Changes in Red Cell Volume, Venous Hematocrit, and Hemoglobin Concentration in Growing Beagles[1] (35564)

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The blood volume (ml/kg body wt) of a number of species of animals is significantly higher at birth than when they become adults (1–4). Whether this is true in the human is less clear because of the variability of the data for the newborn (5–8). In a number of species including the human there is a period during the early part of growth in which there is a continuous decrease in venous hematocrit, hemoglobin content, total hemoglobin, and in the red cell volume per unit body weight (1, 2, 8–11). In the dog, the change in the relationship of these variables to body weight and to each other with growth has received little attention. Therefore, the changes in these parameters were measured in beagles from the day of birth to 1 year of age.

Materials and Methods. The data were gathered from our colony of purebred beagles ranging in age from 1 day to 1 year. Maintenance of the colony has been described elsewhere (12).

All animals were sedated with morphine sulfate subcutaneously or anesthetized with morphine subcutaneously and sodium pentobarbital intraperitoneally or intravenously. The dosage of drugs and route of administration were adjusted to the age (size) of the pup. There were no significant differences between measurements done on morphine sedated or fully anesthetized (morphine and pentobarbital) pups; however, since the 1- to 2-week-old puppies did not tolerate anesthesia well, they were sedated with 0.5 to 1 mg of morphine sulfate alone. Dogs less than 3 months old received 2–5 mg/kg of morphine and 7–15 mg/kg of pentobarbital, and those 3 months or older received the full adult doses of morphine (10 mg/kg) and pentobarbital (15 mg/kg).

Red cells were tagged with ^{51}Cr using a modification of the method of Gray and Sterling (13). A portion of the mother's blood was tagged for pups younger than 7 weeks old (weaning age), a littermate's blood from 7 weeks until they were 3 months old, and the pups' own blood after the age of 3 months. From 10 to 20 ml of whole blood was incubated for 30 min at room temperature with 2 μCi of radioactive sodium chromate/ml of blood. At the end of 30 min, ascorbic acid was added to prevent further tagging and the blood was centrifuged. The plasma was discarded and the cells were washed twice with 0.9% saline. Then the tagged cells were resuspended in saline to the original volume and a standard was prepared for counting. The volume of tagged red cells injected varied from 0.5 ml in very young to 10 ml in 1-year-old dogs. Blood samples (1.2 ml) were drawn into heparinized syringes from the jugular vein either through a catheter or by veni puncture 10, 20, 30, and 60 min after injection. One half to 1 ml of whole blood was pipetted into counting vials and saline was added to bring the total volume to 2 ml. The samples were monitored in a deep-well scintillation counter with a pulse height analyzer, and radioactivity was expressed as counts per minute minus background per milliliter of packed red cells. Red cell volume was calculated from the formula: cell volume = total counts injected $\div$ counts per milliliter of packed red cells. Hematocrits were done on all blood samples by the micromethod. No correction was made for trapping of cells. Hemoglobin concentration was determined by a Spencer Hb meter. The pups were divided into groups

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

TABLE I. Body Weight, Venous Hematocrit, Red Cell Volume, Blood Volume, and Hemoglobin in Beagles from 1 Week to 12 Months of Age.

	Weeks									Months					
	1	2	3	4	5	6	7	8	9	3	4	5	6	9	12
Body wt (kg)	0.356	0.686	0.828	1.42	1.42	1.68	2.20	2.55	3.46	4.97	7.20	8.12	8.62	11.8	12.8
	±0.013[a]	±0.024	±0.039	±0.075	±0.100	±0.071	±0.092	±0.176	±0.124	±0.209	±0.281	±0.457	±0.392	±0.82	±1.37
	(64)[b]	(57)	(19)	(13)	(15)	(13)	(13)	(15)	(19)	(19)	(21)	(16)	(16)	(10)	(6)
Venous HCT (%)	41.2	33.4	29.8	30.2	26.5	29.0	29.2	29.3	31.2	32.4	35.4	44.3	44.1	44.4	44.1
	±1.00	±0.50	±0.59	±1.33	±0.88	±0.83	±0.76	±0.76	±0.73	±0.67	±0.50	±1.51	±0.94	±2.33	±2.05
p values[c]		<0.001	<0.001		<0.02	<0.02					<0.001	<0.001			
Cell vol (ml)	13.3	17.6	18.6	31.7	31.1	40.0	58.2	58.1	84.1	142.6	213.5	306	324	449	496
	±0.44	±0.62	±1.01	±2.08	±2.40	±1.52	±4.00	±3.78	±3.26	±6.07	±9.04	±29.2	±19.4	±25.9	±73.8
Cell vol (ml/kg)	39.2	26.2	22.4	22.3	22.1	24.3	26.4	23.1	24.4	29.0	29.4	37.2	36.8	38.5	38.3
	±1.58	±0.68	±0.68	±0.73	±0.93	±1.08	±1.07	±0.70	±0.51	±0.97	±0.45	±2.34	±1.23	±0.96	±1.73
p values		<0.001	<0.001					<0.05		<0.001		<0.01			
Hemoglobin (g/100 ml)	12.2	9.3	7.6	8.7	7.9	8.9	9.2	10.1	10.2	11.6	12.7	15.2	14.3	15.3	16.4
	±0.28	±0.14	±0.13	±0.38	±0.26	±0.22	±0.29	±0.27	±0.14	±0.09	±0.24	±0.23	±0.50	±0.25	±0.28
	(91)	(68)	(10)	(10)	(11)	(11)	(12)	(11)	(11)	(12)	(10)	(4)	(11)	(10)	(3)
p values		<0.001	<0.001	<0.02		<0.01		<0.05		<0.001	<0.001	<0.001			
Blood vol (ml/kg)	103	86	82	82	82	84	79	77	75	82	69	78	82	82	82

[a] Means ± SE.
[b] Number of cases were the same for each parameter of a particular age group except for hemoglobins.
[c] The standard Student's *t* test was used to determine significant differences between two consecutive age groups.

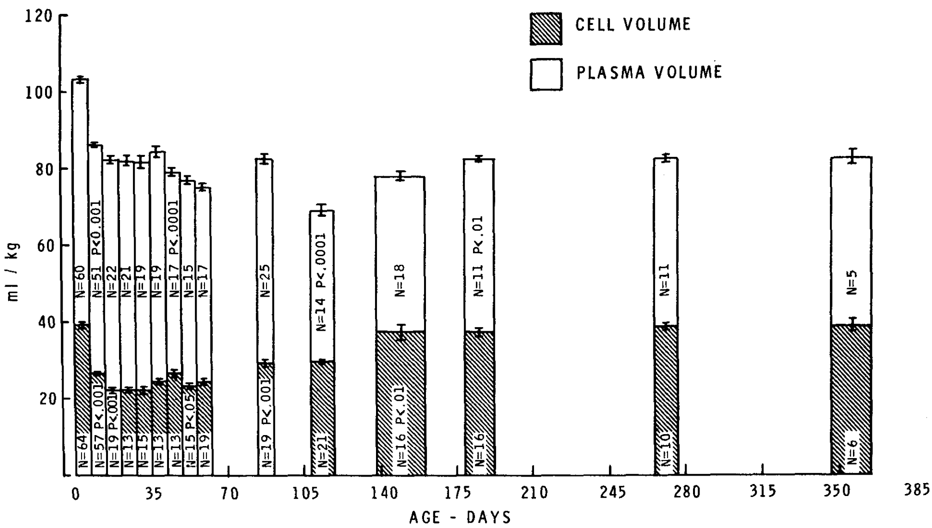

FIG. 1. Mean red cell and plasma volume per unit body weight in beagles: Plasma volume data are from Ref. (12), as explained in text. The width of the columns represent the spread in days of a particular age group. N = number of cases. P values signify the Student's t test differences between a column in which it is recorded and the previous column.

by age, using weekly intervals up to 9 weeks, monthly intervals up to 6 months, and then into 9- and 12-month-old groups.

Results. The data for 291 beagles from 63 litters are presented in Table I.

Red cell volume. Total red cell volume in the beagle increased with age and paralleled the increase in body weight during the 1-year period studied. The red cell volume expressed in milliliters per kilogram of body weight fell significantly from the first to the second week and from the second to the third week. From the third through the ninth week the cell volume varied only slightly. In the third month the red cell volume increased with a further significant increase in the fifth month and no further change through the twelfth month. Red cell volume (ml/kg) was directly related to venous hematocrit. The slope of the line was highly significant with a correlation coefficient of .85.

Venous hematocrit. The mean venous hematocrit of beagle pups declined from the first through the fifth week of age. From the fifth to the sixth week there was a significant increase; then the venous hematocrit varied only slightly up to the fourth month when it again increased significantly. Finally, the adult value was reached in the fifth month.

Hemoglobin. Hemoglobin concentration followed the changes in venous hematocrit. There was a gradual reduction in hemoglobin concentration from the first through the fifth week of age. Starting with the sixth week there was a progressive increase in hemoglobin concentration until the adult level was reached at 5 months of age with no further significant change through the twelfth month.

Blood volume. The blood volume was estimated by taking the present data on red cell volume and combining them with the plasma volume of beagles from our colony having the same age, weight, and venous hematocrit (Fig. 1) (12). The blood volume of beagles 1 week old was higher than that of any other age group. Blood volume decreased sharply during the second week with a downward trend through the ninth week; it then increased in the third month but decreased again in 4-month-old beagles to the lowest volume of any age group. The adult level of blood volume was reached between the fifth and sixth months. The blood volume, when calculated from the red cell volume and the venous hematocrit, followed the same general trend as the sum of cell and plasma volume.

Discussion. In beagles, during the period between birth and 1 year of age, several significant changes were measured in each of the variables. During the first 5 weeks after birth there was a gradual reduction in the venous hematocrit, unit red cell volume (ml/kg), and hemoglobin concentration, and the reductions were parallel to each other. In 5-week-old beagles the venous hematocrit was 26.5 ± 0.88%; red cell volume, 22.1 ± 0.93 ml/kg; and hemoglobin concentration, 7.9 ± 0.26 g/100 ml. These low values confirm that an anemic period exists in the beagle during the early rapid phase of growth, as has been reported for a number of other animals including the human (1, 2, 8–11). The cause of the anemia is not clear. In the rat (2), evidence suggests that, although during the anemic period red cell production was at its maximum, it would not keep up with the very rapid rate of increase in body weight. The administration of iron prevented the early anemia with growth, which was also seen in the pig (10). These data suggest that the anemia during the early period of growth was the result of a deficiency of iron in the milk of the mother, but no one has tested this in the dog.

The increases in venous hematocrit, red cell volume, and hemoglobin concentration which begin in the sixth week were associated with the weaning period, a phenomenon seen in other species (1, 2, 9–11, 14). Other physiological events which appear to be associated with weaning are a significant decrease in plasma volume when expressed in milliliters per kilogram of body weight (12), total body water and extracellular water as a percentage of body weight, biochemical and metabolic maturation of the brain, and finally, a significant change in behavior (15). The next significant increase in venous hematocrit, red cell volume, and hemoglobin was between the fourth and fifth months and established the adult values (15).

Whether these various physiological events, which occur at these periods, have any relationship to each other or occur independently of each other cannot be answered on the basis of present knowledge.

The marked fall in blood volume (ml/kg) between the first and second weeks was the result of a significant decrease in both red cell and plasma volumes (ml/kg) (12). The blood volume ratio (ml/kg) was relatively stable between the second and sixth weeks, as there were no significant changes in either cell or plasma volume ratios, but in the seventh week plasma volume ratio decreased markedly (9), and, as a result, blood volume ratio decreased also. In the third month the increase in blood volume ratio was the result of an increase in red cell and plasma volume ratios, while in the fourth month, when the lowest blood volume ratio was measured, the decrease was a consequence of a significant fall in plasma volume ratio. During the fifth and sixth months the adult blood volume ratio was established due to a significant rise in cell volume ratio in the fifth month and a lesser increase of plasma volume ratio in the sixth month.

Summary. Red cell volume (ml/kg), venous hematocrit, and hemoglobin concentration of beagles decreased from the first through the fifth week of age, then increased gradually till at 5 to 6 months the adult values were reached. The reversal of the downward trend coincided with the weaning period. The changes in red cell volume (ml/kg), hematocrit, and hemoglobin concentration were parallel from the first week to the first year of age. Throughout growth, red cell volume (ml/kg) was linear to venous hematocrit. The blood volume (ml/kg) of 1-week-old beagles was higher than that of any other age group, including adults.

We gratefully acknowledge the technical help of Mrs. Dorothy Barber, Mr. William Sears, and Mr. Edward Crowell. Also, it is a pleasure to thank the Texas Department of Corrections for the maintenance of our beagle colony at the Wynne Unit, Huntsville, Texas.

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Received Nov. 30, 1970. P.S.E.B.M., 1971, Vol. 137.