Body Composition: Studies in the Human Being by the Dilution Principle[1]

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IN A BRIEF COMMUNICATION published in this journal in 1946 (*1*) the principle of isotope dilution was set forth as a means of studying body composition in the living human being. Utilizing this principle, tracer materials were injected intravenously, allowed to reach uniform distribution, and from the dilution achieved at equilibrium certain constituents of the body were measured. Radioactive and stable isotopes were thus used to measure the potassium and sodium in the body, the total body water, and the extracellular phase of body water. Color tracers were used, as they had been for several years, to measure plasma volume by the same dilution principle. As an example of the application of this concept, a series of measurements ("isotopic dissection") in a normal healthy young man was presented.

An approach to the understanding of disease by an investigation of changes in total body composition has been an outgrowth of such methods. The horizon of clinical chemistry has widened from the narrow confines of concentration studies to a broader view of the total aqueous environment of the cells and of the total quantity of solutes in circulation inside and outside of cells.

It is our purpose herewith to review very briefly the history of this work, its general principles, and the significant advances of recent years. As a footnote to the 1946 report we present again the "isotopic dissection" of the same young man, carried out in 1951 by the greatly refined techniques now available.

General Principle and Developmental History

The extent to which a substance is diluted in a solvent constitutes a measure of the volume of the solvent. If one adds to a beaker of distilled water 1 g of sodium chloride and finds after mixing that the concentration is .01 g/ml, it is quite clear that the volume of the beaker is 100 ml. This simple relationship may be expressed as the following equation:

$$V_2 = \frac{C_1 V_1}{C_2}, \qquad (1)[7]$$

where C_1 and V_1 are, respectively, the concentration and volume of the solute before dilution, and C_2 and V_2 are the concentration and volume of the solute after dilution. The concentration and volume of the solute before dilution are known, and the concentration after dilution is experimentally measured. The only unknown in the equation is, therefore, V_2, and the equation is accordingly solved for the unknown volume.

In the study of body composition in the living human by the dilution principle, the subject's body or some fraction thereof is the beaker of our analogy. An easily identified isotope, dye, or other tracer substance is the solute. The extent of dilution achieved measures the volume of some fraction of body water such as the plasma volume, or the weight of some constituent such as potassium. The validity of application of this principle varies with each special use to which it is put. In no instance is the human body or any of the parts truly analogous to a simple beaker. The human beaker is in dynamic equilibrium with other liquid and solid phases; it may be inhomogeneous or multicompartmented, and its volume changes in disease. And, finally, the establishment of dilution equilibrium and its measurement may be a matter of some difficulty in proof or practice.

The dilution principle was first used in the study of total body composition in the living human being in 1915 by Keith, Rowntree, and Geraghty (*2*) when they first explored the use of a red dye to measure the plasma volume. Here the dye was injected intravenously and allowed to circulate for a few minutes, after which multiple plasma samples were taken to measure the concentration after dilution. It was soon discovered that the concentration of the dye after mixing was not constant. It "disappeared" from the blood plasma. To return to our analogy of the salt placed in the beaker full of water, the beaker appeared to have a little fresh water running into it all the time and a little salt water running out the bottom. It was being constantly refilled, and it had a leak. The result was that the concentration of the dye slowly fell. However, by mathematical means a

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[7] This equation is derived from the simple consideration that the product of concentration and volume has the dimension of weight or mass, and within a closed system the mass of the solute is constant regardless of the extent of its dilution; that is:

$$C_1 V_1 = C_2 V_2.$$

reasonable estimate could be made of the volume of plasma in which the dye was initially diluted.

During the next fifteen years progress in this field was made with the aid of a blue dye known as T-1824, signifying that tolidine is coupled with a 1-amino, 8-naphthol, 2,4-disulphonic acid. This dye is also known as Evans Blue, because it was first extensively studied by Herbert M. Evans, of California (*3, 4*). It has come into wide use for plasma volume measurement, and great attention has been given to the exact details of the method, improvements in optical measurements of the dye, and clinical interpretation (*5–7*). But the fundamental problem remains—and still remains with all plasma volume methods—that the beaker has a small leak in it, and a small amount of water is being added all the time. In other words, the dilution curve is not flat but remains sloping until the dye completely disappears.

In 1934 Crandall and Anderson published their classical article on "Estimation of State of Hydration of Body by Amount of Water Available for Solution of Sodium Thiocyanate" (*8, 9*). The concept here was that a larger beaker was being measured, because an ion was used that diluted itself in a larger volume than the blood plasma. This beaker contained, not just the water in the blood plasma, but also the water on the far side of the capillary in the interstitial fluid, the lymph, and indeed all the water of the body outside of cells. This was termed the "available fluid" and was measured by injecting thiocyanate, permitting it to reach a dilution equilibrium, sampling its concentration, and, by application of the dilution formula, calculating the volume of water in which it was diluted. The technical problems here were many, but the principle was sound. Here the beaker of our analogy has a good-sized leak in it, just as in the plasma. The "leak" constitutes movement of thiocyanate into cells and urine. However, there is another complexity with extracellular methods; namely, that the beaker is not homogeneous. The blood plasma may at least be considered to be a single anatomical compartment within the blood vessels. The extracellular phase, by contrast, is widespread and constitutes many different kinds of fluid, including liver lymph, visceral lymph, peripheral subcutaneous lymph, muscle lymph, and many other types of fluid outside cells that will be mentioned in greater detail below. Despite these limitations the thiocyanate method considerably widened the horizon of investigation.

This was about where dilution studies of body composition rested in 1940, when many new methods began to become available. The developments of the past twelve years have brought us new methods not only for plasma and extracellular fluid volume, but also for volume of the red cells, total body water, intracellular water, total exchangeable potassium, total exchangeable sodium, the labile iron pool, and many others. These are all dilution methods resting on the simple analogy of the beaker and the salt. Many involve radioactive and stable isotopes as well as dye and color tracers. They all have intrinsic errors, or problems, that are described in general by the analogy of how big the "leak" is in the beaker, how much water is being added to the beaker per unit time, and whether the material added for the measurement itself alters the volume of the beaker or is metabolized within the beaker.

Plasma and Blood Volume

Advances in technology for direct measurement of blood volume by the dilution principle have taken two main directions. First have been those that are direct descendants of the dye methods and are aimed at measurement of the plasma volume. Second are those that measure the red cell volume directly by diluting a material—usually tagged red cells—within the red cell mass.

The methods for plasma volume have been expanded to include in addition to the blue dye method the use of radiobromine- (*10*) and radioiodine- (*11*) tagged plasma protein. These methods vary in technical detail, but they all present the same essential problem; namely, that the dilution curve is at no time "flat" and some sort of extrapolation must be used to derive the volume of initial dilution. Despite these disadvantages, all the methods have given us useful information, and successive measurements in the same patient show significant variations that are correlated with the clinical course of the patient. There is no evidence that the plasma volume is more nearly a "true value" as measured by any one of these methods than by any other method. Yet the details vary in technical convenience for the particular clinical circumstances in which they are used. Albumin, in contrast to a dye, is a normal constituent of plasma, but halogenated albumin is not. Furthermore, there are large amounts of albumin outside the plasma in the lymph of liver and viscera (*12*). Any valid albumin tracer will equilibrate into these areas outside the plasma. The tagged albumin methods (*13*) have yet to show themselves better in theory or practice than the time-honored dye, which indeed partakes of this same feature by virtue of its preferential affinity for albumin (*14*), with which it unites in stoichiometric proportions. In our opinion, the perfect plasma volume method has yet to be found and may consist of a dilution method based on a large molecule that does not readily cross the visceral capillaries.

The methods for measurement of the red cell volume employ the dilution of tagged red cells. They consist of putting the tag directly into the red cells by *in vivo* synthesis or *in vitro* exchange. A mass of tagged red cells is then infused. The concentration of the tagged red cells discovered in the recipient red cell mass within the first hour or two indicates the dilution and hence the size of the recipient mass. In most instances the tag employed has been a radioactive isotope. Radioactive iron (*15*) has been incorporated into hemoglobin by special donors given the material over a period of months. Radioactive phosphorus (*16*) and chromium (*17*) have been equilibrated into the cells *in vitro*. Carbon monoxide has also been used as a

chemical tag and should be of special utility in small-animal research (*18*).

Here, in the measurement of the red cell mass, there is one helpful variation from the problems previously mentioned. The beaker has a very small leak and nothing new is being added. At least nothing is being added fast enough to alter concentration; red cells are found only within the blood stream, and the rate of synthesis of new red cells is so slow that the dilution curve achieves a truly "flat" value. Indeed, with radio-iron-tagged red cells, once mixing has occurred, the time-concentration curve is essentially flat, demonstrating only an extremely slow fall as new stable iron is added from the diet, and is synthesized into new hemoglobin, which then appears in new red cells. The other isotopic red cell tags (P^{32}, Cr^{51}) show variable rates of leakage of the tag because they are held in the cell by a binding less stable than that of iron in hemoglobin (*16, 17*).

From simultaneous measurement of plasma and red cell volume by two tracers, the concept of the "whole body hematocrit" has emerged as being the erythrocyte fraction of the total blood volume (*15, 19*). There are fewer red cells per unit blood volume in the patient as a whole than there are in a random sample of blood taken peripherally, for example, from the antecubital vein. It has been common practice for many years to measure the "blood volume" by measuring the plasma volume, the hematocrit in a sample of blood from a large vein, and then calculation by the familiar expression:

$$BV = PV \times \left(\frac{100}{100 - Hct}\right). \qquad (2)^{8}$$

The difference between the large vessel hematocrit and the whole body hematocrit (about 10% of the former) introduces a systematic error such that this should not be termed a measure of blood volume at all.

Ironically, there is no escape from this enigma by measurement of the red cell mass and calculation of blood volume from:

$$BV = RV \times \left(\frac{100}{Hct}\right). \qquad (3)$$

The large vessel hematocrit is still a determinant and one in error, and not to be escaped by such further maneuvers as hemolyzing the blood and reading the cell concentration some other way. The fact remains that there are truly more red cells per unit of blood in large vessels than in the body as a whole. In the last analysis a blood volume measurement should be based on the expression, independent of hematocrit, that:

$$BV = RV + PV. \qquad (4)$$

There is general agreement, therefore, that the most accurate *in vivo* measurements of blood volume are based on simultaneous dilution measurement of red cell volume and plasma volume by two distinct tracers.

[8] In formulas 2, 3, and 4, blood volume is indicated as *BV*, plasma volume as *PV*, red cell volume as *RV*, and peripheral large vessel hematocrit as *Hct*.

Any pair of the above-mentioned may be used. The resulting volume for the total blood in circulation is then independent of the error introduced by the difference between the large vessel hematocrit and the "whole body hematocrit." The "whole body hematocrit" can be calculated from the large vessel hematocrit by the use of standard data (*15*). Such corrections are not always valid in the presence of acute disease. Normal blood volume varies from 7 to 9 per cent of body weight in healthy young adult males and from 6 to 8 per cent in young adult females. Certain aspects of this work have recently been reviewed extensively by Gregersen (*20*) and Reeve (*21*).

Extracellular Fluid Volume

Extracellular volume methods have also undergone distinct evolution. In addition to the thiocyanate method mentioned above (*8*), radiosodium (*22*), bromide (*23*), and radiobromide (*24*) have been used for this purpose. Inulin (*25*), sucrose (*26*), and mannitol (*27, 28*) have been explored as well as, more recently, the thiosulfate ion (*29, 30*). All these methods for the measurement of the extracellular phase involve technical difficulties and interpretive uncertainties, because no substance has yet been identified that is a small molecule readily diffusible into all extracellular areas, nonmetabolized, and exclusively extracellular in distribution. In fact, one might name these as the "ideal requirements for an extracellular volume method."

In discussing the methods for plasma volume, it was pointed out that, although the beaker has a leak and some water is always being added, it is in point of fact one beaker. By this analogy it is meant that the plasma volume resides within the blood stream, even though it is actively exchanging water across the capillary. The extracellular volume has a disturbing heterogeneity about it. Although most of it is water that is immediately beyond ordinary capillaries, the other chemical characteristics of this fluid demonstrate its extremely variable nature. The content of protein and fat may vary widely, even though the electrolyte content is essentially in equilibrium. In addition to this obviously *extracellular water*, however, there is water in special compartments of the body such as the cerebrospinal fluid, the water in synovial sacs, the water in glandular lumina, the water of bile and within the lumen of the gastrointestinal tract itself, and the urine in the collecting tubules and bladder. None of this water is within cells. In that sense it might be called "extracellular" water. However, it has had to pass through cells and be processed by cells other than capillaries in order to reach its present site. We have therefore termed this *transcellular water* and prefer to think of it as a special subdivision of body water. It is included in the deuterium technique of total body water measurement, but in general the molecules used to measure extracellular water do not penetrate readily into these areas.

Although no one of these methods of extracellular space determination is ideal any more than any one of

the plasma volume methods is perfect, they have been extremely useful. The amount of water outside of body cells appears to be between 25 and 30 per cent of body weight in the normal. The normal plasma volume of 3–5 per cent is, of course, included in this figure. When plasma volume and transcellular water are subtracted, an interstitial fluid in the general neighborhood of 12–15 per cent of body weight is obtained.

Recent work suggests that the thiosulfate ion does approximate the rigid requirements for a good extracellular method and has certain practical advantages over inulin. The use of this ion is simple, and the volume calculation is based on a single injection. Urine concentrations do not have to be measured. The method consists of a brief infusion (10–20 min) of 10 per cent sodium thiosulfate. Four to six serial blood samples are taken over a 1-hour period. The analyzed values for thiosulfate content are plotted on semilogarithmic coordinates, and the zero time dilution is determined by extrapolation. Graphic correction for renal loss and extrarenal metabolism is thereby achieved (*30*). In 10 normal young adult male subjects the volume of dilution of thiosulfate averaged 16.8 per cent of body weight, and in 13 normal young adult female subjects the volume of dilution averaged 17.5 per cent of body weight (Table 1).

TABLE 1

BODY COMPOSITION AS MEASURED BY DILUTION IN NORMAL YOUNG ADULT HUMANS

Body constituent and method used	Males			Females		
	No. of subjects	Mean	Range	No. of subjects	Mean	Range
Thiosulfate volume of dilution (% of body wt)	10	16.8	15.3–18.8	13	17.5	15.3–21.0
Total body water (D_2O) (% of body wt)	37	62.0	54.5–70.3	19	51.5	45.6–59.9
Exchangeable sodium (Na^{24}) (mEq/kg body wt)	11	41.4	36.1–46.0	3	41.0	31.4–45.9
Exchangeable potassium (K^{42}) (mEq/kg body wt)	33	46.8	35.6–53.6	14	40.7	28.0–47.2

TOTAL BODY WATER

The measurement of total body water with heavy water was first tentatively explored in 1934 (*31*). Our report in 1946 described values in the human being and a simple check on the method based on desiccation studies in animals (*1*). These studies have been considerably extended through the refinement of the falling drop method of analysis (*32*) and by its corroboration in an extensive series of simultaneous measurements carried out on the mass spectrometer (*33, 34*). It has thus been possible to proceed with measurement of the normal human subject throughout the life span in both sexes (*33, 35, 36*). Antipyrine has become available for this method and has been investigated thoroughly in the past three years (*37*); this substance has many attractive technical features, particularly ease of analysis, but falls short of perfection both theoretically and on empirical grounds (*38*).

As shown in Table 1, the total body water in normal males is 62 per cent of body weight and in the female, 51.5 per cent. Both are average figures based on deuterium dilution.

Considered in the light of the blood and extracellular fluid methods, the deuterium total body water method has great theoretical advantages. The beaker has essentially no leak, and very little water is being added—i.e., very little water is lost from the body during the 2 or 3 hours during which the measurement is carried out. Urine secretion in this time may account for only 100 or 200 ml, constituting less than 0.5 per cent of the total body water being measured. Water intake may be restricted to zero, and the water lost through the skin and lungs is less than 150 ml. The solute whose dilution is being measured—namely, heavy water—therefore distributes itself in a very large beaker from which there is little leak and very little is being added. Some deuterium is exchanged, and some is synthetically incorporated into organic molecules during the 2 or 3 hours of the measurement, but this is a tiny fraction of the total hydrogen in the body and during this short time probably accounts for less than 1.5 per cent of the injected deuterium. At high concentrations deuterium is toxic and cannot be considered as an ideal tracer for hydrogen. The mass difference is twofold, and there may be other differences also. However, at the low concentration employed for these studies (the final concentration of deuterium in body water is in the neighborhood of 0.2 atoms % deuterium), the mass effect does not appear to influence dilution, and deuterium oxide is close to ideal as a water tracer. Viewed in the perspective of 6 or 8 hours, the deuterium dilution curve becomes essentially "flat" after mixing. Viewed in the perspective of several days or weeks, there is a slow rate of fall, indicating the rate of total water turnover.

The total body water method, of course, includes a measurement of all the water in the body. This must be considered as including plasma water, red cell water, interstitial water, all the varieties of lymph, all the intracellular water, and all the transcellular water. Extension of this method to clinical problems is in its infancy.

TOTAL EXCHANGEABLE SODIUM AND POTASSIUM

The measurement of solid constituents by the dilution principle is directly related to the measurement of the fluid volumes (*1*). There is only the technical

difference that we employ the dilution of an isotope in the family of naturally occurring isotopes within the body, rather than its dilution within a fluid moiety. For example, radioactive potassium is injected into the body, and the dilution achieved in the body's mixture of potassium isotope is measured and expressed as the "equilibrium specific activity." From this figure the amount of potassium with which the isotope is exchanged may be calculated.

since it is in very dry tissue such as bone cortex, hair, nails, and teeth. It is especially difficult to find where best to measure exchange.

In the case of both sodium and potassium, the plasma surprisingly appears to be the most satisfactory area in which to measure exchange. Despite the fact that the elements in plasma exchange with the isotopes to the same extent as sodium and potassium elsewhere, there are still certain areas of slow or in-

TABLE 2

BODY COMPOSITION AS MEASURED BY DILUTION IN A NORMAL HUMAN BEING*

Body constituent and method used	Dilution volume or mass (liters or mEq)	% Body weight	By surface area (Per m^2)
A—Direct Measurements			
Plasma volume (blue dye)	3.67 liters	5.1	1.92 liters
Extracellular volume (thiocyanate)	16.8 "	23.5	8.80 "
" " (thiosulfate)	13.4 "	18.8	7.03 "
Total body water (deuterium)	40.6 "	57.0	21.3 "
" exchangeable sodium (Na^{24})	3075 mEq	43.2 mEq/kg	1610 mEq
" " potassium (K^{42})	3440 "	48.2 " "	1801 "
B—Derived Data			
"Intracellular" water (by difference)†	27.2 liters	38.1	14.2 liters
Extracellular potassium (4.3 mEq/l × thiosulfate space)	57.6 mEq	0.81 mEq/kg	———
"Intracellular" potassium (by difference)†	3382 "	47.4 " "	———
"Av intracellular" potassium concentration†	124 mEq/liter		
Extracellular sodium (142 mEq/l × thiosulfate space)	1903 mEq	26.7 " "	———
"Intracellular" sodium (by difference)†	1172 "	16.4 " "	———
"Av intracellular" sodium concentration‡	43.2 mEq/liter		

* Subject: D. C.; male; age, 32; weight, 71.4 kg; height, 181.5 cm; surface area, 1.91 m^2.

† Intracellular water and intracellular potassium concentration are dependent on the characteristics of the methods used for estimating extracellular fluid volumes and do not represent exact values in any single tissue. It is particularly important to emphasize that the true intracellular water is not obtained merely by subtracting an extracellular dilution volume from total body water. The transcellular water—at present unknown—must also be subtracted.

‡ This figure for "average intracellular" sodium includes some bone matrix sodium and is an average of all the sodium not in solution in extracellular fluid. It should not be construed as indicating the exact sodium concentration in, for example, muscle or liver cells.

The measurement of solid constituents by this principle has moved forward in the past five years. Total exchangeable potassium has now been measured in a sizable group of normal individuals (*39*). The exchangeable potassium in the body seems to be related in size to the lean body mass and to creatinine excretion. Total exchangeable sodium has been measured with the short-lived isotope Na^{24} (*40*).

The development of these methods for *in vivo* measurement of the weight of sodium and potassium in the body has pointed up the central problem of all such measurements when based on isotope dilution. The distribution of these ions is heterogeneous, and a "fair sample" of the element must be established, which exhibits an isotope dilution representative of that in the body at large.

In terms of our analogic beaker, we are here dealing with a beaker which contains a solid in solution, and we are measuring the weight of the solid rather than the volume of fluid in the beaker. Some of the solid is in solution in extracellular water, some in muscle or liver cells, some in tendon or fascia or skin, and some can hardly be referred to as in solution at all complete exchange (e.g., for potassium, the red cell; for sodium, bone). For this reason the weight of elements measured by isotope dilution is not termed "total," but "total exchangeable." In Table 1 are shown average values for total exchangeable sodium and potassium. The mean exchangeable sodium in young adult males is 41.4 mEq/kg; in females, 41.0. These figures for sodium corroborate the more extensive data of Forbes and Perley (*40*). The mean exchangeable potassium in young adult males is 46.8 mEq/kg; in females, 40.7. By differential precipitation of sodium and potassium from the blood plasma, it is now possible to carry out simultaneous isotopic measurements of total exchangeable sodium and potassium in the same individual.

REMEASUREMENT OF SUBJECT D. C.

In our communication in 1946 (*1*), measurements were tabulated (as an example of the concept) on subject D. C. This helpful person recently returned to our laboratory, and we were offered the opportunity of remeasuring him, with the more refined techniques and concepts now available. These measure-

ments are appended in Table 2. The 1951 data are in interesting contrast to those of 1946. The subject has gained 5.4 kg in body weight, and his plasma volume has increased significantly as measured by Evans Blue dye. The thiocyanate space is essentially the same as before; the thiosulfate volume of dilution (not available as a method in 1946) gives a significantly lower figure.

The total body water of the previous report (47.8 liters) is almost certainly in error in consequence of our use of the gradient tube for the analytic technique. This method, we now realize, was grossly inaccurate (in our hands) as compared with the falling drop or the mass spectrometer (particularly at the low deuterium concentrations observed in the human studies, as opposed to those studies reported in the rabbit, where higher deuterium concentrations could be measured with reasonable accuracy in a gradient tube).

The total exchangeable potassium data were in error not on technical, but on theoretical grounds; namely, in our use of the red blood cell for the measurement of specific activity at equilibrium. We now know that the red blood cell does not reach equilibrium of potassium exchange early enough to be a useful measure of isotope dilution. The specific activity of urine or plasma, on the other hand, is valid as a "barometer" of exchange and dilution at 24 hours, as demonstrated by tissue studies (*39*).

The total exchangeable sodium measurements carried out in 1946 were based on the volume of dilution of Na^{24} at 60 minutes. There is now a wealth of evidence that this early dilution is not a measure of total exchangeable sodium, but that the dilution indicated by the plasma specific activity at 24 hours does measure the total exchangeable sodium. This, however, still does not measure the total amount of sodium in the body because of the lack of exchange of much of the sodium in bone. Because 35–40 per cent of the sodium in the body is in the skeleton, and half of this is not available for exchange and therefore probably not available for metabolic emergencies, the isotope measurement may be of greater clinical interest than the theoretically perfect total body sodium measurement.

The "average intracellular" potassium concentration, calculated as shown in Table 2, is now seen to be significantly lower than the 1946 methods indicated. The same is true, though to a lesser extent, in the case of sodium. The calculation of intracellular components has the shortcoming imposed by the limitation of the particular method used for estimating the extracellular fluid volume. The tracers for water and electrolytes penetrate into many "transcellular" areas such as spinal fluid, intestinal fluid, and glandular ducts, which are largely impermeable to the molecules used for extracellular fluid determinations. Therefore, the contents of these extracellular areas will be included in "intracellular" estimates. Although this source of error is large in determining absolute intracellular composition, relative changes should still be amenable to study by these methods.

Rates of Exchange

As a contribution to these studies, the isotopes are yielding information on rate problems. This also is understandable in terms of our beaker analogy. If we drop a tiny amount of salt into the beaker and repeatedly sample the water where the salt is added, we will find a rapidly decreasing concentration as the salt diffuses throughout the beaker. If the beaker is boiling, the salt will diffuse more rapidly; if it is frozen, more slowly. If there is a cellophane membrane in the middle of the beaker, the diffusion will proceed at a rate determined not only by thermal energy but also by membrane permeability, by chemical forces on the two sides of the membrane, and by the geometry of the system. Such factors do indeed influence the rate of change of isotope concentration in body fluids. For ease of consideration, isotope disappearance curves are considered to embody three phases: pre-equilibrium, equilibrium, and postequilibrium.

The *pre-equilibrium* portion of the isotope disappearance curve has been analyzed in an attempt to gain insight into the internal exchange across rate-limiting boundaries for sodium, chloride, and water (*41–43*). The studies on water kinetics indicate an unexpectedly rapid rate of penetration of water across the capillary membrane. Indeed, in 30 seconds the injected D_2O has reached a volume of dilution equal to, or larger than, the total extracellular fluid phase. The two rates evident in the distribution of heavy water from this time onward to equilibrium are apparently due to two widely separated mean velocities of penetration into cell areas (*42*).

The *equilibrium* value is that used to calculate dilution and the total exchangeable mass of the element in question (*44*).

The *postequilibrium* rate of disappearance of the isotope is a function of "turnover" by the body of the naturally occurring element (*45, 46*). For instance, the elimination of deuterium from the body is a simple exponential function that may be expressed as the "half-time" of water in the body (9–11 days) or as "turnover" rate (3000 ml/day) (*33*). Plotted semilogarithmically, this fall in deuterium concentration becomes a straight line, the slope of which is determined by the total rate of water metabolism. Pathological processes produce interesting changes—for example, in thyroid disease. In thyrotoxicosis the slope is "steep," and water turnover rapid (*46*). "Turnover rate" in the case of nonmetabolized substances refers to exchange of the molecule between the organism and its environment. There is a class of molecules such as albumin, urea, etc., which is synthesized by the organism, and in these cases "turnover rate" is a function of metabolic activity (i.e., internal production and degradation or excretion) rather than exchange between the organism and its environment.

From tissue studies essential to establish the validity of the dilution techniques has come much inter-

esting information about the physical state of body water and solutes, particularly the observation that about 55 per cent of the sodium in bone is "bound," or "unexchangeable" (*40, 47*). It has also been observed that among the various body cell areas, the red cell is remarkably slow in its exchange of potassium, reaching equilibrium only after 60 hours (*39, 48*).

Metabolic Correlations

When body water measurement is supplemented by measurement of the metabolic balance, an accurate account of changes in body composition may be made. This is based on the interrelationship of fat, water, and lean tissue solids (fat-free solids). Pace (*49*) has shown that, when water content of the normal body is known, fat can be calculated from:

$$\% \text{ Fat} = 100 - \frac{\% \text{ water}}{0.732}. \quad (5)$$

This is derived from the fact that lean tissue is 73 per cent water, and that the body consists of water, lean tissue solids, and fat.

Fat can be calculated from specific gravity, and such studies have been carried out by Behnke (*50*). The empirical formula is:

$$\% \text{ Fat} = 100 - \left(\frac{5.548}{\text{Sp gr}} - 5.044\right). \quad (6)$$

Measurements of fat by this method correlate well with calculation of fat from total body water data obtained by deuterium dilution.

Changes in lean tissue can further be measured by nitrogen balance on the basis that:

$$\Delta N \times 30 = \Delta \text{ lean tissue.} \quad (7)$$

Since lean tissue is 73 per cent water it follows that:

$$\Delta N \times 21.9 = \Delta \text{ lean tissue water.} \quad (8)$$
$$\Delta N \times 8.1 = \Delta \text{ lean tissue solids.}^{9} \quad (9)$$

As an example of the application of these methods, one or two recent case studies from our laboratories might be illustrative. Patient L. S., a 66-year-old man, suffered a very complicated course after subtotal gastrectomy for polyploid antral gastritis. Over the course of 51 days he was fed largely by the intravenous route and lost 7.0 kg. His body water only fell from 32.1 liters to 31.5 liters, indicating a rise in the water fraction from 48.3 per cent to 52.8 per cent of body weight. By application of the Pace formula (normal hydration being present at the start and end of the study), he lost about 6.0 kg of fat, the oxidation of which (at 9 cal/g) would contribute 54,000 calories, or an average of 1080 calories/day to his nutrition. During one 13-day period of acute illness this patient lost only 800 g of weight while increasing body water 1.8 liters. This indicates a loss of 2.6 kg of body solids. Metabolic balance demonstrated a loss of 44 g of nitrogen, representing 357 g of lean tissue solids. The remainder of the solid loss (2.2 kg) was therefore fat, utilized for energy at an average rate, during this acutely ill period, of 170 g (1530 cal)/day.

The largest loss of fat we have studied was that of a very obese patient, Mrs. M. N., who lost 8.8 kg of body weight in 120 days, following the removal of a pancreatic islet-cell tumor with associated hyperinsulinism. During this weight loss her body water changed only from 35.9 liters to 35.0 liters. This 900 ml of water would carry 0.3 kg of lean tissue solids, leaving 7.6 kg of her weight loss as fat.

We have observed instances of change in lean tissue and in whole tissue, as well as in fat. An example of whole tissue gain is to be found in patient E. W., who recovered from severe thyrotoxicosis after treatment with radioiodine. She gained 12 kg during a 6-month period. During this time her body water increased 4.6 liters, indicating a gain of 7.4 kg of solids. This much water would carry 1.7 kg of lean tissue solids, leaving 5.7 kg as the fat redeposited during recovery.

As a further example, the unique problems of metabolism in the postoperative patient are being elucidated (*51*). After operation a patient loses weight and gains sodium; his nitrogen balance discloses loss of lean tissue inadequate to account for the weight loss. When total body water is also measured, one thereby determines the rate at which the patient utilizes fat for energy during his brief period of postoperative starvation. The metabolic response to surgery unfolds as a pattern of changing composition characterized chiefly by oxidation of fat, degradation of protein with excretion of nitrogen, loss of potassium, probably transfer of sodium into cells, and a tendency to increase body water. The endocrine determinants of this complex adaptation to trauma are now under study.

Although difficult to apply to sick patients, the body specific gravity methods of Behnke and Pace (*49, 50*) have been of remarkable significance in clarifying concepts of total body composition, particularly with reference to body fat, and in rounding out the data obtained with isotopes. Direct fat methods based on the dilution principle may become available on the basis of dilution of certain inert gases (*52*) soluble in fat.

By no means the least important of the advances made has been the application of dilution principles in the elucidation of "pool" sizes for certain ions and organic molecules. The "labile iron pool" has been measured by Wintrobe (*53*). Sprinson and Rittenberg (*54*) developed a technique for estimating the rate of protein synthesis (or turnover) and the amount of amino acid nitrogen, using N^{15} as the tracer. A similar study of Stetten's group indicates the size of the uric acid "pool" and the uric acid turnover rate in the human, using N^{15}-labeled uric acid (*55*). Within a short time, studies by these principles of such im-

[9] This nitrogen coefficient (8.1) for lean tissue solids is representative of total lean tissue solids including bone, where a heavy salt component increases the weight of tissue solids per unit nitrogen over that found in soft tissues. More data from human tissues such as muscle, liver, lung, and kidney must be experimentally obtained to establish a valid nitrogen coefficient for use in the interpretation of short-term metabolic balance studies where soft tissue changes predominate.

portant organic species as albumin, cholesterol, urea, thyroxine, etc., may confidently be expected to appear.

The continuous support of this work, and the supply of isotopes furnished to the several laboratories by the Atomic Energy Commission, have been gratifying examples of the growth of basic biological information under the sponsorship of our national atomic energy program. Over the long term, the growth of biological knowledge of this type, which leads to sound concepts and effective procedures in the care of sick patients, may be a more important outgrowth of isotope research in the medical sciences than the direct application of the isotopes themselves to patients as therapeutic or diagnostic devices (*56*).

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News and Notes

Scientists in the News

E. D. Adrian, president of the Royal Society, has been elected master of Trinity College, Cambridge. He is also retiring from the chair of physiology in the university, a position he has held since 1937. He will be succeeded by **B. H. C. Matthews,** reader in experimental physiology in the university since 1946, and formerly head of the Physiological Laboratory at Farnborough, where he directed research in aviation medicine.

E. C. Auchter, chairman of the Standing Committee on Crop Improvement in the Pacific Area, and president and director of the Pineapple Research Institute of Hawaii, has been awarded the Wilder Medal for outstanding achievements in the science of horticulture. Awarded by the American Pomological Society, the medal has been given to only 18 persons in the U. S. and Canada in 80 years.

Paul M. Cook, senior chemical and radiation engineer and technical director of several Stanford Research Institute projects for the AEC, will head the new radiation engineering staff. **J. D. Graves,** formerly assistant chief of the Physics Branch, Naval Radiological Defense Laboratory, has joined SRI as senior physicist in the Radiation Engineering Laboratory.

Alfred Gellhorn, associate professor of medicine at the College of Physicians and Surgeons, Columbia University, has been appointed director of the Insti-