would guess from the very brief opportunity to look at the graph (shown by the lantern slide) that it would be concluded that the relationship between the two variables is not significantly different from unity.

A. P. REMENCHIK I would like to caution about inferences from studies of different age groups, selected not at random. I think it is statistically inappropriate to draw any conclusions from studies of patients grouped by age. There are many examples of the inappropriateness of this type of conclusion. One of the first that comes to my mind is the belief of some physicians that blood pressure increased with age. But, when a population was observed for a prolonged period of time (for example, the thousand aviators studied by Greybill in 1942), it was observed that eliminating factors that we knew were responsible for elevating blood pressure resulted in the finding that the blood pressure of the population did not change. I think you (referring to Dr. Krzywicki) indicated that this was a rest haven, and I would put to you an alternative explanation for your observation of an increase in fat content. What you have done is discharge the active individuals into civilian life, and you have kept the fatties in the Army.

C. F. SIERK Dr. Krzywicki, any response to that?

H. J. KRZYWICKI I am sure this is typical. It is very difficult to make a random population selection, but I would also like to add that in these particular samples, especially in the younger age groups (there are a lot of bachelors in the army) are many men who subsist at certain mess halls. I probably didn't make this too clear, but we do survey the ration relative to calories, etc. The populations we have tried to describe include individuals who subsist at particular mess halls. In a way we are eliminating the type of individual we should observe. In other words, these groups do not eat "all over the place." They do add to their diet, certainly from the PX and the like, but when we consider the 40-year olds and older, we are dealing with the married soldiers who subsist at home. We did pick these obese men deliberately because we are not looking for a random population. These were specific groups we wanted to study.

A. BENSADOUN Various people have made certain comments concerning body weight and its use. I would like to underline here that there might be species differences. The gentleman (Dr. Lesser) who raised a question concerning body weight is not here, unfortunately. He might have been referring to our paper on sheep. Their ages ranged from 6 months to 3 years. Thus, we were dealing with a very wide range in terms of ages. I would like to point out that up to a body weight of 30 kg, the correlation is extremely high, but a wider distribution begins to occur as body weight increases above this weight. I would like to remind you that in the first paper in this conference, Dr. Reid made this point very clear—that in the case of ruminants, at least up to 3 or 4 years of age (we haven't worked with aged sheep), one of the major parameters of body composition is body weight. Also, the only situation in

which this was not the case was that in which animals had been starved for long periods. Also, a comparison of very rapidly growing sheep with very slowly growing sheep revealed that it did not really make any difference in terms of composition at a given weight. He showed data for sheep that reached a certain weight after 3 months, whereas others reached this weight after a year, and yet, the composition of the body was identical. To emphasize this point, I think there is the possibility that we might be dealing with a species difference. Although the situation described holds for the sheep, this might not be the case when you are dealing with monogastric animals.

C. F. SIERK Speaking for one of the sponsoring groups of the conference, I think we owe a real debt to Dr. Reid, who has borne the primary responsibility for developing the program, contacting the speakers, and advertising the conference. Also, I wish to acknowledge the very excellent job of Dr. Zobrisky and his committee here at the University of Missouri.

J. T. REID We regret that William Siri of the Donner Laboratory, University of California, was unable to attend this symposium. In conclusion, there are just a few tidbits that I would like to force upon you. Some of these I have obtained in discussions during the conference, especially with Dr. O'Dell of the Agricultural Chemistry Department at the University of Missouri.

He told me that in 1895, H. J. Waters began body-composition work at the University of Missouri. Dr. Waters eventually became Dean, and employed T. F. Trowbridge, an organic chemist, whose name we have already heard. At about the same time, Dr. L. D. Haigh was employed to carry out work in analytical chemistry. Then eventually Moulton, chronologically the third member of the team, joined in the work. Moulton was followed in the same position in the Agricultural Chemistry Department by A. G. Hogan, whom the nutritionists among us will recognize as one of the outstanding contributors to vitamin nutrition knowledge, particularly early in its history.

Now, I would like to come back just a moment to Dr. L. D. Haigh. Of this Moulton-Trowbridge-Haigh team, Dr. Haigh is the lone remaining member. He is approximately 92 years old. He retired in 1945, but he has remained quite active, and, until just recently, has enjoyed very good health.

It seems appropriate that we send a communication to Dr. Haigh from this Symposium.

A unique feature of this Symposium has been the multidisciplinary nature of the audience, the speakers, and the kinds of subject matter presented. We have benefited greatly from the presence of representatives of especially the medical and certain of the physical sciences.

My last comment is that any element of success that this conference has enjoyed is to a great degree due to the audience, not only by its perseverance, but in its direct contribution to the discussion, and also to the speakers. I would like especially to acknowledge the great contributions made to this conference by our overseas speakers, Dr. Widdowson and Dr. Panaretto. I should like also to thank the local committee for the tremendous job they have done in helping to make this conference go.

PARTICIPANTS

E. C. ANDERSON, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico
R. Y. ASFOUR, University of Missouri, Columbia
MILTON E. BAILEY, Department of Animal Husbandry, University of Missouri, Columbia
ALBERT R. BEHNKE, University of California Medical Center, San Francisco
A. BENSADOUN, Department of Poultry Science, Cornell University, Ithaca, New York
B. C. BREIDENSTEIN, Wilson & Co., Inc., Chicago, Illinois
JOSEF BROŽEK, Department of Psychology, Lehigh University, Bethlehem, Pennsylvania
L. S. BULL, Department of Animal Science, Cornell University, Ithaca, New York
T. ROBERT BULLARD, Department of Physiology, School of Medicine, University of Virginia, Charlottesville, Virginia
J. H. BURTON, Department of Animal Science, Cornell University, Ithaca, New York
J. E. CHRISTIAN, Department of Bionucleonics, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Indiana
W. J. COFFMAN, Missouri Agriculture Experiment Station, University of Missouri, Columbia
C. FRANK CONSOLAZIO, Bioenergetics Division, U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado
H. DOORNENBAL, Research Station, Canada Department of Agriculture, Lacombe, Alberta, Canada

MARGARET A. FLYNN, Department of Nutrition and Dietetics, Medical Center, University of Missouri, Columbia
GILBERT B. FORBES, Department of Pediatrics, School of Medicine and Dentistry, University of Rochester, Rochester, New York
G. F. FRIES, Animal Husbandry Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland
W. N. GARRETT, Department of Animal Husbandry, University of California, Davis
P. A. GLEESON, Agricultural Institute, Moore Park, County Cork, Ireland
I. K. HAN, Department of Nutrition, Seoul National University, Suwon, Korea
FIKRI HANNA, Department of Pediatrics, Medical Center, University of Missouri, Columbia
S. L. HANSARD, Department of Animal Science, Louisiana State University, Baton Rouge
R. B. HARRINGTON, Department of Animal Sciences, Agricultural Experiment Station, Purdue University, Lafayette, Indiana
L. N. HAZEL, Department of Animal Science, Iowa State University, Ames
H. B. HEDRICK, Department of Animal Husbandry, University of Missouri, Columbia
D. P. HUTCHESON, Department of Animal Husbandry, University of Missouri, Columbia
L. E. JEREMIAH, Department of Animal Husbandry, University of Missouri, Columbia
D. E. JOHNSON, Department of Animal Science, Cornell University, Ithaca, New York
J. E. JOHNSON, Department of Animal Science, Colorado State University, Fort Collins
Y. D. JOO, Department of Animal Science, Cornell University, Ithaca, New York
M. D. JUDGE, Department of Animal Sciences, Purdue University, Lafayette, Indiana
W. V. KESSLER, Department of Bionucleonics, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Indiana
G. F. KRAUSE, Department of Statistics, University of Missouri, Columbia
HARRY J. KRZYWICKI, Bioenergetics Division, U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado
T. G. LOHMAN, Department of Animal Science, University of Illinois, Urbana
CHARLES H. LONG, Department of Animal Husbandry, University of Missouri, Columbia
RUTH N. LUTZ, Department of Nutrition, University of Missouri, Columbia
G. P. LYNCH, Animal Husbandry Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland
T. G. MARTIN, Department of Animal Sciences, Agricultural Experiment Station, Purdue University, Lafayette, Indiana
W. R. McMANUS, Department of Physiology, University of New South Wales, Kensington, Australia
CHARLES E. MILLER, Health Division, Argonne National Laboratory, Argonne, Illinois
H. W. NORTON, Department of Animal Science, University of Illinois, Urbana
O. L. PALADINES, Inter-American Institute of Agricultural Sciences, La Estanzuela, Uruguay
B. A. PANARETTO, Division of Animal Physiology, The Ian Clunies Ross Animal Research Laboratory, Prospect, Australia
H. E. PARKER, Department of Biochemistry, Agricultural Experiment Station, Purdue University, Lafayette, Indiana
A. M. PEARSON, Department of Food Science, Michigan State University, East Lansing
GROVER C. PITTS, Department of Physiology, School of Medicine, University of Virginia, Charlottesville, Virginia

R. L. PRESTON, Department of Animal Husbandry, University of Missouri, Columbia
R. W. PURCHAS, Department of Food Science, Michigan State University, East Lansing
J. T. REID, Department of Animal Science, Cornell University, Ithaca, New York
E. P. REINEKE, Department of Physiology, Michigan State University, East Lansing
ALEXANDER P. REMENCHIK, Stritch School of Medicine, Loyola University, Hines, Illinois
L. B. SASSER, Department of Animal Science, Colorado State University, Fort Collins
C. F. SIERK, Cooperative State Research Service, U.S. Department of Agriculture, Washington, D.C.
E. G. STANT, JR., Division of Nuclear Medicine and Biophysics, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
H. H. STONAKER, College of Agriculture, Colorado State University, Fort Collins
J. W. STROUD, Department of Animal Science, Cornell University, Ithaca, New York
A. R. TWARDOCK, Department of Veterinary Physiology and Pharmacology, University of Illinois, Urbana
H. F. TYRRELL, Department of Animal Science, Cornell University, Ithaca, New York
B. D. H. VAN NIEKERK, Department of Animal Nutrition, Grootfontein College of Agriculture, Middleburg, South Africa
L. D. VAN VLECK, Department of Animal Science, Cornell University, Ithaca, New York
G. M. WARD, Department of Animal Science, Colorado State University, Fort Collins
G. H. WELLINGTON, Department of Animal Science, Cornell University, Ithaca, New York
E. M. WIDDOWSON, Medical Research Council Infant Nutrition Research Division, Dunn Nutritional Laboratory, University of Cambridge, England
S. E. ZOBRISKY, U.S. Department of Agriculture, CSRS, Washington, D. C.