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EFFECT OF NEMBUTAL ANAESTHESIA ON RESTORATION OF PLASMA VOLUME AFTER HAEMORRHAGE IN DOGS, CATS AND RABBITS

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During an investigation into the effects of haemorrhage on lymph flow and other phenomena in dogs anaesthetized with nembutal, it was observed that after removing about 20% of the animal's blood volume, no haemodilution occurred during the ensuing 4–6 hr. On the other hand, in unanaesthetized dogs, a similar haemorrhage was followed in this time by definite haemodilution. While studying the carbon monoxide and T-1824 disappearance curves in the rabbit, Courtice & Gunton (1949*b*) also noted that nembutal influenced the rate of haemodilution after the removal of small blood samples at intervals during the course of an hour. In the series of nembutalized animals a fall in the haematocrit of approximately 5% occurred, whereas in unanaesthetized rabbits a decrease up to 20% of the original was noted, although similar amounts of blood at the same time intervals were removed.

Since nembutal is commonly used as an anaesthetic for experimental animals, it was decided to investigate its effects on the restoration of the blood volume after haemorrhage in dogs, cats and rabbits.

METHODS

The reabsorption of fluid into the circulation after haemorrhage has been studied by determinations of the haemoglobin percentage, haematocrit, plasma protein concentration and blood volume before and after removal of a known amount of blood. These experiments were performed on unanaesthetized dogs and rabbits, and on dogs, cats and rabbits under nembutal anaesthesia. Anaesthesia was maintained for 4–6 hr. after which the cats were killed, but the dogs and rabbits were allowed to recover and determinations repeated after 24 hr.

Abbot's veterinary nembutal (pentobarbital sodium) containing 1 grain/c.c. was used throughout. It was administered intraperitoneally in dogs and cats, and intravenously in rabbits, in doses of 1 c.c./5 lb. or sufficient to anaesthetize the animal. Repeated smaller doses were given when necessary to keep the animals anaesthetized for the required time.

Dogs, both unanaesthetized and anaesthetized, were bled from the jugular vein by venepuncture and slight suction by negative pressure. The volume removed was 16 c.c./kg. which, according to the figures of Courtice (1943), represents about 20% of the blood volume. The blood samples were also taken from the jugular vein by venepuncture. Cats were bled from a cannula in the femoral artery, and blood samples were taken in the same way. Rabbits were bled from the marginal vein

of the ear. In all experiments food and water were withheld from the unanaesthetized animals for 4–6 hr. after bleeding, the time comparable to the period of anaesthesia in the anaesthetized animals.

Analytical methods. The haemoglobin percentage was determined by the Haldane haemoglobinometer, and the plasma proteins by micro-Kjeldahl digestion and nesslerization. The blood volume in dogs was measured by the dye (T-1824) method described by Courtice (1943) and in rabbits by the CO method described by Courtice & Gunton (1949*b*). The blood volume of anaesthetized cats was also determined by the CO method, using the apparatus for rabbits. Instead of a mask, a tracheal cannula was inserted and connected to the rebreathing circuit. In each of the four cats used, the CO concentration of the blood was estimated at 10, 20 and 30 min. after administration of CO to the closed circuit. The results of these experiments are given in Table 1. In the cat, as in man and rabbit, there is on the average a gradual fall in the CO content of the blood after from 10 to 30 min. The blood volume has been calculated from the 10 min. figure.

TABLE 1. The carbon monoxide content of the blood in anaesthetized cats before and at intervals after the administration of carbon monoxide in the closed rebreathing circuit. Carbon monoxide in c.c. at S.T.P./100 c.c.

Time (min.) ...	0	10	20	30
1	0·09	1·83	1·77	1·68
2	0·06	2·24	2·04	1·96
3	0·18	2·02	2·06	2·07
4	0·12	1·81	1·76	1·76
Mean	0·11	1·98	1·91	1·87

RESULTS

Effect of nembutal anaesthesia on blood of dogs and rabbits. It has been shown by previous investigators (Bollman, Svirbley & Mann, 1938; Hahn, Bale & Bonner, 1943; Jarcho, 1943) that barbiturate anaesthesia causes a decrease in red cell volume, an increase in plasma volume, a fall in plasma protein concentration and an increase in the size of the spleen. These changes have been confirmed in dogs as shown by average results in Table 2. The effect on rabbits is much less pronounced, the degree of dilution being less than that observed in the dog.

TABLE 2. The effect of nembutal anaesthesia on the blood of normal dogs and rabbits

	Before nembutal	Hours after nembutal ½	1	2	4
	(*a*) Mean of four dogs				
Haemoglobin (%)	122	109	105	106	109
Haematocrit	42·5	38·9	—	—	37·3
Plasma proteins (g./100 c.c.)	6·2	6·0	5·8	5·7	5·9
	(*b*) Mean of five rabbits				
Haemoglobin (%)	74	72	73	74	74
Plasma proteins (g./100 c.c.)	5·6	4·9	5·3	5·3	5·5

(*c*) Mean of six dogs

	Before nembutal	½ hr. after nembutal
Total blood vol. (c.c.)	1463	1530
Cell vol. (c.c.)	638	606
Plasma vol. (c.c.)	825	924

The difference in behaviour of these two animals may in part be due to the relative size of the spleen. The dog is known to have a relatively large spleen, so that the deposition of red cells in the spleen after nembutal is probably greater than in the rabbit. That the spleen plays a large part in the effect of nembutal on red cell volume can also be seen in the reaction of splenectomized dogs to nembutal. In these animals no significant fall in haemoglobin percentage occurs after nembutal (Table 2). The action of nembutal on the blood is rapid and, once haemodilution occurs, the haemoglobin percentage remains fairly constant at the lower level for at least 4 hr. with the animal anaesthetized all the time.

Haemorrhage experiments in the dog. Dogs were bled 16 c.c./kg. fairly rapidly, usually 5–15 min. In Table 3 are shown the rates of haemodilution in unanaesthetized and anaesthetized animals. In unanaesthetized dogs the haemoglobin percentage falls to a minimum in 6–24 hr. on the average. In nembutalized dogs, on the other hand, there is no fall, but in individual experiments sometimes a rise in the haemoglobin percentage and haematocrit during the 4–6 hr. of anaesthesia. Next day, when the animal has recovered from the anaesthetic, haemodilution has been observed as expected.

TABLE 3. Effect of bleeding 16 c.c./kg. in normal dogs

(*a*) Unanaesthetized

	Group 1 (mean of 4 dogs)		Group 2 (mean of 6 dogs)		
	Hb (%)	Plasma proteins (g./100 c.c.)	Hb (%)	Haematocrit	Plasma proteins (g./100 c.c.)
Before bleeding	102	6·0	109	39·6	6·0
1 hr. after bleeding	88	5·5	96	—	5·9
2 ,, ,,	87	5·5	96	—	5·7
4 ,, ,,	85	5·3	95	34·4	5·4
6 ,, ,,	82	5·2			
24 ,, ,,	81	5·6			
48 ,, ,,	84	6·2			

(*b*) Anaesthetized

	Group 1 (mean of 7 dogs)		Group 2 (mean of 6 dogs)		
	Hb (%)	Plasma proteins (g./100 c.c.)	Hb (%)	Haematocrit	Plasma proteins (g./100 c.c.)
Before nembutal	—	—	116	41·6	5·7
After nembutal	96	5·4	104	37·3	5·5
1 hr. after bleeding	98	5·0	106	—	5·2
2 ,, ,,	99	5·0	106	—	—
4 ,, ,,	100	4·8	107	37·2	4·9
6 ,, ,,	100	4·8			
24 ,, ,,	88	4·9			
48 ,, ,,	88	5·1			

In two groups of dogs the blood volume has been measured before and 4 hr. after haemorrhage. Table 4 shows that whereas about half the plasma removed

has been restored in this time in unanaesthetized dogs, no restoration has been made in the anaesthetized animals. It will also be observed that in neither group is there an increase in red cell volume, which indicates that with this degree of haemorrhage there is no significant contraction of the spleen. The failure of the haemoglobin percentage to fall after haemorrhage in the anaesthetized dogs is, therefore, not due to a contraction of the spleen. This

TABLE 4. The effect of haemorrhage on the blood volume of unanaesthetized and anaesthetized dogs

	B.V. before bleeding (c.c.)	Blood removed (c.c.)	Theoretical B.V. immed. after (c.c.)	Actual B.V. 4 hr. after (c.c.)
	(*a*) Mean of four unanaesthetized			
Total	1204	238	966	1013
Plasma	724	143	581	648
Cells	480	95	385	365
	(*b*) Mean of four anaesthetized			
Total	1586	351	1235	1224
Plasma	974	215	759	761
Cells	611	136	475	463

can also be shown by observing the effect of haemorrhage in previously splenectomized dogs under nembutal. Here, there is no haemodilution after nembutal, and also no significant restoration of plasma volume after haemorrhage while the animal is anaesthetized. When the anaesthetic has worn off, haemodilution occurs as shown in Table 5.

TABLE 5. The effect of bleeding 16 c.c./kg. in dogs under nembutal anaesthesia for 6 hr. and splenectomized 2–3 weeks previously (mean of 4)

	Hb (%)	Plasma proteins (g./100 c.c.)
Before nembutal	96	5·6
After nembutal	99	5·4
1 hr. after bleeding	95	5·0
2 ,, ,,	94	4·8
4 ,, ,,	95	5·0
6 ,, ,,	95	4·8
24 ,, ,,	77	5·1
48 ,, ,,	79	5·5

Haemorrhage experiments in the cat. Experiments have been carried out only in anaesthetized cats. The blood volume of a cat under nembutal anaesthesia was determined by the CO method, a measured volume of blood removed and the blood volume again determined 2 hr. after haemorrhage. The haemoglobin percentage and haematocrit were further determined at 4 hr. The mean results of four experiments are given in Table 6. It can be seen that no restoration of the plasma volume has been made during this time and no increase in red cell volume has occurred, indicating that the spleen has not added to the circulating red cells. Thus the anaesthetized cat behaves in a manner similar to the dog after haemorrhage.

TABLE 6. The effect of haemorrhage in cats under nembutal anaesthesia (mean of 4)

	Before haemorrhage	Hours after haemorrhage 1	2	4
Haemoglobin (%)	76	77	75	73
Haematocrit	32·6	33·3	32·2	31·6
Total B.V. (c.c.)	187	—	135	—
Plasma vol. (c.c.)	127	—	92	—
Cell vol. (c.c.)	60	—	43	—

Actual blood removed: Total 52 c.c.
Plasma 35 c.c.
Cells 17 c.c.

Haemorrhage experiments in the rabbit. In a preliminary series of experiments, the effects of bleeding approximately 14 c.c./kg. on the haemoglobin percentage and plasma protein concentration were determined in three groups of rabbits: (*a*) unanaesthetized, (*b*) anaesthetized, and (*c*) splenectomized 2–3 weeks earlier and anaesthetized. The average results are given in Table 7. In the anaesthetized groups the animals were kept under nembutal for 4 hr. and then allowed to recover. These results showed a rapid haemodilution in the unanaesthetized group and a slower haemodilution in the anaesthetized groups. Splenectomy appeared to have little or no effect.

TABLE 7. Effect of bleeding 14 c.c./kg. in rabbits (mean of 5 in each group)

Unanaesthetized			Anaesthetized				
			Normal		Splenectomized	
	Hb (%)	Plasma proteins (g./100 c.c.)		Hb (%)	Plasma proteins (g./100 c.c.)	Hb (%)	Plasma proteins (g./100 c.c.)
Before bleeding	82	5·5	Before nembutal	78	4·8	76	5·2
1 hr. after bleeding	71	4·6	After nembutal	75	4·3	74	4·6
2 „ „	66	4·2	1 hr. after bleeding	70	4·1	69	4·2
4 „ „	66	3·9	2 „ „	70	3·9	67	3·9
24 „ „	62	5·6	4 „ „	71	4·0	66	3·8
			24 „ „	61	5·1	59	5·2

In a second series of experiments the blood volume was determined by the CO method. Ten rabbits were used, five unanaesthetized and five anaesthetized with nembutal. Anaesthesia was maintained for 4 hr. and the animals then allowed to recover. As soon as the blood volume was estimated, the animals were bled from an ear vein. This usually required 10–20 min. and an amount of blood equal to approximately 30% of the blood volume was removed in each case. This blood was collected in a flask containing dry powdered heparin, its volume measured and haematocrit determined. Taking the mid-point of the bleeding period as zero time, small samples for haemoglobin and haematocrit determinations were withdrawn at 1, 2, 6 and 24 hr. The amount of blood taken for the preliminary blood volume estimation and for the 1 and 2 hr. haematocrit were included in the calculation for total volume of blood removed. The blood volume determination was repeated at 2 and 24 hr. in each of the ten animals.

Average values for the blood volume in the two groups before and 2 and 24 hr. after haemorrhage are given in Table 8. These experiments show how rapidly the unanaesthetized rabbit restores its plasma volume after haemorrhage. The average plasma volume of five rabbits was 104 c.c. before haemorrhage, and 2 hr. after removing 30 c.c. of plasma, the volume was 118 c.c. and

TABLE 8. The effect of haemorrhage on blood volume, c.c., in unanaesthetized and anaesthetized rabbits

	B.V. before	Volume removed	Theoretical B.V. after	Actual B.V. at 2 hr.	Actual B.V. at 24 hr.
		(*a*) Unanaesthetized (mean of 5)			
Total	153	43	110	153	157
Plasma	104	30	74	118	124
Cells	49	13	36	35	33
		(*b*) Anaesthetized (mean of 5)			
Total	136	40	96	126	159
Plasma	87	27	60	90	124
Cells	49	13	36	36	35

increased to 124 c.c. at 24 hr. The accuracy of the blood volume determinations can be seen from the cell volumes, the actual determined cell volume corresponding very closely to the theoretical cell volume after haemorrhage.

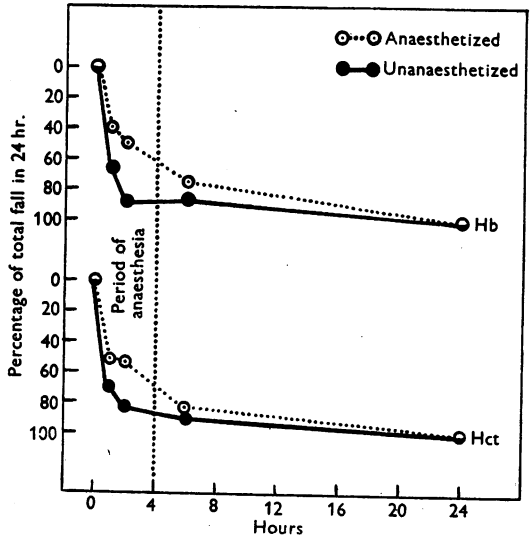

Fig. 1. The fall in haemoglobin percentage and haematocrit after haemorrhage in anaesthetized and unanaesthetized rabbits. The fall is expressed as a percentage of the decrease at 24 hr. Mean of five experiments in each group.

In the anaesthetized group the initial plasma volume was 87 c.c., and 2 hr. after removing 27 c.c. plasma the plasma volume was 90 c.c. and this increased

to 124 c.c. at 24 hr. Thus, although haemodilution occurred in the anaesthetized rabbit after haemorrhage, it was slowed down and increased when the animal recovered from the anaesthetic.

The difference between the two groups can also be well seen in the changes in haematocrit and haemoglobin percentage. In Fig. 1 are plotted the percentage fall in haematocrit and haemoglobin percentage, taking the fall from zero to 24 hr. as 100%.

DISCUSSION

These experiments on haemorrhage suggest several points of physiological interest. The first of these concerns the changes in circulating red cell volume following bleeding. It has been generally recognized that while a large proportion of the body's total red cells are in active circulation, there are reserve depots of red cells in such tissues as the spleen, liver sinusoids and bone marrow where the blood flow is sluggish or even stagnant (Wiggers, 1944). Barcroft and his associates (Barcroft, Harris, Orahovats & Weiss, 1925) focused attention on the spleen as one of the most important blood reservoirs which contracted during muscular exercise and haemorrhage. Other workers have observed splenic contraction and a rise in haematocrit following injection of adrenaline (Izquierdo & Cannon, 1928). There can be little doubt that these phenomena actually occur, but the fact that the spleen contributes a significant volume of red cells to the circulation has largely been inferred. Direct measurement of the red cell volume would provide the only satisfactory proof.

The results presented here do not demonstrate any increase in circulating red cell volume following non-fatal haemorrhage in the rabbit, cat and dog. The observations on rabbits and cats were made by the CO method, those on the dog by the dye method. The red cell volume after haemorrhage was always approximately equal to the original red cell volume less the volume of cells removed during the haemorrhage. It would appear, therefore, that in the degree of haemorrhage used here the reserve depots of red cells play an insignificant part in restoring blood volume. These observations in animals are supported by the work of Ebert, Stead & Gibson (1941) in man, where the red cell volume 2 hr. after bleeding approximately 1000 c.c. was equal to the original value less the amount of cells removed.

Concerning muscular exercise, Courtice & Gunton (1949*a*) showed that moderate work did not affect the red cell volume. Nylin (1947), using radioactively tagged red cells, has reported no increase in circulating red cell volume in man during heavy muscular exercise.

Another point of interest is the rate of replacement of fluid to the circulation following haemorrhage in unanaesthetized and anaesthetized animals. Since there is no replacement of cells, the plasma fraction of the blood is responsible for the early return of the circulating volume to normal. In Fig. 2 the changes in plasma volume in the cat and rabbit at 2 hr., and in the dog at 4 hr., after

haemorrhage are presented schematically. There is a very striking difference between the species. The rabbit replaces in 2 hr. more plasma than was removed in both anaesthetized and unanaesthetized groups, although this overcompensation is greater in the unanaesthetized animals. The unanaesthetized dogs have replaced part of the plasma removed, but the nembutalized cats and dogs have restored no fluid. It is probable that human subjects resemble cats and dogs in the rate of replacement of circulating fluid volume. Blood loss in hospital patients under barbiturate anaesthesia would, therefore, not be compensated for until recovery from the anaesthetic. This phenomenon is probably of practical importance in the choice of anaesthesia for surgical procedures in patients suffering from traumatic shock.

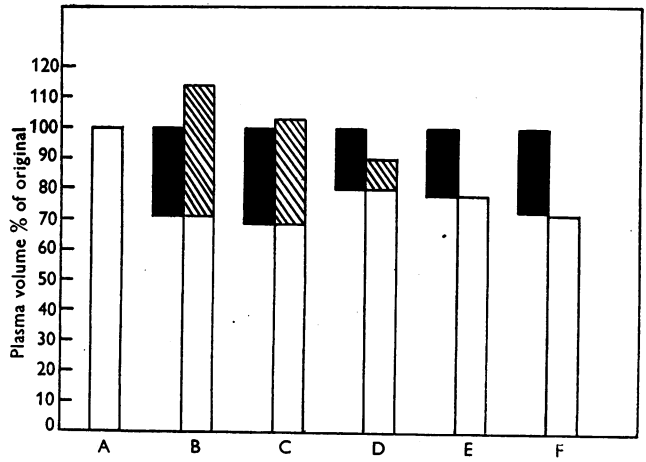

Fig. 2. The changes in plasma volume in rabbits and cats 2 hr. after haemorrhage, and in dogs 4 hr. after haemorrhage. In each case the original plasma volume is taken as 100. The black areas represent the volume of plasma removed in haemorrhage and the shaded areas the amount replaced in the stated times. A, original plasma volume; B, rabbits, unanaesthetized (mean of 5); C, rabbits, anaesthetized (mean of 5); D, dogs, unanaesthetized (mean of 4); E, dogs, anaesthetized (mean of 4); F, cats, anaesthetized (mean of 4).

The mechanism by which nembutal inhibits haemodilution is not clearly understood. Restoration of fluid to the circulation following haemorrhage in unanaesthetized animals is believed to be due to a fall in capillary pressure, with the result that fluid passes into the blood stream from the tissues. The fall in capillary pressure is brought about by peripheral arteriolar constriction, a reaction which maintains arterial blood pressure as blood is lost during haemorrhage. It is known that barbiturates cause arteriolar relaxation through depression of sympathetic tone. This would result in a raised capillary pressure. The osmotic effect of the plasma proteins would then be counterbalanced by the increased hydrodynamic pressure in the capillaries, thus slowing or stopping haemodilution.

The very rapid haemodilution in the rabbit, compared with the dog, is probably due to the more readily available tissue fluid stores of the rabbit. Cameron & Courtice (1946) have shown that in phosgene poisoning the rabbit very rapidly compensates for the loss of fluid into the lungs whereas the dog does not. When the fluid stores are deprived of water for a time, however, the rabbit behaves like the dog in this respect. It is probable that if rabbits were placed on a dry diet for a few days the haemodilution after haemorrhage would also be much slower. This is at present under investigation. Courtice & Gunton (1949*b*) have shown that an increase in water intake above normal significantly increases the plasma volume of rabbits and presumably also the available tissue fluid, so a decrease in normal water intake probably has the reverse effect.

In the rabbits studied in the experiments described above, the plasma volume is above the original level at 2 hr. when the plasma protein concentration is low. At 24 hr., when the plasma protein level is restored to its original value, the plasma volume is only slightly greater than at 2 hr. It is not until the red cells regenerate to restore the red cell volume to normal that the plasma volume falls once more to its original value. This suggests that in rabbits at least the total circulating protein is not only the controlling factor in determining the final plasma volume. It may be that the viscosity of the blood, by affecting the peripheral resistance and therefore the blood pressure, plays a part.

SUMMARY

1. The effect of nembutal anaesthesia on the restoration of fluid to the blood stream after haemorrhage has been studied in dogs, cats and rabbits.

2. In unanaesthetized dogs and rabbits and in anaesthetized dogs, cats and rabbits, there is no evidence of splenic contraction to increase the circulating blood volume after non-fatal haemorrhage.

3. Nembutal anaesthesia inhibits the restoration of the plasma volume after haemorrhage in dogs and cats and slows down this process in rabbits.

4. Rabbits behave differently from dogs in that they very rapidly over-compensate by withdrawal of fluid from the tissues.

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