comments in nutrition

A review of body composition studies with emphasis on total body water and fat[1, 2]

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ABSTRACT Tritiated water measures a volume 4 to 15% of body weight larger than that by desiccation, and, at present, only 0.5 to 2.0% of the overestimation can be explained by the exchange of hydrogen of tritiated water with those of the proteins and carbohydrates of the body. The remainder of the error is unexplained. Water in the lumen of the gut is an appreciable percentage of total body water (TBW) in many mammalian species, with the pig and the human as possible exceptions, and it should be considered an integral part of TBW. Consequently, the exclusion or inclusion of this transcellular water as part of TBW significantly affects the final TBW volume. As tritiated water exchanges with water in the gut, a comparison of the data from the indirect method with the data from the direct method can only be made when water in the gut is included in the desiccation method. Conceptually, the amount of water in lean body mass is a reflection of the actively metabolizing cell mass of the body. However, water in the gut is outside this cell mass, and if included, it significantly overestimates the water associated with the lean body mass compartment. The percentage of water in fat-free wet weight for most mature animals is estimated at 73.2%, although the mean values in the literature range from 63% for the beagle to 80% for the mouse, with the mean for the majority of species between 70 and 76%. If the percentage of water in fat-free wet weight lies between 70 and 76% for most species, then the error in calculating fat using the figure 73.2% in the equation (% fat = 100 − % TBW/0.732) is significant. In the application of this equation, the largest potential error lies in the estimation of TBW with tritiated water. *Am. J. Clin. Nutr.* 32: 630–647, 1979.

Historical background

It is technically simple to remove a small amount of bone from a living animal, analyze it for calcium, and quantitate the results with a high degree of accuracy. It is more difficult, however, to state with an acceptable degree of accuracy the amount of calcium in the skeleton or, for that matter, in the whole animal because there is at present no satisfactory indirect method (with the possible exception of neutron activation analysis) for measuring total body calcium or for estimating the weight of the skeleton. Indirect methods are used for the measurement of some components of the whole body; for example, total body water (TBW), extracellular water, plasma volume, and the electrolytes sodium, potassium, and chloride. The validation of these indirect methods for the human, as well as for other species, is based commonly on a comparison of the results of two indirect methods or by a comparison, particularly in the human, of the results measured indirectly with the very few data available on direct chemical analyses of cadavers. Such comparisons are inadequate, however, and the final confirmation of the validity of an indirect method should depend on its results agreeing, within an acceptable error, with those from chemical analysis of the same animal.

Those interested in earlier literature on body composition should consult the exten-

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sive review by Widdowson and Dickerson (1) of most of the available data up to 1964. Both the historical background and an analysis of some of the methods used in the study of body composition, particularly the indirect estimation of fat, can be found in the articles by Keys and Brožek (2) and Siri (3). Pinson and Langham (4, 5) give an excellent analysis of the physiology and toxicology of tritium, while Coleman et al. (6) provide a useful analysis of the dynamics of distribution of isotopes in the fluid compartments of the body. The techniques currently used for measurement of the various components of body composition are reviewed in a symposium for which Brožek and Henschel served as editors (7). A most provocative book entitled *The Fire of Life* (8) contains useful information on body composition and energetics. Finally, Behnke's work (9) was an important activator of modern interest in the study of body composition, and his Harvey lecture is a milestone in the modern study of body composition.

No attempt will be made to review the large number of indirect methods used for the measurement of various chemical components of the body. This review is confined to available literature in which TBW is measured both directly and indirectly in the same animal and in the primary data used to support the concept that the amount of water in a mature animal is constant when the results are expressed as a percentage of fat-free wet weight (FFWW) or lean body mass (LBM).

Two generalizations commonly used by investigators of body composition are that tritiated water (THO) measures TBW within an error of 0.5 to 2.0% of body weight and that the fat-free body weight (or FFWW) contains 73.2% water (10–16). The attractiveness of these two generalizations lies in the fact that they permit the separation of the body into two compartments: a fat and a lean. The separation is accomplished by the calculation of the percentage of fat using the simple equation $100 - \%\ TBW/0.732$ (15); then the percentage of fat is converted into total body fat (kg) by the equation $\%\ \text{fat} \times \text{body weight (kg)}/100$. This figure is subtracted from the body weight to give the nonfat or lean body compartment, which is also called the LBM (12–14, 17, 18) by many students of body composition. The term LBM also is used synonymously with FFWW by some (17, 19–21). However, LBM does contain fat, principally essential lipids such as lecithin and phospholipids, which was estimated originally at 10% of total fat, but this estimate has slowly declined to about 2% or less at the present time (2, 9, 17). Although there is conceptually a difference between FFWW and LBM, it is difficult technically to distinguish between them as FFWW also contains a small percentage of essential lipids when fat is extracted by the usual chemical methods.

The possible usefulness of measuring an "active tissue mass" was first recognized by Rubner in 1902 (22) and was replaced later by the more specific concept of LBM proposed by Behnke (9). Theoretically, it can be argued that LBM represents more closely the active metabolizing cell mass than either body weight or body surface area. Consequently, it should serve as a better reference standard for physiological measurements related to this cell mass, such as cardiac output, oxygen consumption, red cell and plasma volumes, caloric requirements, and drug dosages. Also, by separation of the body into a fat and a lean compartment, investigations are possible on factors affecting these two compartments, such as growth, the influence of nutritional elements, the gain or loss of energy from the body under specified conditions, the effects of disease, the relationship between body composition and behavior, and the influence of body composition on predisposition to certain metabolic disorders in the adult.

As Moore et al. (17), however, aptly point out, LBM contains an appreciable amount of extracellular tissue, primarily skeleton and extracellular water, whose metabolic activity is significantly less than that of the major cell mass of the body. The extracellular tissue in the 1-year-old beagle, for example, is estimated to be 45% of the body weight, or 53% of the LBM (20). In place of LBM, Moore et al. (17) propose the estimation of an entity called the body cell mass. The size of this actively metabolizing cell mass can be calculated quantitatively using various assumptions. Two of these assumptions are that nearly all the potassium in the body is located in the cells and that ^{42}K, when injected, mixes with the total pool of potassium. Although the concept of body cell mass is intriguing and some of the necessary postulates have been examined critically in both the pig and the beagle (19, 20), in this review, only the literature which relates to the validity of the assumptions used to calculate LBM from TBW is examined.

An interest in body composition antedates this century. In the early and middle parts of the last century, a number of investigators were interested in the composition of the body; among them, perhaps the most widely quoted is von Bezold (23). In 1857, after investigation of a number of species of vertebrates including the human fetus, he concluded that each animal has an approximately constant amount of water, organic matter, and salt typical of its species and age. In addition, he was probably the first to recognize that as an animal grew there was a decrease in its water content. These are conclusions still acceptable to the modern investigator.

Although investigators measuring blood volume do not usually think of themselves as students of body composition, the volume of blood is an important component of the body fluid compartments making up extracellular water and TBW, and, therefore, is a part of body composition. Their work, in addition, provided the theory and methods essential to the measurement of any fluid compartment. In the 1880's, a number of attempts were made to measure blood volume in the human. The earliest of these experiments (24) consisted of exsanguination followed by flushing of the circulatory system with water to remove all red cells for the measurement of total hemoglobin content, a procedure applicable to the human only under unique circumstances. In spite of their technical crudity, the results obtained by Welcker (24) compare favorably with those of recent investigators using more sophisticated techniques, such as cell labeling. The use of the dilution principle to measure a body fluid compartment was probably first made by Gréhant and Quinquand in 1882 (25), who measured blood volume using carbon monoxide as a label for hemoglobin, although they measured the decrease in oxygen content rather than the concentration of carbon monoxide. It is true that earlier (1838) Valentin (26) had measured blood volume indirectly, but the method is more properly classified as a blood dilution method (large amounts of fluid were injected) rather than the dilution principle as it is used at present. Excellent summaries of the historical developments in the methods used in measurement of blood volume can be found in the articles of Gregersen and Rawson (27) and Lawson (28).

The search for suitable substances for the indirect measure of the fluid compartments continued with emphasis on blood volume measurement until Keith et al. (29) reported, in 1915, the successful measurement of plasma volume with the dye Vital Red. Subsequently, in 1920, Dawson et al. (30) introduced the dye T-1824 (Evans blue), and it remained the standard for measuring plasma volume until the introduction of radioisotopes.

Measurement of TBW

The successful measurement of plasma volume using an appropriate indicator substance gave added impetus to the search for substances that would measure extracellular water and TBW, and with the isolation of deuterium oxide (DHO) in 1932 by Urey et al. (31), its potential for measuring TBW was quickly realized. The first investigators to extend the use of DHO to the measurement of TBW were Hevesy and Hofer in 1934 (32), who published data on two rabbits and one human (Table 1). Their report was followed within a few months by an article in 1934 by McDougall et al. (33) on rats that corroborated Hevesy and Hofer's conclusion that DHO measured TBW (Table 1). Only two more attempts were made to measure TBW with DHO in the next decade: that of Hevesy and Jacobsen (34), in 1940, on rabbits (Table 1) and Flexner et al. (35), in 1942, on guinea pigs (Table 1). It was not until 1946 that a comparison of the indirect and direct methods of measuring TBW in the same rabbits was made by Moore (36), and the volume of TBW measured by DHO compared favorably with that of desiccation with a difference of only 0.7% of body weight (Table 2). Also, in the same article, the measurement of TBW by DHO in a human (Table 1) was reported.

TABLE 1
Indirect measurement of total body water

Species	Sex	No	Body wt	Reference	Remarks
			%		
Human	M	1	63.0	32	DHO
	M	1	72.5	36	DHO
	M	1	64.5	18	DHO
		8	51.9 ± 2.1[a]	102, Table 5	Antipyrine
		8	53.3 ± 2.3	102, Table 5	DHO
		2	56.7 ± 1.8	103, Table 1	
		81	61.1	104, Table 2	Antipyrine
	M	17	61.8 ± 0.8	16, Table 1	DHO
	F	11	51.9 ± 1.4	16, Table 1	DHO
	M	35	61.1 ± 0.7	105, Table 5	DHO, ages 17–34 yr
	F	19	51.2 ± 0.9	105, Table 6	DHO
	M	37	62.0	106, Table 1	DHO
	F	19	51.5	106, Table 1	DHO
	M	15	52.1 ± 0.8	107, Table 3	
	M	5	58.9 ± 1.6	107, Table 2B	
	M	17	60.1 ± 1.3	108, Table 1	Antipyrine
		5	62.2 ± 1.5	4, Table 1	
	M	10	54.3 ± 1.4	109, Table 4	DHO
	F	10	48.6 ± 1.5	109, Table 4	DHO
	M	10	70.8 ± 1.1	54, Table 3	
		13	57.3	110, Table 1	DHO, ages 10–15 yr
		5	55.3	111, Table 1	
	M	7	64.2 ± 1.4	112, Tables 1, 2	Sample 4
	M	5	65.2	113, Table 1	
		20	57.8 ± 1.1	114, Table 1	
	M, F	34	58.8 ± 1.6	114, Table 2	Patients
Pig-tailed monkey		10	68.6 ± 1.0	115, Table 4	Method A
Rat		2	66.0	33	DHO
		8	70.2 ± 1.7	116, Table 1	DHO
		10	68.1 ± 1.2	54, Table 3	
		12	59.6 ± 1.2	111, Table 1	
Kangaroo rat		20	62.2 ± 0.5	111, Table 1	
Rabbit		2	74.0 ± 0.3	34, Tables 1, 2	DHO
	M	13	73.3 ± 0.7	68, Table 2	
	F	13	74.8 ± 0.1	68, Table 2	
		7	65.5 ± 2.3	54, Table 3	DHO
		4	58.3 ± 2.6	111, Table 1	
Mouse	F	24	58.5 ± 0.8	111, Table 1	
Guinea pig		5	65.0	35	DHO
		18	75.2 ± 0.9	54, Table 3	DHO
Cat		8	70.6 ± 0.9	54, Table 3	DHO
Dog		5	65.9 ± 0.6	111, Table 1	
	F	5	70.7 ± 4.5	117, Table 1	Controls, recalculated using means
		5	65.2 ± 1.2	6, Table 1	
Horse		7	63.8 ± 0.5	118, Table 2	
		5	55.2 ± 0.5	118, Table 2	
		5	65.7 ± 0.4	111, Table 1	
Pony		11	67.7 ± 2.2	119, Table 1	

[a] SE.

TABLE 1—*Continued*
Indirect measurement of total body water

Species	Sex	No	Body wt	Reference	Remarks
Cattle		8	50.7 ± 1.9	120, Table 3	
		4	69.6 ± 0.3	121, Table 2	
Boran		5	72.0 ± 4.1	75, Table 4	
Pig		24	46.8 0.01	122, Table 1	Antipyrine
		7	45.5 ± 2.2	120, Table 3	
Sheep		6	62.0 ± 10.6	123, Table 3	
		13	57.1 ± 1.5	120, Table 3	
		8	54.6 ± 1.8	46, Table 1	
		40	64.7 ± 0.8	93, Table 1	Ages 1-1½ yr
Kangaroo					
Macropus	2M, 4F	6	78.0 ± 2.5	74, Table 4	
giganteus	4M, 1F	5	77.6 ± 2.7	74, Table 4	
Macropus	2M, 4F	6	72.5 ± 2.2	74, Table 4	
robustus	3M, 3F	6	60.8 ± 1.9	74, Table 4	
Megaleia rufa	3M, 3F	6	58.8 ± 2.3	74, Table 4	
Macropus eugenii					
Potorus tridactylus					
Camel		3	69.2	124, Figure 2	Hydrated
Hartebeest		2	84.3	75, Table 4	

This article was followed by one by Pace et al. (18), in 1947, on TBW measured both directly and indirectly in 2 rabbits (Table 2) and indirectly in 1 human (Table 1).

After 1946, the number of reports in which TBW was measured with DHO increased rapidly. In addition, during the late forties and early fifties the use of antipyrine as an indicator substance for measuring TBW was tried, but, because of conflicting results, its use was stopped in favor of THO. With the advent of THO (isolated by Alvarez and Cornog in 1939 (37)), the development of liquid scintillation counting (38, 39), and the demonstration that THO had distribution properties similar to DHO (4, 16, 39–42), THO became the preferred substance for the measurement of TBW.

An often overlooked source of data on many aspects of body composition by students interested in this field is the literature on animals of economic importance; cattle, pigs, sheep, and goats. The nutritionists' and breeders' viewpoints in the study of body composition in these animals are epitomized by the title of an article by Hammond in 1933 (43), "Pigs for Pork and Pigs for Bacon," a title expressing the view that there is an inherent biological plasticity in animals that can be modified by breeding practices and nutrition. The primary goal of these investigators is to produce an animal of marketable size from the cheapest source of feed in the shortest possible time and with a body composition acceptable to the consumer. Any progress toward these goals has clear economic advantages for the producer. Consequently, the animal nutritionists are actively involved in the study of body composition, although, because of their specialized goals, the data are often presented in a manner that is not applicable to the purposes of this review. Many of the data that are amenable to the organization of this review are included in the tables. An added difficulty in selecting the data was the bewildering terminology used to describe the condition of the animal analyzed; for example, "shrunk body," "carcass," "eviscerated," "empty body," and "normal" may mean the same or different conditions. In examining the articles for this review, when one of these terms was used and its meaning not clear, the data were not tabulated.

Whereas the emphasis in this review is on the measurement of TBW with THO, data on DHO are included because studies on diffusion coefficients, arterial disappearance rates, transit times in muscle and lungs of dogs, and distribution properties of these two substances do not differ significantly from each other (3–5, 33–36, 39–42). In addition, a few articles on antipyrine and one on sulfanilamide are included in the tables because of their historical interest and because they are often cited.

It was necessary to establish a format for the presentation of the available data. In the literature, variability of the data was expressed as either the standard deviation or the standard error, but for the five tables presented in this review, all standard deviations are converted to ±1 standard error of the mean. When no estimate of variability was given, but the data were available, the SEM was calculated. If the indicator for measuring TBW is not specified under "Remarks," THO was used.

Although the volumes of the body fluid compartments and their changes during growth have been described in detail over the 1st year of growth for the beagle and over the first 12 weeks for the pig, as well as over restricted periods of growth for other species (11, 21, 44–52), only data in which direct and indirect measurements of TBW were made in the same animal are presented in Table 2. In these experiments, after estimating the volume of TBW with an indicator substance, the animals were killed and the TBW estimated

TABLE 2
Total body water estimated directly and indirectly

Species	No animals	Indirect: Body wt	Indirect: FFWW	Direct: Body wt	Direct: FFWW	Reference	Remarks
			%				
Rat	10	68.1 ± 1.2[a]	ND[b]	63.9 ± 1.0	ND	54, Table 2	
	7	70.8 ± 1.3	ND	66.5 ± 0.8	ND	54, Table 2	
	32	74.4 ± 1.8	83.5 ± 1.4	66.4 ± 0.3	75.8 ± 0.5	55, Table 1	
	21	71.4 ± 0.4	ND	70.2 ± 0.3	ND	53	
		11%, 14%[c]	ND	ND	ND	56	
Rabbit	9	73.5 ± 0.5	ND	72.8 ± 1.1	ND	36, Table 1	DHO
	2	55.7	ND	55.9	ND	18, Table 3	
	4	74.2 ± 1.9	ND	73.8 ± 1.6	ND	76, Table 1	Antipyrine
	17	1162.5 ± 36.8[d]	ND	1162.9 ± 37.1	ND	77, Table 1	Whole body
	8	62.4 ± 2.6	ND	61.4 ± 2.7	ND	78, Table 2	Antipyrine
Pig	29	44.4 ± 0.9	ND	45.5 ± 0.7	ND	57, Table 4	Antipyrine
	26	65.8 ± 0.9	ND	66.9 ± 2.1	ND	59, Table 4	DHO, ages day 0–65
	11	56.1 ± 0.5	ND	55.5 ± 0.2	ND	60, Table 4	27 kg
	16	66.8 ± 1.2	ND	56.5 ± 0.7	ND	60, Table 4	55 kg
	13	61.0 ± 1.1	ND	52.9 ± 0.9	ND	60, Table 4	90 kg
	15	71.9 ± 1.3	ND	72.3 ± 1.3	ND	58, Table 1	DHO, ages days 2–102
	55	78.6 ± 1.0	88.6 ± 0.9	72.1 ± 0.4	83.7 ± 0.1	49, Table 1	Ages day 0–wk 12
Dog	5	65.9 ± 2.3	ND	62.5 ± 2.3	ND	79, Table 4	Sulfanilamide
Beagle	25	82.0 ± 1.5	93.9 ± 2.2	66.6 ± 1.9	74.4 ± 0.7	61	Ages day 0–mo 3
Beagle	18	64.2 ± 1.0	78.7 ± 3.5	53.1 ± 0.9	63.0 ± 0.7	61	Ages mo 4–1 yr
Cattle							
Holstein	20	68.0 ± 1.2	ND	67.9 ± 1.2	ND	63, Table 1	Antipyrine, ages 16–80 weeks, gut emptied
Angus	13	80.5	ND	68.9	ND	62, Table 1	
Jersey	13	75.4	ND	66.5	ND	62, Table 1	
Goats	11	65.8 ± 3.1	ND	64.9 ± 1.7	73.8 ± 0.5	80, Table 6	Reduced in direct figure by 3%
	10	67.5 ± 1.9	ND	66.8 ± 1.9	75.6 ± 0.4	69, Table 2	
Sheep	9	61.1 ± 1.1	ND	60.8 ± 4.2	77.0 ± 1.6	69, Table 2	

[a] Standard error. [b] Not determined. [c] THO 11% higher for lean rats, 14% for fat rats. [d] Grams.

again by desiccation. The majority of data presented in Table 2 were obtained on mature animals; the exceptions are noted under "Remarks" section.

When the means of TBW (percentage body weight) measured by the two methods are compared, the indirect method may measure a volume approximately the same or significantly larger (up to 15%) than that measured by the direct method (Table 2). In addition, the data suggest the possibility of a species difference in the success with which THO measures TBW. For the rabbit, excluding the data for antipyrine, the agreement between the means for the remaining three groups is excellent. Although the data are limited for the goat and sheep, the means are also in good agreement. In contrast to these species, the difference in the means is significant for the rat, pig, beagle, and cattle (with the exception of the holsteins). In only one out of the five groups of experiments on the rat, is the difference between the means obtained by the two methods insignificant, and that is the recent data of Culebras et al. (53) where the difference was 1.2% of body weight. In the other investigations, the difference between means was approximately 4%, reported by Foy and Schnieden (54) for two groups of rats, 8% by Tisavipat et al. (55), and 10% for lean rats and 14% for fat rats by Bell and Stern (56). The results for the pig are not too different from those for the rat; disregarding the data on Clawson et al. (57) where antipyrine is used, there is satisfactory agreement between the means of the two methods (58–60) in three of the experiments, but in the remaining three groups the differences between the means range between 6.5 and 10.3% of body weight (49, 60). Similar differences between means are found for the beagle and cattle; for the beagle the difference was approximately 15% of body weight for pups from day 0 to month 3 and 11.0% from month 4 to 1 year (61); for a group of jerseys, the difference was 8.6%; and for Angus, 11.6% (62). For a group of holsteins (63), however, there is a difference of only 0.1% between the means, but this was with the use of antipyrine for estimation of TBW.

It is to be expected that both THO and DHO will measure a larger space than the desiccation method because of the exchange of hydrogens of THO and DHO with proteins and carbohydrates of the body (5). An analysis of the available data presented in Table 2 shows that this expectation is met with the exception of the rabbit and possibly the sheep and goat. In addition, there is tentative evidence that there may be a species difference in the amount of error with which THO estimates TBW. The pig seems to be an exception because, in three experimental groups, the means of TBW measured by the two methods are in satisfactory agreement, but they are significantly different in another three groups, with the indirect method measuring a body weight of 6.5 to 10.3% greater than the direct method. At present, no explanation for a difference of such magnitude is possible. It is impossible to assess the degree to which technical errors may have affected the differences between the two methods of measuring TBW in these different investigations. However, in support of these data, it can be said that in all instances the methods and techniques used were standard ones and that many of the investigators whose data are quoted have considerable experience in studies of body composition.

With the finding that TBW may be overestimated by as much as 15% with THO in some species, the question arises as to the source of the often quoted 0.5 to 2.0% error in measurement of TBW. These figures are based on the assumptions that: *1*) protein constitutes 15% of body weight, 6% of protein is hydrogen, and 6 to 15% of its hydrogen atoms exchanges readily; *2*) carbohydrate constitutes 0.5% of body weight, and 6% of the carbohydrate is hydrogen with 35% of its hydrogen atoms exchanging readily; and *3*) fat has no rapidly exchanging hydrogen atoms. From these data, the usually quoted figures of 0.5 to 2.0% of body weight for total exchangeable hydrogen are calculated (5). Presumably, then, when TBW is measured with THO, the result should be decreased by 0.5 to 2.0% of body weight to compensate for the hydrogen exchange, but this is done rarely. The 0.5 to 2.0% overestimation of TBW by THO, then, is not the final measure of the error in measuring TBW, although many investigators assume it is, but is, instead, an estimate of the probable amount of exchange of hydrogen atoms of THO with those of the organic compounds of the body. The final decision on the degree of accuracy with which THO measures TBW depends, therefore, on a comparison in the same animal of the volume of TBW estimated with THO and by desiccation; these data are presented in Table 2.

Inasmuch as the exchange of hydrogen between THO, protein, and carbohydrate of the body accounts for only a small fraction of the possible overestimation of TBW with THO, other explanations need to be found. Losses of THO through the lungs and skin and into the gastrointestinal tract and urine do occur, but these combined losses over the period of equilibration of THO add only a minor correction to the total overestimation of TBW (4). At present, then, it is not possible to provide an explanation for the overestimation of TBW by THO.

Fat

The assumption that water constitutes 73.2% of the fat-free body in the adult appears to be questionable when this figure is used for the calculation of fat from TBW measured either by the direct or indirect method for some of the adult animals in Table 2. When TBW is measured by the indirect method and the mean figures of 74.4% of body weight for adult rats (55), 80.5% for Angus cattle (62), and 75.4% for Jersey cattle (62) are used for the calculation of fat using the figure of 73.2% for water in the fat-free body, the highly improbable condition of a negative value for fat is obtained. Calculation of fat for nine rabbits (36) whose TBW is 73.5% of body weight by the indirect method yields essentially a fatless animal. Nor does the use of the mean 72.8% for TBW measured directly in the same rabbits appreciably improve the results; it is true that this time there is some fat, but it is only 0.5% of body weight. Similarly for rats, if the mean figure of 70.2% of Culebras et al. (53) is used, mean fat is only 2% of body weight. Unfortunately, fat was not measured in any of these experiments, but a more likely figure for fat for many laboratory animals would range between 10 and 15% of body weight if they were adequately fed.

Even with the direct measurement of TBW (Table 2), the range of mean values within a species is considerable: in the adult rabbits for different groups, mean TBW ranged from 55.9 to 73.8% of body weight; and in the adult rats, from 63.9 to 70.2%. The explanation usually offered for this range in means for TBW in animals of the same species and age is a difference in fatness. Consequently, with the indirect method, to the large and perhaps species-dependent error, in measuring TBW with THO must be added, also, the variation in measuring TBW resulting from an unknown amount of fat.

In addition, some of the range in means for the same species may be accounted for by an interesting experiment by Dawson et al. (64). They established three lines of mice: *1*) a control line; *2*) a line selected for heavier body weight; and *3*) a line selected for a particular body conformation. Each line was bred for 15 generations. Some of the rats were killed at the 12th generation and others at the 15th generation and their body composition analyzed. At the 12th generation, there was a statistically significant difference from the control values for water, fat, and ash with the difference greater at the 15th than at the 12th generation. This experiment could account for some of the differences between means in the literature when the different lines of a particular species used in different laboratories are considered. It seems improbable, however, that such an explanation can completely account for the magnitude of the differences reported in the literature.

Total body fat was measured chemically for some of the animals listed in Table 2 and the percentage of water in the fat-free body calculated. The mean percentage of water measured by desiccation in FFWW of mature animals is 63.0% for beagles 4 months to 1 year, 75.8% for one group of rats, 73.8% for one group of goats, 75.6% for another group of goats, and 77.0% for sheep, all, with the exception of the beagle, with a greater percentage of water in FFWW than the commonly used figure of 73.2%. Whereas the mean value for the rat of 75.8% (55) differs only by 2.6% from 73.2%, when the percentage of fat is calculated with the equation: 100 − % TBW/0.732, this small difference significantly affects the estimated amount of fat. For a 200 g rat with a TBW of 66.4% (Table

2), and 73.2% as the constant for FFWW, calculated fat is 18.6 g; but if, instead, the measured value of 75.8% water in FFWW (Table 2) is used, the estimate is 24.8 g of fat, a difference of 6.2 g, and a difference of objectionable magnitude if the object of the experiment is, for example, to measure the total energy stores of a rat. Also, Sheng et al. (65) calculated fat using the actual percentage of water measured by both desiccation and THO in the fat-free body and, also, the usually used value of 73.2% and compared the results with measured fat in beagles 4 months to 1 year and in 0 to 12-week-old pigs. For the beagle, agreement is unsatisfactory between measured fat and fat calculated from THO with either the calculated constant of 78.7 or 73.2%, even after the volume of TBW was relatively stabilized; but with desiccation and the calculated value of 62.9% for water in FFWW, the agreement between measured and calculated fat is good, but with the constant 73.2%, whereas directional changes are followed, the error for absolute values is large (calculated fat is larger by approximately 10% of body weight than measured fat). A comparison of measured and calculated fat for the pig differs from that of the beagle because the pig was still in a period of rapid growth (day 0 to week 12), while the major part of the growth period for the beagle was over and the percentage of water in FFWW was relatively stabilized. For the pig, when fat is calculated from TBW measured by desiccation and the constant 73.2% is used, the directional changes in calculated fat follow those of the measured fat, but calculated fat is consistently lower than measured fat by about 10% of body weight. However, with desiccation and the calculated value of 83.7% for water in FFWW, the agreement between measured and calculated fat is excellent. In contrast to the latter result are those with THO: with the constant 73.2%, the majority of pigs have negative values for calculated fat, and if 88.6% is used as the constant, although the directional changes in calculated and measured fat are followed, the correlation is poor. Thus, for the beagle and pig, the errors involved in calculation of total fat are the use of the constant 73.2% for the percentage of water in the fat-free body and, also, the potential error in estimation of TBW with THO.

The concept that the amount of water in FFWW of an animal decreases during part of the growth period and then stabilizes and remains relatively constant after maturation was first clearly stated by Moulton (66) from his studies on a number of species, but primarily economically important animals. It was he who coined the phrase "chemical maturity." The portion of the generalization that water in FFWW stays relatively constant after maturation received major confirmation in the work of Pace and Rathbun on guinea pigs (15). Although, one of the difficulties in using the assumption of a constant amount of water in FFWW is our lack of knowledge about the age at which "chemical maturity" occurs for any species.

The major part of available data in which TBW is measured by desiccation is expressed as a percentage of body weight and FFWW when fat is measured chemically. These results are summarized in Table 3. The most widely quoted paper in support of a constant amount (73.2%) of water in FFWW is that of Pace and Rathbun (15), with later supporting data by other investigators (Table 3). Pace and Rathbun calculated a mean of 72.4% water in FFWW for 50 guinea pigs, whereas the mean of 73.2% comes from combining the mean for the guinea pigs with the limited data available at that time for the rat, rabbit, cat, dog, and monkey (15). Several criticisms can be made of their data. Inasmuch as the animals of Pace and Rathbun were eviscerated, which means their body weight was reduced by approximately 25%, it is necessary to assume, if these data are to be applied to the whole animal, that the tissues removed had the same concentration of water as the carcass. Such an extrapolation is not supported by Cizek's data (67) on the guinea pig, rabbit, and a number of other species. His results on the rabbit were confirmed later by Gotch et al. (68). Cizek's choice of the noun carcass to describe the condition of his animals is unfortunate, and it has led to a misunderstanding of his results. The word carcass is used most commonly to mean the condition of the animal after the viscera are removed, but in Cizek's usage, the weight of the carcass is the body weight less the water in the lumen of the gut, and the word is used with this meaning in discussing his results. He reports a sex difference in the amount of

TABLE 3
Direct measurements of total body water

Species	No/sex	Body wt	FFWW	Reference	Remarks
		%			
Rat	2	65.3	72.0	81, Table 7	
	6	67.6	72.2	82, Table 1	Gut emptied
	7	61.5	72.6	82, Table 1	Gut emptied
	20	63.5 ± 0.8[a]	74.4 ± 0.4	83, Table 1	Final body wt
	8 M	58.2	69.5	84, Table 1	Gut emptied
	5 F	60.6	74.2	84, Table 1	Gut emptied
	M	60.4	ND[b]	85, Table 1	Food ad libitum
	F	58.8	ND	85, Table 1	Food ad libitum
	6 M	65.0	75.0[c]	86	Estimated from figs. 2 and 3
	6 F	60.0	75.0	86	Estimated from figs. 2 and 3
	50 M	64.3	ND	87, Table 1	Controls, eviscerated
	12 F	66.6 ± 0.6	ND	67, Table 3	Food and water
	12 M	68.3 ± 0.4	ND	67, Table 3	Food and water
	6 F	65.6 ± 1.5	ND	67, Table 3	No food or water for 24 hr
	6 M	68.3 ± 0.8	ND	67, Table 3	No food or water for 24 hr
	16 F	ND	72.2 ± 0.2	71, Table 1	
	7 M	ND	73.0 ± 0.4	71, Table 1	Controls
	28	55.8	67.0	10, Table 1	Calculated using FFWW
	28	55.6	67.0	10, Table 1, 11C	Calculated using FFWW
	30	57.7	66.7	10, Table 1, 11C	Calculated using FFWW
	26	55.0	69.0	10, Table 1, 11C	Calculated using FFWW
		62.4	70.6	88, Table 1	Controls
	15	65.4	72.6	89, Table 11	
Mouse	39	76.6	79.9	86, Tables 1 and 3	
	14 F	ND	74.0 ± 1.2	71, Table 1	Controls
	6 F	68.5 ± 0.6	ND	67, Table 5	
	6 M	72.7 ± 0.8	ND	67, Table 5	
	12	67.4 ± 0.1	72.9 ± 0.7	11, Table 2	Ages 31–97 days
	10	61.1	72.8	64, Table 1	12th generation, line CL
	10	59.0	73.0	64, Table 1	12th generation, line HL
	9	65.0	72.7	64, Table 1	12th generation, line LL
	9	62.3	72.7	64, Table 1	12th generation, line KL
	10	68.4	74.4	64, Table 1	12th generation, Quakenbush
	20	61.6	75.2	64, Table 2	15th generation, line CL
	20	65.5	75.0	64, Table 2	15th generation, line HL
	20	65.0	75.0	64, Table 2	15th generation, line LL
	20	64.0	75.0	64, Table 2	15th generation, line KL
Rabbit	3	74.3 ± 0.6	76.3 pm 0.8	90, Table 1	Gut emptied, mean used for calculation
	1 M	69.0	75.0	86, Tables 2 and 3	Estimated
	1 F	62.0	70.0	86, Tables 2 and 3	Estimated
	8 F	66.8 ± 0.8	ND	67, Table 1	
	8 M	70.4 ± 1.0	ND	67, Table 1	
	4 M	70.6 ± 1.0	ND	67, Table 1	
	4 F	74.1 ± 1.3	ND	67, Table 1	
	2	ND	72.8	86, Table 9	
	20 M	ND	72.8 ± 0.2	78, Table 4	
	17 F	ND	72.5 ± 0.2	78, Table 4	
Guinea pig	50	63.5 ± 0.8	72.4 ± 0.3	15, Table 3	Eviscerated
	6 F	71.8 ± 0.6	ND	67, Table 2	
	6 M	72.7 ± 0.3	ND	67, Table 2	
	6 F	73.0 ± 0.8	ND	67, Table 2	
	6 M	74.1 ± 0.4	ND	67, Table 2	
Hamster	6 M	68.0 ± 0.4	ND	67, Table 5	

[a] Standard error. [b] Not determined. [c] Also given as 72% FFWW from Table 11, Reference 21.

TABLE 3—*Continued*
Direct measurements of total body water

Species	No/sex	Body wt	FFWW	Reference	Remarks
	6 M	67.4 ± 0.6	ND	67, Table 5	
	34 M	58.4 ± 0.5	73.3 ± 0.1	91, Table 1	
Cat	3	ND	76.9	86, Table 9	
Dog	2	59.5 ± 4.5	74.4 ± 0.5	90, Table 1	Gut emptied, mean used for calculation
	5 F	59.3 ± 1.5	ND	67, Table 4	
	5 M	64.8 ± 1.4	ND	67, Table 4	
	5 F	63.6 ± 3.2	ND	67, Table 4	
	5 M	64.6 ± 1.7	ND	67, Table 4	
Monkey	2	68.5 ± 0.2	73.2 ± 0.2	90, Table 1	Gut emptied, mean used for calculation
Goat	10	66.8 ± 1.9	75.6 ± 0.4	69, Table 1	
	10	62.7 ± 2.1	72.6 ± 0.4	69, Table 1	Gut emptied
	1	67.0	ND	67, Table 5	
Sheep	9	66.6 ± 0.9	76.5	92, Table 2	Age 8 mo, gut emptied
	9	60.6 ± 4.2	75.6 ± 0.5	69, Table 1	
	9	57.4 ± 4.0	73.6 ± 0.3	69, Table 1	Gut emptied
	9	58.1 ± 1.2	76.3	92, Table 3	Age 20 mo, gut emptied
	221	ND	74.9 ± 0.2	89, Table 3	Gut emptied, ages 90–895 days
	40	64.7 ± 8.5	ND	93, Table 1	Ages 1–1½ yr
Pig	2	ND	75.6	86, Table 9	
	8	52.5 ± 4.3	76.7 ± 1.9	57, Table 5	Body wt 98 kg, gut emptied
Chuffy	15	45.0	76.5	89, Table 10	Gut emptied
Intermediate	16	45.0	76.6	89, Table 10	Gut emptied
Rangy	20	47.0	76.1	89, Table 10	Gut emptied
YC	6	35.6	58.5	94, Table 2	Mean body wt 91 kg
YD	6	33.5	59.6	94, Table 2	Mean body wt 91 kg
	8 M	40.0 ± 1.1	77.0 ± 0.3	95, Table 1	Groups 10, 11, 12
	8 F	42.9 ± 1.8	77.0 ± 0.1	95	Body wt 95.7–133 kg
Cattle	7	64.9 ± 3.2	76.5 ± 0.2	96, Table 1	
	256	62.7	72.9 ± 0.1	89, 97, Table 3	Ages 1–4860 days

gut water in the guinea pig: in the male, gut water is 20% of TBW, and in the female, 17%. Using only data for the male, TBW less gut water is 70.5% of carcass weight and 58.2% of body weight, whereas TBW is 72.7% of body weight; thus, carcass water is a smaller percentage of carcass weight than TBW is of body weight. Although the difference in the amount of water as a percentage of body weight and as a percentage of carcass weight is small (2.2%), when these figures are used to calculate fat with 73.2% water in FFWW and a TBW of 72.7% body weight (Table 3), there is 1.4 g fat in a 200 g guinea pig, and with water as 70.5% of carcass weight, there is 7.4 g fat, an appreciable difference in the amount of fat for a 200 g guinea pig.

A similar effect of gut water on the measurement of TBW can be calculated from Panaretto's data on sheep and goats (69). For 9 mature sheep (Table 3), mean TBW is 60.6 ± 4.2% of body weight, but, with the elimination of gut water, it decreases by 3.2% to 57.4 ± 1.9%; or when expressed as a percentage of FFWW, the mean for TBW is 75.6 ± 0.5%, but without gut water, it is 73.6 ± 0.3%. Similarly, for 10 goats, mean TBW is 66.8 ± 1.9% of body weight, but with the exclusion of gut water, it is 62.7 ± 2.1%, whereas the percentage of water in FFWW is 75.6 ± 0.4% including gut water, but with its exclusion, 72.6 ± 0.4%.

Most of the commonly used laboratory species maintain an appreciable fraction of TBW in the gut; the amount differs between the species, and it is influenced little by the usual laboratory watering and feeding regimen (67). This water is measured by THO (5). Consequently, for the results given in Table 3, if the animals are eviscerated or the gut emptied, there is, for a number of species, an underestimation of TBW as a percentage of body weight or FFWW by approximately 2 to 3%. From Panaretto's data, for example, a more appropriate figure for water in FFWW is 75.6% for sheep and goats, rather than the usual figure of 73.2%. Apparently, the human, and perhaps the pig, is different because the amount of water in the gut is negligible: less than 1% of TBW for the human (68) and 3% or less for the pig (14, 58). Thus gut water in the human, aside from the error in measurement of TBW with THO and assuming the correctness of 73.2% water in FFWW, would have a minor influence on the calculated figure for fat.

It is questionable whether the water in the lumen of the gut should be included in LBM, a biological concept that directly associates the TBW with the metabolically active tissues in the body. The effect of the water in the gut of the guinea pig, which approximates 20% of TBW, on estimates of the amount of water in the LBM is considerable: a 200 g guinea pig with a TBW of 66% and a calculated fat of 10% has 73.3% water in the LBM, but, if the 20% TBW that is in the gut and outside of the LBM is subtracted from TBW, water in the LBM is 59%.

Another criticism of Pace and Rathbun's work was made in 1963 by Wedgwood (70), who subjected their data to regression analysis and found that as the weight of the carcass increased, the percentage of water in FFWW increased significantly. A recalculation of Pace and Rathbun's data gave results similar to Wedgwood's, with the slope of the line significant at the $P < 0.01$ level. At a carcass weight of 200 g there was a mean of 70.2% water in FFWW, and, at a weight of 600 g, the mean was 74.3%. Consequently, if the constant 73.2% is used to calculate fat instead of the actual value of 70.2%, at a mean carcass weight of 200 g and a TBW of 66% of body weight, the amount of fat is overestimated by approximately 8 g, and at a mean carcass weight of 200 g and 74.3% water in FFWW, the amount of fat is underestimated by 4 g. Clearly, it is an oversimplification of Pace and Rathbun's data to conclude that the amount of water in FFWW is constant at 73.2%. Further, 90% of the individual values for the guinea pig ranges from 68.6 to 76.7% (2, 15), and approximately 10% of the guinea pigs could be expected to exceed this range; and, in addition, it is not unreasonable to assume that the range of these values, if the animals were intact, would be greater than for the eviscerated animals.

In a review article in 1953, Keys and Brožek (2) argued correctly that the concentration of water in FFWW must be independent of the fat content of the body if fat is to be calculated from TBW. On the basis of a low positive correlation between water as a percentage of FFWW and total body fat that they obtained from Pace and Rathbun's guinea pig data, they concluded that the water in FFWW is not constant, and they state that this assumption is at best an approximation. However, we recalculated Pace and Rathbun's data, and found that the amount of water in FFWW of the guinea pigs was independent of the amount of fat in the body, a conclusion in agreement with Babineau and Pagé's (10) data for rats, Anneger's (71) for mice, and the data of Sheng et al. (65) for the beagle from month 4 to 1 year, but not during the growth period from day 0 to month 3, or for the pig from day 0 to week 12. Thus, there are data that support the concept that, at maturity, a fat-free portion of the body exists that contains all the water in the body and a fat compartment that varies independently of the fat-free portion. Consequently, total fat can be estimated with an adequate degree of accuracy if the water in the fat-free portion is 73.2% or some other known constant and TBW is measured with a sufficient degree of accuracy by an indirect method.

This analysis of Pace and Rathbun's data is not meant to detract from their important contribution to the investigation of body composition, but to place their work in the proper perspective and to help define the boundaries of the constant 73.2%.

In addition to the early data on laboratory animals that contributed to the concept that the amount of water in the LBM was 73.2% and that it remained relatively constant after chemical maturity was reached, further credence was given to the concept by chemical analyses of a small number of humans. Most summaries of the human include data for only five of the seven cadavers; the two excluded have a higher TBW as a percentage of FFWW than the remaining five. Recently Moore et al. (72) added data on an eighth cadaver, a female whose TBW as a percentage of FFWW is high, 80.8%. The data for all eight human cadavers appear in Table 4. It is difficult to judge to what degree their terminal illnesses may have influenced the volume of TBW and, thus, to what extent any of these results can be considered normal. For the five cadavers, the mean is 71.6 ± 0.78% of FFWW, and the mean for the eight cadavers is 74.8 ± 1.68%. Both means differ from 73.2 by 1.6%, with the mean for the five cadavers below and the mean for the eight cadavers above the value of 73.2%, undoubtedly a coincidence. If the data for the five cadavers are considered to be representative of normal, then the suggestion that the constant 73.2% be replaced by 71.2% for the human is closer to arithmetic reality (36). Also, mean body fat is calculated not only with 73.2% as water in FFWW, but with the 74.8% for all the cadavers and 71.6% for the usually quoted five. These results are given in Table 5. For the eight cadavers and with the constant 0.732, calculated fat was underestimated by 1.9 kg (21% of total fat), and with the constant 0.748, calculated fat was underestimated by 0.7 kg (8% of total fat). Calculated body fat for the five cadavers was overestimated by 0.9 kg (8% of total fat) with the constant 0.732, but underestimated by 0.2 kg (2% of total fat) with the constant 0.716. If to the uncertainty that exists on the amount of water in LBM present in the human and other species is added the significant overestimation of TBW with THO which occurs in many species (and may well include the human, although there is a lack of data for the latter), the use of the equation 100 − % TBW/0.732 for the estimation of fat can at best be a gross approximation.

Total body water has been measured indirectly in a number of species of animals, and a fairly complete sample of these data, with the exception of the human, are presented in Table 1. Unless stated differently in "Remarks" section, the data, according to the different investigators, were obtained on healthy, normal humans between the ages of

TABLE 5
A comparison of mean total measured and calculated fat of human cadavers[a]

No	Body wt	Constant	Measured fat	Calculated fat	Difference
	kg			kg	
8	60.8 ±11.3	0.732	9.1 ±2.1	7.2 ±3.3	−1.9
8	60.8 ±11.3	0.748	9.1 ±2.1	8.4 ±3.2	−0.7
5	61.2 ±12.0	0.732	11.1 ±2.9	12.0 ±3.3	+0.9
5	61.2 12.0	0.716	11.1 2.9	10.9 3.4	−0.2

[a] Data from references in Table 4.

TABLE 4
Direct estimations of total body water in adult humans

Age	Sex	Weight	TBW body wt	TBS FFWW	Reference	Remarks
		kg	%			
35[a]	M	70.5	67.8	77.6	98	Decompensation and failure
25	M	71.8	61.8	72.5	99	Uremia
42	F	45.1	56.0	73.2	99	Suicide by drowning
48[a]	M	63.8	81.5	82.0	99	Infectious endocarditis
46	M	53.8	55.1	69.4	100	Cerebral injury
60	M	73.5	50.5	70.1	101	Cardiac failure
48	M	62.0	70.8	73.0	101	Cardiac and vascular failure
67[a]	F	46.2	73.7	80.8	72	Carcinoma

[a] Usually omitted.

15 and 50 years. These data, of course, provide no evidence for the accuracy with which THO measures TBW, but do provide comparative information on mean TBW for a number of species.

Excluding the data on Moore et al. (36) for one human where TBW was 72.5%, the means range from 48.6 to 70.8%. Using these means, calculation of the mean percentage fat for a 70.0 kg man with the constant 73.2% and a mean TBW of 70.8% gives the figure of 3.3% (2.3 kg) fat, and with a mean TBW of 48.6, a figure of 33.6% (23.5 kg) fat. For groups of individuals characterized as normal and healthy, this disparity in the estimated mean percentage of fat is large, particularly as the human is considered to be one of the fatter species. Slightly over half of the means for the human in Table 1 fall between 51.5 and 59.1%: assuming a mean of 55% water and a mean body weight of 70 kg, calculation gives a mean fat content of 25% or 17.5 kg. This may or may not be a reasonable percentage of fat for a normal, healthy, and relatively young man, but it is definitely a greater percentage of fat than the 14% for a 70 kg standard man or the 15.3% for the standard body postulated by Brožek et al. (73).

The range of TBW as a percentage of body weight estimated indirectly (Table 1), raises again the unanswerable spector of differences in technique and, in addition, poses the dilemma of how to deal with anomalous data. For example, the mean figure of Moore (36) for the rabbit of 72.8% water in FFWW is an accepted figure, but should his figure of 72.5% of body weight for water in the human and an estimated fat of 0.9% if the constant 73.2% is used to calculate fat be accorded the same degree of acceptance? Both sets of data appear in the same article (36). Or should we accept Foy and Schnieden's often quoted data (54) on the rat of 63.9 and 66.5% of body weight because the values seem appropriate (Table 2), and then reject their data of 70.8% water as a percentage of body weight for a group of 10 humans and 75.2% for 18 guinea pigs as inappropriate (Table 1) because these values are thought too high?

A sampling of data for species other than the human reveals that the spread of mean values of TBW as a percentage of body weight is of the same order as that found for the human, with the exception of the truly startling figures for the mean values of three species of kangaroos (Table 1) of 78.0, 77.6, and 72.5% of body weight (74) and 84.3% for the hartebeest (75). Denny and Dawson (74) postulate that the high values for TBW as a percentage of body weight for three species of kangaroos are the result of a relatively high ratio of gut-to-body weight characteristic of ruminants. At present, according to these investigators, kangaroos are classified as ruminant-like animals. At best, this can be only a partial explanation as the values for the three species of kangaroos are definitely greater than those for other ruminants, such as the sheep, goat, and cattle (Tables 2 and 3).

Addendum

Two recent papers from Dr. Moore's laboratory were overlooked by us in preparing this review. They are: *1*) Culebras, J. M., and F. D. Moore. Total body water and the exchangeable hydrogen. I. Theoretical calculation of nonaqueous exchangeable hydrogen in man. Am. J. Physiol. 232: R54, 1977, and *2*) Culebras, J. M., G. F. Fitzpatrick, M. F. Brennan, C. M. Boyden and F. D. Moore. Total body water and the exchangeable hydrogen. II. A review of comparative data from animals based on isotope dilution and desiccation, with a report of new data from the rat. Am. J. Physiol. 232: R60, 1977.

In the first paper, using the calculated exchangeable hydrogen based on normal body composition of a 70-kg man with 20% fat, Culebras et al. computed that the theoretical maximum nonaqueous exchangeable hydrogen is 5.2% of total exchangeable hydrogen. In the second paper, they expand the data given in Reference 53 of this review. Their conclusion is that the discrepancy between total body water measured by tritium and total body water measured by desiccation in the rat (Tisavipat et al. (55)) and in the beagle (Sheng and Huggins (61)) is the result of technical errors. However, there are reports by other investigators of the indirect method measuring a volume more than 5% larger than that by desiccation (see Table 2 of this review).

Also, an interesting paper by F. E. Ruch, Jr. and M. R. Hughes on "The effects of hypertonic sodium chloride injection on body water distribution in ducks (*Anas platyrhynchos*), gulls (*Larus glaucescens*), and roosters (*Gallus domesticus*)," Comp. Biochem. Physiol. 52A: 21, 1975, should be cited. TBW was measured with THO, and for the seven ducks drinking tap water, TBW was 68.5% of body weight; and for the seven drinking 0.48 M NaCl, TBW was 64.0% of body weight. For the two gulls drinking tap water, TBW was 87.9% of body weight; and for the two roosters also drinking tap water, TBW was 54.3% of body weight.

References

1. Widdowson, E. M., and J. W. T. Dickerson. Chemical composition of the body. In: Mineral

Metabolism, edited by C. L. Comar and F. Bronner. New York: Academic Press, 1964, part A, vol. 2, Chapt. 17, p. 1–347.
2. KEYS, A., AND J. BROŽEK. Body fat in adult man. Physiol. Rev. 33: 245, 1953.
3. SIRI, W. E. Body composition from fluid spaces and density: analysis of methods. In: Techniques for Measuring Body Composition, edited by J. Brožek and A. Henschel. Washington, D.C.: Natl. Acad. Sci., Natl. Res. Council, 1961, p. 223–244.
4. PINSON, E. A., AND W. H. LANGHAM. Physiology and toxicology of tritium in man. J. Appl. Physiol. 10: 108, 1957.
5. PINSON, E. A. Water exchanges and barriers as studied by the use of hydrogen isotopes. Physiol. Rev. 32: 123, 1952.
6. COLEMAN, T. G., R. D. MANNING, JR., R. A. NORMAN, JR. AND A. C. GUYTON. Dynamics of water-isotope distribution. Am. J. Physiol. 223: 1371, 1972.
7. BROŽEK, J., AND A. HENSCHEL. Techniques for Measuring Body Composition. Washington, D.C.: Natl. Acad. Sci., Natl. Res. Council, 1961.
8. KLEIBER, M. The Fire of Life. Huntington, N.Y.: Robert E. Kreiger Publ. Co., 1975.
9. BEHNKE, A. R. Physiological studies pertaining to deep sea diving and aviation, especially in relation to the fat content and composition of the body. Harvey Lect. 37: 198, 1941–1942.
10. BABINEAU, L.-M., AND E. PAGÉ. On body fat and body water in rats. Can. J. Biochem. Physiol. 33: 970, 1955.
11. BAILEY, C. B., W. D. KITTS AND A. J. WOOD. Changes in the gross chemical composition of the mouse during growth in relation to the assessment of physiological age. Can. J. Animal Sci. 40: 143, 1960.
12. DARROW, D. C., AND S. HELLERSTEIN. Interpretation of certain changes in body water and electrolytes. Physiol. Rev. 38: 114, 1958.
13. FORBES, G. B. Methods for determining composition of the human body with a note on the effect of diet ‹ n body composition. Pediatrics 29: 477, 1962.
14. HANSARD, S. L. Radiochemical procedures for estimating body composition in animals. Ann. N.Y. Acad. Sci. 110: 229, 1963.
15. PACE, N., AND E. N. RATHBUN. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158: 685, 1945.
16. SCHLOERB, P. R., B. J. FRISS-HANSEN, I. S. EDELMAN, A. K. SOLOMON AND F. D. MOORE. The measurement of total body water in the human subject by deuterium oxide dilution. J. Clin. Invest. 29: 1296, 1950.
17. MOORE, F. D., K. H. OLESEN, J. D. MCMURREY, H. V. PARKER, M. R. BALL AND C. M. BOYDEN. The Body Cell Mass and Its Supporting Environment. Philadelphia: W. B. Saunders, 1963.
18. PACE, N., L. KLINE, H. K. SCHACHMAN AND M. HARFENIST. Studies on body composition. IV. Use of radioactive hydrogen for measurement in vivo of total body water. J. Biol. Chem. 168: 459, 1947.
19. NAIBORHU, A., M. SETIABUDI, H. P. SHENG AND R. A. HUGGINS. Relationship between red cell mass, body weight, lean body mass, and body cell mass in growing pigs and beagles. Growth 40: 147, 1976.
20. SHENG, H. P., AND R. A. HUGGINS. Body cell mass and lean body mass in the growing beagle. Proc. Soc. Exptl. Biol. Med. 142: 175, 1973.
21. SHENG, H. P., AND R. A. HUGGINS. Changes in water, protein, sodium, potassium, and chloride in tissues with growth of the beagle. Growth 39: 137, 1975.
22. RUBNER, M. Die Gesetze des Energieverbrauchs bei der Ernährung. Leipzig and Wien: Deutiche, 1902.
23. VON BEZOLD, A. Untersuchungen über die Vertheilung von Wasser, organische Materie und anorganischen Verbindungen im Thierreiche. Zeitschr. f. wissenschaftliche Zoologie, Bd. 8, 1857.
24. WELCKER, H. Bestimmungen der Menge des Körperblutes und der Blutfarbekraft, sowie Bestimmungen von Zahl, Maass, Oberfläsche und Volume des einzelnen Blutkörperchens bei Thieren und bei Menschen. Zeitschr. Med. 4: 145, 1859.
25. GRÉHANT, J. L., AND E. QUINQUAND. Mesure du volume de sang contenu dans l'organisme d'un mammifère vivant. Compte Rend. Acad. Sci. 94: 1450, 1882.
26. VALENTIN, G. G. Versuche über die in dem thierischen Körper euthaltene Blutmenge. Repertor. Anat Physiol. 3: 281, 1838.
27. GREGERSEN, M. I., AND R. A. RAWSON. Blood volume. Physiol. Rev. 39: 307, 1959.
28. LAWSON, H. C. The volume of blood—a critical examination of methods for its measurement. In: Handbook of Physiology. Circulation, edited by W. F. Hamilton and P. Dow. Washington, D.C.: American Physiological Society, Sect. 2, vol. 1, Chapt. 3, 1962, pp. 23–49.
29. KEITH, N. M., L. G. ROWNTREE AND J. T. GERAGHTY. A method for the determination of plasma and blood volume. Arch. Internal Med. 1‹: 547, 1915.
30. DAWSON, A. B., H. M. EVANS AND G. H. WHIPPLE. Blood volume studies. III. Behavior of large series of dyes introduced into the circulating blood. Am. J. Physiol. 51: 232, 1920.
31. UREY, H. C., F. G. BRICKWEDDE AND G. M. MURPHY. A hydrogen isotope of mass 2. Physiol. Rev. 39: 164, 1932.
32. HEVESY, G., AND E. HOFER. Elimination of water from the human body. Nature 134: 879, 1934.
33. MCDOUGALL, E. J., F. VERZÁR, H. ERLENMEYER AND H. GAERTNER. Heavy water in the animal body. Nature 134: 1006, 1934.
34. HEVESY, G., AND C. F. JACOBSEN. Rate of passage of water through capillary and cell walls. Acta Physiol. Scand. 1: 11, 1940.
35. FLEXNER, L. B., A. GELLHORN AND M. MERRELL. Studies on rates of exchange of substances between the blood and extravascular fluid. J. Biol. Chem. 144: 35, 1942.
36. MOORE, F. D. Determination of total body water and solids with isotopes. Science 104: 157, 1946.
37. ALVAREZ, L. W., AND R. CORNOG. Helium and hydrogen of mass 3. Physiol. Rev. 56: 613, 1939.
38. LANGHAM, W. H., W. J. EVERSOLE, F. N. HAYES AND T. T. TRUJILLO. Assay of tritium activity in

body fluids with use of a liquid scintillation system. J. Lab. Clin. Med. 47: 819, 1956.

39. Leibman, J., F. A. Gotch and I. S. Edelman. Tritium assay by liquid scintillation spectrometry. Circ. Res. 8: 907, 1960.
40. Anbar, M., and Z. Lewitus. Rate of body-water distribution studied with triple labelled water. Nature 181: 344, 1958.
41. Enns, T., and F. P. Chinard. Relative rates of passage of $H^1H^3O^{16}$ and of $H^1{}_2O^{18}$ across pulmonary capillary vessels in the dog. Am. J. Physiol. 185: 133, 1956.
42. Chinard, R. P., and T. Enns. Relative rates of passage of deuterium and tritium oxides across capillary walls in the dog. Am. J. Physiol. 178: 203, 1954.
43. Hammond, J. Pigs for pork and pigs for bacon. Royal Agric. Soc. of England, 1933, p. 15.
44. Deavers, S., E. L. Smith and R. A. Huggins. Changes in red cell volume, venous hematocrit, and hemoglobin concentration in growing beagles. Proc. Soc. Exptl. Biol. Med. 137: 299, 1971.
45. Huggins, R. A., S. Deavers and E. L. Smith. Growth in beagles: changes in body weight, plasma volume, and venous hematocrit. Pediat. Res. 5: 193, 1971.
46. Anand, R. S., and H. R. Parker. Total body water and water turnover in sheep. Am. J. Vet. Res. 27: 899, 1966.
47. Chuntananukoon, S., A. Naiborhu, M. Setiabudi, H. P. Sheng and R. A. Huggins. Growth of the pig: patterns of changes in electrolytes, water, and protein. Growth 40: 99, 1976.
48. Lowrey, L. G. The growth of the dry substance in the albino rat. Anat. Rec. 7: 143, 1913.
49. Setiabudi, M., S. Kamonsakpithak, H. P. Sheng and R. A. Huggins. Growth of the pig: changes in body weight and body fluid compartments. Growth 39: 405, 1975.
50. Setiabudi, M., H. P. Sheng and R. A. Huggins. Growth of the pig: changes in red cell and plasma volumes. Growth 40: 127, 1976.
51. Sheng, H. P., and R. A. Huggins. Growth of the beagle: changes in chemical composition. Growth 35: 369, 1971.
52. Sheng, H. P., and R. A. Huggins. Growth of the beagle: changes in the body fluid compartments. Proc. Soc. Exptl. Biol. Med. 139: 330, 1972.
53. Culebras, J. M., G. F. Fitzpatrick, M. F. Brennan, C. M. Boyden and F. D. Moore. A comparative study of total body water (TBW) as measured by isotope dilution (tritium space) and body desiccation in the rat. Federation Proc. 35: 450, 1976.
54. Foy, J. M., and H. Schnieden. Estimation of total body water (virtual tritium space) in the rat, cat, rabbit, guinea-pig and man, and of the biological half-life of tritium in man. J. Physiol. (Lond.) 154: 169, 1960.
55. Tisavipat, A., S. Vibulsreth, H. P. Sheng and R. A. Huggins. Total body water measured by desiccation and by tritiated water in adult rats. J. Appl. Physiol. 37: 699, 1974.
56. Bell, G. E., and J. S. Stern. Evaluation of body composition of young obese and lean Zucker rats. Growth 41: 63, 1977.
57. Clawson, A. J., B. E. Sheffy and J. T. Reid. Some effects of feeding characteristics and the body composition of swine and a scheme for the resolution of the body composition. J. Animal Sci. 14: 1122, 1955.
58. Flynn, M. A., F. Hanna, C. H. Long, R. Y. Asfour, R. N. Lutz and S. E. Zobrisky. Deuterium-oxide dilution as a predictor of body composition in children and pigs. In: Body Composition in Animals and Man, edited by J. Brŏzek. Washington, D.C.: National Academy of Sciences, Publication 1598, 1968, pp. 480–491.
59. Groves, T. D. D., and A. J. Wood. Body composition studies on the suckling pig. II. The in-vivo determination of total body water. Can. J. Animal Sci. 45: 14, 1965.
60. Kay, M., A. S. Jones and R. Smart. The use of tritiated water, 4-aminoantipyrene and N-acetyl-4-aminoantipyrene for the measurement of body water in living pigs. Brit. J. Nutr. 20: 439, 1966.
61. Sheng, H. P., and R. A. Huggins. Direct and indirect measurement of total body water in the growing beagle. Proc. Soc. Exptl. Biol. Med. 137: 1093, 1971.
62. Carnegie, A. B., and N. M. Tulloh. The *in vivo* determination of body water space in cattle using the tritium dilution technique. Proc. Soc. Aust. Soc. Animal Prod. 7: 308, 1968.
63. Wellington, G. H., J. T. Reid, L. J. Bratzler and J. I. Miller. Use of antipyrine in nutritional and meats studies with cattle. J. Animal Sci. 15: 76, 1956.
64. Dawson, N. J., S. K. Stephenson and D. K. Fredline. Body composition of mice subjected to genetic selection for different body proportions. Comp. Biochem. Physiol. 42B: 679, 1972.
65. Sheng, H. P., S. Kamonsakpithak, A. Naiborhu, S. Chuntananukoon and R. A. Huggins. Measured and calculated fat during growth in the pig and beagle. Growth 41: 139, 1977.
66. Moulton, C. R. Age and chemical development in mammals. J. Biol. Chem. 57: 79, 1923.
67. Cizek, L. J. Total water content of laboratory animals with special reference to volume of fluid within the lumen of the gastrointestinal tract. Am. J. Physiol. 179: 104, 1954.
68. Gotch, F., J. Nadell and I. S. Edelman. Gastrointestinal water and electrolytes. IV. The equilibration of deuterium oxide (D_2O) in gastrointestinal contents and the proportion of total body water (T.B.W.) in the gastrointestinal tract. J. Clin. Invest. 36: 289, 1957.
69. Panaretto, B. A. Body composition *in vivo*. III. The composition of living ruminants and its relation to the tritiated water spaces. Aust. J. Agric. Res. 14: 944, 1963.
70. Wedgwood, R. J. Inconstancy of the lean body mass. Ann. N.Y. Acad. Sci. 110: 141, 1963.
71. Annegers, J. Total body water in rats and in mice. Proc. Soc. Exptl. Biol. Med. 87: 454, 1954.
72. Moore, F. D., J. Lister, C. M. Boyden, M. R. Ball, N. Sullivan and F. J. Dagher. The skeleton as a feature of body composition. Human Biol. 40: 135, 1968.
73. Brožek, J., F. Grande, J. T. Anderson and A.

KEYS. Densitometric analysis of body composition: revision of some quantitative assumptions. Ann. N.Y. Acad. Sci. 110: 113, 1963.

74. DENNY, M. J. S., AND T. J. DAWSON. Comparative metabolism of tritiated water by macropodid marsupials. Am. J. Physiol. 228: 1794, 1975.

75. MALOIY, G. M. O., AND D. HOPCRAFT. Thermoregulation and water relations of two East African antelopes: the hartebeest and impala. Comp. Biochem. Physiol. 38: 525, 1971.

76. SOBERMAN, R. J. A comparison of total body water as determined by antipyrine and desiccation in rabbits. Proc. Soc. Exptl. Biol. Med. 71: 172, 1949.

77. REID, J. T., C. C. BALCH AND R. F. GLASCOCK. The use of tritium, of antipyrene and of N-acetyl-4-amino-antipyrene in the measurement of body water in living rabbits. Brit. J. Nutr. 12: 43, 1958.

78. PANARETTO, B. A. Body composition *in vivo*. I. The estimation of total body water with antipyrine and the relation of total body water to total body fat in rabbits. Aust. J. Agric. Res. 14: 594, 1963.

79. PAINTER, E. E. Total body water in the dog. Am. J. Physiol. 129: 744, 1940.

80. PANARETTO, B. A., AND A. R. TILL. Body composition *in vivo*. II. The composition of mature goats and its relationship to the antipyrine, tritiated water, and N-acetyl-4-aminoantipyrine spaces. Aust. J. Agric. Res. 14: 926, 1963.

81. HATAI, S. Changes in the composition of the entire body of the albino rat during the life span. Am. J. Anat. 21: 23, 1917.

82. LIGHT, A. E., P. K. SMITH, A. H. SMITH AND W. E. ANDERSON. Inorganic salts in nutrition. XI. Changes in composition of the whole animal induced by a diet poor in salts. J. Biol. Chem. 107: 689, 1934.

83. ASHWORTH, U. S., AND G. R. COWGILL. Body composition as a factor governing the basal heat production and the endogenous nitrogen excretion. J. Nutr. 15: 73, 1938.

84. SCHEER, B. T., E. STRAUB, M. FIELDS, E. R. MESERVE, C. HENDRICK AND H. J. DEUEL, JR. The effect of fat level of the diet on general nutrition. J. Nutr. 34: 581, 1947.

85. HALDI, J., G. GIDDINGS AND W. WYNN. The effect of vitamin B complex deficiency on the water content of the body and various organs of the albino rat. Am. J. Physiol. 141: 83, 1944.

86. SPRAY, C. M., AND E. M. WIDDOWSON. The effect of growth and development on the composition of mammals. Brit. J. Nutr. 4: 332, 1950.

87. DA COSTA, E., AND R. CLAYTON. Studies of dietary restriction and rehabilitation. J. Nutr. 41: 597, 1950.

88. COHN, C. Feeding frequency and body composition. Ann. N.Y. Acad. Sci. 110: 395, 1963.

89. REID, J. T., A. BENSADOUN, L. S. BULL, J. H. BURTON, P. A. GLEESON, I. K. HAN, Y. D. JOO, D. E. JOHNSON, W. R. MCMANUS, O. L. PALADINES, J. W. STROUD, H. F. TYRRELL, B. D. H. VAN NIEKERK AND G. W. WELLINGTON. Some peculiarities in the body composition of animals. In: Body Composition in Animals and Man, edited by J. Brŏzek. Washington, D.C.: National Academy of Sciences, Publication 1598, 1968, pp. 19–44.

90. HARRISON, H. E., D. C. DARROW AND H. YANNET. The total electrolyte content of animals and its probable relation to the distribution of body water. J. Biol. Chem. 113: 515, 1936.

91. KODAMA, A. M. In vivo and in vitro determinations of body fat and body water in the hamster. J. Appl. Physiol. 31: 218, 1971.

92. REID, J. T., A. BENSADOUN, O. L. PALADINES AND B. D. H. VAN NIEKERK. Body water estimations in relation to body composition and indirect calorimetry in ruminants. Ann. N.Y. Acad. Sci. 110: 327, 1963.

93. WADE, L., JR., AND L. B. SASSER. Body water, plasma volume, and erythrocyte volume in sheep. Am. J. Vet. Res. 31: 1375, 1970.

94. MARTIN, T. G., E. G. STANT, JR., W. V. KESSLER, M. D. JUDGE AND J. E. CHRISTIAN. Associations of body potassium, chemical composition, and carcass measurements with fat-lean composition determined by physical separation. In: Body Composition in Animals and Man, edited by J. Brŏzek. Washington, D.C.: National Academy of Sciences, Publication 1598, 1968, pp. 428–451.

95. DOORNENBAL, H. Growth, development and chemical composition of the pig. III. Bone, ash and moisture. Growth 39: 427, 1975.

96. MOULTON, C. R. Biochemical changes in the flesh of beef animals during underfeeding. J. Biol. Chem. 43: 67, 1920.

97. REID, J. T., G. H. WELLINGTON AND H. O. DUNN. Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. J. Dairy Sci. 38: 1344, 1955.

98. MITCHELL, H. H., T. S. HAMILTON, F. R. STEGGERDA AND H. W. BEAN. The chemical composition of the adult human body and its bearing on the biochemistry of growth. J. Biol. Chem. 158: 625, 1945.

99. WIDDOWSON, E. M., R. A. MCCANCE AND C. M. SPRAY. The chemical composition of the human body. Clin. Sci. 10: 113, 1951.

100. FORBES, R. M., A. R. COOPER AND H. H. MITCHELL. The composition of the adult human body as determined by chemical analysis. J. Biol. Chem. 203: 359, 1953.

101. FORBES, R. M., H. H. MITCHELL AND A. R. COOPER. Further studies on the gross composition and mineral elements of the adult human body. J. Biol. Chem. 223: 969, 1956.

102. SOBERMAN, R., B. B. BRODIE, B. B. LEVY, J. AXELROD, V. HOLLANDER AND J. M. STEELE. The use of antipyrine in the measurement of total body water in man. J. Biol. Chem. 179: 31, 1949.

103. HOLLANDER, V., P. CHANG AND C. TUI. Deuterium oxide and thiocyanate spaces in protein depletion. J. Lab. Clin. Med. 34: 680, 1949.

104. OSSERMAN, E. F., G. C. PITTS, W. C. WELHAM AND A. R. BEHNKE. *In vivo* measurement of body fat and body water in a group of normal men. J. Appl. Physiol. 2: 633, 1950.

105. EDELMAN, I. S., H. B. HALEY, P. R. SCHLOERB, D. B. SHELDON, B. J. FRIIS-HANSEN, G. STOLL AND F. D. MOORE. Further observations on total body water. I. Normal values throughout the life span. Surg. Gynecol. Obstet. 95: 1, 1952.

106. EDELMAN, I. S., J. M. OLNEY, A. H. JAMES, L. BROOKS AND F. D. MOORE. Body composition: studies in the human being by the dilution principle. Science 115: 447, 1952.

107. PRENTICE, T. C., W. SIRI, N. I. BERLIN, G. M. HYDE, R. J. PARSONS, E. E. JOINER AND J. H. LAWRENCE. Studies of total body water with tritium. J. Clin. Invest. 31: 412, 1952.

108. WEDGWOOD, R. J., D. E. BASS, J. A. KLIMAS, C. R. KLEEMAN AND M. QUINN. Relationship of body composition to basal metabolic rate in normal man. J. Appl. Physiol. 6: 317, 1953.

109. PARKER, H. V., K. H. OLESEN, J. MCMURREY AND B. FRIIS-HANSEN. Body water compartments throughout the lifespan. In: Ciba Foundation Colloquia on Ageing, edited by G. E. W. Wolstenholme and M. O'Connor. Boston: Little, Brown & Co., 1958, vol. 4, pp. 102–115.

110. FRIIS-HANSEN, B. Body water compartments in children: changes during growth and related changes in body composition. Pediatrics 28: 169, 1961.

111. RICHMOND, C. R., W. H. LANGHAM AND T. T. TRUJILLO. Comparative metabolism of tritiated water by mammals. J. Cell Comp. Physiol. 59: 45, 1962.

112. CARDUS, D., W. G. MCTAGGART AND C. L. YOUNG. Effect of exercise on determination of total body water by tritium oxide. J. Appl. Physiol. 27: 1, 1969.

113. PRICE, W. F., J. B. HAZELRIG, R. A. KREISBERG AND C. K. MEADOR. Reproducibility of body composition measurements in a single individual. J. Lab. Clin. Med. 74: 557, 1969.

114. HOFFER, E. C., C. K. MEADOR AND D. C. SIMPSON. Correlation of whole-body impedance with total body water volume. J. Appl. Physiol. 27: 531, 1969.

115. KODAMA, A. M. Total body water of the pig-tailed monkey, *Macaca nemestrina*. J. Appl. Physiol. 29: 260, 1970.

116. HAIGH, C. P., AND H. SCHNIEDEN. Virtual deuterium oxide space (total body water) in normal and protein-deficient rats. J. Physiol. (Lond.) 131: 377, 1956.

117. MULCAHY, J. J., R. L. MALVIN AND W. P. GEIS. The effects of cardiac denervation on body fluids. Proc. Soc. Exptl. Biol. Med. 143: 265, 1973.

118. JULIAN, L. M., J. H. LAWRENCE, N. I. BERLIN AND G. M. HYDE. Blood volume, body water and body fat of the horse. J. Appl. Physiol. 8: 651, 1956.

119. DEAVERS, S., J. P. ROSBOROUGH, H. E. GARNER, R. A. HUGGINS AND J. F. AMEND. Blood volumes and total body water in the domestic pony. J. Appl. Physiol. 34: 341, 1973.

120. HANSARD, S. L. Total body water in farm animals. Am. J. Physiol. 206: 1369, 1964.

121. ASCHBACHER, P. W., T. H. KAMAL AND R. G. CRAGLE. Total body water estimations in dairy cattle using tritiated water. J. Animal Sci. 24: 430, 1965.

122. KRAYBILL, H. F., E. R. GOODE, R. S. B. ROBERTSON AND H. S. SLOANE. *In vivo* measurement of body fat and body water in swine. J. Appl. Physiol. 6: 27, 1953.

123. TILL, A. R., AND A. M. DOWNES. The measurement of total body water in the sheep. Aust. J. Agric. Res. 13: 335, 1962.

124. MACFARLANE, W. V., R. J. H. MORRIS AND B. HOWARD. Turn-over and distribution of water in desert camels, sheep, cattle and kangaroos. Nature 197: 270, 1963.