Growth of the Beagle: Changes in the Body Fluid Compartments[1] (36137)

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The volumes of total body water (TBW) and extracellular water (ECW) expressed as a percentage of body weight for a number of species of animals appear to be less for the adult than for the newborn, but the variability in the data on the intracellular water (ICW) compartment permits only the tentative conclusion that there may be either a small increase or no change with growth (1–3). Data on the volumes of the body fluid compartments are available also for various ages between birth and maturity for several species of animals including the human (1–4). However, the number of individuals studied at any age and the number of different ages studied during this time period are insufficient to permit determinations of the pattern and rate of change in the body fluid compartments with growth. These data for the beagle are presented in this paper.

Materials and Methods. The beagles came from a colony maintained at the Wynne Unit of the Texas Department of Corrections at Huntsville. Details of the management of the colony have been published earlier (5).

The fluid compartments of the body were determined using the dilution principle. TBW and ECW compartments were measured simultaneously by tritiated water and sodium thiocyanate, respectively, in beagles ranging in age from 0 day to 5 years. The dogs were anesthetized with a combination of morphine sulfate given subcutaneously and sodium pentobarbital given intraperitoneally or intravenously. The dosage varied with the age and weight of the dogs. In very young puppies only 1 mg of morphine was given. Dogs between 1 and 6 months of age were given 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 dog was laid in a supine position, and one of the external jugular veins was exposed for the insertion of a catheter into the right heart. After a control blood sample was drawn, known amounts of tritium oxide (10 μCi/kg) and 5% sodium thicyanate solution (0.5 ml/kg) were injected through the catheter and flushed with 0.9% saline. Equilibration of the tritiated water and thiocyanate was achieved in 1.5 (younger pups) to 2.5 hr (older dogs). Heparinized blood samples of 1.5 to 8 ml (depending on the size of the dog) were collected at either 1.5 or 2.5, 3, and 4 hr after the injections. The blood samples were centrifuged and the plasma was analyzed for tritium and thiocyanate by the techniques of Udekwu *et al.* (6) and Eder (7), respectively. A tritium standard was prepared for each dog by adding 0.1 ml of 1:100 dilution of the injected tritium to 0.4 ml of the control plasma. This standard was treated and counted in the same manner as the plasma samples. A standard curve was plotted for thiocyanate relating various concentrations of thiocyanate solutions with their optical densities. The techniques were modified for young puppies. The plasma proteins were precipitated by 10% trichloroacetic acid. After centrifugation, for analysis of tritium, 0.5 ml of the supernatant was pipetted into 15 ml of scintillation fluid for counting in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.); for analysis of thiocyanate, 2 ml of the supernatant was mixed with 1.5 ml of ferric nitrate reagent and the color formed was read at 460 mμ in a Bausch and Lomb Spectronic 20 colorimeter. The plasma concentrations of tritium and

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thiocyanate were plotted against time on semilogarithmic paper; the curves were extrapolated back to zero time and the concentrations of these two substances were read at the intercept. TBW, ECW, and ICW were calculated as follows:

$$\text{TBW (ml)} = \frac{\text{total counts injected/min}}{\text{counts in 1 ml of plasma/min}},$$

$$\text{ECW (ml)} = \frac{\text{amount of thiocyanate injected (mg)}}{\text{mg of thiocyanate/ml of plasma}},$$

$$\text{ICW (ml)} = \text{TBW}-\text{ECW}.$$

The dogs were grouped according to age as shown in Table I, and at least two different litters were represented in a group. Lines of best fit for the different plots were calculated using the data for each dog. The equation $y = ax + b$; where y is the dependent variable; x, the independent variable; a, the slope of the line; and b, the intercept, was used (8). 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 ($p < .05$) from each other, and from that of a single line, they were considered valid lines.

Results. The increase in mean body weight with age (Fig. 1) was described best by three significantly different rates: the first extended from the day of birth (0 day) through 1.5 months, the second from months 2 through 5 and the third from the age of 5 months through year 5. The rate of increase in body weight was most rapid between months 2 and 5, after which the rate of increase was relatively small, but was still significantly different from 0 ($p < .001$).

The mean volumes of TBW, ECW, and ICW increased with age and with increasing body weight (Table I). The volumes of TBW and ECW increased with an increase in body weight at three significantly different rates, but ICW increased at only one rate (Table II). The most rapid rate of increase

TABLE I. Mean Volumes (liters) of Body Fluids at Various Ages.

Age	No. of dogs	Body wt (kg)	Body fluid compartments		
			Body water[a]	Extracellular[b]	Intracellular[c]
0 day	9	0.32 ± 0.02[d]	0.27 ± 0.02[d]	0.17 ± 0.01[d]	0.10 ± 0.01[d]
1 day	9	0.28 ± 0.01	0.24 ± 0.01	0.18 ± 0.01	0.06 ± 0.004
8 days	10	0.48 ± 0.04	0.41 ± 0.03	0.25 ± 0.02	0.16 ± 0.01
9–10 days	10	0.56 ± 0.04	0.45 ± 0.04	0.27 ± 0.02	0.18 ± 0.02
2 weeks	10	0.92 ± 0.04	0.71 ± 0.03	0.44 ± 0.02	0.27 ± 0.02
4 weeks	8	1.40 ± 0.12	1.21 ± 0.11	0.61 ± 0.06	0.60 ± 0.06
5 weeks	10	1.69 ± 0.07	1.43 ± 0.04	0.95 ± 0.03	0.49 ± 0.03
6 weeks	10	2.3 ± 0.2	1.63 ± 0.10	1.07 ± 0.09	0.56 ± 0.03
8 weeks	9	2.2 ± 0.1	1.59 ± 0.11	0.87 ± 0.08	0.72 ± 0.05
2.5 months	5	2.7 ± 0.1	1.98 ± 0.05	1.00 ± 0.03	0.99 ± 0.04
3 months	10	4.6 ± 0.1	3.12 ± 0.12	1.69 ± 0.05	1.43 ± 0.08
4 months	16	7.2 ± 0.3	4.64 ± 0.18	2.59 ± 0.12	2.05 ± 0.09
5 months	16	8.9 ± 0.4	5.49 ± 0.24	2.63 ± 0.11	2.86 ± 0.16
6 months	14	8.9 ± 0.6	5.60 ± 0.30	2.88 ± 0.13	2.72 ± 0.19
9 months	9	9.5 ± 0.4	5.82 ± 0.28	2.81 ± 0.15	3.01 ± 0.28
1 year	9	10.2 ± 0.5	5.99 ± 0.25	2.74 ± 0.19	3.25 ± 0.22
2–5 years	9	12.5 ± 0.7	6.24 ± 0.18	2.90 ± 0.09	3.34 ± 0.13

[a] Tritium space.
[b] Thiocyanate space.
[c] Difference between tritium and thiocyanate space.
[d] Mean ± 1 SEM.

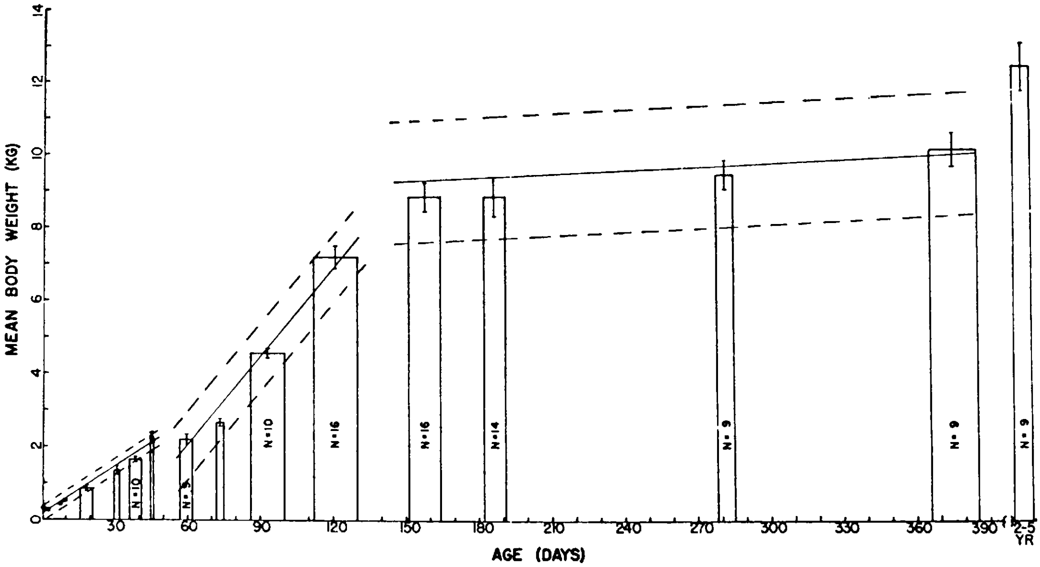

FIG. 1. The width of the columns represents the range in age, and the vertical line through the mean, ± 1 SEM. Where the columns were too narrow to include the number (N) of dogs, or where dots were used to represent the mean body weight, the number of dogs starting at 0 day were successively: $N1 = 9$, $N2 = 9$, $N3 = 10$, $N4 = 10$, $N5 = 10$, $N6 = 8$, $N8 = 10$, and $N10 = 5$. The three regression equations are: for line 1 (0–46 days) $y = 0.042(x) + 0.201$; for line 2 (57–130 days) $y = 0.080(x) - 2.710$; for line 3 (150 days–5 years) $y = 0.003(x) + 8.676$; (- -) ± 1 SE of the line. The slope of line 1 was significantly different from the slopes of lines 2 and 3; the slope of line 2 from line 3; and the slope of line 3 from 0; $p < .001$ in each case.

TABLE II. Regression Equations for Growth of Body Fluid Compartments.

Variables				
Body wt (kg) x	Compartment (liters) y	Slope a	Intercept b	± 1 SEE[a]
0.2–3.0 (92)[b]	TBW	0.714	0.073	0.093[c]
3.5–8.0 (34)	TBW	0.631	0.205	0.276[c]
8.1–16.5 (51)	TBW	0.345	2.448	0.479[c]
0.2–3.0 (92)	ECW	0.409	0.066	0.110[d]
3.5–8.0 (34)	ECW	0.319	0.223	0.213[d]
8.1–16.5 (51)	ECW	0.112	1.741	0.378[d]
0.2–16.5 (177)	ICW	0.298	0.032	0.308

[a] One standard error of estimate of the line.

[b] Number of dogs.

[c] TBW: The slopes of the lines differed significantly from each other ($p < .0001$).

[d] ECW: The slopes of the lines differed significantly from each other ($p < .05$ between body weights of 0.2–3.0 and 3.5–8.0 kg, while $p < .0001$ between body weights of 3.5–8.0 and 8.1–16.5 kg).

in volume for both TBW and ECW was from birth to a body weight of 3 kg, the second most rapid rate was between a body weight of 3 and 8 kg, while the slowest rate of increase was above a body weight of 8 kg.

The fluid compartments when expressed as a percentage of body weight decreased with an increase in body weight (Table III). Again, three significantly different rates best described the decrease in TBW and ECW, with the most rapid decrease occurring between a body weight of 0.2 and 3.0 kg, after which the rates of decrease were relatively slow. Although the ICW compartment decreased at a constant rate of 0.02%/kg throughout growth, the slope was not different from 0 ($p > 0.8$); thus, the ICW compartment remained a constant percentage of the body weight.

Discussion. In the beagle, the volume of TBW measured by tritiated water was larger than that measured by desiccation of the whole carcass by a mean of 13.8 ± 1.04% of the body weight throughout the growth period (9). Sodium thicoyanate also overestimated the volume of ECW, but it was difficult, on the basis of published data, to state with precision the extent of the overestimation (10). However, on the basis of the results of other investigators (1) and in the present investigation, as both substances gave statistically reproducible results with repetitive determinations in the same animal and for dogs within the same age group, the directional changes in these volumes with growth would appear to be valid.

In spite of the variability of the data in the human, the volume of TBW expressed as a percentage of body weight clearly decreased with growth, but the amount of decrease cannot be determined. However, the data of the present investigation permitted a

TABLE III. Regression Equations for Body Fluid Compartments as a Percentage of Body Weight.

Variables				
Body wt (kg) x	Compartment (% body wt) y	Slope a	Intercept b	± 1 SEE[a]
0.2–3.0 (92)[b]	TBW	—5.000	86.6	6.03[c]
3.5–8.0 (34)	TBW	—0.480	69.6	4.18[c]
8.1–16.5 (51)	TBW	—1.834	77.6	4.31[c]
0.2–3.0 (92)	ECW	—5.971	57.3	7.35[d]
3.5–8.0 (34)	ECW	—0.545	39.1	3.13[d]
8.1–16.5 (51)	ECW	—1.395	42.9	3.76[d]
0.2–16.5 (177)	ICW	—0.022	30.6	6.37

[a] One standard error of estimate of the line.

[b] Number of dogs.

[c] TBW: The slopes of the lines differed significantly for body weights between 0.2–3.0 and 3.5–8.0 kg ($p < .0001$), while the slopes were not different for body weights between 3.5–8.0 and 8.1–16.5 kg ($p > .05$).

[d] ECW: The slopes of the lines differed significantly for body weights between 0.2–3.0 and 3.5–8.0 kg ($p < .0001$), while the slopes were not different for body weights between 3.5–8.0 and 8.1–16.5 kg ($p > .1$).

statistical analysis of the rates of decrease in the body fluid compartments expressed as a percentage of body weight with growth of the beagle. The volumes of TBW and ECW expressed as a percentage of body weight decreased at three significantly different rates with an increase in body weight. The change in rates occurred at body weights of 3.0 and 8.0 kg, corresponding to the ages of 1–2 and 4–5 months, respectively. At the age of 5 months, the volume of ECW, which up to this time had been larger than that of ICW, now occupied a space less than that of ICW. A similar finding has been reported for the human, although at a different age (3). However, in the human, the change in the relationship of the percentage of ECW and ICW was the consequence of a disproportionate decrease in ECW, while in the beagle it was the consequence of a proportionate decrease in both the volume of TBW and ECW with no change in ICW. The volume of ICW remained a constant percentage of the body weight throughout the growth period.

Friis-Hansen (3) has published considerable data on TBW measured indirectly by deuterium dilution and reported a mean value of 78.7% of the body weight with a range from 71.2 to 83.5% for the newborn. Other investigators have used tritiated water to measure the volume of TBW of the newborn: one group reported a mean of 87% of body weight for males and 84% for females (6) while another reported a means of 74% for 11 males and 79% for 7 females (11).

The number of newborn in which TBW was determined directly by desiccation was even fewer. A summary of the older data gave a mean of 71.4% of the body weight with a range from 66.0 to 80.0% (3), while more recently Fee and Weil (12) reported a mean of 84.1% with the range from 77.4 to 91.1% on 13 babies that died in the immediate neonatal period or were stillborn. Clearly there is a discrepancy of some consequence in the measurement of the volume of TBW in the newborn as the spread of the data was from 71 to 87% of the body weight for the indirect method and from 66 to 91% for the direct method. Further, the data for the direct method should be treated with caution because of the pathology involved in the deaths of the newborn. The differences in the physical status of the newborn, for example the degree of fatness, might account for some of the differences between the data collected by the indirect method, but certainly not for all; nor did a review of the methodology used by the different investigators suggest that technical errors of any significance would account for differences of this magnitude.

For the adult human, direct determination of the volume of TBW has been reported on five adult cadavers, four males and one female (13, 14). The mean volume of TBW was 59% of the body weight with a range from 51 to 70%. When tritiated and deuteriated water were used to measure the volume of TBW, the means ranged from 54.5 to 62.2% of the body weight with a greater range for the individual values [(14), Table III].

Summary. In the present investigation in the beagle, there was a decrease in both the TBW and ECW compartments expressed as a percentage of body weight with growth, but the ICW compartment did not change significantly. The decrease in the TBW and ECW compartments occurred at three different rates: the first significant change in rate occurred between the ages of 1 and 2 months and the second between 4 and 5 months. The volumes of TBW and ECW calculated as a percentage of body weight were 85 and 56%, respectively, in the newborn and 60 and 29%, respectively, in the 1-year-old, while ICW was 30.6% throughout growth.

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