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CHAPTER 2

Isotopic Determination of Body Composition in Man

By NATHANIEL I. BERLIN, M.D. AND WILLIAM E. SIRI

THE USE OF RADIOACTIVE ISOTOPES in physiologic and clinical studies has made possible a number of measurements of various aspects of body composition. Studies of body composition have had four principal orientations. The first has been the study of a population to determine the changes in body composition during the life span and to assess nutritional status. The second goal has been to relate body composition to other physiologic measurements, particularly metabolic rate.¹⁴ The third goal has been to study patients with various diseases to determine the effect of disease upon body composition.⁶⁰ A fourth goal has been to determine the relationship between concentration of a particular substance in the blood and the total quantity in the body. This has been particularly investigated for electrolytes³⁴ and for red cells and plasma.^{20,62} It has been amply demonstrated that for total red cell and plasma volume, and particularly for serum electrolytes, the measurement of the concentration in the peripheral blood need not be directly correlated to the total body content and often is misleading. This failure to have adequate information can have important clinical consequences.

In terms of gross body composition, the body can be divided into four chemical compartments: fat,* water, minerals, and proteins. The total quantity of macromolecular carbohydrates and nucleic acids is small and in this review will not be considered. Other systems of classification are discussed below. Historically, measurement of the fat content has been of greatest interest probably because, in the normal, it is the greatest variable and because of the pioneering work of Behnke and his collaborators in the measurement of body density and in formulating the concept of a lean body mass and a fat compartment.^{12,15,16,65} This

*This should be distinguished from adipose tissue, and in this review will always be taken as fat in the chemical sense.

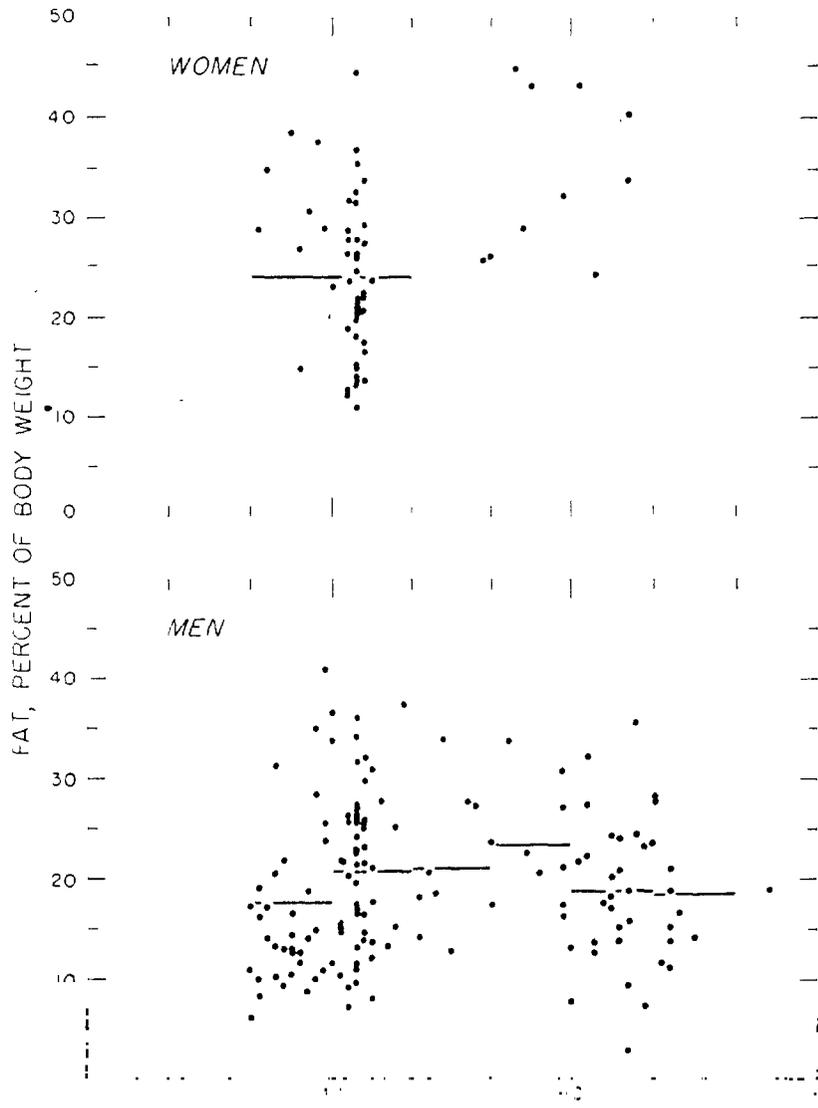


Fig. 1. The variation in body fat as determined by constant body water - body density method is a function of age and sex.

that range in body fat content is a function of both age and sex and race. Studies have shown that the body fat content of males is generally higher than that of females. The mean body fat content of males is approximately 15% and that of females is approximately 25%.

the determination of body water and electrolytes. The development of a gas dilution device⁵⁶ has made possible the convenient measurement of body volume. Analytical reviews of the concepts and principles of body composition measurements^{46,54,55} have been published. This review will be concerned with the measurement of body composition in man[†] by isotopic means but must, of necessity, include at least reference to body volume and body density. Two comprehensive reviews of body composition were published in 1963. The book by Moore et al.⁶⁰ contains a detailed description of the methods developed by his group, their data in normal subjects, together with a compilation of the data from the literature and an extensive report of the application to a variety of clinical problems. The New York Academy of Sciences, Volume 110, (September 26, 1963) contains an extensive review of many body composition studies.

Principles of Isotope Dilution: All measurements of body composition by isotopic means depend upon the use of suitable tracer substances and the measurement of their distribution in a specific biochemical or anatomical compartment. The principles of isotope dilution have been used in a number of studies and are widely known. The principle equation is as follows:

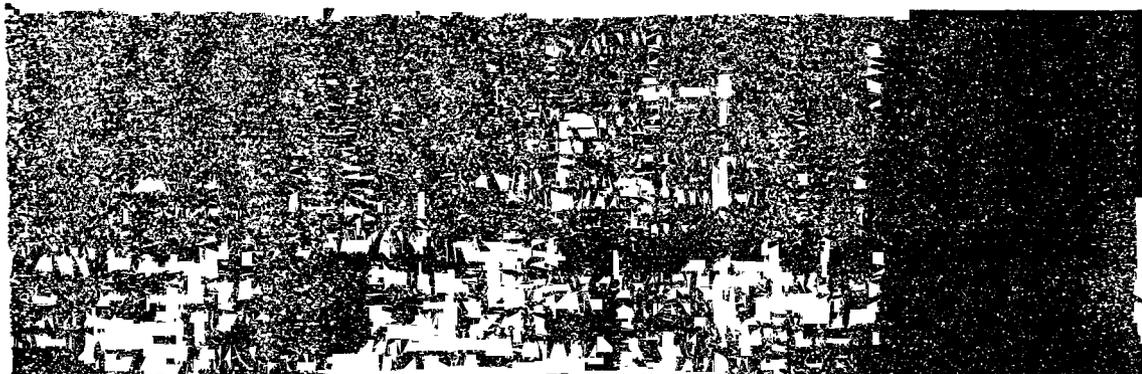
$$C_1V_1 = C_2V_2 \quad \text{where } C_1 = \text{Conc. of isotope in Vol. } V_1 \\ C_2 = \text{Conc. of isotope in Vol. } V_2,$$

or alternatively, where C represents the specific activity (in terms of isotope/unit mass) and V represents mass. However, the requirements for a satisfactory determination of quantity by the isotope dilution method are much less frequently discussed. These are as follows: (1) for the measurement of any particular body component, there must be a suitable isotopic tracer, either radioactive or an enriched stable atom, as in the case of deuterated water; (2) it must be possible to place this atom quantitatively in the compartment under study; (3) it must be uniformly distributed within the compartment; (4) the rate of loss of the label from the compartment under study should be measurable and preferably slow when compared to the rate of mixing; and (5) facilities for obtaining an adequate sample must be available. These requirements are fully met for only a limited number of the body constituents.

Various methods of analysis of isotopic dilution data are possible.

[†]Reference to studies in experimental animals will be included where data for man are not available or where implications for studies in man are apparent.

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Generally, after mixing is completed, the system may be sampled repeatedly and the specific activity extrapolated to the time of administration. If the isotope is excreted by a single route, or by known routes, and can be collected quantitatively, then the amount excreted can be subtracted from that administered and the volume of dilution calculated from:

$$V = \frac{Q - q}{\text{dpm/cc}}$$

$Q =$ amount of isotope administered (in dpm)
 $q =$ amount of isotope excreted to time t ,
 when dpm/cc of compartment under study
 is measured.

As knowledge regarding the distribution and transport of specific chemical compounds has increased, it has become necessary to develop more complex metabolic models. This subject has been reviewed in The New York Academy of Sciences, Volume 108 (May 10, 1963). These models generally treat the compound being studied as being transported within a series of compartments. These models have had extensive application in iron and albumin metabolism.^{11, 44, 52, 68, 81} Models of calcium metabolism have also been developed.^{5, 41} The application of these models has led to more complex methods for determination of rate of transfer between various anatomical and biochemical compartments and have also yielded information regarding the size of the compartments under study.

Of the major body constituents, only total body water can be measured directly with a suitable isotopic diluent either in the form of deuterated or tritiated water. In terms of the mineral components of the body the following can be measured: exchangeable sodium, exchangeable potassium, exchangeable chloride, iron, and exchangeable calcium. In terms of the fluid compartments, the total body water can be measured. Methods of determining exchangeable for measurement of extracellular water and by reference to total body water, intracellular water, water in connective tissue, the plasma volume, and the total amount of protein can be measured. A number of other plasma proteins can be measured and the total of these proteins measured. The total of the amino acids and total amino acids can be measured. The total of the primary amino acids can be measured and the total of these amino acids can be measured. A number of other amino acids can be measured and the total of these amino acids can be measured. The total of these amino acids can be measured.

Fluid Compartments

The measurement of the body fluid compartments has been reviewed by Edelman and Leibman³³ and by Levitt and Gaudino.³¹ This section will review the measurement of total body water, extracellular fluid, and blood volume.

Measurement of Total Body Water: The requirements for measurement by an isotope dilution technique can be met for total body water. Total body water may be measured by the dilution of deuterated water,^{32,42,59,99} tritiated water,^{66,69} labelled urea,^{77,78} antipyrine, and N-acetyl-4 amino antipyrine.^{88,97} An iodinated antipyrine derivative has also been prepared with I¹³¹.⁹⁷ Unfortunately, both antipyrine and iodoantipyrine undergo relatively rapid catabolism or excretion of the test substances, which precludes their use in patients with loculated fluid collections, such as in ascites and hydrothorax or in edematous states in which the diffusion of these molecules is slow. In contrast, tritiated and deuterated water are distributed throughout all fluid spaces as rapidly as natural water and share the same relatively long excretion half-time of approximately 10 days. The tritium method requires a measurement of the isotope concentration of tritium which emits a very weak β particle. The liquid scintillation spectrometer, however, has made the measurement of tritium reliable and technically easy.²² For the measurement of deuterium, a mass spectrometer or a falling drop apparatus is required.^{79,99} The cost of instrumentation and the exacting requirements of technique of both the mass spectrometer and the liquid falling drop measurement are such as to make the use of tritium an attractive alternative. However, it should be recognized that tritium is radioactive and where it is desired to avoid the use of radioisotopes, deuterium does afford a satisfactory method for measurement of total body water. A small amount of labelled water may be given either intravenously, intramuscularly, or orally and adequate time, usually several hours, allowed for mixing. Either urine or plasma may be analyzed for isotope content. If plasma is used for radio assay, it is suggested that samples be taken at about 3, 6, and 9 hours and the specific activity extrapolated to the time of administration. In the case of urine, a similar procedure can be used but care must be taken to insure emptying of the bladder 1 hour before the first sample is taken.

The values obtained for total body water by deuterium and tritium agree well with that obtained by antipyrine^{98,98} but appear to be slightly



larger due to exchange of the hydrogen isotope with other hydrogen containing compounds.^{9,17} While a large number of values of total body water have been reported²⁰ as a function of age and sex, the wide range of total body water in the normal generally precludes an unequivocal interpretation of any single measurement. When expressed as percentage of body weight, total body water diminishes with increasing fat content. Consequently, in an individual with normally hydrated lean tissue, the body water can range from 35-65 per cent. The studies of Pace and Rathbun²¹ indicated that in the guinea pig the fat-free carcass (lean tissues) had a mean water content of 72.4 ± 2.1 per cent. This was extended to man and a number of workers utilized this factor to obtain the total lean tissue mass from a measured total body water. However, in man the range of hydration of the lean tissue is greater and the error in calculating the lean tissue mass is thus larger than in the guinea pig.^{22,23,100}

The methods of determining extracellular water fall into two classes. The first group consists of dilution (Benedict²⁴ and Gellhorn²⁵) methods. These studies are possibly overestimate extracellular water. In the second group is the dye dilution method. Such methods use a known quantity of dye to equilibrate with the extracellular water. The methods of determining extracellular water volume can also be divided into "kinetic" and "equilibrium" methods. In the kinetic method is isotopic dilution. The dye dilution method is administered, allowed for mixing and the concentration usually in blood is extrapolated to the time of administration by the equilibration methods. The material quality is selected to cause a constant level in the extracellular fluid from which the volume of dilution can be obtained.

None of the methods of estimating extracellular water is free from significant error. The extracellular fluid volume varies from approximately 15 per cent of body weight to approximately 24 per cent of body weight depending upon the sex and age. The error in the dilution method is relatively great and the error in the dye dilution method is a function of the dye used. The dye dilution method is a very water soluble dye which is not normally present in the body.

Flow Methods. The flow methods consist of the volume of fluid entering the cells and the volume of fluid leaving the cells. The flow may be estimated by measuring the volume of fluid which may be estimated by measuring the volume of fluid which is

phosphorus (P^{32}) or radiochromium (Cr^{51}).²⁰ In the normal person, the white blood cells and platelets constitute less than 1 per cent of the blood volume and are generally not considered. The measurement of total red cell volume with labelled cells is independent of the hematocrit of the blood sample. There are no theoretic objections to the measurement of total red cell volume with labelled red cells. Fifteen minutes is usually allowed for mixing at which time it is difficult to demonstrate further mixing by studying peripheral blood. A considerably longer period, 30 to 40 minutes, is actually required for mixing in the normal person.²² In some patients, particularly those with splenomegaly,²⁶ a large component of mixing continues for 45 minutes.

The measurement of plasma volume is more difficult. Opinions differ as to what actually constitutes the plasma volume and how it should be measured. Historically, the oldest method is based upon the dilution of the volume of a dye, the most satisfactory of which is the Evans blue dye (T-1824). This dye is bound to albumin²¹ and in essence the volume of distribution of the albumin dye complex is measured. Analysis of the early disappearance of the dye from plasma shows a rapid phase lasting about 10 to 15 minutes followed by several slower rates of disappearance. Generally, the most rapid of the slower phases has been extrapolated to the time of administration and the plasma volume calculated. Comparable values for plasma volume are obtained with I^{131} labelled albumin. However, when labelled fibrinogen is used in the dog,⁷ or gamma globulin in man,³ the measured plasma volume is significantly smaller. It appears likely, at least in part, that some of this early phase attributed to mixing within the plasma volume is actually due to disappearance of the dye-protein complex from the plasma. For this reason, the plasma volume as measured by T-1824 or I^{131} albumin, is probably overestimated.

Mineral Components

Exchangeable Potassium: There are two short-lived isotopes of potassium, K^{42} ($t_{1/2} = 12$ hours) and K^{43} ($t_{1/2} = 22$ hours). Radiopotassium⁴³ does not appear to be widely used. In man, the bulk of potassium is intracellular. Radioactive potassium equilibrates within approximately 24 to 40 hours with virtually all of the total body potassium.^{1,29,34} The requirements for a satisfactory measurement of total body potassium are met. However, the short half-life of K^{42} makes it necessary to have at hand either a production facility or to receive frequent shipments.

Rubidium⁸⁶ has been suggested as a substitute but does not appear to be satisfactory.^{24,27} Total body potassium (K^{40}) can also be measured in total body counting facilities. In general, the measurement of total exchangeable potassium with K^{42} and total potassium with K^{40} are in good agreement.^{1,75,94} Calibration of total body counters and geometric factors relating to the distribution of the K^{40} in the body and the position of the crystal or crystals are the principal factors determining the precision of the measurement of total body K^{40} .⁵⁵

Exchangeable total potassium can be used to calculate lean body mass (LBM) from

$$\text{LBM} = \frac{\text{mEq K}}{k} \quad \text{where } k \text{ has a value of } 60\text{-}70 \text{ mEq/Kg.}^{55}$$

The division by Moore et al.⁵⁰ of the tissues into K rich and K poor groups, and also physiologically into actively metabolizing, supporting, and transporting categories, provides an additional usefulness for total body potassium measurements. They have been used as a reference for other physiologic measurements, such as basal caloric expenditure and total red cell volume.^{1,63}

Exchangeable Chloride: Exchangeable chloride has been measured with Cl^{36} ($t_{1/2} = 3 \times 10^5$ years).^{39,95} These values are generally taken as representing the volume of extracellular water. Because of the long physical half-life of Cl^{36} and the short physical half-life of the other isotopes of chlorine, radioactive bromine (Br^{82}) has been substituted and appears to be equivalent.^{52,64}

Exchangeable Sodium: Two isotopes of sodium are available (Na^{22} , $t_{1/2} = 2.6$ years and Na^{24} , $t_{1/2} = 15$ hours). Sodium is generally administered intravenously (as the chloride) and 18 to 24 hours allowed for mixing. However, at the end of this time, mixing is not complete within the total body sodium and the quantity measured is called exchangeable sodium.^{25,37,45} There is a slowly exchanging component but most of it is in the extracellular space and is usually provided by its presence there. In addition, the exchangeable sodium is not necessarily in the extracellular space. A large portion of the exchangeable sodium is in the intracellular space. The distribution of the exchangeable sodium is not uniform and the measurement is not a true measure of the total body sodium. The measurement of exchangeable sodium is a useful parameter.

Exchangeable Calcium: Exchangeable calcium is usually measured with Ca^{45} . The half-life of Ca^{45} is 163 days. The measurement of exchangeable calcium is a useful parameter. The measurement of exchangeable calcium is a useful parameter.

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at least four exponential components to the plasma Ca^{47} disappearance curve. The extrapolation of the slowest component to t_0 has been used to calculate both exchangeable calcium pool and to estimate bone formation rate. A longer lived radioactive isotope of calcium, Ca^{45} ($t_{1/2} = 160$ days), is also available, but not as useful as Ca^{47} because it can not be used for external monitoring over bone and because it is difficult to prepare for radio-assay.

Iron: In the normal person, approximately 75 per cent of the total body iron is in circulating red cells. Smaller amounts are present in erythropoietic cells in the marrow: in spleen, liver, and bone marrow as storage iron, and even smaller quantities occur in other iron containing compounds such as catalase and transport iron. A measurement of total body hemoglobin with carbon monoxide serves to set a lower limit to body iron content. If the storage iron could be measured, then a good approximation of total body iron could be obtained. The complex models of iron metabolism^{14, 65, 51} yield information regarding storage iron but with considerable uncertainty. Gale et al.⁵² have measured the quantity of storage iron in the bone marrow with Fe^{59} .

Magnesium: Magnesium²⁸, with a half-life of 21.3 hours, has been used to measure exchangeable magnesium. The exchangeable magnesium was less than one-fifth the total body magnesium.⁹

Plasma Proteins

A number of plasma proteins have been labelled with radioactive iodine (I^{131} and I^{125}) and their total body content and turnover have been measured.⁵⁶ While it is not difficult to label proteins with radioactive iodine, considerable care is required to avoid denaturation. The proteins studied include albumin,¹⁰ 6.6 S gamma globulins,²³ fibrinogen,⁵³ transferrin,¹¹ ceruloplasmin,¹¹ and macroglobulin.¹⁰ Iodinated proteins have been used to measure plasma volume, catabolic rate and total body content of the labelled protein. The last measurement requires supplementary information or more elaborate analysis of the experimental data.

There are four methods for analyzing the data from iodine labelled protein studies. These are: (1) extrapolation of the slowest component of the plasma disappearance curve to the time of administration;⁹⁰ (2) measurement of the ratio of serum isotope content to whole body isotope content;⁵⁷ (3) the equilibrium time method of Campbell et al. in which



the ratio of whole body content to plasma content is determined when the isotope content of the extravascular fluid is at a maximum:²⁶ (4) resolution of the plasma radioactivity curves into a series of exponential curves, that are matched to mathematical models, from which the ratio of intravascular to extravascular protein can be determined.^{11,56,72}

In principle, the last of these methods is the most reliable but it also is the most difficult because it requires extensive computation and experimental data that is sufficiently reliable to permit application of a complex model of protein metabolism.

The total hemoglobin can be measured by the dilution of carbon monoxide.^{27,74,75,101} This includes both the hemoglobin in red cells and hemoglobin in marrow. The data available indicate that this measurement does not appear to include much myoglobin which can bind carbon monoxide but, under the experimental conditions used, does not appear to do so.⁷⁴

Uric Acid: Total exchangeable uric acid has been measured with both N¹⁵ and C¹³ labelled uric acid. This measurement is probably satisfactory in the normal subject.^{17, 23,80} The labelled uric acid does not mix uniformly with solid deposits in gouty subjects.¹⁷

Urea and Creatinine: Urea and creatinine are uniformly distributed within the total body water and therefore the total body urea and creatinine can be measured with labelled molecules. Labelled urea and creatinine have been used to measure total body water.^{77,78}

Glucose: The glucose pool has been determined in normal and diabetic subjects with C¹⁴-labelled glucose. The volume of distribution⁷² appears to be in the approximate range reported for extracellular fluid. The total quantity is dependent principally upon the concentration, since in the diabetic the volume of distribution is only moderately increased.

Calculation of Body Fat: While a number of the body constituents can be measured, the problem of determining the total amount of chemical fat present still remains. This has been approached in two ways. One is by direct measurement of the uptake of a gas (cyclopropane or krypton) within total body lipids.^{10, 70} The second is by calculation from other body components.

The measurement of total body fat by the gas absorption technique has been carried out in a limited number of individuals using either cyclopropane or krypton. The subjects are exposed to a known quantity of gas in a closed system and the total fat is determined

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of cyclopropane in the gas phase of the system was followed to equilibrium. From this, the amount of gas taken up by the body is calculated. If the relative solubility of the gas in lipid and tissues is known then an estimate of total body fat can be made.

In man, the rate of change of the concentration of the gas was not followed to equilibrium because the time required was in excess of 8 hours. This made it necessary to extrapolate mathematically to an equilibrium value.

The following formulae have been used to estimate body fat. These formulae have been evaluated by Keys and Brozek,⁴⁸ Siri,⁸⁴ and Behnke.¹³ In each instance, f represents the fat content as a fraction of the body weight.

$$(1) f = \frac{k_1 - k_2}{d} \quad \text{where } d = \text{body density}$$

$$(2) f = 1 - \frac{\text{TBW}}{k_3} \quad \text{where TBW} = \frac{\text{total body water}}{M \text{ (body weight)}}$$

$$(3) f = \frac{k_4}{d} - k_5 \text{ TBW} - k_6$$

$$(4) f = \frac{k_7}{d} - k_8 \text{ ECW} - k_9 \quad \text{where ECW} = \frac{\text{extracellular water}}{M}$$

$$(5) f = k_{10} \text{ ECW} - k_{11} \text{ TBW} + k_{12}$$

$$(6) f = 1 - \frac{K}{k_{13}}$$

$$(7) f = 1 - m - \frac{k_{14} K}{M} \quad \begin{array}{l} K = \text{total body potassium} \\ M = \text{mineral mass} \end{array}$$

$$(8) f = 1 - k_{15} \text{ TBW} - k_{16} K$$

The second class of methods for calculating body fat are based on one or more of the following measurements: body density, body water, extracellular fluid measurement, and total potassium. In principle, the methods based upon body water depend upon an assumed constant hydration of the nonfat portion of the body. The body density methods depend upon an assumption of a two-compartment system: one of high density, the hydrated lean tissues ($d = 1.10$), and one of low density ($d = 0.90$), the fat compartment.¹⁵ The use of combined measurements of body water and body density, in essence, takes into account the contri-



butions due to water and again assumes a two-compartment-system: one consisting of the dry, lean tissue constituents and the second fat, again with very different densities.²⁷ In the normal, the best estimation of total body fat is derived from a combined measurement of body density and body water rather than either alone.²⁴ The body density method alone or the body water method alone both assume constancy for the hydration of the lean tissue or a constant composition of the lean tissue, and thus are not applicable in disease states affecting hydration.

The constants of the equations are listed in Table 1. Often in the determination of these constants other empirical constants or experimental methods are used that are not entirely free of significant criticism. Thus, the numerical values in some instances can be considered only approximations.

There are a number of assumptions underlying these equations that affect in varying degrees their reliability. They are as follows:

Equation 1—assumes that the lean tissue (lean body mass) has a constant composition. Since water is the principal component, this is tantamount to assuming a constant hydration of the lean tissue. In general, variations in this mineral-protein ratio are probably less significant in their effect on the value of the constants used.

Equation 2—requires the same assumptions as equation 1.

Equation 3—assumes a mineral-protein ratio of 0.35. Parenthetically, it should be noted that if a standard deviation of this ratio of 0.10 is applied, the calculated fat content is not appreciably affected.²⁴ Allen et al.² have modified this equation by subtracting the volume and mass associated with bone mineral. Good agreement in the calculated fat content was obtained when the constants proposed by Allen et al. were compared to those previously published. This was, in a sense, to be anticipated because of the small effect a large variation in mineral-protein ratio has on the calculated fat content.

Equation 4—the principal assumption is normal hydration of cells.

Equation 5—this method has been evaluated²⁴ and does not appear to be of value. In fact, in the normal the factors used cancel out one measurement or the other.

Equation 6—assumes a constant potassium content of all lean tissues.

Equation 7—implies a constant potassium content of selected tissues (the body cell mass of Moore et al.)²⁸ and requires a measurement of the mass of the other tissues for which an independent direct method of

Table 1. Constants for Body Composition Formulae

Constant	Values	Reference	Values	Reference	Values	Reference	Values	Reference	Values	Reference
k_1	4.950	84	5.548†	70	4.340	65	4.206	46	4.206	46
k_2	4.500	84	5.044†	70	3.983	65	3.817	46	3.817	46
k_3	0.724	67	0.718	65	0.714	100	0.71	51	0.707	48
k_4	2.118	84			2.516	1				
k_5	0.78	84			0.740	1				
k_6	1.354	84			1.793	1				
k_7	5.118	*84								
k_8	0.573	84								
k_9	4.612	84								
k_{10}	0.596	84								
k_{11}	1.620	84								
k_{12}	1.041	84								
k_{13}	68.1	35	0.354 - 8.2 x 10 ⁻⁴ males	1						
			0.33 - 8 x 10 ⁻⁴ females	1						
k_{14}	8.33	60								
k_{15}	1.88	*								
k_{16}	0.045	*								

*Calculated from data of Forbes and Lewis,²⁸ using model of Anderson.¹

†Instead of d use specific gravity. The other values of constants k_1 and k_2 cited represent recalculation of these values.

Table 2. Body Compartment Systems

	Fat	Fat	Fat	Fat	Fat	Fat	Fat	Standard Man
	Lean body mass and essential lipids	Water	Body cell mass	Water	Muscle	Cells (water, mineral, protein)		Fat (F) water (W) mineral (M) protein (P) in fixed proportion
	Protein (also called cell solids)	Mineral	Supporting and transport	Dry body mass (total body solids)	Muscle Free lean (MFL)	Bone mineral	Excess adipose tissue	Fat, water, mineral, and protein in variable proportion
	16	2, 16, 85	60	61	1	10	16, 53	
								Extracellular fluid

measurement is not available, but which Allen et al. calculates from measurement of height and inter-condylar dimensions of elbows, knees, wrists, and ankles.²

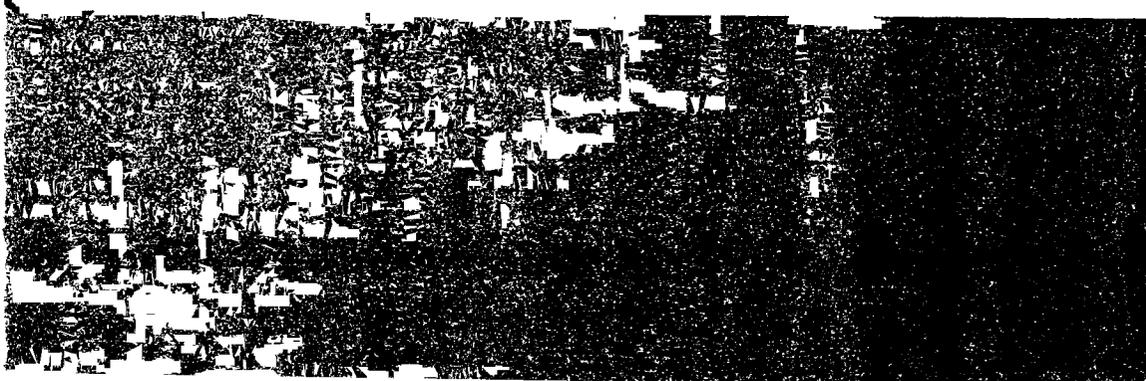
Equation 8—divides the lean tissues into muscle and muscle-free lean (MFL) with different potassium and water contents.⁴

Body Compartment Systems

Systems of classification of the components of the body have proliferated rapidly and with confusing terminology (see Table 2). These systems have had their origin in the measurement of body water alone, body density alone, a combination of body water and body density, a combination of chemical and anatomical terms and attempts to divide the body into physiological or functional divisions.^{16,60} Each has had separate goals. Perhaps the oldest classification is that of the division of the body into two compartments, the fat free body mass, consisting of minerals, proteins, water and a lipid component.¹³ Various modifications of the fat free body mass have been made. Behnke added a small amount of fat which was considered to be essential lipids,¹⁶ principally the lipids of the central nervous system, and to this applied the term "lean body mass." There has been a tendency to use interchangeably the terms "lean body mass" and "fat free body mass." As defined by Behnke, the difference is small but real. Recently, Moore and colleagues⁶⁰ and Anderson⁴ have developed a three compartment system. The "body cell mass" of Moore et al.⁶⁰ is a chemically homogeneous mass containing approximately three milliequivalents of potassium per gram of nitrogen and consists of tissues that require oxygen, produce CO₂, and burn glucose. To this is added a second compartment consisting of extracellular fluid and supporting tissues. These are tissues that contain much less potassium. The third compartment is fat.

Anderson⁴ has proposed a somewhat similar division into muscle and muscle free lean (MFL) tissues and fat based upon measurement of potassium and water content by Forbes and Lewis.³⁶

There is a continually recurring theme of a reference man^{2,40,54} which consists essentially of an ideal person with the mineral, protein, and fat content that would exist in a healthy young male adult. To this framework, adipose tissue is added. Unfortunately adipose tissue, while principally lipid, contains mineral, protein, and water in variable concentrations.²⁵



A reasonable goal of studies of body composition would be to sort out these various systems of compartmentalization of body components and evolve an acceptable scheme. Perhaps the best would be either a separation into fat, water, mineral, and protein as chemical entities, or in a physiologic sense, a system consisting of a body cell mass, supporting structures, and fat. From the quantitative standpoint, there does not appear to be much advantage to the use of a reference man to which is appended "excess" adipose tissue or fat. For anthropometric or nutritional studies where it is desired to have some measure of the adequacy of caloric intake, a measurement of the excess adipose tissue could be useful.

Changes in Gross Body Composition: Changes in gross body composition in a given individual can be estimated in two ways, either from metabolic balance data^{47,61,72} or changes in body density,^{19,47} body water, or total body potassium. The net change in body nitrogen, derived from metabolic balance data, can be used to calculate the protein gained or lost. From this and an assumed hydration of the lean tissue, the total lean tissue mass gained or lost can be estimated:⁷³

$$\Delta \text{ fat} = \Delta M + k\Delta N$$

where $k = 6.25$ Gm protein, gm N

$M =$ body weight

$N =$ nitrogen (Gm)

If the change in total body water is also measured, a more satisfactory measurement of change in gross body composition can be obtained from:^{47,61}

$$\Delta \text{ fat} = \Delta M + 6.25\Delta N + \Delta \text{TBW}$$

where TBW = total body water (Gm)

In a study of changes in body composition with weight reduction, it was estimated that changes of the order of approximately 500 Gm of body fat can be determined with body density-body water determination.¹⁹

The Future

It should be recognized that only a few laboratories have attempted to measure body composition in terms of more than one or two components. Studies of body density, body water, and blood volume have been reported (Huff and Feller,¹⁹ Siri⁵³). Moore and his colleagues⁶⁰ measure total red cell volume, plasma volume, total body water, extracellular water, total exchangeable sodium, and potassium. This requires

a considerable effort and supporting staff and utilizes procedures that are not currently utilized in day to day clinical medicine. For these reasons, relatively few laboratories have engaged in studies of body composition involving all these measurements. However, with improvements in technique, clinical investigators may undertake these studies with the goal that the basic tenets that emerge can be widely utilized clinically.

At present, probably the most significant contribution to body composition studies would be a simple and reliable means of directly determining body fat. The gas dilution methods^{12,19} do not appear to meet the criterion of simplicity and they have not been sufficiently studied to determine their reliability. In general, the relatively low blood-perfusion rate and the low efficiency of extraction of fat-soluble diluents from the blood by adipose tissue preclude the development of a simple direct determination of body fat content by the isotope dilution principle. An entirely new approach is needed for the direct determination of body fat.

A promising application of neutron-activation analysis *in vivo* for estimating total quantities of certain elements in the body has been tested recently by Anderson and his associates at Harwell, England.^{1A} The method calls for exposure of the subject to a uniform dose of about 0.1 rad of 14 MeV neutrons and subsequent determination of the induced radioactivities with a whole-body counter. Total quantities of sodium, chlorine, and calcium were readily estimated in preliminary tests on two human subjects. Estimates of the quantities of nitrogen, potassium, and possibly other elements may also become practicable with further improvements in technique.

REFERENCES

1. ALLEN, T. H., ANDERSON, E. C., AND LANGHAM, W. H.: *J. Gerontol.* 15: 348, 1960.
2. —, WELCH, B. E., TRUJILLO, T. T., AND ROBERTS, J. E.: *J. Appl. Physiol.* 14: 1009, 1959.
3. ANDERSEN, S. B.: *Clin. Sci.* 23: 221, 1962.
4. ANDERSON, E. C.: *Ann. N. Y. Acad. Sci.* 110: 189, 1963.
- 4a. ANDERSON, J., OSBORN, S. B., TOMLINSON, R. W. S., NEWTON, D., RUNDO, J., SALMON, L., AND SMITH, J. W.: *Lancet* 2: 1201, 1964.
5. AUBERT, J. P., BRONNER, F., AND RICHELLE, L. J.: *J. Clin. Invest.* 42: 885, 1963.
6. AWAL, M., AND BROWN, E. B.: *J. Lab. & Clin. Med.* 61: 363, 1963.
7. BAKER, C. H., AND WYCOFF, H. D.: *Am. J. Physiol.* 201: 1159, 1963.
8. BAKER, N., SHREEVE, W. W., SHIPLEY, R. A., INCEFY, G. E., AND MILLER, M.: *J. Biol. Chem.* 211: 575, 1954.

9. BARNES, B. A., AND BROWNELL, G.: Proc. 2nd Int. Conf. Peaceful Uses of Atomic Energy 26: 204. 1958.
10. BARTH, W. F., WOCHNER, D., WALDMANN, T. A., AND FAHEY, J. C.: J. Clin. Invest. 43: 1036. 1964.
11. BEEKEN et al.: J. Clin. Invest. 41: 1312. 1962.
12. BEHNKE, A. R.: Harvey Lect. 37: 198. 1942.
13. —: In Brozek, J. and Henschel (Eds.): Techniques for Estimating Body Composition. Washington, D.C., NAS-NRC. 1961. p. 118.
14. —: Ann. N. Y. Acad. Sci. 56: 1095. 1953.
15. BEHNKE, A. R., JR., FEAN, B. G., AND WELHAM, W. C.: J.A.M.A. 118: 495. 1942.
16. BEHNKE, A. R., OSSERMAN, E. F., AND WELHAM, W. C.: Arch. Int. Med. 91: 585. 1953.
17. BENEDICT, J. D., FORSHAM, P. H., ROCHE, M., SOLOWAY, S., AND STEPHEN, D. W., JR.: J. Clin. Invest. 29: 1104. 1950.
18. BENEDICT, J. D., FORSHAM, P. H., AND STETTEN, D. W., JR.: J. Biol. Chem. 181: 183. 1949.
19. BERLIN, N. I., GEVIRTZ, N. R., AND WATKIN, D. M.: Metabolism 11: 302. 1962.
20. —, LAWRENCE, J. H., AND ELMINGER, P. J.: Blood 12: 147. 1957.
21. BISHOP, C., GARNER, W., AND TALBOTT, J. H.: J. Clin. Invest. 30: 879. 1951.
22. BOLING, E. A.: Ann. N. Y. Acad. Sci. 110: 246. 1963.
23. BROZEK, J., GRANDE, F., ANDERSON, J. T., AND KEYS, A.: Ann. N. Y. Acad. Sci. 110: 113. 1963.
24. BURCH, G. E., THREEFOOT, S. A., AND RAY, C. T.: J. Lab. & Clin. Med. 45: 371. 1955.
25. BURCH, G. E., RAY, C. T., AND THREEFOOT, S. A.: Acta med. Scandinav. (Supp. 266) 142: 329. 1952.
26. CAMPBELL, R. M., CUTHBERTSON, D. P., MATTHEWS, C. M., AND MCFARLANE, A. S.: Int. J. Appl. Rad. & Isotopes 1: 66. 1956.
27. CARDUS, D., LUFT, U. C., AND BECH, B. J.: Lab. & Clin. Med. 61: 944. 1963.
28. COHEN, S., AND FREEMAN, T.: BIOCHEM. J. 76: 475. 1960.
29. CORSA, L., JR., OLNE, J. M., JR., STENBERG, A. W., BELL, R. W., AND MOORE, F. D.: J. Clin. Invest. 29: 1280. 1950.
30. COTLOVE, E., AND HOGBEN, C. A. M.: Chloride, Chap. 27. In Comar, C. L., and Bronner, F. (Eds): Mineral Metabolism, An Advanced Treatise, Vol. II. The Elements, Part B: New York, Academic Press. 1962.
31. CROOKS, J., BLUHM, M. M., AND MULDOWNEY, F. P.: J. Clin. Sci. 18: 175. 1959.
32. EDELMAN, I. S., HALEY, H. B., SCHLOERB, P. R., SHELDON, I. B., FRIIS-HANSEN, B., STOLL, G., AND MOORE, F. D.: Surg. Gynec. & Obst. 95: 1. 1952.
33. —, AND LEIBMAN, J.: Am. J. Med. 27: 256. 1959.
34. —, —, O'MEARA, M. P., AND BIRKENFELD, L. W.: J. Clin. Invest. 37: 1236. 1958.
35. FORBES, G. B., AND HURSH, J. B.: Ann. N. Y. Acad. Sci. 110: 255. 1963.
36. —, AND LEWIS, A. M.: J. Clin. Invest. 35: 596. 1956.
37. —, AND PERLEY, A.: J. Clin. Invest. 30: 558. 1951.
38. GALE, E., TORRANCE, J., AND BOTHWELL, T.: J. Clin. Invest. 42: 1076. 1963.
39. GAMBLE, J. L., JR., ROBERTSON, J. S., HANNIGAN, C. A., FOSTER, C. G., AND FARR, L. E.: J. Clin. Invest. 32: 483. 1953.
40. GRANDE, F.: In Brozek, J. and Henschel, A. (Eds.): Techniques for Measuring Body Composition, Washington, D.C., NAS-NRC. p. 168. 1961.
41. HEANEY, R. P., AND WHEDON, G. D.: J. Clin. Endocrinol. & Metab. 18: 1246. 1958.

5013053

42. HEVESY, G., AND HOFER, E.: *Nature* 134: 879, 1934.
43. HUFF, R. L., AND FELLER, D. D.: *J. Clin. Invest.* 35: 1, 1956.
44. —, AND JUDD, O.: *Adv. Viol. & Med. Physics* 4: 223, 1956.
45. JAGGER, P. I., HINE, G. J., CARDARELLI, J. A., AND BURROWS, B. A.: *J. Clin. Invest.* 42: 1459, 1963.
46. KEYS, A., AND BROZEK, J.: *Physiol. Rev.* 33: 245, 1953.
47. KYLE, L. H., WERDEIN, E. J., CANARY, J. J., AND PACHUTA, B.: *J. Clin. Invest.* 38: 1475, 1959.
48. LESSER, G. S., KEEMAN, I., AND STEELE, J. M.: *Ann. N. Y. Acad. Sci.* 110: 578, 1963.
49. LESSER, G. T., PERL, W., AND STEELE, J. M.: *J. Clin. Invest.* 39: 1791, 1960.
50. —, AND ZAK, G.: *Ann. N. Y. Acad. Sci.* 110: 40, 1963.
51. LEVITT, M. F., AND GAUDINO, M.: *Am. J. Med.* 9: 208, 1950.
52. LEWALLEN, C. G., BERMAN, M., AND RALL, J. E.: *J. Clin. Invest.* 38: 66, 1959.
53. LJUNGGREN, H.: *Acta Endocrinol.* 25: (Suppl. 33) 1957.
54. MCCANCE, R. A., AND WIDDOWSON, E. M.: *Proc. Roy. Soc., Ser. B*, p. 115, 1951.
55. MCFARLANE, A. S.: *J. Clin. Invest.* 42: 346, 1963.
56. — *In* Munro, H. N., and Allison, J. B. (Eds.): *Metabolism of Plasma Proteins: Mammalian Protein Metabolism*. New York, Academic Press Inc., 1963c.
57. MARTIN, M. M., AND WALKER, G.: *Nature* 181: 705, 1958.
58. MILLER, C. E., AND REMENCHIK, A. P.: *Ann. N. Y. Acad. Sci.* 110: 175, 1963.
59. MOORE, F. D.: *Science* 104: 157, 1946.
60. —, OLESEN, K. H., McMURREY, J. D., PARKER, H. V., BALL, M. R., AND BOYDEN, C. M.: *The Body Cell Mass and Its Supporting Environment*. Philadelphia, W. B. Saunders, 1963.
61. —, HALEY, H. B., BERING, E. A., BROOKS, L., AND EDELMAN, I. S.: *Surg. Gynec. & Obst.* 95: 155, 1952.
62. MULDOWNNEY, F. P., AND WILLIAMS, R. T.: *Am. J. Med.* 35: 768, 1963.
63. —, CROOKS, J., AND BLUHM, M. M.: *J. Clin. Invest.* 36: 1375, 1957.
64. NICHOLSON, J. P., AND ZILVA, J. F.: *Clin. Sci.* 19: 391, 1960.
65. OSSERMAN, E. F., PITTS, G. C., WELHAM, W. C., AND BEHNKE, A. R.: *J. Appl. Physiol.* 2: 633, 1950.
66. PACE, N., KLINE, L., SHACKMAN, H. K., AND HARFENIST, M.: *J. Biol. Chem.* 168: 459, 1947.
67. —, AND RATHBUN, E. N.: *J. Biol. Chem.* 158: 685, 1945.
68. POLLYCOVE, M., AND MORTIMER, R.: *J. Clin. Invest.* 40: 753, 1961.
69. PRENTICE, T. C., SIRI, W. E., BERLIN, N. I., HYDE, G. M., PARSONS, R. J., JOINER, E. E., AND LAWRENCE, J. H.: *J. Clin. Invest.* 31: 412, 1952.
70. RATHBUN, E. N., AND PACE, N.: *J. Biol. Chem.* 158: 667, 1945.
71. RAWSON, R. A.: *Am. J. Physiol.* 138: 708, 1943.
72. REEVE et al.: *J. Lab. & Clin. Med.* 60: 923, 1962.
73. REIFENSTEIN, E. C., JR., ALBRIGHT, F., AND WELLS, S. L.: *J. Clin. Endocrinol.* 5: 367, 1945.
74. ROOT, W. S., ALLEN, T. H., AND GREGENSEN, M. I.: *Am. J. Physiol.* 175: 233, 1953.
75. —, ROUGHTON, F. J. W., AND GREGENSEN, M. I.: *Am. J. Physiol.* 146: 739, 1946.
76. ROVNER, D. R., AND CONN, J. W.: *J. Lab. & Clin. Med.* 62: 492, 1963.
77. SAN PIETRO, A., AND RITTENBERG, D.: *J. Biol. Chem.* 201: 445, 1953.
78. SCHLOERB, P. R.: *Am. J. Physiol.* 199: 661, 1960.
79. —, FRIIS-HANSEN, J. B., EDELMAN, I. S., SOLOMON, A. K., AND MOORE, F. D.: *J. Clin. Invest.* 29: 1296, 1950.

934.
35: 1, 1956.
4: 223, 1956.
BURROWS, B. A.: J. Clin. Invest.
5: 1953.
PACHUTA, B.: J. Clin. Invest. 38:
Ann. N. Y. Acad. Sci. 110: 578,
Clin. Invest. 39: 1791, 1960.
40: 1963.
5: 208, 1950.
6: J. Clin. Invest. 38: 66, 1959.
7: 1957.
8: Roy. Soc., Ser. B, p. 115, 1951.
9: 63.
10: Metabolism of Plasma Proteins:
Academic Press Inc., 1963c.
11: 705, 1958.
12: Y. Acad. Sci. 110:175, 1963.
13: R. H. V., BALL, M. R., AND BOY-
porting Environment. Philadelphia,
14: AND EDELMAN, I. S.: Surg. Gynec.
15: J. Med. 35: 768, 1963.
16: Invest. 36: 1375, 1957.
17: 19: 391, 1960.
18: C., AND BEHNKE, A. R.: J. Appl.
19: ARFENIST, M.: J. Biol. Chem. 168:
20: 8: 685, 1945.
21: Invest. 40: 753, 1961.
22: DE, G. M., PARSONS, R. J., JOINER,
23: 31: 412, 1952.
24: 158: 667, 1945.
25: 43.
26: 962.
27: ELLS, S. L.: J. Clin. Endocrinol. 5:
28: 1: Am. J. Physiol. 175: 233, 1953.
29: 1: Am. J. Physiol. 146: 739, 1946.
30: lin. Med. 62: 492, 1963.
31: Chem. 201: 445, 1953.
32: 960.
33: LOMON, A. K., AND MOORE, F. D.:
34: 80. SÉEGMILLER, J. E., GRAYZEL, A. I., LASTER, L., AND LIDDLE, G. L.: J. Clin. Invest.
40: 1304, 1961.
35: 81. SHARNEY, L., WASSERMAN, L. R., SCHWARTZ, L., AND TENDLER, D.: Ann. N. Y.
Acad. Sci. 108: 230, 1963.
36: 82. SHREEVE, W. W., BAKER, N., MILLER, M., SHIPLEY, R. A., INCEFY, G. E. AND
CRAIG, J. W.: Metabolism 5: 22, 1956.
37: 83. SIRI, W. E.: To be published.
38: 84. —: Body Composition from fluid spaces and density: analysis of methods. In
Brozek, J. and Henschel, A. (Eds.): Techniques for Measuring Body Compo-
sition. Washington, D.C., NAS-NRC, 1961.
39: 85. —: Adv. in Biol. & Med. Physics 4: 239, 1956.
40: 86. —: Rev. Scientific Instruments 21: 729, 1956.
41: 87. —, AND EVERS, J.: Tritium Exchange in Biological Systems, Vol. II, p. 71.
Tritium in the Physical and Biological Sciences, International Atomic Energy
Agency, Vienna, 1962.
42: 88. SOBERMAN, R., BRODIE, B., LEVY, B., AXELROD, J., HOLLANDER, V., AND STEELE,
J. M.: J. Biol. Chem. 179: 31, 1949.
43: 89. STEINFELD, J. L.: J. Lab. & Clin. Med. 55: 904, 1960.
44: 90. STERLING, K.: J. Clin. Invest. 30: 1228, 1951.
45: 91. STERNLIEB, I., MORELL, A. G., TUCKER, W. D., GREENE, M. W., AND SCHEINBERG,
I. H.: J. Clin. Invest. 40: 1834, 1961.
46: 92. STRAJMAN, E., BERLIN, N. I., ELMLINGER, P. J., AND ROBINSON, J.: Acta med.
Scandinav. 157: 263, 1957.
47: 93. TALSO, P. J., LAHR, T. N., SPAFFORD, N., FERENZI, G., AND JACKSON, H. R. O.:
J. Lab. & Clin. Med. 46: 619, 1955.
48: 94. —, MILLER, C. E., CARBALLO, A. J., AND VASQUEZ, I.: Metabolism 9: 456, 1960.
49: 95. THREEFOOT, S. A., BURCH, G. E., AND RAY, C. T.: J. Lab. & Clin. Med. 42: 16,
1953.
50: 96. TIZIANELLO, A., AND PANNACCIULLI, I.: Acta Hemat. 21: 346, 1959.
51: 97. WALSER, M.: Proc. Soc. Exper. Biol. & Med. 79: 372, 1952.
52: 98. —, SELDIN, D. W., AND GROLLMAN, A.: J. Clin. Invest. 32: 299, 1953.
53: 99. WENTZEL, A. D., IACONO, J. M., ALLEN, T. N., AND ROBERTS, J. E.: Physics in
Med. & Biol. 3: 1, 1958.
54: 100. WERDEIN, E. J., AND KYLE, L. H.: J. Clin. Invest. 39: 626, 1960.
55: 101. WIKLANDER, O.: Acta Chir. Scandinav. Suppl. 208, 1956.
Note: This bibliography is not intended to be inclusive. Where possible an initial re-
port and a current study have been cited.

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