

The Gross Composition of the Body¹

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	<i>Page</i>
I. Introduction.....	239
II. Body Water.....	242
III. Measurement of Total Body Water.....	246
1. Evaluation of Methods.....	246
2. Antipyrine.....	249
3. Deuterium.....	250
a. Falling Drop Method.....	250
b. Mass Spectrometer.....	251
4. Assay of Tritium.....	252
IV. Measurement of Extracellular Fluid.....	254
V. Fat.....	257
VI. Measurement of Fat.....	262
1. Densitometry.....	262
a. Principles.....	262
b. Density by Archimedes' Principle.....	264
c. Helium Dilution Method.....	267
2. Fat Estimated from Total Body Water.....	269
3. Combined Methods.....	269
4. Fat-Soluble Gases.....	272
VII. Protein and Mineral.....	272
References.....	278

I. INTRODUCTION

The gross composition of the body can and has been described in a variety of ways, ranging from a tabulation of its elemental constituents to a description of the proportions of its specific organs. Between these extremes from atoms to organs, a description of more immediate physiological and clinical interest is one given in terms of the body's chief functional constituents: water, fat, protein, and minerals. Except for a few hundred grams of carbohydrate and still smaller amounts of other organic substances, these four constituents form the entire bulk of the body, and, hence, their relative proportions and alterations must have a significant role in the physiology of normal and disease states. Genetics, environment, nutritional habits, age, sex, and disease all influence the gross composition of the body, which consequently may be expected to

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undergo alteration during the life span and to vary widely among individuals of the same species; more widely, in fact, than the differences that may exist among at least the mammalian species. On the other hand, it would be misleading to suggest that the dependence of body composition on these factors is well understood. Qualitative conclusions may be derived from the general fund of physiological and clinical information, but careful quantitative studies of the body's constituents, particularly in humans, have been confined with few exceptions to water and fat under relatively few conditions.

If at times, however, the investigation of body composition seems to have attracted perhaps less than its appropriate share of objective scientific study, the same cannot be said of the consuming attention it has received from the layman. Height, weight, musculature, and fatness are everyone's concern, and the universal desire for the proper proportions in these quantities according to prevailing aesthetic standards has aided and often fostered vast commercial enterprises. There is hardly a human activity in which body composition does not feature prominently, if only tacitly. Its bearing on athletic performance is evident, but, if it appears any less important to the housewife and executive, it is only because the reasons are less directly associated with maximal physical performance and more concerned with general health, degenerative disease, and longevity.

Despite the obvious usefulness of a complete analysis of the body into its principle constituents, whether it is from a lay or a medical point of view, the assessment of the quantities and distribution of water, fat, protein, and mineral in the living body has been severely handicapped by practical limitations. There are, in fact, no adequate methods for complete *in vivo* analysis, and there are, moreover, none in sight. Water is the only constituent amendable to direct measurement while fat, which from many points of view is the most important substance to measure reliably, can be estimated only by indirect means, either from the total body water or from the mean density of the entire body. At the present time, protein and mineral can only be lumped together as fat-free residue and its quantity inferred as that which is left after subtracting total body water and fat.

Even careful analysis of cadavers encounters severe technical difficulties because of the quantity of material that must be handled. Less than half a dozen careful analyses have been reported, and they hardly provide average values for normal humans for obvious statistical reasons and even more because of the circumstances leading to death.

The problems of body gross composition remains consequently in large part unsolved and to some extent unresolved. Where it has been

met, it has necessarily been reduced to the question: What constituents can be measured; and more often than not, the more troublesome query: What is it that was measured. The latter question is one that still can be asked of present techniques for measuring extracellular water and of the methods for estimating body fat.

Ambiguities that still resist attempts to formulate precise definitions of body components arise in part from our inability to measure directly all the components and in part from uncertainties in the interpretation of indirect methods of analysis. Even in a direct measurement, such as that of extracellular fluid, the space that is observed depends upon how it is measured, and, while it may be said that the most reliable methods give a reasonable value for extracellular fluid, the error is still comparable to the magnitude of changes and differences that are of most interest. Fat, too, has often been used without clear indication of what it is, although to those investigating the problem it means lipid substance, the ether extractable residue. Adipose tissue, particularly, is not fat in this sense but a combination of lipid, water, and protein.

The practical difficulties in evaluating body composition are also augmented in another way. In a laboratory experiment some semblance of control is maintained over factors other than the one investigated, but this kind of control over the proportions of constituents in the living body is impossible. Except for minor and transient changes in water, an alteration in any one constituent is accompanied by change in the others, and not always in a predictable manner. Changes in body weight, the only controllable factor, reflect a reshuffling in the proportions of all the body's components.

In some measure, the approach to a description of body composition has also been guided by the end that was sought. Behnke found it useful for some purposes to introduce the concept of the "lean body mass" (2,4), hoping to find in it a relatively constant structure, a common denominator of all body forms, with which comparisons would be meaningful and to which physiological factors such as BMR could be reduced. He defined lean body mass as the whole body minus nonessential, or excess, lipids. The description is in reality qualitative for it cannot, within the framework of the definition, represent a body of constant composition. The quantity of essential lipid is estimated to be two per cent of the lean body weight, but it is not known precisely and it may not be constant. This is a small detail in any case and may not be important but there remains a more cogent reason for suspecting the constancy of lean body mass. Adipose tissue consists of fat, water, and protein, and if fat alone is subtracted then a substantial quantity of adipose water and protein that varies with fatness remains as part of the lean body mass.

The difficulty is not removed by subtracting the whole of adipose tissue because its composition is not well established and may not be constant. In spite of the ambiguity in its detailed description or, more exactly, in its compositional variability, Behnke's concept of lean body mass has been and doubtless will continue to be put to useful purpose. It certainly is a better basis than body weight for correlation and intercomparison of physiological measurements.

Ideally, it would seem desirable to measure separately the total quantities of water, lipids, protein, and mineral, but this much information may not, for many purposes, be needed. Skin fold and anthropometric measurements and somatotyping can hardly be expected to give a precise quantitative picture of body gross composition, yet they may nevertheless prove to be highly useful indices where they show a workable correlation with physiological, nutritional, or clinical factors under study.

Although the value of these methods is recognized, they are outside the scope of the subject material presented here. The following sections are confined to discussions of water, fat, protein, and mineral in the adult human body, and of the techniques employed for their estimation. Wider aspects of these problems, especially where they relate to fat, are covered in the comprehensive review of Keys and Brožek (37), to which this author has had frequent occasion to refer.

II. BODY WATER

Water, the substratum for all living processes, constitutes the largest fraction of the basic materials of cells. Equally important, water constitutes the external environmental medium for the cell, and, except in the lowest phyla of life, it forms an integral part of the animal—an extracellular fluid that the animal must not only support but regulate within reasonable limits. In the most general terms, the function and distribution of water define the two major fluid compartments which together constitute the total body water: The intracellular water, which is contained within the confines of cellular walls; and the extracellular water, which is distributed throughout the serous cavities and vessels.

The intracellular fluid space would seem to require no further subdivision beyond recognition that the proportion of water may vary among cells of very different tissues. The extracellular fluid, which serves more diverse functions, also has a more complex distribution. For the most part it is distributed throughout the interstitial spaces of tissues and organs, but it is also contained in distinct anatomical pools which include the blood vascular system, the lymphatic system, the intestinal tract, cerebro-spinal fluid, and numerous smaller accumulations such as the renal glomeruli and tubules and vesicles of glands. For some pur-

poses, extracellular fluid is considered as two compartments: Namely, vascular and interstitial. The only fluid space that cannot be regarded as part of the total body water is that defined by the renal pelvis, ureters and the bladder. Fluid contained in these "dead" spaces has been separated by the kidney from the body's functional water pool and no longer takes part in physiological processes associated directly with water metabolism.

Each fluid space is physiologically real in the sense that it performs specific functions and contains highly specific solutes. Water itself, however, is insensible to such compartmentalization of the body and diffuses rapidly from one space to another as though cell walls were nearly nonexistent. Under normal conditions there is, of course, no large net

TABLE I
Approximate Ranges in Fluid Spaces of Adult Humans (and Mammals)

Compartment	Per Cent of Body Weight
Total body water	40-70
Extracellular water	15-20
Blood	4- 7.5
Interstitial and other	10-15

shift in water from one space to another. Water loss by excretion from the kidneys, in feces, by perspiration, and by evaporation from the skin and lung surfaces is balanced with water gained by absorption from the intestine and from metabolism, but the rates of redistribution are far faster than the rates of gain and loss by these processes.

Although a steady state is maintained between fluid spaces, the rapid transfer of water between them is demonstrable with deuterium- or tritium-labeled water. When these isotopes are given intravenously, their concentration in serum decreases rapidly until equilibrium is reached, not only in the vascular system but also in the whole of the body water. The plasma isotope disappearance can be characterized by the sum of two exponential terms: One with a half time of 30-60 sec. and the other with a half time of 2-10 min. (24, 41, 61). Even at the slowest rate, 99 per cent of equilibrium is attained in about 1½ hr. Although these constants do not give an unambiguous value for water turnover in the vascular system, they at least suggest that a quantity of water equal to the whole plasma volume exchanges in only 2-5 min. When labeled water is ingested, equilibration is attained in less than twice the time that is required by the vascular route of administration (61), which again indicates an exchange of water between the gastrointestinal tract and the rest of the body's water pool at a rate equal to its volume in 5-10 min.

The exchange, or turnover, rate of water with the external environment is very much slower than the transfer rates between fluid spaces. Labeled water, which cannot be sensibly different from unlabeled water in this respect, is lost from the body exponentially because of simple replacement dilution with a half time of about 9 days (61); hence, a volume of water equal to the total body water is gained and lost in about 13 days (turnover time). Anything that alters the rate of water excretion, such as extremes in environment, correspondingly changes the turnover time, although the total quantity of water remains constant.

TABLE II
Approximate Percentage of Water in Various Organs

Organ	Per Cent Water
Muscle	75
Bone	25
Blood	
Plasma	92
Red cells	60
Liver	70
Nerve tissue	75
Connective tissue	60
Adipose tissue	10-30

The amount of water relative to body weight in the normally hydrated body is dependent primarily upon the quantity of depot fat and diminishes with increasing obesity. Between the extremes from emaciation to gross obesity, the total body water may differ by a factor of two. In the leanest possible body, one devoid of storage fat, total body water constitutes about 72% of body weight (52) of which about one-fourth—18% of body weight—is extracellular water. At the other extreme, water has been observed to constitute as little as 38% of body weight (63).

Between these extremes, from roughly 40-70%, water has a complicated and poorly understood dependence on fat. Adipose tissue is known to contain 10-30% water, but the exact proportion is not known nor is it certain that the proportion is necessarily constant for varying degrees of obesity. If, in a first approximation, the body can be pictured as a basic lean structure to which fatty tissue (not pure fat) is appended, the total body water expressed as a proportion of body weight is related to the proportion of fatty tissue, A , by

$$w = \alpha(1 - A) + \beta A$$

where α is the water fraction of the lean body, and β the water fraction of A . The proportion of water obviously depends primarily upon A , but unless the quantitative relationship of water to the other major constituents is constant, neither are α and β .

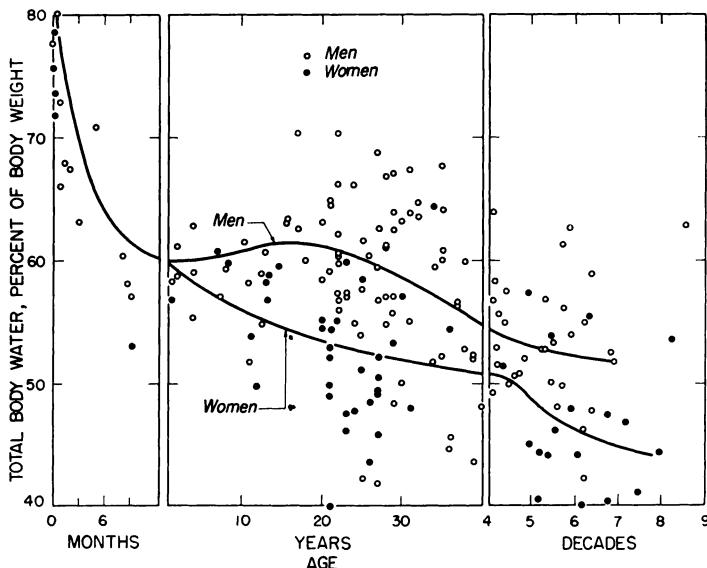


FIG. 1. Total body water in the human as a function of age. An estimated trend in the proportion of water for men and women is indicated by the solid lines. Data are taken from references (19, 63, 67).

An estimate of the variability of water for a given percentage of fat is gained from the differential of the formula above

$$\Delta w = (1 - A)\Delta\alpha + A\Delta\beta$$

The factor α varies from about 0.68 to approximately 0.73; hence, a reasonable value for its range of variation is $\Delta\alpha = \pm 0.03$. The range of variation in β is not known with certainty but for the present purpose is estimated to be about $\Delta\beta = \pm 0.05$. For persons in whom $A = 0.3$, the range of variability in total body water is then $\Delta w = \pm 3.6\%$ of body weight, or about $\pm 6.5\%$ of the total body water.

The foregoing estimate of variability is not intended to define the limits of normal hydration because the quantities that enter into the calculation are at best rough approximations. It does, however, suggest the order of magnitude of variation in total body water that cannot be attributed to differences in fat.

Between birth and the first year of age, the proportion of total body

water in the body decreases rapidly from 80% to about 60% of body weight. In men, there appears to be a slight gain, on the average, until the early twenties, when total body water begins to decrease very slowly with age. The latter decline seems to reflect merely the slow accumulation of storage fat during adult life. From the data now available, women do not seem to gain water relative to body weight in youth, but, rather, continue to show a steady decrease during the life span (Fig. 1). Both this behavior and the generally lower total body water of women as compared with men are attributable to differences in storage fat with age and with sex.

III. MEASUREMENT OF TOTAL BODY WATER

1. *Evaluation of Methods*

The quantitative determination of the total water in the body with *in vivo* techniques now available is a considerably more confident measure of a specific body constituent than is that of the extracellular fluid space. The greater confidence, however, does not stem particularly from more precise analytical techniques but rather from the fact that the test solutes actually measure total body water and that their validity can be tested directly by desiccation of the animals. Nonetheless, some of the uncertainties attendant upon nearly all *in vivo* dilution methods are still present to a small extent in the most reliable of solutes for body water. Intercomparisons between solutes agree within a few per cent and, in turn, the fluid space they measure agrees within a similar error with total body water measured in animals, at least by desiccation. Experimental error accounts for a substantial part of the uncertainty in individual measurements, but there is reason to believe that the test solute spaces differ from total body water by amounts in the order of 1% of the volume, or about $\frac{1}{2}$ liter for a lean 70 kgm. man. Since the best experimental error is of the same order of magnitude, existing data do not clearly indicate which of the acceptable test solutes measures most precisely the true total body water.

The three most widely used and, consequently, the most carefully tested agents for measuring body water are antipyrine and water labeled with either tritium or deuterium. A fourth test solute, N¹⁵-labeled urea, is omitted from this group only because it has received less attention and rarely has been used in humans. There is no factual evidence to suggest it is any less valid a solute than labeled water or antipyrine.

None of the many alternative solutes proposed are free of serious objections and have seen little use beyond initial investigations for validity. Among these substances are urea (unlabeled) (20, 39, 44, 48,

55, 71), thiouria (16), sulfanilamide (55, 78), alcohol, and glycerol. Taken as a group, the factors which invalidate these substances as test solutes for total water are principally a rapid rate of metabolism or excretion compared to the rate of distribution in the body water, unequal distribution throughout the fluid space, accumulation in nonfluid sites, e.g., uptake in fat if fat-soluble, and, particularly for urea, a variable endogenous production of the solute and possibly a diuretic effect. Suitable corrections might conceivably be found for any one of these solutes, but the added complexity seems hardly justified where labeled water or even antipyrine methods are practicable.

The validity of antipyrine as a measure of total body water has been tested directly by comparison with values obtained from desiccation, but it rests on rather few data. Soberman (66), in view of the substantial analytical error in the method, found remarkable agreement between the two methods in rabbits, dogs, and monkeys where the mean difference was only 0.3% of the body water. Comparison with other methods is necessarily less conclusive although suggestive. Simultaneous determinations of body water with antipyrine and deuterium- (26, 67) or tritium-labeled water (56) agree on the average within 1 or 2 per cent. Antipyrine consistently leads to the smaller of the two spaces, but since this difference is of the same magnitude as the overestimate expected with labeled water because of hydrogen exchange, it suggests that antipyrine, fortuitously or otherwise, indicates a space that is more nearly equal to true total body water.

These validations seem remarkable in view of the fact that antipyrine appears to be slightly soluble in fat and fat solvents (31, 59) and hence should ultimately find its way into a "space" substantially larger than the total body water. This may, in fact, happen, but, if the process is slow, as the comparable process with fat-soluble gases would indicate, then it cannot be distinguished from other mechanisms which contribute to the disappearance of antipyrine. The customary procedure of extrapolating the plasma disappearance curve would then compensate for the absorption of antipyrine in fat as well as for its degradation and excretion.

Although the utility of antipyrine is amply demonstrated for the normally hydrated body, it is still questionable if antipyrine reliably measures total body water in patients with edema, ascites, or pleural effusion. The turnover rate even of tritium-labeled water, which would not differ significantly from unlabeled water, in large volumes of transudate is slow compared to the transfer and mixing rates within the rest of the body (57). Antipyrine may be expected to have a still slower turnover rate because of its significantly lower rate of diffusion. The long mixing time under these circumstances presents no difficulty in the use of deu-

terium- or tritium-labeled water, whose biological half time in the body is in the order of 10 days, but it does raise a troublesome obstacle for antipyrine, whose disappearance half time is only 10 hr. The slow mixing in the transudate as well as the normal rate of loss of antipyrine are both reflected in the serum antipyrine "disappearance" curve by a complex interrelationship, and the two processes cannot be differentiated by a simple extrapolation procedure, which here could result in large discrepancies such as found by Hurst *et al.* (35). At best, the disappearance curve would have to be followed, from serial blood samples, for at least two half times (20 hr. or more) and then analyzed in detail by a laborious analytical procedure which, it may be noted, involves considerably more than extrapolating a second straight line portion of the curve if, indeed, it appears.

The choice between antipyrine, deuterium-, and tritium-labeled water for measuring total body water of lean, normally hydrated persons has been largely a matter of personal preference and often dictated by the availability of the necessary equipment. With modern techniques deuterium and tritium are no more, and by some methods, less difficult to assay than antipyrine, although they require special apparatus. Both deuterium and tritium can be measured with greater accuracy than antipyrine, but, on the other hand, there is a systematic overestimate of about one per cent from hydrogen exchange. In some circumstances, labeled water may have a distinct advantage in that it can be given orally and assayed in urine, thus avoiding the need for sterile solutions and the inconvenience of venipunctures.

The advantages accrue more decidedly to deuterium and tritium where total body water is determined in subjects with substantial edema and ascitic fluid volumes. The antipyrine method would then be needlessly complicated, and it is even doubtful that reliable values could be obtained.

Deuterium- and tritium-labeled water are very nearly, if not quite, the ideal test solutes which Hevesy and Hofer (33) thought them to be. Both deuterium oxide and tritium-labeled water form ideal solutions with water and despite their slightly greater molecular weight, 19 for HDO and 20 for HTO, diffuse throughout the body at rates which cannot be sensibly different from that of H₂O. The early fears that an isotope effect would selectively modify the excretion, metabolism, and, possibly, the distribution of the labeled molecules is unfounded, at least insofar as it affects measurement of total body water. After equilibrium is reached, deuterium concentrations in urine, gastric juice, cerebrospinal fluid, and sweat are identical to those in serum (33, 60). Moreover, the turnover time of about 13 days ($T_{1/2} = 9$ days) for deuterium (60) and tritium

(63) is the same as for total body water estimated from water balance. Even a slight differential in the kidney filtration rate for labeled and unlabeled water would be reflected in a marked difference in their turnover time as well as a difference in serum and urine concentration, and it may be concluded that both isotopes accurately characterize the distribution and kinetics of body water since these differences do not occur.

Deuterium and tritium behave in the body fluids exactly as normal water, but this very property leads to a systematic overestimate of total body water. Hydrogen in water, and consequently deuterium and tritium as well, exchanges with labile hydrogen atoms in cellular material, mainly in carboxyl, hydroxyl, and other groups in which hydrogen is not bound to carbon (62). The deuterium or tritium space, while identical to that of normal hydrogen in water, is nonetheless greater than total body water by the same proportion in which exchange occurs.

Concurrent measurements of water with antipyrine and deuterium (61) and antipyrine and tritium (57) always result in a higher value indicated by the hydrogen isotopes amounting to 0.5–2.0% of body weight. This is, of course, no test of the assumed systematic error due to exchange because there is ample reason to suspect the validity of antipyrine, but it is the same order of magnitude that is derived on more direct evidence. Schloerb and associates (61) estimated on theoretical grounds that deuterium gave total body water values about 2% of body weight greater than the actual value. A more direct estimate with tritium obtained by Prentice *et al.* (57) from analyses of rabbit tissues also places the error in the range of 1–2% of body weight. The latter studies also suggested that the precise value of the correction depends upon the relative amount of lean tissue, and, hence, the error relative to body weight would be smaller in obese and edematous persons than in the lean individual.

In observing changes in total body water within a person, the error due to exchange is unimportant because it will remain constant. For absolute water measurements, however, a correction of about 1% of body weight is needed, but, more important, a more precise estimate of the correction is necessary before much can be gained from improved precision in assaying deuterium and tritium.

2. Antipyrine

Antipyrine (2,3-dimethyl-1-phenylpyrazolone-5) is perhaps more widely known as an analgesic and antipyretic. It is nontoxic in the amount required for body water determination, and, equally important, it is quickly and uniformly distributed throughout the body tissues and fluids of normally hydrated man and other mammals (66, 67).

Antipyrine in man is excreted very slowly, only 0.3–0.6% in 4 hr., but it undergoes degradation at a rate of about 6% hr.⁻¹ (67). In rabbits, dogs, and monkeys the disappearance rate is very much greater, ranging from 20–50% hr.⁻¹ (66, 67). Uniform distribution of antipyrine in the whole of the body water of normally hydrated persons is complete in 1–2 hr., after which its disappearance is accurately characterized by a single exponential term, i.e., by a linear decrease with time on a semi-logarithmic scale, with a half time in the order of 10 hr. At least three blood samples, spaced at intervals of several hours, are needed to establish the antipyrine disappearance curve, which is then extrapolated to the time of injection to secure the plasma level and, hence, the dilution factor corrected for disappearance.

In practice, approximately 1 gm. of antipyrine is given intravenously as a 5% aqueous solution. Blood samples are subsequently taken at suitable intervals, e.g., 2, 4, and 6 hr., to establish the plasma antipyrine disappearance curve. The antipyrine is assayed in the blood samples by the method of Brodie *et al.* (8). The plasma filtrate is deproteinized with zinc hydroxide, and the antipyrine concentration determined spectrophotometrically at 350 m μ by extrapolation between standards prepared from serial dilutions.

From tests for consistency in the chemical assay of antipyrine, Keys and Brožek (37) report a replicate standard deviation of $\pm 0.8\%$ of the mean value of antipyrine in serum from duplicate measurements. This appears to represent the result of careful refinement in technique and probably is smaller by a factor of 2 or 3 than can be expected from the more casual user. The overall consistency of the method was also estimated by Keys and Brožek (37) from their own and other published data on duplicate measurements of body water. They report replicate standard deviations ranging from $\pm 2.8\% - \pm 5.18\%$ of the mean body water.

3. Deuterium

a. *Falling Drop Method.* Hevesy and Hofer (33) first reported the use of deuterium for measuring total body water in 1934, but, because of scarcity of the isotope and the great difficulties in its assay at that time, deuterium oxide (D_2O) was not fully exploited for this purpose until more recent years. The isotope is now readily available in large quantities at moderate cost, and its assay with modern instruments is no more difficult than many ordinary laboratory procedures.

Deuterium oxide is usually given in very nearly pure form, 99.8% D_2O , either by mouth or by intravenous infusion. With administration of 100 cc., the equilibrium concentration in body water of a lean, 70 kgm. man is then about 0.25%, which lies in a range conveniently measured by

either of the two methods most widely used for assaying deuterium: Namely, the mass spectrometer and the falling drop method. In clinically normal humans, equilibrium is reached in 1-2 hr. (19, 61) after which venous samples are taken and assayed for serum deuterium concentration or, alternatively, urine samples may be used provided the bladder is first emptied of water excreted before equilibrium was attained.

The falling drop method (23, 60, 64) depends on the slight increment in density contributed to water by HDO that is present in excess of its concentration of about 1:6000. The rate of descent of small drops of water in an immiscible fluid, usually orthofluorotoluene whose density is slightly less than that of water, is directly related to the density of the drop and, hence, to the concentration of HDO. Direct estimates of the sample HDO concentration are made by comparison with the rate of fall of drops from serial dilutions of the original stock solutions.

In experienced hands the method is rapid and accurate, but it demands meticulous care. Drops of about 25 mm.³ delivered by micropipette must be consistent in volume to 0.1%, the temperature of the orthofluorotoluene column must be held within $\pm 0.001^\circ\text{C}$, and purity of water is of the utmost importance since traces of solvent or organic material produce entirely disproportionate changes in density. The last of these factors is more easily dealt with by assaying DHO in serum than it is in urine. Purification of serum water in a two-stage vacuum distillation train suffices, whereas urine requires chemical purification (60, 64) as well as multiple distillation and may still contain traces of contamination.

The falling drop method as practiced by Schloerb *et al.* (60, 61) had an accuracy of $\pm 0.5\%$ (S.D.) in the determination of volume per cent HDO in serum samples, which for a lean adult corresponds to ± 200 cc. in total body water. However, they estimate an overall standard deviation of ± 400 cc., which includes all sources of experimental error in the determination of total body water. This does not, of course, include systematic error that may be inherent in the use of deuterium due to hydrogen exchange.

b. Mass Spectrometer. The mass spectrometer was invented and primarily developed to measure stable isotope abundance; consequently, it is the ideal method—in principle if not always in practice—to measure deuterium. At its best, the mass spectrometer is the most precise means for measuring deuterium concentration, but, offsetting this advantage, the great cost of the instrument and its maintenance often precludes its use in body water studies except where such facilities are already available.

The procedure for analyzing deuterium has been described in detail for its specific application to total body water measurement (68). Water

distilled *in vacuo* from either serum or urine samples is first reduced to H₂ and HD by passing the water vapor over hot zinc. The concentration of deuterium, and thus the amount by which it has been diluted in the total body water, is derived from the ratio of the hydrogen ion currents observed at masses 2 and 3, corresponding to H⁺ + D⁺ and (HD)⁺ + H₃⁺. A calibration curve of deuterium concentration as a function of the mass 3/mass 2 ratio is usually needed to compensate for the interference from H₃⁺, whose formation in the ion source is sensitive to the instrument parameters.

In the same fashion that background radiation affects the ultimate precision with which radioisotopes can be assayed, the natural abundance of deuterium in water sets the limit on the permissible dilution of deuterium and contributes its error in measurement to that of the deuterium determination in serum and plasma samples. In estimating the error of measurement Solomon (68) and associates found that serial determinations yielded a standard deviation in volume per cent deuterium of $\pm 0.13\%$ and, if the error in measuring natural concentration is comparable, the error is still only $\pm 0.18\%$. The total analytical error of the mass spectrometer procedure, however, is estimated to be $\pm 0.5\%$, while the overall error in the total body water determination, not counting a systematic error because of hydrogen exchange, is stated as $\pm 1\%$ of body water, or about ± 400 cc. in a lean, 70 kgm. man.

4. Assay of Tritium

Tritium, the sole radioactive isotope of hydrogen, is assayed by detection of the beta particle emitted in the course of radioactive decay to helium. The extremely low energy of the radiation, which averages only 7 kev., makes tritium one of the most difficult to measure of all the radioisotopes used in tracer applications. Since the range of the radiation in tissue and water is about a micron, tritium cannot be detected by most of the conventional instruments that are used to measure other radioisotopes. In general, it is necessary to place the tritium from a biological sample directly into the sensitive region of a radiation-detecting device in a form that voids self-absorption.

In the early studies of body water with tritium, Pace and associates (53) introduced tritium-labeled water vapor directly into a Geiger-Müller tube as part of the filling gas. Counter tubes do not tolerate appreciable quantities of either water vapor or hydrogen, and it was necessary, therefore, to limit the water vapor pressure within the tube to only 1 or 2 mm. of mercury. The overall sensitivity that can be achieved is relatively low, and this, combined with the intricacy of the technique

and the too frequent failure of counter tubes, led to the abandonment of this method in favor of others that were less demanding.

The proportional counter has had some success in measuring tritium, particularly when the tritium can be synthesized into an organic gas that does not interfere with operation of the counter. The device still has severe technical limitations, however, in applying it to routine studies of total body water and has rarely been used for this purpose.

The simplest as well as the least expensive device for assaying tritium has been the ionization chamber although it did not come into use for water studies until recent years. Operation of the ionization chamber is not affected by the kind of filling gas that is used, and it is practicable, therefore, to achieve a high sensitivity by filling the chamber entirely with hydrogen reduced from a body water sample. With a sensitive current-measuring device, such as the vibrating reed electrometer or the Lindemann electrometer, the ionization chamber has the highest overall sensitivity of the conventional means for detecting tritium.

Water from urine, plasma, or other body fluid is reduced to hydrogen by the well-known method of passing the vapor over hot zinc. Quantitative conversion of the water is necessary in order to avoid an isotope effect from differential evaporation rates.

An alternative method used by the author (57) for converting water samples to hydrogen is faster and simpler for routine analysis of tritium in body water studies. A saturated solution of lithium-aluminum hydride (LiAlH_4) in diethyl "Carbitol" is used to reduce the water sample *in vacuo*. The procedure is extremely rapid, but it has one disadvantage in that tritium generated from the sample is diluted by a factor of two by hydrogen contributed by the hydride. Unless the reaction takes place in an excess of the hydride, a significant isotope effect also occurs, which can introduce serious error.

The analytical error in the ionization chamber method is approximately $\pm 1\%$ (S.D.) for serial determinations with standard solutions. The overall error in measuring total body water, including analytical, volumetric, and standard solution specific activity errors, is about ± 0.7 liters, or $\pm 1.8\%$ of body weight in a lean, 70 kgm. man. This does not, of course, include the hydrogen exchange error.

With further development it appears likely that the liquid scintillation counter will supersede the ionization chamber as the method of choice for assaying tritium. The small proportion of water that can be added to the liquid scintillator without interfering with its function still limits somewhat its sensitivity, but the chief obstacle to its widespread use at present is the complexity of the electronic circuits (34) and the consequent high cost of the apparatus and its maintenance. Nevertheless,

the liquid scintillation counter has been successfully used in several laboratories for tritium analysis, and it promises to be the simplest and most accurate technique for measuring total body water.

The amount of tritium administered to a subject depends upon the compromise that is reached between sensitivity, counting time, and statistical accuracy. In practice, the dose may vary from 0.2–2.0 mc., and it may be given orally or intravenously. Equilibration with the total body water in normally hydrated persons is attained in 2 hr. or less by either route. The tritium may then be assayed either in blood or urine. If urine is analyzed, a somewhat longer sampling time—3–4 hr.—is preferable and subjects should void several times before a sample is taken for analysis. It is not necessary to establish a disappearance curve since the turnover time for tritium is in the order of 13 days and, if several fluid samples are taken during the first 6 hr., their tritium specific activities need only be averaged.

In patients with large accumulations of transudate—edema, ascites, etc.—equilibration takes place less rapidly. In such patients, 3 or more fluid specimens should be taken during the interval from 8–24 hr. after administration of tritium. The values obtained over an interval this long after administration must be extrapolated to the time of administration to compensate for the disappearance of tritium (about 7% day⁻¹).

IV. MEASUREMENT OF EXTRACELLULAR FLUID

The first measurements of extracellular fluid achieved a result, fortuitous or otherwise, that is in good agreement with the best of modern methods. From histological examination of frozen preparations of frog muscle, Fenn (22) obtained values from 15–17% of the muscle weight. However, the procedure obviously was not acceptable for *in vivo* studies in humans and would in any event be confined to relatively few tissues. In the subsequent search for less traumatic *in vivo* methods, extracellular fluid space has posed a challenging problem which still is not resolved to everyone's satisfaction. Dilution techniques in one form or another are the only direct approach to *in vivo* measurement, but they require a solute that diffuses rapidly across capillary membranes—rapidly compared to excretion and metabolism of the substance—and yet is strictly barred from diffusion through cellular walls.

The early belief that sodium and chloride ions were confined almost wholly to the extracellular fluid, together with high permeability of capillary walls to these ions, led very naturally to the use of the radio-isotopes Na²⁴ and Cl³⁸ to measure extracellular space. Radioactive sodium, as interpreted in such studies, indicated spaces ranging from 25–35% of the body weight whereas Cl³⁸ tended to distribute in a small

space, 18–25% of body weight. Neither ion, however, is a valid indicator of extracellular fluid volume. They penetrate the cellular domain in significant and variable quantity; sodium is taken up in bone; and the ratio of sodium to chloride is lower in some tissues, e.g., red cells, connective tissue, and in gastric and intestinal mucosa than it is in serum. Nevertheless, sodium may still be clinically useful in estimating the degree of ascites and edema since the sodium space appears to increase in proportion to these alterations in extracellular fluid (77).

Bromide has also been used (5, 9, 30, 74), but it is subject to the same criticism as the chloride and, in addition, is concentrated somewhat in the thyroid.

Perhaps the most widely used substance has been thiocyanate (15, 43). It equilibrates rapidly, and renal excretion is slow, but, like chloride, it enters the red cells, gastric mucosa, and other tissues, and in some pathological conditions cellular permeability to thiocyanate is markedly increased. Consequently, the thiocyanate measures a space, assuming a uniform concentration of the solute in water, that is 22–30% of body weight. Similar results—and objections—are met in the use of thiosulfate (12, 28), ferrocyanide (11), and unlabeled sulfate (6, 43). These substances also undergo rapid renal excretion, and unlabeled sulfate must in addition be corrected for endogenous serum sulfate as well as for that excreted.

In the search for solutes to which cell walls are impermeable, three large molecules proved more successful than the foregoing small ions in that they indicate spaces more in accord with acceptable extracellular fluid volumes. However, they are not without serious disadvantages. Two of these substances, sucrose (42, 43) and mannitol (18, 21, 51), give spaces ranging from 16–25% of body weight, which, however, is still somewhat too high. Their use is complicated by the unfortunate combination of slow penetration through capillary walls with very rapid renal excretion, and both are metabolized to an indeterminate extent by the body.

The third of these substances, inulin (5, 13, 27, 40, 47, 80), distributes itself into a smaller space and is believed to give a reasonable volume of extracellular fluid, ranging from 12–about 20% of body weight. Inulin, because of its large molecular weight does not penetrate red cells, cells of the renal tubules or, by inference, cells of other tissues. It is not an electrolyte and hence does not affect fluid balance between compartments; it is lipid-insoluble and, finally, since it is recovered quantitatively in urine, may be assumed to be metabolically inert.

The large molecular weight of inulin, however, delays its entry and distribution into the whole of the extracellular space, while at the same time it is rapidly excreted. For this reason, equilibration of inulin cannot

be achieved with administration of a single dose except in renal insufficiency and in nephrectomized animals. To circumvent this difficulty, inulin is continuously infused into a vein until the serum level remains constant. A steady state is reached in 2 hr. in dogs and about 5 hr. in man. After attaining a constant serum level, infusion is stopped, the bladder completely emptied, and the total inulin collected in subsequent renal excretion. The extracellular space is then given by the ratio of total inulin excreted to its concentration in serum.

Two sources of error may, however, still persist. The first stems from urine retained in the tubules, pelves, and ureters. Inulin in high concentration, withheld in these dead spaces after cessation of infusion and emptying the bladder, is subsequently passed in the urine where, accumulated as part of the postinfusion inulin recovery, it introduces an error as great as half a liter unless the urine flow rate is high, i.e., in excess of 3 cc. min.⁻¹. The second arises from the large molecular weight, which may possibly deny access to some compartments of extracellular space. This effect has not, however, been verified.

The inulin infusion method, though complicated, seems valid in subjects with normal hydration, but it is not a useful method in the presence of edema or ascites. Inulin diffuses too slowly to reach equilibrium in such discrete accumulations of fluid, even after 30 hr. (5). This is understandable in view of the 8 hr. or more required for equilibration of labeled water between plasma and ascitic fluid (57).

The sulfate ion labeled with S³⁵ appears to offer distinct advantages over the preceding substances as a test solute for extracellular fluid space. The method, which has been investigated in both man and in dogs by Walser (75, 76), is simple and rapid compared to the inulin infusion method, and it appears to indicate a space in very close agreement with that of inulin when both methods were used concurrently. Walser (76) reports a ratio of radiosulfate to inulin space in 9 normal persons of 0.95 ± 0.11 .

In practice, 100 mc. of carrier-free S³⁵ is injected intravenously as H₂SO₄ and the blood serum level of S³⁵ then followed for 5–6 hr. During the initial period of 15–20 min. following administration of S³⁵ in man (25–30 min. in dogs), the specific activity in serum exhibits the characteristic mixing components common to all nonphagocytizable substances injected into the blood system. Once mixing throughout the extracellular space is completed, which for a rapidly diffusing substance such as sulfate is controlled predominantly by the transport time in the vascular system, the S³⁵ serum activity continues to decrease but less rapidly and as a single exponential with an average half time of 5.9 hr. in man and 7.5 hr. in dogs. The slow disappearance of radiosulfate, compared to its rapid

mixing in the extracellular space, justifies a simple extrapolation of the curve back to the time of injection to determine the volume in which the administered dose was diluted. The fact that the serum radiosulfate disappearance may reflect a slow penetration of cellular walls as well as renal excretion is unimportant since this effect is compensated in the extrapolation of the disappearance curve to the time of injection.

The radiosulfate space in man, as measured by Walser, varied from 12–21% of body weight, with an average of 14.6%. For dogs, the space varied from 16–24%, with an average value of 20.1% of body weight.

None of the methods now available, including radiosulfate, can be expected to give a reliable indication of extracellular space when it is augmented by edema, ascites, or other accumulations of tissue exudate. The time required for even the most mobile ion to reach a steady state between the blood vascular system and anatomically distant fluid masses where diffusion is the controlling factor is so long compared to loss through other channels that it is unlikely for the solute ever to approach a uniform distribution. The urine excretion curve contributes little additional information because it does not aid in differentiating between equilibration in edema or ascitic fluid and the loss by penetration of cell walls, metabolism, and exchange.

V. FAT

Fat is the most variable of the body's major constituents, both in the individual and within the population. Bone remains relatively constant throughout adult life, and the total protein can be altered only by heroic measures and disease, but the quantity of fat depends upon a simple everyday experience—the difference between energy output and caloric input. Partly because of its variability, fat commands special interest in studies of body composition. From its alteration in the individual undergoing weight change, it provides important clues that are needed in estimates of the remaining constituents. This is not, however, the most important reason for interest in fat as a body component. It is fundamental to the widespread nutritional problems associated with both inanition and obesity; it is an important factor in health and longevity; and, economically, fat is significant in the meat industries since its deposition by animals is inimical to efficiency in converting animal feeds to edible protein.

Fat is a chronic preoccupation of much of the adult population, though possibly for different reasons in different places. In most parts of the world, fat is regarded as aesthetically undesirable when it becomes superficially evident. There is, however, good justification for this point of view on other grounds. Overlooking the highly motivated "Battle of

the Bulge" to achieve an acceptable cosmetic effect, there is the more compelling reason for concern over excess fat for its adverse influence on longevity and, more specifically, on the degenerative diseases.

This fact has long been recognized by life insurance companies in their reliance on standard tables of height and weight derived from actuarial-mortality statistics. The tendency to overweight is common and altogether human in areas where food is plentiful and the standard of living high; it is also in such areas that degenerative diseases often set the pace of mortality rates because of the effective control of infectious disease. Under these conditions the substantially higher death rate among overweight people is clearly evident. Perhaps fat cannot be held directly responsible for a greater incidence of diabetes, cardiovascular-renal diseases, coronary diseases, cirrhosis of the liver, etc., but insurance statistics again show not only a higher mortality rate from these diseases among overweight people but also a striking correlation of death rate with the degree of obesity.

In frank obesity the great bulk of depot fat can be a physical handicap as well as a potential hazard in disease. It is not too uncommon, particularly in middle age, to find persons burdened with as much as 100 lb. of fat-bearing tissue. When this represents one-half of the body weight, its constant mechanical support and maintenance become a major effort, and there remains little net vitality beyond the consuming demands of the fat burden.

At the other extreme of the emaciation-obesity scale there appears to be no deleterious effect when total lipids constitute only a few per cent of body weight in normal, adequately nourished persons. This need not represent necessarily an ideal to be achieved, however, since there is insufficient evidence to suggest that depot fat amounting to 10 or even 15% of body weight is particularly harmful, and it is a proportion easily maintained. The more obvious questions then present themselves: What is the ideal weight for each individual? What, indeed, is the criterion for ideal weight? And, assuming an ideal weight were defined, what per cent of it is depot fat? The last question is no less important than the others because depot fat is the only constituent that can be altered by diet alone. The insurance companies' standard tables of height and weight (1, 17) were for many years the only source of extensive statistical data relative to this problem and, in the absence of something better, were often construed as tables of "best" weights for given age and height. Aside from questions of accuracy and of statistical validity when applied to populations other than that subscribing to life insurance, the tables represent average values of weight and consequently show progressive increase in weight with age, a trend which can only reflect a steady

and undesirable increase in fat deposit. Perhaps more important, the standard tables do not take into account differences in body weight which are not due to fat but rather to body build.

On the other hand, the more recent (1942) Metropolitan Life Insurance Company "Table of Desirable Weights for Adults" (70), which has as its criterion life expectancy, recognizes these factors and provides a better first approximation of best body weight. The table recommends weights which depend upon size of frame—small, medium and large—as well as on height, and the weights remain constant after the age of 25 years. Although, doubtless, it is the more reliable table in terms of its hygienic value, it is not likely that the best solution to the problem of ideal individual weight is to be found in tables of this kind. Ultimately it may depend upon more exact estimates of depot fat or even of body composition.

Throughout the discussion here and in following sections, fat is construed as lipids or "pure fat" to distinguish it from adipose tissue, "obesity tissue," or some popular connotation of fat. It includes the ether-soluble extract of tissue: Phospholipids, glycolipids, sterols, and neutral fats or glycerides. Apart from the bulk of fat consisting of glycerides, lipids—especially phosphatides—form an essential constituent of cell walls and protoplasm and are present in all tissue, although in somewhat greater amount in viscera than in skin and muscle. The quantity of these lipids remains nearly constant in cells and is little affected by body weight changes and fasting. To distinguish them from the neutral or storage fat, the lipid constituents of cells are referred to variously as "specific constituent fat" (46), "élément constant" (73), and, perhaps more concisely, "essential lipids" (4). The depot, or nonessential fat that is stored in fat cells forms the variable and, except in the leanest persons, by far the largest fraction of the body's total lipids.

The total quantity of essential lipid is not well established. Behnke *et al.* (4) estimate that it constitutes 2% of the lean body, but this probably represents the upper limit in view of the known concentrations of essential lipids in separate organs; e.g., 2–3% in visceral organs, but considerably less in muscle and skin which form the greater fraction of the body weight.

Storage fat varies over a remarkably wide range. The body reduced by severe undernourishment may be totally devoid of stored fat while, at the other extreme, the fat content of clinically normal persons studied by the author was found to be as high as 50% of their body weight. Few generalizations are possible regarding the proportion of fat in the normal person. Even in the study by Behnke *et al.* (4) on young naval personnel, for whom the median value of storage or "excess fat" was 16.5% of

body weight, the range extended from 1.5%–38.5%. This was a highly selected test group and neither the median nor the range is likely to be representative of the general population.

The difference in fat between the sexes and the trend with increasing age was known long before fat could be estimated *in vivo*. The sex difference in adults is now well established, at least qualitatively, by the tendency of women to have lower densities and a smaller proportion of total body water, both of which are quantitatively related to fat. The trend with age, however, appears to be roughly the same for both sexes after passing the age of 25. Deposition of fat continues steadily during most of the adult life of both sexes, apparently with no significant alteration in the other components of the body, at least until 45–55 yr. of age. The trend is not so clear, however, during senescence. A relatively greater fraction of persons who live to advanced age may never have been obese but, on the other hand, fatty degeneration gradually takes place in visceral organs, bone marrow, and possibly in muscle as well. The overall trend, including statistical and physiological factors, is not known.

The density of human fat is essential to the correct interpretation of the densitometric method for estimating the total fat. Fat density differs appreciably among animals of various species and may even change in a single animal with alteration in diet and environment. While the data are still relatively meager, the evidence suggests that variation in the density of human fat is considerably less. Keys and Brožek (37), citing the work of Fidanza, Keys, and Anderson, report that the mean value for the density of fat in the adult human is 0.9007 gm. cm.⁻³ at 36° C. Although fat was taken from various locations in the body, the range in density extended only from 0.8996–0.9015 gm. cm.⁻³. The older value of 0.9179 gm. cm.⁻³ is also correct but, as noted by Keys and Brožek, it refers to specific gravity of fat at 15° C compared to water at 15° C and, hence, leads to significant error if used in densitometric formulas.

When a person undergoes a change in weight, the tissue that is gained or lost is not pure fat. Behnke *et al.* (4) observed that the change in whole body density could not be accounted for by the difference in weight unless the density of tissue involved in the weight change was substantially higher than that of fat. Keys and Brožek (37) investigated these changes in a series of experiments with men who gained weight ranging from 2.5–21.0 kgm. by overeating. The mean value for the density of the obesity tissue gained in this study was 0.9478 gm. cm.⁻³ at 36–37° C with apparently little variability.

Adipose tissue is well known to consist in part of water, variously stated as 10–30% of the tissue weight. Even if the highest value is taken, however, the combined density of 30% water and 70% fat would be only

0.928 gm. cm.⁻³; hence, it is evident that unless adipose tissue consists also of protein whose density is 1.34 gm. cm.⁻³ the observed density would require nearly 50% of the weight as water.

From measurements of changes in extracellular fluid space combined with densitometric considerations, Keys and Brožek (37) estimated the composition of obesity tissue, the tissue gained by men in overeating, as 62% fat, 24% cellular matter, and 14% extracellular fluid. If the cellular matter, in turn, is essentially the same as lean tissue and consists of 70% water, the composition of obesity tissue is then 62% fat, 31% water, and 7% protein.

The foregoing estimate of the composition of fat-bearing tissue is probably as good as any that is possible with available data and techniques. It can, of course, only be assumed that it is representative of all fatty tissue in the body, but it is also necessary to bear in mind that it is, in the strictest sense, an estimate only of the composition of tissue gained. There is, on the other hand, suggestive evidence that the proportion of fat in fatty tissue may not be constant but may depend rather on the total amount of excess fat. At the lower end of the emaciation-obesity range, the Minneapolis Starvation Experiment (38) showed clearly that fat forms a smaller proportion of the tissue that is lost from undernutrition. At the other extreme, in frank obesity, fat may constitute a still larger proportion than 62% of the obesity tissue. While there is, of course, no more direct confirmation for this than there is for the reverse effect in emaciation or, for that matter, of the estimated composition of obesity tissue, still the excessive reduction in total body water in obesity seems convincing evidence. Among several hundred persons whose body composition was investigated by the author, total body water as low as 38% of body weight was observed and more than a dozen had total body waters less than 45%. None of these low values could be accounted for on the basis of the composition of obesity tissue proposed by Keys and Brožek.

It is perhaps more realistic to regard fat-bearing tissue not as tissue of fixed composition but rather, if it is necessary to consider it at all aside from its content of fat, to assume that its composition varies in such a manner that both the absolute and relative amount of fat increase together. This would be the more reasonable view if fat cells accumulated fat droplets without producing a proportionate increase in protoplasm and extracellular fluid. It can only be said in conclusion that the composition of fat-bearing tissue, or cells, is not well understood and that estimates based in part on extracellular space measurement, particularly during weight change, are subject to the same uncertainties attending extracellular space.

VI. MEASUREMENT OF FAT

1. Densitometry

a. Principles. An indirect method for estimating the body's content of fat is suggested by the fact that water, fat, protein, and mineral have distinctly different densities. This property of the constituents can be utilized in relating the proportion of fat to the density of the whole body provided there is a predictable relationship among the constituents. This possibility was recognized by Stern (72) and Spivak (69), but they lacked the means for implementing it because their density measurements lacked the necessary accuracy and no direct estimates of fat were available. Twenty years later Behnke and associates (3) perfected the method of underwater weighing to determine density and were able to show a clear qualitative relationship between body density and fat.

With advances in technique and better understanding of body structure, densitometry became widely accepted as the most reliable indirect method for estimates of fat content. Nevertheless, densitometry, or more correctly, the interpretation of body density, is still beset with many uncertainties. The entire range in body density extends from about 1.1 gm. cm.⁻³ to a little less than 1 gm. cm.⁻³, a variation of only 10%, and for this same range, fat varies from 1 or 2% to about 50% of body weight. Water and fat, which together constitute about 75% of the total weight in most adults, differ in density by only 10%. Because the difference in fat and water density is so small, large variations in their proportions produce only small changes in body density. Moreover, gross density is meaningful only if there exist determinable and consistent relationships between the quantities of water, fat, protein, and mineral, in addition to the completely general but indeterminate relations

$$1 = w + f + p + m$$

and

$$\frac{1}{d} = \frac{w}{d_w} + \frac{f}{d_f} + \frac{p}{d_p} + \frac{m}{d_m}$$

where d is the combined density, i.e., the whole body density, and w , f , p , and m are the proportions of the constituents. Fortunately, to a first approximation at least, an interdependence does exist, but, on the other hand, the variability is sufficiently great to render estimates of absolute fat content no more reliable than perhaps $\pm 3\%$ of body weight at best.

The estimate of fat from the body density is based upon a relation of the form $f = (a/d) + b$, where a and b are empirical constants and d is the whole body density. The formula was obtained empirically by Pace and Rathbun (54). The values of the constants were initially derived

from studies on eviscerated guinea pigs whose "specific gravities" were determined by underwater weighing, and the lipid independently estimated by petrol ether extraction. For this group of animals, a least squares analysis of the data gave values for the constants of $a = 5.135$ and $b = -4.694$. Rathbun and Pace (58) then proposed a similar formula for estimating fat in humans, adjusting the constants to fit a lean-body-mass specific gravity of 1.1 and a human fat density of $0.918 \text{ gm. cm.}^{-3}$. This procedure yielded the widely used formula

$$f = \frac{5.548}{d} - 5.044$$

In the light of more recent developments, it would seem that the constants derived by Pace and Rathbun cannot give a correct estimate of fat. The true density of human fat is $0.9007 \text{ gm. cm.}^{-3}$ instead of 0.918 ; specific gravity is indicated but without specifying the water temperature at the time of underwater weighing or the reference temperature. The most serious objection, however, is the implicit assumption that the body consists of a lean mass of fixed composition to which pure fat is added to account for any density (or in their terms, specific gravity) less than 1.1 gm. cm.^{-3} .

If corrections are made for the first two discrepancies (with water and body specific gravity referred to body temperature) as was done by Keys and Brožek (37), the constants are then $a = 5.120$ and $b = -4.684$.

This does not take into account the basic difficulty—the assumption that the proportions of body constituents are either constant or exactly predictable. The composition of the tissue gained or lost in weight change appears to depend upon how much excess fat there was to begin with. Any formulation of the type $f = (a/d) + b$ is a severely limited form of the more general relationship between constituents and hence describes a hypothetical man consisting of a basic lean body of fixed density to which tissue of a different but constant composition and density is appended.

Keys and Brožek (37) proposed to circumvent this difficulty, at least in part, by abandoning the attempt to estimate the absolute proportion of fat from this formula and, instead, by modifying it to permit estimates of differences in fat content. The constants were adjusted to represent the difference in fat between the subject under measurement and that of a standard man derived from a careful study of a selected group of young men whose mean density was $1.0649 \text{ gm. cm.}^{-3}$, and fat estimated to be about 14% of body weight. The constants are derived on the assumption that obesity tissue consists of 62% fat, 14% extracellular fluid, and 24% cellular matter, with a density of $0.9395 \text{ gm. cm.}^{-3}$. The difference in

pure fat is then given by

$$\Delta f = \frac{5.427}{d} - 5.106$$

For an unspecified range near 14% fat, this may be the best means for estimating changes in fat. Unfortunately, it contains the same limitations as the preceding formulas, for if the composition of obesity tissue does not remain constant over the obesity range, then neither do the constants in the formula. In estimates of absolute fat content, the difficulties are further compounded by the uncertainty in the precise fat fraction in the standard man. This factor alone appears to be in the order of $\pm 2\%$ of body weight.

In view of the indirect way in which constants of this equation are derived and of its inability to cope with variations in body constituents other than fat, it is difficult to justify preference for one pair of constants over the others in spite of the apparent differences in depth of insight exercised in their derivation.

In spite of what has been said in outlining the limitations of fat estimates from body density alone, it is still the most reliable single *in vivo* method when applied to the normally hydrated adult human. Obviously, if there are gross differences from the norm in any of the constituents except fat, the formulas are no longer valid. It cannot, for example, be applied with any expectation for rational results in patients with edema, ascites, or other transudations.

b. Density by Archimedes' Principle. The oldest, in principle the simplest, and until recently, the only practical method for measuring body density is an adaptation of Archimedes' famous experiment in hydrostatic weighing. The method was first developed into a useful technique for humans and extensively used by Behnke and his associates (3) and later refined and put to intensive use by Keys and Brožek (10, 37). It was with this technique also that Rathbun and Pace (58) first demonstrated a quantitative relationship between whole body density and fat.

In principle the method is no more involved than Archimedes' original experiment. The weight of the body in air compared to its weight in water when totally submerged is a direct comparison of the overall density of the body with that of the water it displaces. The relationship between these quantities is expressed in the simple formula $d = d_w W_a / (W_a - W_w)$, where W_a and W_w are the body weights in air and in water, and d_w is the density of water for the temperature at which W_w was obtained. This is not, however, the density of the tissue mass but rather a mean value of body density that includes air left in the respiratory passages and lungs, and gas in the gastrointestinal tract. If these gas spaces constitute a

volume v at the moment the submerged body weight is taken, the true tissue density is then

$$d = \frac{d_w W_a}{W_a - W_w - vd_w}$$

In practice, correction for gas volume is made only for respiratory air spaces. There is still no reliable direct means for estimating the intestinal gas volume, but several methods are available for measuring the air still enclosed in the respiratory system when the subject is underwater. When the volume is measured before or after underwater weighing, as is most frequently done, it is essential, obviously, to reproduce as nearly as possible the air volume contained at the time of weighing. This is accomplished by forced maximal expiration which leaves a residual air volume of about 1500 cc. and which, with practice, is reproducible with a standard deviation in the order of ± 100 cc., including the analytical error in its measurement as well as the variability in absolute gas volume.

A better procedure and one that is less demanding on the subject is described by Brožek *et al.* (10). The residual air is measured at the same time as the underwater weighing; hence, as may be seen from the formula above, it is immaterial what phase of the respiratory cycle is used or what absolute value the lung volume may have, provided it is determined with the requisite accuracy. Neither compression of the residual air by hydrostatic pressure on the thorax and abdomen (10) nor variability in residual air after full expiration should then affect the value of the correction.

The absolute value of residual air is most frequently determined by volumetric analysis of nitrogen following washout with oxygen in an open-circuit system (14). Following maximal expiration, or immediately following weighing if an underwater measurement is made, the subject breathes oxygen on demand and the expired air is collected in a Tissot gasometer or Douglas bag. The residual air volume is calculated from the total volume and nitrogen concentration of expired gas collected over a 7 min. interval (14). Closed-circuit systems utilizing hydrogen (49) and helium (29) are also widely employed for lung volume measurement and, when used for estimating residual air, give absolute volumes and errors comparable to those obtained with the open-circuit nitrogen dilution system.

Gas in the gastrointestinal tract still cannot easily be estimated with accuracy even by roentgenological examination, and it is usually disregarded in correcting body density for residual gas. Under some conditions, particularly after ingesting gas-producing foods, and in gastrointestinal disturbances, the volume of gas may exceed several hundred cc., but it is also likely that a quantity of gas this large would produce distress

and excessive flatus which would be sufficient reason to disqualify a subject for measurement. Moreover, roentgenological examination would show clear evidence of gas accumulation in amounts this large since it is not found in humans under ordinary circumstances; with the subject in a basal condition, the gas volume would appear to be substantially smaller, but its variability is not known with certainty for more general conditions. On the basis of their own studies and a survey of the published data, Keys and Brožek are led to the conclusion that 50–100 cc. may represent the upper limit of gastrointestinal gas in subjects who are screened roentgenologically and who discharge flatus. This appears to be the best available estimate for selected subjects under optimal conditions, but the problem nevertheless persists in studies of large numbers of individuals where it is not always practicable to measure body density under the most suitable conditions or to screen them carefully for intestinal gas.

The error in density that is introduced by an uncertainty Δv in gas volume can be estimated from the differential of the formula above. In terms of relative error it may be stated as

$$\frac{\Delta d}{d} = \frac{100d\Delta v}{W_a} \%$$

For an uncertainty of 100 cc. in the volume of a 70 kgm. subject whose density is 1.05 and residual air 1500 cc., the error in density is only 0.15%, an error which is entirely acceptable for most purposes. The error in the estimate of residual lung volume and the quantity of gas in the gastrointestinal tract are of the same order of magnitude but the former is random, the latter systematic, and, consequently, the two errors may half the time be additive. Unless residual air is estimated from the average of multiple tests, the error in density may exceed $\pm 0.3\%$ from this cause alone. The accuracy that can be achieved by experienced operators under the most carefully controlled conditions is substantially better, at least so far as internal consistency of the method is concerned. For an early series of studies on selected test subjects—young men living under highly controlled conditions—Keys and associates (38) report a replicate standard deviation in absolute value of ± 0.0015 gm. cm.⁻³ (about $\pm 0.15\%$); for a later group studied by Carlson and Chen in their laboratory, a replicate standard error of estimate of ± 0.0004 gm. cm.⁻³ is cited. This represents the ultimate in precision that has been reported to date, but it is not indicative of the overall error to be found in routine studies of gross density. A more realistic appraisal is also given by the same authors for the overall error in more routine circumstances with random subject material. They estimate the upper limit in standard deviation to be

about $\pm 0.005 \text{ gm. cm.}^{-3}$, which includes variability in body composition, residual gases, and error in measurement. The corresponding error in fat estimate is about $\pm 2\%$ of body weight.

c. Helium Dilution Method. The search for a method of measuring body density that would circumvent the limitations of the underwater weighing technique, particularly in attempting to perform such measurements on patients and aged subjects, has led to the investigation of a multitude of ingenious devices. Some of these diverse approaches, aimed mainly at measuring body volume directly, include a variety of gas-dilution techniques, steady-state as well as pulsating pressure changes in

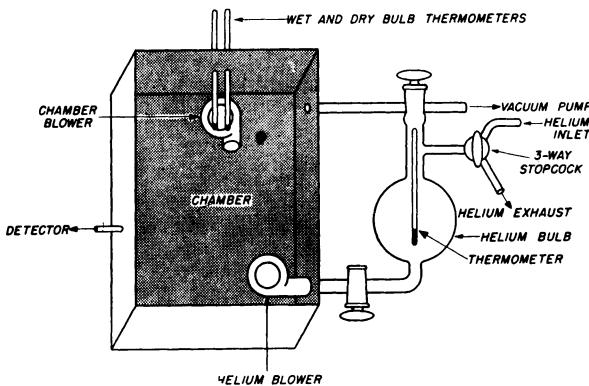


FIG. 2. Diagram of an apparatus for measuring body volume by helium dilution (63).

closed chambers, changes in acoustic resonance in chambers, and a host of devices for detecting these effects. Most of these efforts fall short on reliability and long on intricacy for the very good reason that nearly all such devices are also affected by the heat, water vapor, and carbon dioxide produced by the body.

A gas-dilution technique has been developed (63, 65), however, in which the effects of body heat and respiratory gases are fully corrected to yield a measurement of body volume with the requisite reliability, and the method serves the primary need by placing no demands on the subject.

In principle, body volume is determined from the dilution of helium in a chamber by a quantity of air that is inversely proportional to the volume of the subject. The apparatus, illustrated in Fig. 2, consists of two chambers connected to form a closed system. With the subject placed in the larger chamber and the smaller filled with pure helium, the gases in the two chambers are mixed with no net change in pressure or total volume. The initial volume of helium v is diluted by a volume of air—or, more correctly, air slightly modified by respiration—equal to the differ-

ence between the chamber and body volumes, $V - V_0$, hence its concentration after complete mixing is

$$c = \frac{v}{V - V_0 + v}$$

From among the many techniques for detecting helium, the thermal conductivity cell was selected for its high sensitivity and stability. In the form of a wheatstone bridge it is capable of measuring differences in helium concentration as small as $1:5 \times 10^{-5}$. The nonlinear characteristics of the device, however, restrict its use to changes in helium concentration of only a few per cent. This is not a practical disadvantage, however, because of the great sensitivity of the device, and it permits considerable simplification in the apparatus. The instrument used by Siri employs helium and chamber volumes of 12.5 and 413 liters; hence, for a range in body volume from 50–150 liters, the corresponding range in helium concentration extends only from 3.5–5%, or 0.015% per liter difference in body volume.

Systematic errors are eliminated and the residual nonlinearity compensated by calibrating the instrument with inert reference volumes that have been measured by independent methods and whose volumes bracket the range in body volume. This does not eliminate corrections for helium and chamber gas temperature nor the continuous effect of respiration on the composition and, hence, the thermal conductivity of the chamber gas. The latter effect is readily compensated for by a simple extrapolation since the rate of drift of the thermal conductivity unit in response to the steady accumulation of carbon dioxide and consumption of oxygen remains constant before and after the injection of helium.

No correction, obviously, is required for air in the lungs and respiratory passages because helium comes to equilibrium in these spaces in less time than is required to mix the gases in the chamber. If gas in the gastrointestinal tract amounts to about 100 cc., as the best estimates seem to indicate, its contribution to body volume is very nearly cancelled by the fortuitous absorption of very nearly the equivalent amount of helium in the body fluids and tissues (36).

The precision of the helium dilution method is dependent primarily upon the uniformity of the gas temperature and the accuracy with which it can be measured. For an uncertainty of $\pm 0.02^\circ\text{C}$ in temperature the corresponding error in the determination of body volume is less than ± 0.1 liter. However, when combined with other instrumental errors, the standard deviation for a single measurement is about ± 0.15 liter.

2. Fat Estimated from Total Body Water

The total water content of the leanest body and of the fat-free body is near 72% of the fat-free body weight. If it is assumed, as in the densitometric method, that the body composition is essentially constant except for greater or lesser amounts of fat, then the proportion of fat is obviously what is left after subtracting the fat-free body from the total. The lean body component of a fat person is then: (total body water) \div 0.72, and the proportion of fat

$$f = 1 - 1.39w$$

Again, the same objection to the validity of this formula can be raised as for the densitometric formula; it greatly understates the complexity of the interdependence of the body constituents, and it allows for no variability in their proportions. As was noted in the preceding section, the body cannot be regarded as a lean mass on which pure fat or even obesity tissue of fixed composition is hung.

Some improvement would seem at first to be realized by starting with a lean body consisting of 72% water, 21% protein, and 7% mineral, and then considering fat to be added as obesity tissue. According to the estimate of Keys and Brožek (37), the latter contains 62% pure fat, 31% water, and 7% protein. The total body water that is measured directly is then the sum of that associated with the basic lean mass and that associated with the fat, or $w = 0.72(1 - f/0.62) + 0.31f/0.62$. Combining these proportions, the resulting formula is

$$f = 1.09 - 1.51w$$

but this is not significantly different from the preceding expression, in view of the uncertainty in the fraction of the lean body—and obesity tissue—that consists of water. Nor is this method independent of the densitometric estimate of fat, since both are derived from exactly the same and necessarily fixed proportions in constituents. Also, as before, an estimate of fat based on total body water is no longer valid in edema and ascites.

3. Combined Methods

Earlier it was noted that two completely general relationships exist among the principle constituents irrespective of their relative proportions. The first is the obvious statement that the sum of the proportions of the constituents must equal unity: $1 = w + f + p + m$. The other relates the fractions of constituents and their separate densities to the

density for the whole body

$$\frac{1}{d} = \frac{w}{d_w} + \frac{f}{d_f} + \frac{p}{d_p} + \frac{m}{d_m}$$

From these two relations, it is evident that the quantities of any two substances may be derived uniquely from the body density and direct measurements of the remaining two constituents without resorting to empirical constants. With existing techniques, however, only gross density and total body water are amenable to independent measurement, leaving three "unknown" quantities and, consequently, an indeterminate system.

The problem is resolved, though at the expense of information and an increase in the possible error, by lumping protein and minerals as a single constituent, which may be termed nonfat solids, $s = p + m$, and whose density is $s/d_s = p/d_p + m/d_m$. The proportion of fat contained in the body can then be expressed in terms of the whole body density and total body water by combining these relationships with the general equations above

$$f = \frac{d_f}{d_s - d_f} \left[\frac{d_s}{d} - w \left(\frac{d_s - d_w}{d_w} \right) - 1 \right]$$

The values for the densities of fat and water at body temperature are now well-established: $d_f = 0.9007 \text{ gm. cm.}^{-3}$ and $d_w = 0.9933 \text{ gm. cm.}^{-3}$. The density d_s is the factor that introduces the greatest uncertainty into this method. If it is assumed that the highest ratio of mineral to protein is 0.5, their combined density is $d_s = 1.64 \text{ gm. cm.}^{-3}$. On the other hand, the proportion of protein may increase in obesity. If, judging from the available data, the mineral to protein ratio in a fat person decreases to $\frac{1}{3}$, the combined nonfat solids' density decreases to about $1.56 \text{ gm. cm.}^{-3}$. If this is the whole range of variation to be expected, the error in the estimate of fat from this uncertainty is in the order of 1% of the body weight. Using the median value of 1.6 gm. cm.^{-3} , the equation for estimating fat then becomes

$$f = (2.057/d) - 0.786w - 1.286$$

The overall error in fat estimate depends, of course, not only on the variability of d_s but on errors in density and water measurement as well. An estimate of this error can be found by applying the law of propagation of errors to the equation for fat. When evaluated for a subject with a density of $1.05 \text{ gm. cm.}^{-3}$ and total body water equal to 55%, the standard deviation, σ_f , in the estimate of fat in terms of errors in d , w , and d_s ,

is obtained from

$$\sigma_f^2 = 3.490\sigma_d^2 + 0.596\sigma_w^2 + 0.088\sigma_a^2$$

Reasonable values for the experimental errors in density and water and for the variability in d_s , are: $\sigma_d = \pm 0.005 \text{ gm. cm.}^{-3}$, $\sigma_w = 0.02$ of body weight, and $\sigma_a = \pm 0.04 \text{ gm. cm.}^{-3}$, which yield a standard deviation in fat of $\sigma_f = \pm 1.7\%$ of the body weight. For a 70 kgm. man with the specifications above, the standard deviation in the determination of his fat content is $\pm 1.2 \text{ kgm.}$

The results of this analysis are intended only to indicate the order of magnitude of the error. It would appear to be, in any case, smaller than could be hoped for by estimates from density or water separately. It also gives a more confident measure of the absolute proportion of fat since the combined measurements leave fewer chances for unaccounted-for variations in the body's components. A particularly important consideration in its application to clinical studies is the method's complete independence of the state of hydration. It is equally valid for normal subjects and for patients with gross accumulations of transudate as in edema and ascites.

Another approach to estimating fat from combined measurements was derived by Keys and Brožek (37). With the aid of specifications for their "standard reference man," they derived an equation for fat in terms of body density and extracellular fluid expressing not the absolute quantity but rather the difference in fat between the reference man and a subject. This difference Δf is given by

$$\Delta f = (5.359/d) - 0.256T - 4.982$$

where T is the extracellular water fraction of the body.

It is obviously a more highly determined system than that in which only density or water is measured; consequently, the formula in principle is also valid in subjects with altered hydration, provided there is no alteration in intracellular water—an assumption which may not be wholly justified.

The main objection to relating fat to extracellular water lies in the grave uncertainty that the extracellular space measured corresponds to anything like the actual extracellular fluid. Moreover, the uncertainty is compounded further by the equation's implicit dependence on the extracellular fluid of the reference man, which Keys and Brožek assumed to be 70% of the thiocyanate space that was measured. Because of the dependence on a reference man whose composition is not known exactly and the unknown error in extracellular fluid measurement, it does not seem possi-

ble to assess the reliability of this method for estimating fat or fat difference.

4. *Fat-Soluble Gases*

Many gases differ in their solubility in water and fats. In a mixture of the two substances, the partition of a fat-soluble gas, as in dilution techniques, gives a direct measure of fat content.

Nitrogen exchange in the tissues has been investigated the most thoroughly of any gas because of its important role in bends and caisson disease. Nitrogen is about five times more soluble in fat than in tissue fluids, and, combined with the low vascularity of fatty tissue, it is washed out of the fat depot at a very much slower rate than from the rest of the body when the subject is placed on pure oxygen. Fat could be estimated, then, by observing the nitrogen elimination curve for a sufficiently long time until its rate of washout corresponded to its removal from fat alone. Unfortunately, the half time for removal from fat is in the order of 2-3 hr. (36), and it would be necessary for the subject to respire oxygen for 12 hr. or more before the removal of nitrogen from fat could be differentiated accurately from muscle and skin for which the process is only slightly faster.

A more effective method was investigated by Lesser and associates (45), who used cyclopropane. The gas is about 26 times more soluble in fats than in tissue fluids so that the differential rates of absorption are more easily measured or, alternatively, the relative amounts at equilibrium are more accurately measured than for nitrogen. Good agreement was found between fat from ether extraction and that obtained with cyclopropane in a closed-system respirometer in studies with rats. However, since the equilibration time depends primarily on the blood perfusion rate of tissues, it does not seem likely that in man cyclopropane would reach equilibrium in fat much faster than nitrogen. The extreme hazard of the gas in concentrations greater than 5% in air cannot be overlooked in using the gas in man.

VII. PROTEIN AND MINERAL

Total body water can be measured *in vivo* with an accuracy of about 2% of the body weight, lipid constituents may be estimated with comparable error, and, hence, by difference, the remaining bulk of the body's constituents can be estimated with only slightly larger error. This constitutes very nearly the extent to which body gross composition is known or can be analyzed quantitatively by *in vivo* methods now available. Beyond this point, an attempt to estimate the proportions of protein and bone mineral that constitute the dry, fat-free fraction of the body is supported only by the most circuitous analyses.

Data from direct analyses of human cadavers are so few that they scarcely suggest more than an order of magnitude in the proportions that may be expected for total protein and mineral. Table III reproduced from Keys and Brožek (37), summarizes the best of modern data that has been reported. Although it may be argued that the percentages of fat, water, protein, and ash found in these analyses may not be representative of the clinically "normal" person because of the circumstances leading to death, the great differences in the ash to protein ratios strongly suggest that

TABLE III
*Body Composition from Direct Analysis of Cadavers**

Age	Sex	Height, cm.	Weight, kgm.	Per Cent Total Weight				Per Cent Fat-Free Weight			Ash/ Protein
				Water	Fat	Pro- tein	Ash	Water	Pro- tein	Ash	
42 ¹	F	169	45.1	56.0	23.6	14.4	7.6	73.2	18.8	9.9	.527
46 ²	M	168.5	53.8	55.1	19.4	18.6	5.4	68.4	23.1	6.7	.290
35 ³	M	183	70.6	67.9	12.5	14.4	4.8	77.6	16.5	5.5	.333
25 ⁴	M	179	71.8	61.8	14.9	16.6	7.5	72.6	17.5	8.8	.503
48 ⁵	M		63.8	81.5	1.1	12.8	4.9	82.4	12.9	5.0	.388

* Table and description reproduced from Keys and Brožek (37).

¹ Suicide by drowning; ²died of cerebral injury; ³died of "an acute heart attack (decompensation or failure)"; ⁴died of uremia; ⁵died of infective endocarditis and the body was wasted and grossly edematous.

similar, if not larger, variations are to be expected in the population at large. In these 5 cadavers the ash to protein ratio ranged from 0.29–0.53; yet there seems to be no obvious correlation with the other constituents.

Direct chemical studies of laboratory animals, on the other hand, are at variance with this, both in magnitude and degree of variation. As indicated by the data in Table IV, both the intra- and interspecies' ratios of ash to protein are remarkably consistent in view of the apparent variation in humans. Ash constitutes only about 5% of the fat-free body, compared to the 5–10% which direct analysis of human cadavers yields, and in animals it consistently forms about one-fourth the amount of protein or 20% of the combined weight. The relatively greater uniformity is not wholly unexpected because laboratory animals are maintained in controlled conditions, and generally a test group consists of uniform stock. This does not explain, however, the interspecies consistency in ash protein ratio nor the great difference between lower mammals and man.

Behnke (4) and Keys and Brožek (37) conclude that the difference in

ash, or bone mineral, between man and the lower animals is real and suggest a value of 7% for the mean proportion of ash (mineral) in the fat-free human body. This appears to be based primarily upon the results of the direct analyses of the 5 humans reported by Widdowson *et al.* (79), Mitchell *et al.* (50) and Forbes *et al.* (25), summarized in Table III. There is, of course, no *a priori* reason to believe that the ash-protein ratio in humans need be comparable to that in other mammals, but the evidence for believing it 50% greater in humans is also inconclusive.

TABLE IV
*Percentage of Water, Protein and Ash in the Fat-Free Body of Adult Laboratory Animals**

Animal	Water		Protein†		Ash		Ash/Protein
	Range	Mean	Range	Mean	Range	Mean	
Rat	71.8		18.2		3.3		
		73.1		20.5		4.2	.21
Guinea pig	74.4		22.2		5.1		
	72.4		19.3		3.9		
Rabbit	74.1			19.6		4.8	.25
	75.6		19.9		5.6		
Cat	72.8		19.3		4.0		
	74.2			21.2		5.6	.27
Dog	76.3		23.2		7.2		
	72.4		21.0		4.6		
Monkey	73.4			21.4		5.2	.24
	74.4		21.9		5.8		
Dog	69.9						
	72.2						
Monkey	74.5						
	67.4						
	69.4						
	71.6						

* Revised from Behnke *et al.* (4) and Pace and Rathbun (54).

† Based on 16% N₂ in protein.

The percentage of mineral in the fat-free body can also be estimated from gross body density and water, as outlined below, but, because of the assumptions that are involved, such estimates are admittedly without great weight. Nevertheless, they are not wholly without foundation, either, and are of interest because they lead to values in accord with those in Table III and further illustrate the variability of mineral and protein.

Precisely what is meant by mineral is not always clearly defined. If it includes only inorganic crystalline material, then evidently it is con-

fined wholly to bone. If it includes inorganic salts in the fluid spaces and metals associated with protein, then bone accounts for something like 85% of the body's total mineral store while the remainder is distributed throughout the body. The contents of ash obtained by direct analysis depend somewhat on the ashing procedure and also consist, in part, of material that should properly be regarded as constituents of protein. Similarly, bone mineral may be altered by ashing, and what is left is no longer mineral as it exists in the body, but something less, together with cellular residue. Aside from the uncertainty this leaves in a detailed description of fat-free solids, it also influences the empirical constants used in densitometric analysis of body composition. For the present purpose it will be assumed that minerals are present only in bone where they exist as a crystalline substance with a density of about 3 gm. cm.^{-3} (7).

Two characteristics of protein are essential to estimating its total quantity by methods now available. In direct analysis by ashing, nitrogen is assumed to constitute, on the average, 16% of protein by weight. Each gram of nitrogen recovered in ashing corresponds then to 6.25 gm. of protein analyzed. This value was used in estimating protein in Tables III and IV. For densitometric analysis, an average density is needed for protein as it exists in cells. Density is not easily defined for proteins except in the dry crystalline state, in which most proteins have a density of $1.25 \text{ gm. cm.}^{-3}$. Their specific volume decreases inversely with hydration until solution is reached at which the specific volume is $0.75 \text{ cm.}^3 \text{ gm.}^{-1}$ (32). The reciprocal of this, or "apparent density," of $1.34 \text{ gm. cm.}^{-3}$ is the best available estimate for protein in the living cell, although it can be considered only a tentative value since it is derived from isolated protein.

Although these values for bone-mineral and protein density cannot be accepted wholly without reservation, they are the only means, short of direct analysis, for estimating the proportions of mineral and protein in humans. Even this is possible only when total body water and fat (lipids) are known with reasonable confidence. The body composition approached in the upper limit of leanness perhaps best serves this purpose. The highest values of body density that have been reported, or that have been observed among several hundred subjects studied by the author, approach the limit of 1.1 gm. cm.^{-3} . Similarly, the upper limit of body water in normally hydrated persons is about 72% of the body weight. If it is assumed that 2% of the weight is essential lipids, then fat-free solids constitute 26% of body weight in the leanest humans. The proportions of mineral and protein can then be estimated from relations $1 = w + f + p + m$ and $\frac{1}{d} = \frac{w}{d_w} + \frac{p}{d_p} + \frac{m}{d_m} + \frac{f}{d_f}$ from which the mineral

proportion can be expressed as a function of water, fat and density

$$m = \frac{d_m}{d_m - d_p} \left[\frac{d_p - d_w}{d_w} w + \frac{d_p - d_f}{d_f} f - \frac{d_p - d}{d} \right]$$

Using the values given above, bone minerals constitute 7.8% of the body weight and protein 18.2%. The ratio $m/p = 0.43$ is about twice that for the animals in Table IV, but of the same order of magnitude as the higher values from direct analysis of humans.

It is instructive to estimate also the influence that alterations in water and fat will have on these values for the lean individual. The standard deviation in m obtained by applying the law of propagation of errors to the equation for m above has the form $\sigma_m^2 = 0.400\sigma_w^2 + 0.781\sigma_f^2$, assuming the densities are constant. Reasonable estimates of the variability of total body water and fat in a body of density 1.1 gm. cm.⁻³ are $\sigma = \pm 0.02$ and $\sigma_f = \pm 0.005$ parts of body weight; hence, the corresponding deviation in mineral is $\sigma_m = \pm 1.4\%$ of body weight, or total mineral may be $7.8 \pm 1.4\%$. Similarly, the variability in protein is $18.2 \pm 3.3\%$. It may be emphasized that the foregoing calculations apply only to the extreme in lean body composition and the estimated deviation in mineral is the result of only small alterations in water and fat but none in body density.

Indirect estimates of body composition based on a combined density-water-fat formulation contain explicitly the density of fat-free, dry solids. There is no direct means for its evaluation, but it can be derived from the familiar expression relating densities and quantities of mineral and protein, $1 = p + m$, and $d_s = (pd_m + md_p)/d_p d_m$, and the ratio calculated above: $m/p = 0.43$. This procedure yields a combined mineral-protein density of 1.61 gm. cm.⁻³. Using the calculated values for variability of mineral and protein, the corresponding standard deviation in d_s is $\sigma_{d_s} = \pm 0.12$ gm. cm.⁻³.

Thus far, the calculations, insofar as they have any validity, apply only to the extreme in body composition—the leanest possible man. When adipose tissue is added, the proportions of constituents, even in the fat-free fraction of the body, are no longer preserved. Lipids, water, and cellular material are all increased in absolute amount, although water diminishes relative to body weight. If, for the purpose of analysis, Keys' and Brožek's estimate of the composition of adipose tissue is accepted, lipids form 62%, total water 31%, and cell solids 7%. The last of these is considered here to be protein, for, although ashing may yield a residue, part will be derived from protein and part from inorganic salts, which constitute a negligible fraction of the whole weight, particularly in view

of the uncertainty in the exact—and probably variable—composition of adipose tissue.

Apart from the adipose tissue in the fat individual, we may assume that the relative proportions of water, fat, protein, and mineral remain the same as in the preceding analysis; a rough estimate of the total constituents can be computed for the whole body when, for example, 22% of its weight is lipid. Since 2% is essential lipid, the added lipid is 20%, and, hence, adipose tissue accounts for $20/0.62$ or 32.3% of body weight. From this the total protein—18% of the lean body and 7% of adipose tissue—is 14.5% of the gross body weight. The mineral content, whose absolute value has presumably not changed significantly, is now reduced in relative value to 5.4% of body weight, and with it, of course, its ratio to protein, which becomes 0.37. The common density of fat-free solids also decreases to $1.58 \text{ gm. cm.}^{-3}$, reflecting the relatively greater protein fraction.

The preceding discussion, which is derived almost wholly from densitometric considerations, cannot do more than show orders of magnitude and indicate trends. As the smallest and most dense of body constituents, both protein and mineral necessarily undergo disproportionate alteration both in ratio and in relation to body weight for even small changes in water and lipid. The great accuracy achieved in measuring body density is not sufficient for decisive analysis of fat-free solids because of the attendant uncertainties in the values of other quantities that are mixed into the analysis. While the absolute values that are derived, including the composition of adipose tissue, must then be viewed with reservation, such methods are still useful in demonstrating body components resulting from weight gain or loss.

At the present time it is possible only to speculate on the variability of protein and mineral in man. Except in certain pathological states, the quantity of bone mineral remains relatively stable in the adult individual, but in the population the skeletal mass varies widely for a given height, and it seems unlikely that skeletal muscle mass must necessarily parallel bone mass; certainly skin, brain, and visceral masses do not. Skeletal muscle, which contains the largest fraction of the total protein, is far more stable than fat, but it is not, like bone, wholly inert. Its hypertrophy in exercise, and hypotrophy from lack of it, or in wasting diseases and starvation, are obvious evidence of muscle's and hence protein's variability. The precise evaluation of these changes in terms of water, fat, and cellular material does not await improvement in current densitometric and water dilution techniques but rather the introduction of altogether new methods.

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