Tolerance to Blood Loss in the Growing Beagle[1] (35747)

S. NAGY,[2] S. DEAVERS, AND R. A. HUGGINS[3]

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

There are few studies available on the effects of hemorrhage in the young dog (1–4). Survival after a period of hypovolemia followed by reinfusion has been studied in two experiments (1, 4) in which a high mortality was observed in puppies less than 4 weeks old. There are, however, no data available on the ability of puppies of different ages to survive a large unreplaced blood loss. In the experiments reported here it was found that within a certain age range the growing beagle has a remarkably high tolerance to blood loss when compared to data (5–7) available for adult dogs.

Materials and Methods. The experiments were performed on 90 pure-bred beagles with ages ranging from 7 days to more than 6 months (adult). Sodium pentobarbital anesthesia was used in a dose of 30 mg/kg body wt given intravenously. In puppies less than 2 weeks old the dose was somewhat reduced and individually adjusted to reach a light level of anesthesia. A carotid artery was exposed and cannulated with polyethylene tubing which was used for blood pressure measurement, bleeding, and sampling for hematocrit. In experiments where plasma volume was measured, a jugular vein was also cannulated for the injection of labeled protein. Arterial blood pressure was measured with either a Statham P23AA transducer or a mercury manometer. The transducer was calibrated against a column of mercury every 30 min throughout the experiments. In dogs more than 1 month old, in addition to blood pressure, a lead II ECG and respiration was recorded, the latter with an impedance pneumograph. All recordings were made on a physiograph (E & M Instrument Co.). Five hundred units of heparin per kilogram body weight were given intravenously.

After a 20-min control period the animals were bled from a carotid artery into a graduated glass reservoir. An attempt was made to lower mean arterial blood pressure to 30 mm Hg as fast as possible. After the first few minutes when the rate of spontaneous bleeding slowed down, blood was withdrawn into glass syringes. When the blood pressure reached 30 mm Hg (usually in less than 10 min), the time was noted and the pressure was regulated between 30 and 35 mm Hg for exactly 30 min by additional withdrawal or injection of small amounts of blood when necessary. This period was designated as controlled hypotension. In the next phase, which we called the compensation period, no more blood was taken from the animals. Recordings were continued for 3 hr from the beginning of the compensation period. The blood taken from the animals was not reinfused. The cannula was then removed, the artery ligated, and the skin incision sutured using clean but not aseptic techniques. Eighty units of penicillin and 100 mg of streptomycin were injected intramuscularly per kilogram body weight. The animals were then returned to the vivarium where food and water were available to them, but no specific treatment was given. Animals living 48 hr after completion of the experiments were considered survivors.

[1] This study was supported by National Institutes of Health Grant USPH HE-11395.

[2] Dr. Nagy was a Visiting Assistant Professor, Department of Physiology, Baylor College of Medicine, supported by National Institutes of Health Grant USPH RR-05425. Present address; Institute of Experimental Surgery, University Medical School, Postafiók 464, Szeged, Hungary.

[3] Address reprint requests to Dr. R. A. Huggins, Department of Physiology, Baylor College of Medicine, 1200 Moursund Avenue, Houston, Texas 77025.

TABLE I. Mean Carotid Arterial Blood Pressure.

Group	1 Week		2 Weeks		1 Month		2 Months		3 Months		4 Months		Adult	
	C[a]	P[b]	C	P	C	P	C	P	C	P	C	P	C	P
Mean (mm Hg)	67	20	80	21	85	33	110	55	111	65	119	80	122	62
20th percentile (mm Hg)	58	12	70	12	72	15	101	32	101	51	110	44	110	32
80th percentile (mm Hg)	76	42	92	34	99	53	120	70	124	84	129	114	133	84

[a] C = Control (prehemorrhage).
[b] P = Posthemorrhage (3 hr after end of bleeding period).

In a second series of experiments plasma volume determinations were performed in the control period and again 1 hr after the beginning of the compensation period to estimate the amount of compensatory fluid shift into the circulation in response to blood loss. ^{131}I-labeled albumin was used as the tracer. To minimize blood loss due to sampling, the dilution space of the tracer was calculated from the activity of a single sample taken 6 min after the injection. A control sample was taken immediately before the second injection to correct for the background radioactivity resulting from the first dose. The volume of each sample was 3 ml. Total proteins in the plasma were calculated from the specific gravity as determined by the falling drop method of Barbour and Hamilton (8).

Nonparametric methods were used for the evaluation of statistical significance (9, 10) and variation (11).

Results. The animals were divided into seven age groups designated as 1 week (7 days), 2 weeks (13–14 days), 1 month (30–35 days), 2 months (60–69 days), 3 months (83–96 days), 4 months (117–129 days), and adult (over 6 months). The blood pressure values in the control period and 3 hr after the beginning of the compensation period are shown in Table I. There was a gradual increase in arterial pressure with age. Three hours after the completion of bleeding, blood pressure was below the control value in each group, but a partial compensation was achieved except in the 1- and 2-week-old groups where pressure declined from the level maintained in the controlled hypotensive period, whereas little change occurred in the 1-month-old group.

Bleeding volumes are shown in Table II. There was a decrease, but without statistical significance ($p>0.1$), in bleeding volumes from 1 week to 1 month. Between 1 and 2 months there was a significant ($p<0.02$) increase in bleeding volumes, which thereafter stabilized at a level near 50 ml/kg.

Survival data are presented in Table III. There were no survivors in the two youngest age groups. Survival rate increased to a maximum of 60% in the 2- and 3-month-old groups and then decreased to 20% in adults.

The amount of fluid entering the circulation after blood loss was less in the adult than in the 3-month-old beagles. There were large variations, however, and the difference is not significant statistically ($p>0.1$). Plasma protein concentration decreased in both groups during the bleeding period and a further decrease occurred in the compensation period. The hematocrit showed little change

TABLE II. Bleeding Volume.

Group	1 Week	2 Weeks	1 Month	2 Months	3 Months	4 Months	Adult
Mean (ml/kg)	46.3	44.3	41.3	49.0	49.6	50.1	49.5
20th percentile (ml/kg)	40.8	40.5	36.2	44.0	44.0	45.8	43.5
80th percentile (ml/kg)	49.3	49.6	47.3	55.0	56.3	52.7	55.2

TABLE III. Survival.

Group	1 Week	2 Weeks	1 Month	2 Months	3 Months	4 Months	Adult
Number of animals	8	10	10	10	10	10	10
Number of survivors	0	0	3	6	6	4	2

from the control values at the end of the bleeding period and some increase at 1 hr from the beginning of the compensation period (Table IV).

Heart rates initially were between 150 and 180 beats/min. Bleeding in the puppies was followed by a relative bradycardia with heart rates of 100–120 beats/min, returning to or above control levels in the compensation period. No bradycardia was observed in the adult group, and heart rates tended to be higher, but not significantly, in the late compensation period than in the younger age groups. There was no difference in heart rates among surviving and nonsurviving animals. Elevation of T waves in the ECG and increased respiratory rates and volumes were frequently observed after hemorrhage.

Discussion. The high survival rate in the 2- and 3-month-old group is surprising considering the low tolerance to hemorrhage reported in younger puppies (1, 4) and confirmed in the present study. Rowe and Arcilla (4) found 59% mortality in 2-hr to 9-day-old puppies after bleeding them 35% of their blood volume and reinfusing it 30 min later. Their animals had to live only 105 min to be classified as survivors. These authors' impression was that if the shed blood were not returned or the observation period prolonged, few animals would have survived. Hollmann, Schaaff, and Schmidt (1) observed a 100% mortality in 16 puppies aged 14–26 days bled to a blood pressure of 40 mm Hg, maintaining this pressure for an average of 62 min and followed by reinfusion.

Comparison of our results with data in the literature on survival of adult dogs of large unreplaced or late replaced blood losses again shows the remarkable ability of the young beagle (2–3 months old) to withstand hemorrhage. Ehrlich, Kramer, and Watkins (5) bled dogs 42% of their blood volume. Mortality was 100% and the mean time of death 298 min. In the experiments of Schmidt and Schmier (6) hemorrhagic shock was produced by the withdrawal of an average of 29.2 ml/kg of blood with reinfusion delayed till between 1 hr and 20 min to 12.5 hr. Only 1 out of 13 animals survived 48 hr. Swan *et al.* (7) found the mean lethal hemorrhage (LH_{50}) causing 50% mortality to be 41.6% of the measured blood volume while LH_{80} was 43.6%.

An approximate idea about the magnitude of blood loss in relation to total blood volume in our experiments can be obtained from data on plasma (12) and red cell and blood (13) volumes in the growing beagle from our colony. Using these data as a basis for calculation, the blood volume would be about 75 and 82 ml/kg, respectively, in the 2- and 3-month-old beagles. This gives a 65 and 60% blood loss in the present experiments. We have not found evidence in the literature for survival of hemorrhage of this order of magnitude in the dog.

The mechanism of the increased tolerance to blood loss is not clear. An increased mobilization of extravascular fluid into the circulation after hemorrhage could serve as a possible explanation. This is, however, unlikely as there was no clear-cut difference in mobilized volumes between the 3-month-old and adult groups 1 hr after the completion of bleeding. The mobilized fluid accounted for only 13.8 and 9.2% of the blood lost in the 3-month-old and adult dogs, respectively. The possibility exists that the effect of an early, more rapid fluid shift into the circulation in the younger animals was obscured by a gradual loss in the mobilized fluid in the later period when the measurement was made. Such a phenomenon was observed by others (1). Lister *et al.* noted a tendency in puppies under 10 weeks of age to overcompensate first the plasma volume deficit and then to reduce plasma volume (2). They concluded, however, that plasma volume expansion follows a basically similar pattern to

TABLE IV. Fluid Shift, Plasma Protein Concentration, and Hematocrit.

Group	3 Months (12 animals)						Adult (10 animals)					
	Fluid shift (ml/kg)	P_1[a]	P_2	P_3	H_1[b]	H_2	H_3	Fluid shift (ml/kg)	P_1	P_2	P_3	H_1	H_2	H_3
Mean	6.6	4.97	4.44	4.35	29.4	28.7	33.2	3.8	5.22	5.01	4.56	40.9	41.5	43.6
20th percentile	1.1	4.02	3.92	3.71	28.0	28.0	28.2	0.3	4.95	4.78	4.24	36.0	39.8	40.0
80th percentile	17.2	5.06	4.97	4.83	32.0	30.3	37.4	9.5	5.37	5.27	4.85	44.6	44.6	48.6

[a] Plasma protein concentration (g/100 ml). P_1 = Control; P_2 = end of bleeding period; P_3 = 1 hr after end of bleeding period.
[b] Hematocrit (%). H_1 = Control; H_2 = end of bleeding period; H_3 = 1 hr after end of bleeding period.

that observed in the adult dog and in man. In any case, it would be difficult to attribute the increased survival rate in puppies to any short-lived differences in the speed of refilling in the early posthemorrhagic period. The refilling process takes 24–48 hr to be complete (2).

The small rise in the hematocrit after bleeding in the present experiments is most probably due to contraction of the spleen in response to blood loss. The lower initial hematocrit in the puppies could play a role in their increased survival rate because increasing the hematocrit above 35% has been shown to decrease the resistance to the development of hemorrhagic shock (14).

Therefore, on the basis of available data, the mechanism involved in the unusual resistance to hemorrhagic shock of 2–3-month-old dogs is not known, but investigations of possible mechanisms are being continued.

Summary. Beagles ranging in age from 7 days to adult were bled to a mean arterial blood pressure of 30–35 mm Hg. After 30 min at this pressure level no more blood was taken and the blood lost was never replaced. In the following 3 hr, blood pressure further declined in puppies 1 and 2 weeks old while there was a partial restoration of pressure in older dogs. Average bleeding volumes ranged from 49 to 50.1 ml/kg in 2-month-old and older dogs and from 41.3 in younger animals. Forty-eight-hour survival rate was zero for 2-week-old or younger animals and was 20% in the adult group. A peak survival rate of 60% was observed in 2- and 3-month-old puppies. There was no statistically significant difference in the magnitude of compensatory fluid shift into the circulation between 3-month-old and adult beagles. Tolerance of 2- and 3-month-old beagles to an acute, large unreplaced blood loss seems to be greater than that of adult beagles and other dogs.

1. Hollmann, G., Schaaff, A., and Schmidt, H. D., Z. Exp. Chir. **1**, 330 (1968).

2. Lister, J., Altman, R. P., Win, M. S., and Hanelin, L. M., J. Pediat. Surg. **2**, 241 (1967).

3. Rowe, M. I., and Arcilla, R. A., Ann. Surg. **172**, 76 (1970).

4. Rowe, M. I., and Arcilla, R. A., J. Pediat. Surg. **3**, 278 (1968).

5. Ehrlich, F. E., Kramer, S. G., and Watkins, E., Surg. Gynecol. Obstet. **129**, 1173 (1969).

6. Schmidt, H. D., and Schmier, J., Pflugers Arch. Gesamte Physiol. Menschen. Liere **298**, 336 (1968).

7. Swan, H., Blavier, J., Marchioro, T., Jenkins, D., and Montgomery, V., Arch. Surg. Chicago **79**, 176 (1959).

8. Hamilton, W. F., *in* "Medical Physics" (O. Glasser, ed.), Vol. 1, p. 427. Year Book Pub., Chicago (1944).

9. Wilcoxon, F., Biometrics **1**, 80 (1945).

10. Wilcoxon, F., Katti, S. K., and Wilcox, R. A., "Critical Values and Probability Levels for the Wilcoxon Rank Sum Test and the Wilcoxon Signed Rank Test." American Cyanamid Co., Pearl River, N.Y. (1963).

11. Herrera, L., J. Lab. Clin. Med. **52**, 34 (1958).

12. Huggins, R. A., Deavers, S., and Smith, E. L., Pediat. Res. **5**, 193 (1971).

13. Deavers, S., Smith, E. L., and Huggins, R. A., Proc. Soc. Exp. Biol. Med. **137**, 299 (1971).

14. Crowell, J. W., Bounds, S. H., and Johnson, W. W., Amer. J. Physiol. **192**, 171 (1958).

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