Direct and Indirect Measurement of Total Body Water in the Growing Beagle[1] (35734)

HWAI-PING SHENG[2] AND RUSSELL A. HUGGINS[3]

Department of Physiology, Baylor College of Medicine, Texas Medical Center,
Houston, Texas 77025

The validity and the precision of the indirect method for determination of the TBW compartment can be judged best by comparison with a direct method such as desiccation of the whole animal, and if possible, the same animal should be used for both determinations. Not surprisingly, because of the tediousness and the unesthetic aspect of the desiccation method, relatively few comparisons between the tritium and desiccation methods have been done. There are data on the TBW compartment of the newborn human measured either by tritium, deuterium, or by desiccation (1, 2), but none where both methods of measurement were made on the same individual. However, there are reports where the volume of the TBW compartment was determined by direct and indirect measurements on a few animals of several species (3–6).

While available data leave no question that for a number of species of animals the percentage of water, expressed on either a body weight or fat-free wet weight basis, is more in the newborn than in the adult (1, 7), little attention has been given to the rate of decrease of the water content during the period of growth.

In the present investigation TBW was measured indirectly by tritiated water (^3H_2O) and then directly by desiccation on the same dogs and over a range of body weights from 0.2 kg (newborn) to 13.2 kg (adult).

Materials and Methods. The dogs used in these experiments came from a beagle colony which is maintained at the Wynne Unit of the Texas Department of Corrections at Huntsville, Texas. Huntsville lies outside the Gulf Coast area where heartworm is a serious problem. Details of the management of the colony have appeared in an earlier publication (8).

Forty-three dogs ranging in age from newborn to adult were used in this investigation. The dogs were weighed and usually anesthetized with a combination of morphine sulfate given subcutaneously and sodium pentobarbital given either intraperitoneally or intravenously. The dosage given varied with the age and weight of the dog. In very young pups 1 mg of morphine only was given. Dogs between 1 and 6 months of age received 3–5 mg/kg of morphine and 7–10 mg/kg of pentobarbital, and those over 6 months old 10 mg/kg of morphine and 15 mg/kg of pentobarbital.

The anesthetized dog was laid in a supine position and an external jugular vein exposed for the insertion of a catheter into the right heart. A control blood sample (1.5 ml in puppies under 2 kg and 8 ml in those over) was withdrawn into a heparinized syringe. Then a known volume of tritiated water (10 μCi/kg) was injected through the catheter which was then flushed with 0.9% saline. Preliminary experiments had shown that equilibration of the tritiated water was achieved in 1½ hr in younger puppies and in 2½ hr in the older dogs. After injection of tritiated water, 1.5- or 8-ml blood samples (depending on the weight of the dog) were collected at

---

[1] This study was supported by Grants HE-11395 and HE-05435 from the National Heart Institute.

[2] Present address: Department of Pharmacology, University of Hong Kong Medical School, Hong Kong.

[3] Address reprint requests to Dr. R. A. Huggins, Department of Physiology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77025.

1½ or 2½ hr, then at 3 and 4 hr. The blood samples were centrifuged and the plasma analyzed for tritium by the technique of Udekwu *et al.* (9). The technique was modified for young puppies because of the small volume of blood withdrawn: 5 ml of 10% TCA was added to 0.5 ml of plasma to precipitate the plasma protein. After centrifugation, 0.5 ml of the supernatant fluid was pipetted into 15-ml scintillation fluid and counted in a Tri-Carb liquid-scintillation spectrometer (Packard Instrument Co.). A tritium standard was prepared for each dog by taking 0.1 ml of 1 to 100 dilution of the injected concentration of tritium and adding it to 0.4 ml of the control plasma. This sample was treated and counted in the same manner as the plasma samples.

The plasma counts per minute of tritium were plotted against time on semilogarithmic paper; the curve was extrapolated back to zero time and the counts were read at the intercept. TBW was then calculated: TBW (ml) = total counts injected/min ÷ counts in 1 ml plasma/min.

Our recovery of tritium was tested by putting a known volume of water or blood into a beaker and adding a measured amount of tritiated water. The mean difference between the calculated and measured was ±1%. Next, the analytical reproducibility on multiplicate samples of tissue was tested and a mean of ±1.2% was obtained. Finally, TBW was measured with tritium in a group of dogs of different ages and then redetermined the next day. The paired determinations agreed within a mean of ±2.5% of the body weight.

After the determination of TBW by tritium, the dogs were bled to death and the volumes of blood measured and stored separately in the deep freeze. Then the dogs were shaved closely with clippers and skinned. The skins were weighed, and representative samples from different parts of the body were washed with deionized water, blotted dry, then ground in a sausage grinder and placed in vials in the deep freeze. The heart, lung, gut, liver, kidney, skeletal muscle, brain, and a category labeled "miscellaneous," which included the remainder of the dog except the skeleton, were individually weighed, washed with deionized water, and blotted dry. Next they were homogenized in a blender with a known volume of deionized water. Three portions of each homogenized sample were stored in separate vials in the deep freeze. The whole skeleton was weighed, and pieces of bone from representative parts of the skeleton were ground to a powder in a ball-mill and stored in the deep freeze.

A frozen sample of each organ was removed from the deep freeze, thawed, weighed in a crucible, and then dried to a constant weight at 100° in an oven to obtain its dry weight. The fat was extracted from another sample of tissue with methylene chloride. Fifty milliliters of methylene chloride were added to each gram of the weighed tissue in a separatory funnel and mixed in a shaker for 20 min. The mixture was allowed to stand until the two layers separated. The methylene chloride was drained off and the procedure repeated twice. Then the tissue was transferred to an evaporating dish and dried in an oven at 100° to a constant weight. The fat-free samples were then weighed. The amount of fat in an organ was the difference between the dry weight and the fat-free dry weight. The TBW and the fat-free wet weight of a dog were then obtained by adding the appropriate data for all tissue samples. Lines of best fit were calculated from the data of each dog. The equation $y = ax + b$, where y is the dependent variable; x, the independent; a, the slope of the line; and b, the y intercept, was used. Where breaks in the data suggested that more than one line would fit the data, the slopes of these lines were calculated, and if they differed significantly from that of a single line, $p < .05$, they were considered valid lines (10).

Results. The paired data are shown in Table I. The data on the volume of TBW obtained by the two methods were plotted against body weight, and for both sets of data there was an increase in TBW with an increase in body weight (Fig. 1). With the desiccation method the volume of TBW increased at a single rate, 0.521 liters/kg, while with the tritium method the increase in volume of TBW was best described by two different rates with a change in the rate at a

TABLE I. Paired Determinations of TBW by Tritiated Water and by Desiccation.

Dog no. and sex	Age (days)	Body weight (kg)	TBW$_1$[a] (liters)	TBW$_2$[b] (liters)	TBW$_1$-TBW$_2$ (liters)
BL1 ♂	0	0.32	0.22	0.28	—0.06
BL2 ♀	0	0.36	0.27	0.31	—0.04
AQ3 ♀	1	0.29	0.21	0.26	—0.05
BD3 ♀	1	0.27	0.20	0.24	—0.04
BC1 ♂	8	0.42	0.28	0.35	—0.07
BH5 ♂	8	0.33	0.23	0.30	—0.07
BJ5 ♀	8	0.50	0.37	0.44	—0.07
BG6 ♀	9	0.38	0.25	0.28	—0.03
BK1 ♂	10	0.58	0.42	0.50	—0.08
BK3 ♀	10	0.48	0.35	0.42	—0.07
AY1 ♂	16	1.13	0.69	0.77	—0.08
AX1 ♂	18	0.77	0.52	0.61	—0.09
AW1 ♂	21	1.02	0.65	0.81	—0.16
BG2 ♀	31	1.16	0.69	1.02	—0.33
BI4 ♀	32	1.6	1.05	1.53	—0.48
BF3 ♂	33	1.7	1.26	1.54	—0.28
AY4 ♀	37	1.5	0.89	1.30	—0.41
AX2 ♂	39	1.6	0.98	1.41	—0.43
AW2 ♂	42	1.5	1.03	1.68	—0.65
AK1 ♂	46	2.5	1.73	1.94	—0.21
AK6 ♂	46	2.5	1.50	1.79	—0.29
AZ3 ♀	46	1.3	0.69	0.99	—0.30
BE7 ♀	62	2.0	1.54	1.45	+0.09
AS4 ♀	63	2.5	1.43	1.84	—0.41
AE4 ♀	88	4.5	2.60	3.35	—0.75
AD2 ♂	89	4.5	2.53	2.94	—0.41
AC1 ♂	107	4.5	2.57	3.24	—0.67
TT4 ♀	133	6.0	2.88	3.73	—0.85
WW8 ♂	133	6.5	3.11	4.34	—1.23
UU5 ♀	135	6.0	2.79	4.56	—1.77
OO1 ♂	163	8.5	4.91	4.93	—0.02
NN5 ♂	167	12.0	6.52	6.30	+0.22
KK7 ♂	177	7.0	3.61	5.76	—2.15
KK3 ♀	184	6.0	3.03	4.73	—1.70
PP6 ♀	185	9.0	4.31	5.65	—1.34
JJ2 ♀	198	8.0	3.66	5.03	—1.37
T1 ♂	279	7.5	3.68	5.45	—1.77
S4 ♂	289	8.0	4.84	4.51	+0.33
R5 ♀	292	7.0	3.90	4.57	—0.67
I7 ♂	379	8.7	4.78	5.64	—0.86
G6 ♀	380	8.5	4.71	5.20	—0.49
G1 ♂	383	9.0	5.11	5.72	—0.61

[a] Total body water by the desiccation method.

[b] Total body water by the tritium method.

body weight of 3.5 kg. Between a body weight of 0.2 and 3.5 kg the volume of TBW increased at a rate of 0.738 liters/kg, and the rate was significantly different ($p < .001$) from the rate measured by desiccation over the same weight interval. Above a body weight of 3.5 kg the volume measured by tritium increased at a rate of 0.552 liters/kg, and there was a significant difference between this rate and the rate for body weights between 0.2 and 3.5 kg ($p < .001$), but there was no significant difference in the rates mea-

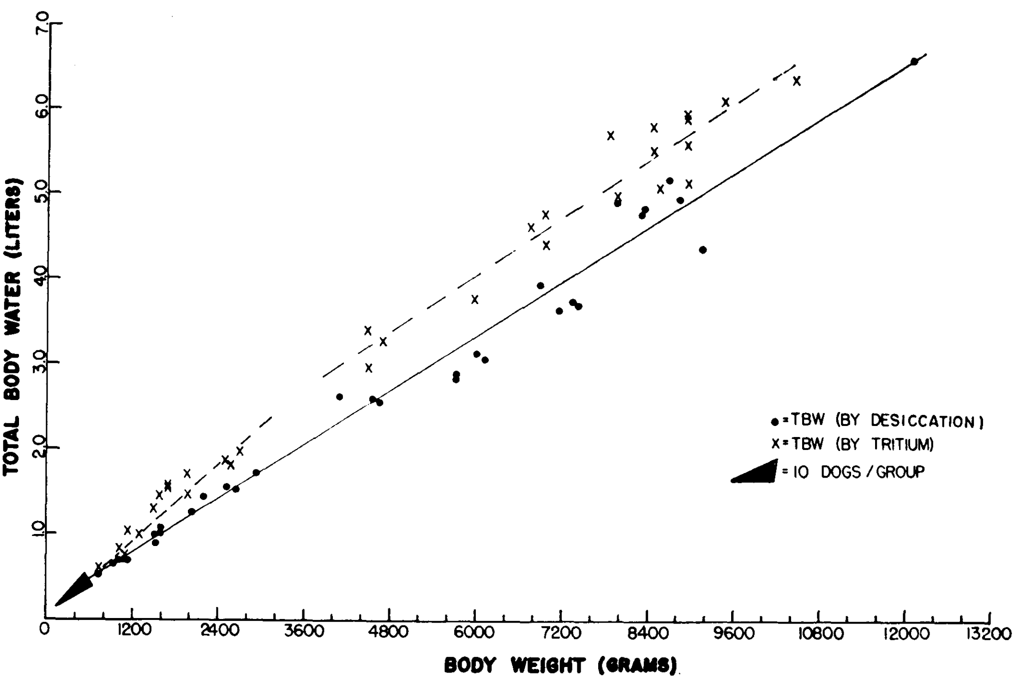

FIG. 1. TBW was first determined with tritium and then by desiccation on the same dog. Paired data on TBW of 42 dogs were plotted. Solid triangle represents paired data for 10 dogs. Two lines were drawn to describe the rate of increase in the volume of TBW determined by the tritium method with body weight. For line 1 (0.2–3.5 kg body weight), $A = 0.738$ liters/kg body weight, $B = 0.070$ liters, $SEE = \pm 0.105$ liters; and for line 2 (3.5–10.5 kg body weight), $A = 0.552$, $B = 0.677$, $SEE = \pm 0.290$. Slope 1 was significantly different from slope 2 ($p<.001$). One line was used to describe the rate of increase in the volume of TBW determined by the desiccation method with body weight: $A = 0.521$, $B = 0.166$, $SEE = \pm 0.208$. Between a body weight of 0.2 and 3.5 kg, slope 1 of TBW determined by the tritium method was significantly different from that determined by the desiccation method ($p<.001$). Between a body weight of 3.5 and 13 kg, slope 2 of TBW determined by the tritium method was not different from that determined by the desiccation method ($p>.6$).

sured by the two methods. However, the mean volume of TBW measured by tritium was 1 liter larger than that measured by desiccation, and the difference was significant, $p<.001$.

The volume of TBW expressed as a percentage of body weight decreased with an increase in body weight (Fig. 2). With the tritium method, a single rate best described the data; the volume of TBW was 84% of the body weight at birth and decreased at a rate of 2.6%/kg. With the desiccation method, the decrease in the volume of TBW expressed as a percentage of body weight was first described by two different rates with the change in rate at a body weight of 3.5 kg, the rates being 6.2%/kg and 0.1%/kg, respectively, with a significant difference between them, $p<.05$. However, the two rates were not significantly different from a single rate (2.0%/kg) calculated from all the data. With a single rate, the body water content calculated by the desiccation method was 68% at birth and decreased with an increase in body weight at a rate of 2.0%/kg. This rate was not significantly different ($p>.9$) from that measured by the tritium method. Throughout growth there was a mean difference of 13.8% ($p<.001$) between the two methods.

The data from both methods were recalculated on a percentage fat-free basis and plotted against body weight (Fig. 3). Again, there was no statistical difference between the two rates of decrease ($p>.1$), but the triti-

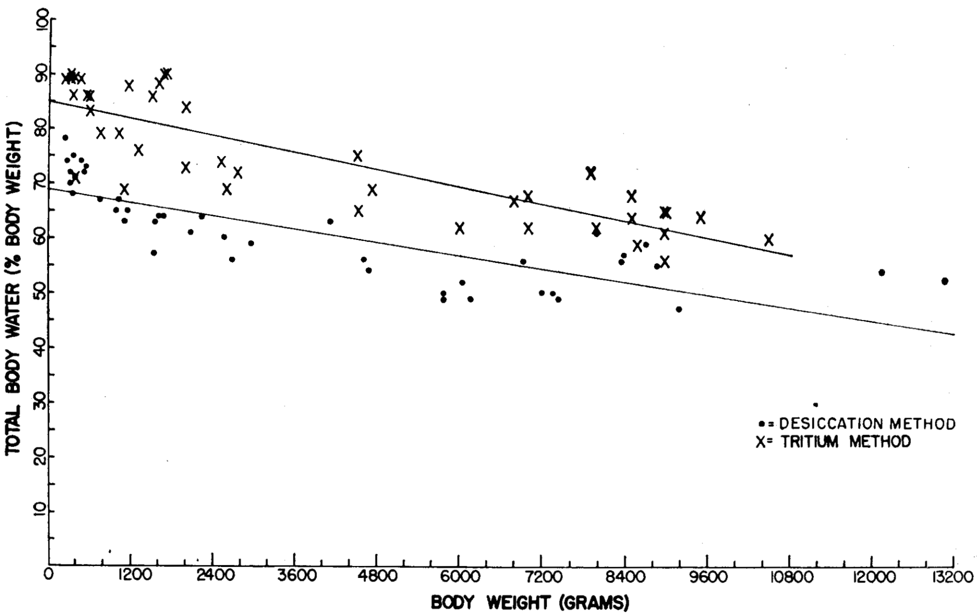

FIG. 2. Single lines were used to show the rates of decrease in the volumes of TBW expressed as a percentage of body weight with body weight by the tritium and the desiccation methods. For the tritium method, A = −2.62% body weight/kg body weight, B = 85.1% body weight, SEE = ±5.8% body weight. For the desiccation method, A = −2.00, B = 68.7, SEE = ±5.2. There was no difference between the two slopes ($p > .9$).

um method measured a volume of 15.6% greater than the desiccation method throughout the growth period. The difference was statistically different from zero ($p < .001$).

Discussion. Aside from exchanging with the hydrogen in water, there is an exchange between tritium and the hydrogen of other molecules in the body, such as fat, protein, and carbohydrate, and, as a consequence, tritium can be expected to overestimate the volume of TBW by 4% of the body weight (6). Another potential source of error in the measurement of TBW with tritiated water which has received little attention is that of water with a highly restricted motional freedom ("bound water"). If there is a portion of water with which the tritium does not exchange under the conditions of our experiment, the volume of body water would be underestimated. For skeletal muscle, it was estimated that approximately 10% of the muscle water did not exchange with deuterium in 24 hr (11), while Ling *et al.* calculated that 5% of muscle water was "tightly bound" (12). As skeletal muscle constituted 17–45% of the body weight of the beagles in this study, and assuming that "bound water" in the muscle was 10% of muscle water, the underestimation of TBW by tritium could range between 2 and 4% of the body weight. The underestimation of the volume of TBW caused by "bound water" apparently would be counterbalanced approximately by the overestimation caused by the exchange of hydrogen between tritium and macromolecules. If "bound water" exists in tissues other than skeletal muscle, the underestimation of TBW by tritium would be greater than postulated. This area is in need of further investigation.

The direct method, too, has its errors in the measurement of TBW. These are principally four in number: first, weighing errors; second, the loss of tissue during dissection; third, loss of water from the tissue during dissection; and fourth, errors during homogenization of the tissue. The total effect of these errors as calculated for these experiments was not more than ±4%. "Bound water" may also cause an underestimation of TBW by desiccation, but the magnitude of

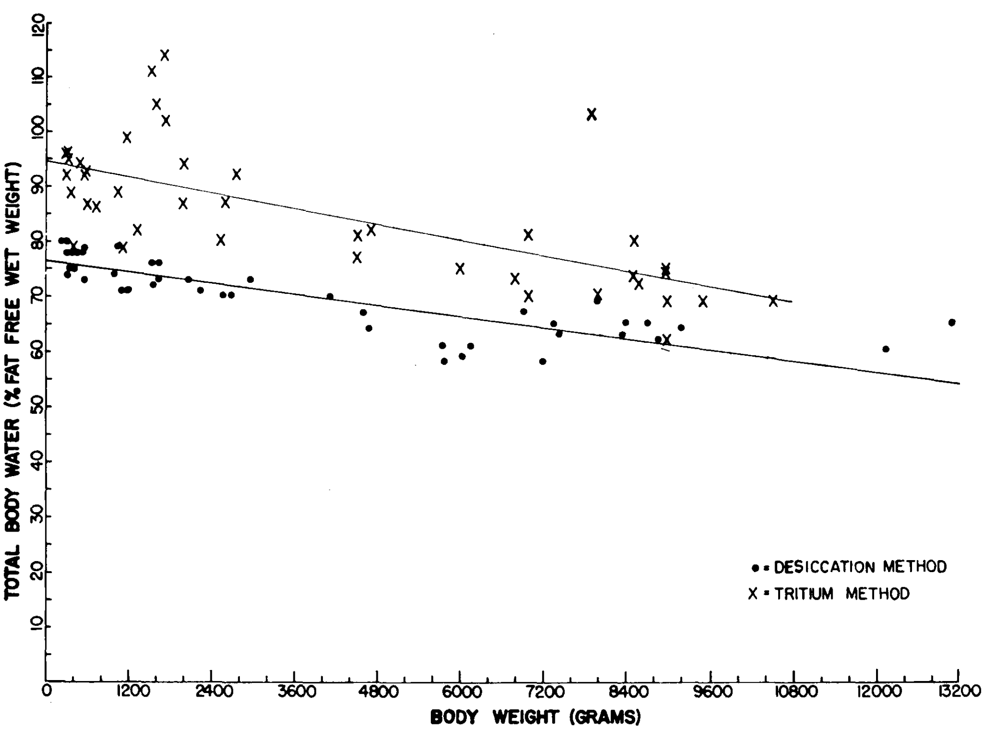

FIG. 3. Single lines were used to show the rates of decrease in the volumes of TBW expressed as a percentage of fat-free wet weight with body weight by the tritium and the desiccation methods. For the tritium method, A = −2.42% fat-free wet weight/kg body weight, B = 94.8% fat-free wet weight, SEE = ±8.9% fat-free wet weight. For the desiccation method, A = −1.73, B = 76.3, SEE = ±3.5. There was no difference between the two slopes ($p > .1$).

the underestimation is not known. However, because the tissues were maintained at 100° for at least 18 hr, its effect would probably be negligible, although we cannot be certain.

The two methods, tritium and desiccation, measured a significantly different volume of body water: the difference between them when expressed as an absolute amount averaged 1 liter for dogs over a body weight of 3.5 kg, and when expressed as a percentage of body weight and fat-free wet weight, averaged 13.8% and 15.6%, respectively, from a body weight of 0.2–13 kg. The difference in results between the two methods definitely exceeded the experimental and technical errors of the methods, and suggests that the "labile hydrogen pool" which exchanged with tritium may exceed the 4% previously reported (6). In spite of the significant difference between the two methods the rates of decrease of TBW were parallel throughout growth when the data were expressed either on a body weight or fat-free weight basis, but this was true only for dogs above a body weight of 3.5 kg if the TBW was expressed in absolute volumes. Therefore, the volume measured by tritium will reasonably approximate those obtained by desiccation if correction factors of 14% and 16% are applied to the tritium volumes when they are expressed as percentage of body weight and percentage of fat-free wet weight, respectively. If the dogs weigh more than 3.5 kg and the volumes measured by tritium are expressed as absolute volumes, a correction factor of 1 liter can be used. However, below a body weight of 3.5 kg, a single correction cannot be applied because from a body weight of 0.2–3.5 kg there was a gradually increasing discrepancy between the two measurements until the mean difference reached 1 liter at a body weight of 3.5 kg. The present data are insufficient to provide a final answer to the cause of the discrepancy between the two methods, but the data do suggest a direction for further investigation to resolve the reason for the difference.

If we assume that both methods measure the water in the body, then if TBW is expressed as a percentage of the fat-free wet weight, the SEE of the regression line should be less than that expressed as percentage of body weight. This is so because, of the tissues comprising body weight, fat is the most variable from animal to animal and contains less water than skeletal muscle (13). As predicted, the SEE of the regression line for the desiccation method decreased from 5.2% when the TBW volumes were expressed as a percentage of body weight to 3.5% when they were expressed on a fat-free wet weight basis. However, when the data for tritium were expressed on a fat-free wet weight basis, the SEE for the regression line was 8.9%, a decided increase from the SEE of 5.8% when the data were expressed as a percentage of body weight.

A significant difference between the measurement of the volume of TBW by tritium and desiccation was not found in comparable experiments on the rat, pig, sheep, and goat, although the number of comparisons for any of the species were few. While in our experiment there were more data over the growth period than in previous investigations, we do not know why the results for the beagle differed from those of the other species studied.

Summary. Total body water (TBW) was measured by tritium and desiccation in 43 beagles between 0.2 and 13 kg body weight. With desiccation, TBW increased at a single rate of 0.521 liters/kg body weight while with tritium there were two rates of increase, 0.738 liters/kg between a body weight of 0.2 and 3.5 kg and 0.552 liters/kg above a body weight of 3.5 kg. Above 3.5 kg, tritium measured a mean volume 1 liter larger than desiccation ($p < .001$). The TBW measured by tritium was 84% of the body weight at birth and decreased at a rate of 2.6%/kg, while with desiccation the TBW was 68% at birth and decreased at a rate of 2.0%/kg. The rate of decrease between methods was not statistically different; the mean difference between them was 13.8% ($p < .001$). On a fat-free wet weight basis there was no significant difference between the rates of decrease, but tritium measured a 15.6% larger volume than desiccation ($p < .001$).

We acknowledge the technical help given us by Mrs. Dorothy Barber and Mr. William Sears. Also, it is a pleasure to thank the Texas Department of Corrections for permitting and assisting us to maintain our dog colony at Wynne Unit at Huntsville, and particularly Lieutenant Percy Crooks. Finally, without the thoughtfulness and constant help of Mr. Edward Crowell the completion of this study would have taken much longer.

---

1. Friis-Hansen, B., Acta Paediat. **46**, Suppl. 110 (1957).

2. Fee, B. A., and Weil, W. B., Jr., Ann. N.Y. Acad. Sci. **110**, 869 (1963).

3. Flynn, M. A., Hanna, F., Long, C. H., Asfour, R. Y., Lutz, R. N., and Zobrisky, S. E., *in* "Body Composition in Animals and Man," p. 480. Nat. Acad. Sci., Washington, D.C. (1968).

4. Wood, A. J., and Groves, T. D. D., Can. J. Anim. Sci. **45**, 8 (1965).

5. Panaretto, B. A., *in* "Body Composition in Animals and Man," p. 200. Nat. Acad. Sci., Washington, D.C. (1968).

6. Foy, J. M., and Schneiden, H., J. Physiol. **154**, 169 (1960).

7. Mattila, M. A. K., Acta Anaesth. Scandinav. Suppl. **37**, 24 (1970).

8. Huggins, R. A., Deavers, S., and Smith, E. L., Pediat. Res., in press (1971).

9. Udekwu, F. A. O., Kozoll, D. D., and Meyer, K. A., J. Nuclear Med. **4**, 60 (1963).

10. Diem, K. (Ed.), "Documenta Geigy. Scientific Tables," 6th Ed., p. 173–177. Geigy Pharmaceuticals, Ardsley, N.Y. (1962).

11. Hazelwood, C. F., and Nichols, B. L., Nature (London) **222**, 747 (1969).

12. Ling, G. N., and Negendank, W., Physiol. Chem. Phys. **2**, 15 (1970).

13. Widdowson, E. M., *in* "Body Composition in Animals and Man," p. 71. Nat. Acad. Sci., Washington, D.C. (1968).

Received Feb. 8, 1971. P.S.E.B.M., 1971, Vol. 137.