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THE EFFECTS OF HAEMORRHAGE IN THE UNANAESTHETIZED RABBIT

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SUMMARY

1. The circulatory response following acute loss of 26% of the blood volume was examined in unanaesthetized rabbits. The groups of animals studied were normal rabbits; adrenalectomized rabbits; animals subjected to prolonged treatment with guanethidine in which peripheral adrenergic nerve transmission is blocked, but which can reflexly liberate adrenal medullary hormones; animals subjected to combined adrenalectomy and guanethidine treatment with no functional adrenergic effectors; in each case with or without administration of atropine. The responses of animals with section of the carotid sinus and aortic nerves were also examined.

2. The spontaneous rate of replacement of the blood volume after haemorrhage by reabsorption of extravascular fluid was the same in all the above preparations, the blood volume returning to normal 3–4 hr after bleeding.

3. The 'passive' effects of haemorrhage were examined in animals without functioning autonomic effectors and include a large fall in right atrial pressure and cardiac output, arterial hypotension, no significant change in total peripheral resistance, and a bradycardia of gradual onset. Reflex autonomic effector activity in normal animals minimizes the fall in atrial pressure, cardiac output and arterial blood pressure, and produces a significant increase in total peripheral resistance and tachycardia. Increased sympathetic nerve activity and secretion of adrenal medullary hormones each play an important and complementary part in the normal circulatory response to haemorrhage of the rabbit. There is also reflex reduction in vagal efferent activity.

4. Reflexes from the carotid sinus and aortic arch limit the fall in arterial pressure for the first 4 hr after haemorrhage. These reflexes also account for the tachycardia normally observed after haemorrhage. The

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baroreceptor reflexes rather than the chemoreceptors appear to be dominant in these responses.

5. Twenty-four hours after haemorrhage the haemodynamic pattern is similar in all preparations irrespective of their autonomic effector status: blood volume, right atrial pressures and cardiac outputs are all elevated, and the arterial pressure has virtually recovered, consistent with the development of hypervolaemic anaemia at this time.

INTRODUCTION

The effects of haemorrhage have been extensively studied in man and other species, but there has been greater emphasis on the mechanisms which result in the production of irreversible shock, than on the mechanisms of normal reflex control (Cournand, Riley, Bradley, Breed, Noble, Lauson, Gregersen & Richards, 1943; Barcroft, Edholm, McMichael & Sharpey-Schafer, 1944; Barcroft & Edholm, 1945; Warren, Brannon, Stead & Merrill, 1945; Brannon, Stead, Warren & Merrill, 1946; Wiggers, 1950; Heymans & Neil, 1958; Guyton & Crowell, 1961; Gauer & Henry, 1963; Guyton, 1963; Fine, 1965). In the present experiments some of the reflex mechanisms of circulatory control have been studied in the unanaesthetized rabbit following removal of a definite fraction of the blood volume. The role of the sympathetic nerves, the adrenal medullary hormones and the parasympathetic effectors in the circulatory response to haemorrhage was assessed by studying the responses of animals with different degrees of autonomic control. The groups investigated were: normal animals in which a reflex increase in orthosympathetic activity could occur through increased sympathetic nerve activity as well as through a release of adrenal medullary hormones; adrenalectomized animals in which this could only occur through sympathetic nerves; animals subjected to prolonged treatment with guanethidine in which adrenergic nerve transmission was blocked, and tissue catecholamine stores were severely depleted (Cass, Kuntzman & Brodie, 1960) but in which there was reflex secretion of adrenal medullary hormones in response to haemorrhage; and animals adrenalectomized and treated with guanethidine without functioning orthosympathetic pathways. In addition the responses of similar groups were examined following administration of atropine. Furthermore the role of the arterial baroreceptors and chemoreceptors has been assessed by comparing the circulatory responses in normal animals with those of animals with section of the carotid sinus and aortic nerves.

METHODS

Animals. New Zealand White rabbits, cross-bred with the New Zealand Giant strain, were used in these experiments. Mean body weight was 2·56 kg (range: 2·0–3·2 kg).

Preparation of animals. Preliminary operations for the insertion of aortic thermistor catheters, for section of the carotid sinus and aortic nerves, and for bilateral one-stage adrenalectomy were carried out as described previously (Korner, 1965; Korner & White, 1966; White, 1966). The adrenalectomized animals were maintained on 1 mg cortisone acetate and 1·5 mg deoxycorticosterone acetate I.M. daily. Depletion of tissue catecholamines and peripheral sympathetic nerve block was achieved by the administration of guanethidine for a period of 7 days (Korner & White, 1966). Administration of guanethidine to adrenalectomized animals resulted in preparations without functional orthosympathetic control. In each of the above preparations cholinergic block was produced by giving atropine sulphate I.V. at an initial dose of 2 mg, followed by alternate doses of 1 and 0·5 mg at 15 min intervals through the experiment.

On the day of the experiment catheterization of both central ear arteries, and right atrium, and insertion of a tracheotomy tube were carried out using local anaesthesia as described previously (Korner, 1965). In animals which were studied for 24 hr after haemorrhage, tracheotomy was not carried out.

Measurement of various circulatory and respiratory parameters. The cardiac output was measured using the thermodilution technique (Fegler, 1954; Korner, 1965) and mean ear artery pressure, mean right atrial pressure and heart rate were determined as described previously (Korner, 1965; Fig. 1). Ventilation was measured by collecting the expired air, and was expressed as l./min dry gas at s.t.p. (Edwards, Korner & Thorburn, 1959). Respiratory rate was counted either from the right atrial pressure record, or from the respiratory movements recorded by means of a small mercury-in-silastic strain gauge placed round the animal's chest (Fig. 1). Arterial pH, P_{CO_2} and P_{O_2} were determined using a model 113 Instrumentation Laboratory Inc. blood gas analyser and pH meter (Chalmers & Korner, 1966).

Measurement of blood volume. Blood volume was determined from the estimated red cell volume, and arterial haematocrit ratio, after applying corrections for plasma trapping and for the (mean body)/(large vessel) haematocrit ratio, based on separate red cell volume and plasma volume determinations in preliminary experiments.

In each animal one determination of red cell volume was carried out during the initial control period after injecting 1 ml. of the animal's own red cells, freshly labelled with ^{51}Cr, and determining the dilution of this volume from the 10 min sample (Lajtha, 1961). At this time haemoglobin was also determined using the method of Drabkin & Austin (1935), and the arterial haematocrit was measured, using Wintrobe tubes spun at 3000 rev/min for 60 min at 13·5 cm radius. A correction of 3 % for plasma trapping was used (Chaplin & Mollison, 1952). The ratio of mean body haematocrit/large vessel haematocrit was determined in nine animals (three normals, three guanethidine-treated adrenalectomized rabbits and three animals with section of the carotid sinus and aortic nerves). The ratio was similar in each of the groups, averaging $0{\cdot}86 \pm 0{\cdot}017$ (S.E. of mean). In these experiments T-1824 was used as plasma label, and samples were obtained 10, 15 and 20 min after the simultaneous injection of labelled red cells and dye. In view of the doubts expressed by Zizza & Reeve (1958) concerning the validity of T-1824 as a plasma protein label in the rabbit, three preliminary experiments were carried out in which decay curves of simultaneously injected T-1824 and [^{131}I] labelled human serum albumin (Radiochemical Centre, Amersham) were obtained. In these animals samples were obtained at 10, 20, 30, 40 and 50 min after injection of the indicators. Plasma concentration of each indicator at zero time was obtained by semilogarithmic extrapolation and the subsequent plasma concentrations expressed as a percentage of this figure. For T-1824 the values at the above

times were respectively 94·2, 91·1, 86·6, 83·6 and 80·7%, whilst for ^{131}I the corresponding values were 96·0, 90·1, 88·4, 83·8 and 83·0. The results indicate that T-1824 can be validly used as a plasma protein label in the rabbit.

After bleeding, the volume of red cells remaining in the animal was calculated as the difference between initial red cell volume and the red cell volume removed. Subsequent replacement of fluid volume was studied by serial haematocrit determinations. In order to minimize blood sampling all subsequent haematocrits after the initial control value were calculated from progressive dilution of the radioactivity from the original injection of labelled red cells, in 0·5 ml. samples of blood. The formula used was:

Haematocrit (Ht) = (counts/min in sample) × (control Ht)/(counts/min control). Thirteen comparisons in three animals were carried out between calculated and observed haematocrits, using the above formula. Values obtained were: Observed haematocrit = 26·87%;

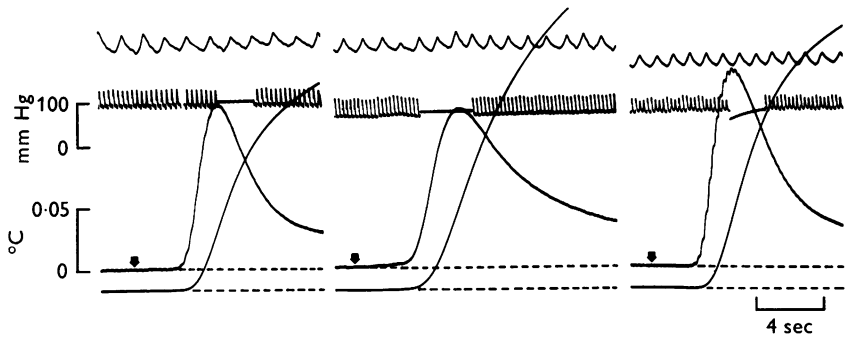

Fig. 1. Records obtained during initial control period, 20 min after start of bleeding, and 2 hr after haemorrhage. In each record, from above down: respiration, ear artery pressure (mean in middle of tracing), aortic temperature change (thermodilution curve) and integrated thermodilution curve. The arrow marks the time of injection of indicator into the right atrium.

Calculated haematocrit = 26·93%; S.D. of difference (within-animal comparisons) ±0·85%. The findings suggest that the assumption underlying the use of the above formula that there is no significant addition of red cells to the original circulating volumes from untagged depots, is valid in the rabbit after haemorrhage. If significant splenic emptying occurred after haemorrhage, the serial haematocrit method for assessing changes in total blood volume would be subject to systematic error. Recent studies by Mott (1965) have shown that splenic emptying plays only a small role in the changes in blood volume after haemorrhage in the rabbit, and its contribution to the blood volume changes has been neglected in the present experiments. A further assumption of the method is that the body haematocrit ratio is unaltered by haemorrhage. This assumption seems reasonable in animals bled 26% of their blood volume, since Gibson, Seligman, Peacock, Fine, Aub & Evans (1947) have demonstrated that very severe degrees of shock are required to produce marked changes in body haematocrit ratio.

Conduct of experiments. Following completion of the minor operative procedures on the day of the experiment, the rabbit was placed inside a large rabbit box where it sat comfortably without restraint. Recording commenced 1 hr later, and the animal was given 500 i.u./kg heparin I.V. The initial blood volume determination was carried out, and the various parameters were measured for an initial control period of 26 min (e.g. Fig. 3). The animal was then bled a fixed fraction of 26% of its blood volume into a burette from the catheterized ear artery, at a rate of approximately 2 ml./min. The duration of bleeding

averaged 20 min. Observations were continued for 4 hr after haemorrhage in all animals, and two animals from each subgroup were studied again 24 hr after haemorrhage and were allowed access to food and water in their cages overnight.

The timing of the various measurements was always the same in different animals. Two to four values of arterial pressure, cardiac output, right atrial pressure, heart rate, ventilation and respiration rate were obtained and averaged for each animal, during each of the selected time intervals (e.g. Fig. 3). In a given set of experiments the mean value for each time interval was determined for each parameter for all the animals in the group, and the standard error of the mean of each time interval estimated by analysis of variance (Mather, 1949) as described previously (Chalmers, Isbister, Korner & Mok, 1965). In some instances in order to facilitate comparison of the response of different groups of animals with different initial control values, the average changes in cardiac output, arterial pressure and heart rate were expressed as percentages of the mean initial control value, and the changes in right atrial pressure as the absolute difference from this value. In each case appropriate standard errors of a single time interval were calculated for each parameter by analysis of variance.

RESULTS

Blood volume changes after haemorrhage

There were no significant differences in the initial blood volumes of normal rabbits, adrenalectomized rabbits, rabbits subjected to guanethidine and rabbits with section of the carotid sinus and aortic nerves (Table 1). However, the animals with combined adrenalectomy and guanethidine-treatment had initial blood volumes about 10% greater than normal (Table 1), despite considerable overlap between the two groups ($P = 0{\cdot}07$).

The rate of restoration of the blood volume by reabsorption of extravascular fluid was similar in all groups after the initial removal of 26% of this volume (Fig. 2). The autonomic effector status of the animals was thus without effect on the rate of spontaneous blood volume replacement. On the assumption that the body haemotocrit ratio remained unchanged by this degree of haemorrhage, the blood volume returned to initial control values 3–4 hr after bleeding (Fig. 2). In the thirty-three animals studied (i.e. pooling results from all the groups) the blood volume was $101 \pm 1{\cdot}0$ (S.E. of mean) % (range 90–116%) 4 hr after haemorrhage. Eight of these animals (two normal, two adrenalectomized, two guanethidine-treated, two guanethidine-treated adrenalectomized rabbits) were studied for 24 hr after haemorrhage (Table 3). In this group the blood volume at 4 hr was 101% (i.e. similar to that of the larger group). After 24 hr it has increased significantly by $11 \pm 3{\cdot}3$ (S.E. of mean) % above the value reached after 4 hr. The animals at this time had a hypervolaemic anaemia with a haemoglobin concentration of $7{\cdot}4 \pm 0{\cdot}4$ (S.E. of mean) g/100 ml. of blood.

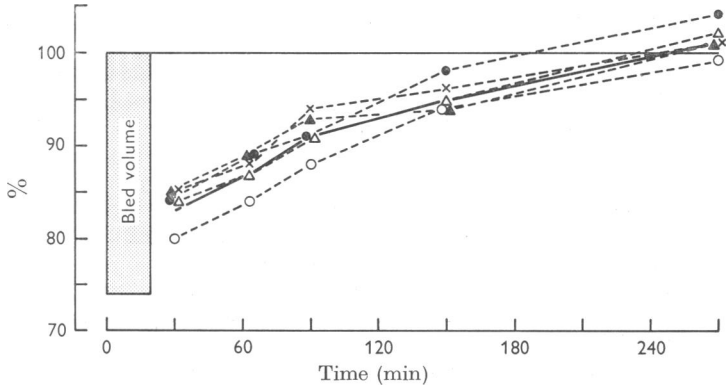

Fig. 2. Changes of blood volume in different groups following removal of 26 % of the initial control volume between times 0–20 min; dotted lines joining various symbols indicate mean value of each group, continuous line is mean of all animals. The standard error of the mean of all animals at any given time interval is ±1·0 %. Symbols: ● normal; ○ adrenalectomy; ▲ guanethidine-treated; △ guanethidine + adrenalectomy; × carotid and aortic nerve section.

TABLE 1. Initial control values of red cell volume (RCV), total blood volume (TBV) and arterial haematocrit in the different groups. Results are given as mean and standard error of mean. Results of normal, adrenalectomized, guanethidine-treated and guanethidine-treated adrenalectomized rabbits include animals given atropine

Group	No.	Wt. (kg)	Haematocrit (%)	RCV (ml.)	TBV (ml.)	TBV (ml./kg)
Normal	10	2·59 ± 0·12	36·8 ± 0·5	44·0 ± 2·7	139 ± 7·3	53·7 ± 1·5
Adrenalectomy	8	2·77 ± 0·09	34·6 ± 0·6	43·5 ± 2·6	147 ± 9·5	53·3 ± 3·4
Guanethidine	9	2·49 ± 0·10	34·3 ± 0·8	41·5 ± 2·4	141 ± 7·4	56·6 ± 2·6
Guanethidine + adrenalectomy	6	2·61 ± 0·14	33·4 ± 1·1	43·8 ± 3·2	152 ± 10·3	58·4 ± 2·1
Carotid + aortic nerve section	3	2·53 ± 0·09	39·1 ± 1·8	42·9 ± 4·1	129 ± 17·6	50·8 ± 5·5

Circulatory findings in normal animals

The effects of removing 26 % of the blood volume in six normal rabbits with intact autonomic effector pathways are shown in Fig. 3 (left panel). Soon after the start of bleeding the right atrial pressure fell significantly. This was associated with a fall in both cardiac output (to 66 % of control) and in arterial blood pressure (to 84 % of control), and a rise in total peripheral resistance. The heart rate also increased by about 30 beats/min during bleeding. After haemorrhage recovery of the right atrial pressure and cardiac output was somewhat slower than the rate of spontaneous restoration of the blood volume (cf. Figs. 2 and 3), but 4 hr after bleeding all three parameters had returned close to their initial control values. The recovery in right atrial pressure, cardiac output and blood volume contrasted with the response of the arterial pressure which remained at the immediate post-haemorrhage value of about 75 % of control for 4 hr after

bleeding (Fig. 3). The total peripheral resistance remained significantly ($P = 0{\cdot}05$) elevated for about 2 hr after bleeding and then returned to normal.

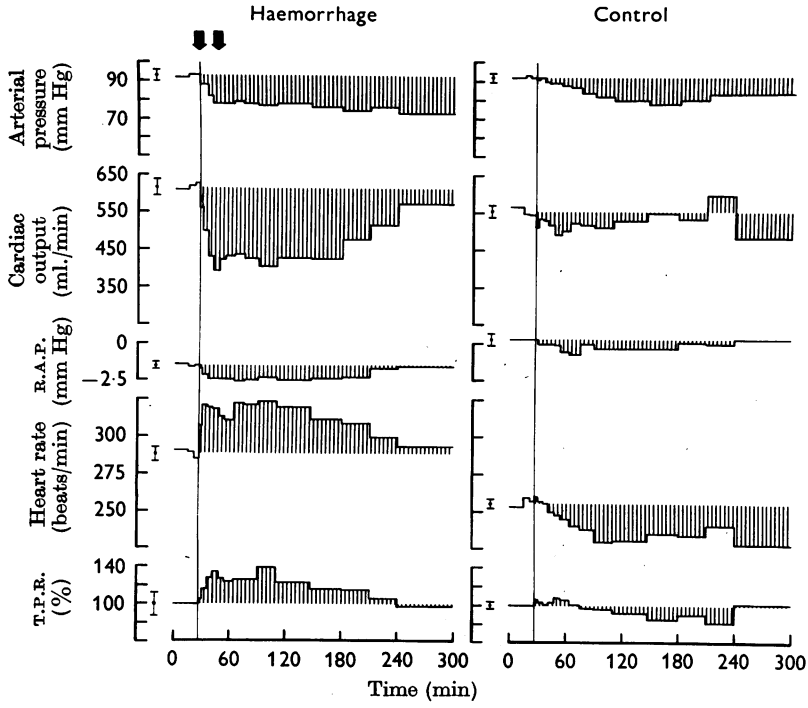

Fig. 3. Mean circulatory changes in arterial pressure (mm Hg), cardiac output (ml./min), right atrial pressure (R.A.P.; mm Hg), heart rate (beats/min) and total peripheral resistance (T.P.R.; % of mean control value) in six normal animals bled 26% of the blood volume (left panel), and in three normal animals not subjected to bleeding (right panel). Bleeding occurred between the arrows. The vertical line denotes the end of the initial control period and subsequent hatching denotes deviation of the various parameters from initial control values. Symbol on left of each parameter: mid point denotes mean control value and the distance above and below this point equals ±1 S.E. of the mean of a single time interval.

Since the experiment involved a prolonged period of observation with the rabbits at rest, the significance of the circulatory changes, such as those in the arterial pressure, was assessed by comparison with the results in a group of unbled animals (Fig. 3, right panel). In this control group the arterial pressure fell gradually during the first $2\frac{1}{2}$ hr of observation. In the next 2 hr the blood pressure remained steady $10 \pm 1{\cdot}7$ (S.E. of difference) mm Hg below the initial value of 92 mm Hg. In the bled animals the arterial pressure fell from an initial level of 93 mm Hg to an average value of 75 mm Hg (difference $18 \pm 1{\cdot}8$ (S.E.) mm Hg). Therefore

the value of the arterial pressure during the last hour of the experiment in the bled animals was significantly lower than in the control group. In the latter there were significant fluctuations in cardiac output superimposed on a slight reduction in this value; the right atrial pressure also fluctuated slightly about an average value of $0{\cdot}4 \pm 0{\cdot}4$ (S.E. of difference) mm Hg below the initial control value. However, in the bled animals there was a larger and more uniform reduction of $0{\cdot}8 \pm 0{\cdot}2$ (S.E. of difference) mm Hg in the mean right atrial pressure for 3 hr after haemorrhage. In the control group the heart rate fell gradually by about 25 beats/min

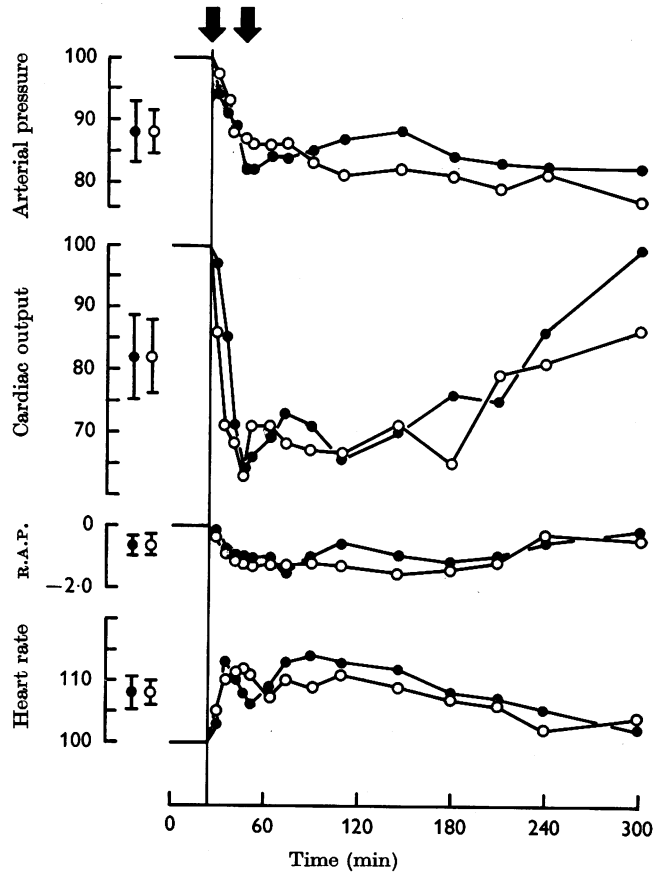

Fig. 4. Mean circulatory response in two groups of three normal rabbits after removing 26 % of the blood volume. Changes are expressed as percentage of the initial control value for the arterial pressure, cardiac output and heart rate, and as deviations from this value for the right atrial pressure (R.A.P.). Standard errors of the mean were obtained by analysis of variance: the symbol on the left of each parameter distinguishes the two groups (open and closed circles) and its height represents 2 S.E. of the mean of a single time interval of each group.

during the first 2 hr and then remained relatively steady. The total peripheral resistance fell slightly in the control group, and the fluctuations in this measurement were smaller than the changes observed after haemorrhage.

Since the present analysis involves comparison of responses of animals with varying degrees of reflex control it is important to determine the reproducibility of the response of a given group of animals subjected to a standard haemorrhage of 26% of their blood volume. The effects of bleeding two groups of three normal rabbits in this way were compared, and the results are shown in Fig. 4. The rate of spontaneous blood volume replacement was the same in the two groups, and the circulatory responses to haemorrhage were similar. The reproducibility of responses was particularly close during the first hour after the start of bleeding.

Circulatory findings in animals without sympatho-adrenal control

The initial control values of normal rabbits and of rabbits without sympatho-adrenal control are shown in Tables 1 and 2. The main differences between these groups include a lower arterial pressure and heart rate, and a higher right atrial pressure and blood volume in the guanethidine-treated adrenalectomized rabbits.

The importance of activation of the orthosympathetic and parasympathetic effector pathways in the normal circulatory response to removal of 26% of the blood volume is seen by comparing the percentage changes occurring in normal rabbits and in guanethidine-treated adrenalectomized animals with or without atropine (Fig. 5). During the first 40 min after the start of bleeding the fall in right atrial pressure was considerably greater in both groups of animals without sympatho-adrenal control (Fig. 5). In these animals the mean right atrial pressure fell by $2{\cdot}1 \pm 0{\cdot}21$ (S.E. of difference) mm Hg during this period, compared to $0{\cdot}7 \pm 0{\cdot}12$ (S.E. of difference) mm Hg in the normal group. This difference in response in the right atrial pressure could result from the differences in initial blood volumes between normal and guanethidine-treated adrenalectomized animals (see Table 1). Although a constant fraction of the blood volume was removed in each preparation, a different effect on the central venous pressure could result if each lay on a different part of the venous pressure–volume diagram. There was considerable overlap in the initial blood volumes of these two groups, and the results from four normal and four guanethidine-treated adrenalectomized animals in which the initial blood volumes were matched have been compared in Table 4. The magnitude of the difference in atrial pressure changes in these two groups was the same as the differences observed in the larger groups, and was again statistically significant ($P < 0{\cdot}05$). The fall in cardiac output

was somewhat greater than normal in animals without orthosympathetic control, but the difference in response was statistically significant ($P = 0{\cdot}05$) only in guanethidine-treated adrenalectomized animals given atropine ('de-efferented' rabbits) (Fig. 5). The fall in arterial pressure in both groups of guanethidine-treated adrenalectomized animals was greater than normal during the first 40 min after the start of haemorrhage, and

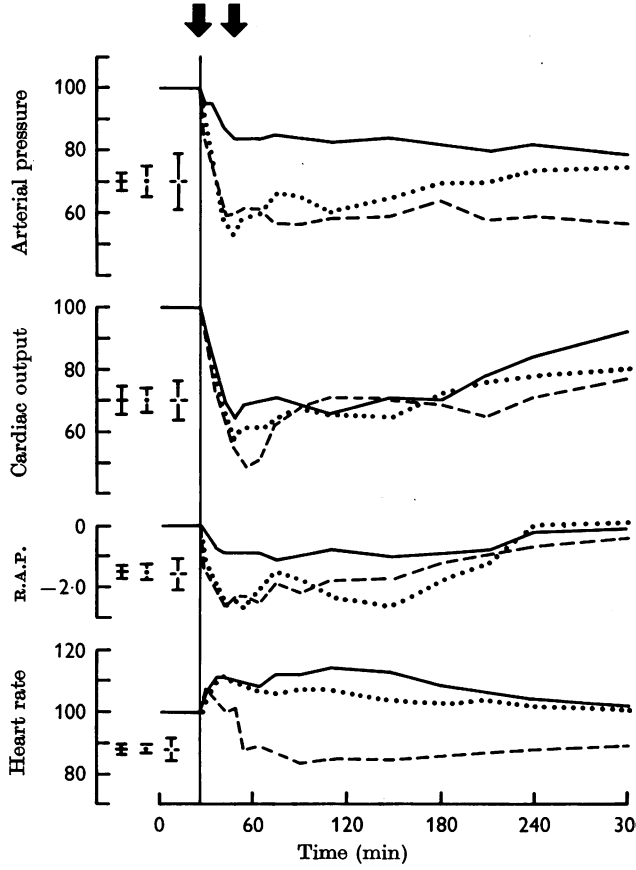

Fig. 5. Mean circulatory effects of removing 26% of the blood volume observed in six normal animals (continuous lines), four guanethidine + adrenalectomy (dotted lines), two 'de-efferented' animals (guanethidine + adrenalectomy + atropine; interrupted lines). Notation as in Fig. 4. Symbols on left are twice the standard errors of the mean of each time interval for (from left to right) normal animals, guanethidine + adrenalectomy animals and 'de-efferented' animals.

was of the same magnitude as the fall in cardiac output. In these animals, in contrast to normal rabbits, there was no consistent change in total peripheral resistance. The changes in heart rate in the early period following the start of bleeding were almost the same in guanethidine-treated

TABLE 2. Initial control values of the various cardiovascular parameters in the different groups. The results are given as mean and standard error of mean. 'De-efferented' rabbits = guanethidine-treated adrenalectomized animals given atropine

	Normal 6	Adrenalectomy 5	Guanethidine 6	Guanethidine + adrenalectomy 4	'De-efferented' 2	Carotid + aortic nerve section 3
Body weight (kg)	2·71 ± 0·17	2·80 ± 0·14	2·51 ± 0·16	2·63 ± 0·14	2·57 ± 0·43	2·53 ± 0·09
Cardiac output (ml./min)	619 ± 53	642 ± 52	633 ± 13	638 ± 33	568 ± 35	609 ± 98
Arterial pressure (mm Hg)	92 ± 5·8	91 ± 3·8	79 ± 2·6	79 ± 3·1	88 ± 16·0	120 ± 5·9
Heart rate (beats/min)	288 ± 14·4	271 ± 14·3	202 ± 2·1	219 ± 11·5	248 ± 6·0	341 ± 7·7
Rt. atrial pressure (mm Hg)	−1·6 ± 0·5	−1·6 ± 0·2	+1·0 ± 0·8	−0·3 ± 0·4	+0·1 ± 0·9	−0·3 ± 0·5

TABLE 3. Changes in the circulation and in blood volume 4 hr and 24 hr after haemorrhage. in two animals of each group. *C*—initial control observations

	Cardiac output (ml./min)			Heart rate (beats/min)			Arterial pressure (mm Hg)			Rt. atrial pressure (mm Hg)			Blood volume (%)	
	C	4 hr	24 hr	*C*	4 hr	24 hr	*C*	4 hr	24 hr	*C*	4 hr	24 hr	4 hr	24 hr
Normal	517	589	719	304	316	319	82	60	74	−2·0	−1·5	−1·5	103	107
	589	494	577	270	257	217	75	60	72	−0·8	−0·8	+0·7	112	119
Adrenalectomy	481	403	628	322	291	240	75	69	62	−1·5	−0·7	+0·8	111	130
	771	541	862	264	289	214	89	51	86	−0·9	−3·0	+1·4	100	100
Guanethidine	596	524	827	193	211	201	71	54	73	−2·0	−0·5	+2·0	97	121
	567	541	857	204	227	246	71	51	70	+1·9	+1·4	+0·9	97	98
Guanethidine + adrenalectomy	720	485	815	204	229	214	71	50	73	+0·3	−1·5	+0·2	99	112
	645	534	730	215	207	200	80	65	73	−0·1	−0·1	+1·0	94	114

adrenalectomized animals with vagus intact, as in normal animals. However in completely 'de-efferented' rabbits the heart rate fell significantly 15 min after the start of bleeding.

After 4 hr the right atrial pressure had returned to initial control values in all three groups in parallel with the changes in blood volume. In the completely 'de-efferented' animals the return in cardiac output was somewhat slower than normal. The arterial pressure remained significantly

TABLE 4. Changes in right atrial pressure following haemorrhage in four normal animals and four guanethidine-treated adrenalectomized animals with comparable blood volumes. Δ = difference between mean control measurements (eight observations) and the mean of measurements taken during the 40 min period immediately following the commencement of haemorrhage (twelve observations) in each animal

	Total blood volume (ml./kg)	Right atrial pressure (mm Hg)		
		Control	'40 min'	Δ
Normal	53·5	−0·8	−2·0	1·2
	53·5	−0·5	−1·1	0·6
	60·1	−2·0	−2·6	0·6
	56·4	−2·0	−2·8	0·8
Mean	55·9	—	—	0·8
S.E. of difference	—	—	—	±0·14
Guanethidine + adrenalectomy	53·5	+0·3	−1·4	1·7
	60·5	+1·9	−1·4	3·3
	52·0	−1·7	−2·9	1·2
	59·0	−1·1	−3·3	2·2
Mean	56·2	—	—	2·1
S.E. of difference	—	—	—	±0·45

below that of the normal group throughout the 4 hr observation period, and bradycardia was also present during this time. The blood pressure changes were less marked in guanethidine-treated adrenalectomized animals *not* given atropine.

The results indicate that reflex activity after haemorrhage minimizes the circulatory effects of bleeding, particularly the initial ones, and helps to maintain a better arterial perfusion pressure throughout the recovery phase.

Circulatory findings in animals with partial sympatho-adrenal control

The magnitude of the circulatory changes following haemorrhage differed quantitatively in animals with only one intact orthosympathetic pathway from the responses of normal rabbits with all reflexes intact on the one hand, and of de-efferented animals deprived of any reflex activity on the other (Fig. 6).

In adrenalectomized animals with intact sympathetic nerves the initial fall in right atrial pressure was identical with that of normal rabbits and the magnitude of the fall in cardiac output was similar. However, the arterial pressure was less effectively maintained than normal for 4 hr after haemorrhage, and fell about halfway along the 'scale of reflex activity' delimited by the response of normal and 'de-efferented' animals as shown in the background shading of Fig. 6. There was more pronounced tachycardia in this group for about 1½ hr after the start of haemorrhage, probably owing to greater baroreceptor activity evoked by the greater fall in arterial pressure.

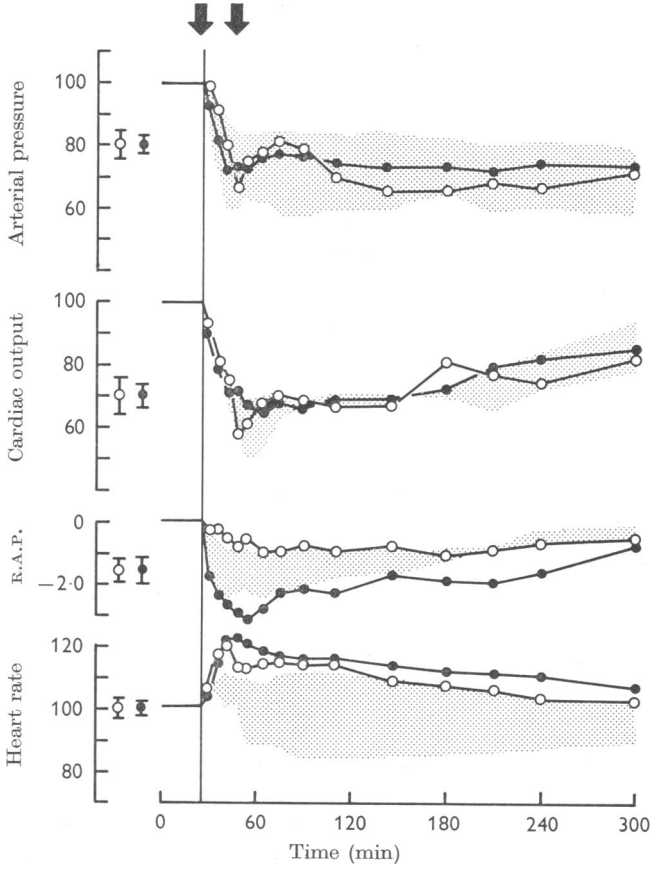

Fig. 6. Mean circulatory effects of removing 26% of the blood volume observed in five adrenalectomized (open circles) and six guanethidine-treated rabbits (closed circles). The stippled background for each parameter represents the normal range of reflex control, the upper edge representing the response of normal animals, and the lower edge that of 'de-efferented' rabbits, taken from the results shown in Fig. 5. Notation as in Fig. 4.

In guanethidine-treated animals the right atrial pressure fell to the level of the 'de-efferented' group after bleeding (Fig. 6). It should be noted that in the guanethidine-treated animals the heart is hypersensitive to the action of reflexly liberated adrenal medullary hormones (Gaffney, Bryant & Braunwald, 1962; Korner & White, 1966) which may account for the normal maintenance of the cardiac output after haemorrhage. However, in these animals arterial pressure is again less effectively maintained than normal. In the guanethidine-treated rabbits the magnitude of the tachycardia following haemorrhage was also greater than the response of normal animals, and was even greater ($P < 0{\cdot}05$) than the response of adrenalectomized rabbits.

Role of the vagus

In animals without sympatho-adrenal control the vagus exerts reflex effects on the heart after haemorrhage resulting in tachycardia. In these animals the cardiac output did not drop significantly further than in the normal group in the first 40 min after haemorrhage, but did in the completely 'de-efferented' group (Fig. 5). The recovery of the arterial pressure was also more rapid and complete after this time in animals with vagus intact. Its role in animals with some degree of sympatho-adrenal control was examined by comparing the response of one group of six rabbits given atropine, with the response of a similar group of animals not given this drug (Fig. 7). Each group comprised two normal, two adrenalectomized and two guanethidine-treated rabbits. The percentage increase in heart rate after bleeding was slightly smaller in atropinized animals. However, their initial heart rates were elevated by about 7 %, so that the absolute heart rates after haemorrhage were similar in both groups. The fall in cardiac output during haemorrhage was somewhat greater ($6 \pm 2{\cdot}25$ (S.E. of difference) %) than normal in atropinized rabbits, but there was no significant difference in the responses of the right atrial and arterial pressures of the two groups.

Response of animals with section of carotid sinus and aortic nerves

The resting arterial pressure and heart rate were significantly elevated in animals with section of the carotid sinus and aortic nerves but the cardiac output and blood volume did not differ significantly from normal values (Table 2). Bleeding these animals by 26 % of their blood volume resulted in a fall in right atrial pressure of normal magnitude. However, the initial reductions in cardiac output and arterial pressure during haemorrhage were greater than observed in normal animals with intact reflexes, and were as large as in 'de-efferented' animals without autonomic effectors. In animals with section of the carotid sinus and aortic nerves the early

elevation in total peripheral resistance observed in normal animals was abolished, as were the changes in heart rate observed normally during and after haemorrhage (Fig. 8). However, in contrast to the 'de-efferented' animals there was evidence of some reflex autonomic activity in animals with section of the carotid sinus and aortic nerves. Thus, following the

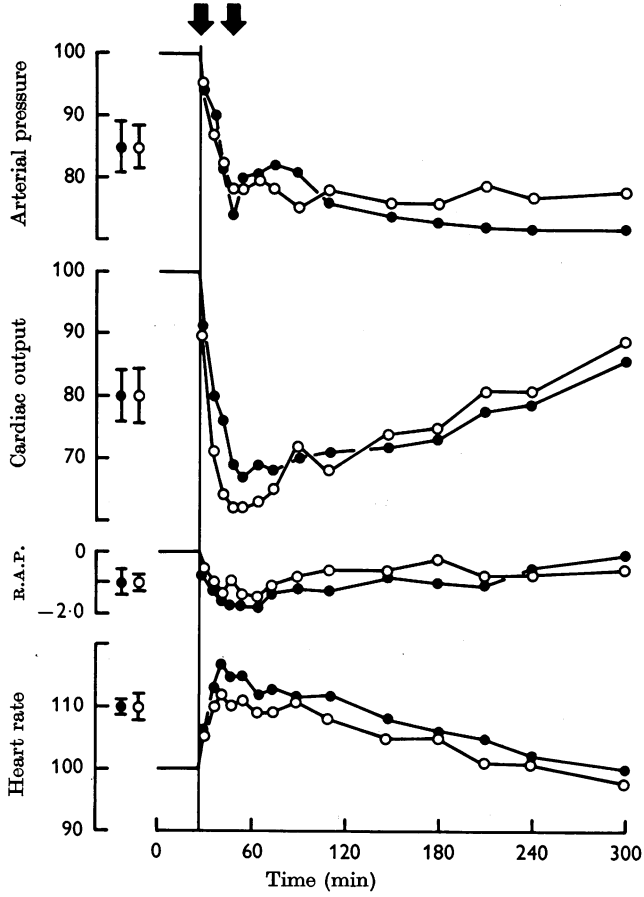

Fig. 7. Mean circulatory effects of removing 26 % of the blood volume in two groups of six animals with some degree of sympatho-adrenal control as described in text. Open circles—animals given atropine; closed circles—no atropine. Notation as in Fig. 4.

initial marked fall in arterial pressure and cardiac output soon after the commencement of haemorrhage there was considerable recovery in these measurements towards normal for about 1 hr after cessation of bleeding. This recovery was not sustained, however, and the blood pressure declined again below 'de-efferented' values 3–4 hr after bleeding. The ability of

animals with section of the carotid sinus and aortic nerves to sustain blood pressure is thus less effective than normal throughout the recovery phase, as well as during haemorrhage.

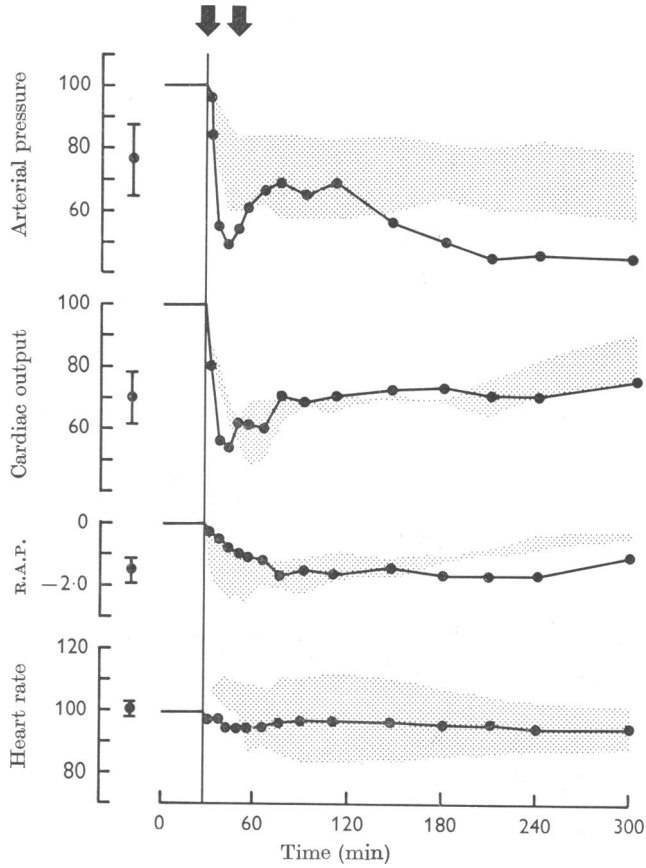

Fig. 8. Mean circulatory effects of removing 26 % of the blood volume in three animals with section of the carotid sinus and aortic nerves. The stippled background represents the limits of normal reflex control, the top edge representing the response of normal animals, and the bottom those of 'de-efferented' rabbits taken from the results shown in Fig. 5. Notation as in Fig. 4.

Circulatory findings at 4 hr and 24 hr after bleeding

In all normal animals, bled 26 % of their blood volume, the arterial pressure was still below initial control values 4 hr after haemorrhage, even though the blood volume, right atrial pressure and cardiac output had recovered at this time (Fig. 3; Table 3). The same degree of haemorrhage resulted in greater depression of the arterial pressure 4 hr after bleeding in animals with varying impairment of the orthosympathetic effector

pathways and was particularly striking in completely 'de-efferented' animals in which the cardiac output was also below normal values (Fig. 5).

In eight animals (two normal, two adrenalectomized, two guanethidine-treated, two guanethidine-treated adrenalectomized rabbits) the circulatory effects were also studied 24 hr after haemorrhage (Table 3). However, after 24 hr the arterial pressure recovered almost to initial values in all groups, and the cardiac output, right atrial pressure and blood volume were significantly elevated at this time.

Respiratory findings

Respiratory minute volume did not change significantly after bleeding in normal rabbits, and in adrenalectomized and guanethidine-treated animals with or without atropine but there was a gradual increase in respiration rate in all groups (Table 5). Only in animals without functioning orthosympathetic effectors and in rabbits with section of carotid sinus and aortic nerves (i.e. groups with the greatest fall in arterial pressure) was there a significant increase in ventilation (Table 5). The transient reduction in P_{CO_2} observed in all groups following haemorrhage probably reflects changes in $\dot{V}_A/Q_c$ ratio (where $\dot{V}_A$ is the alveolar gas minute volume and Q_c is the capillary blood flow) and reduction in metabolism rather than absolute changes in alveolar ventilation. There was no arterial hypoxia or evidence of acidosis in any group after removing 26 % of the blood volume (Table 6).

DISCUSSION

The present results indicate that reflex autonomic effector activity minimizes the circulatory effects of severe haemorrhage in the rabbit, and hastens the rate of recovery during the first 4 hr after bleeding. However, even animals without orthosympathetic control eventually recovered from the effects of loss of 26 % of their blood volume, and after 24 hr the haemodynamic findings were similar in normal, adrenalectomized, guanethidine-treated animals, and animals without any functioning orthosympathetic effectors.

The differences in the spontaneous rate of restoration of the blood volume between the various groups were minimal, and the degree of autonomic activity did not thus appear to influence the rate of reabsorption of extravascular fluid after blood loss. These findings indicate that the reduction in mean capillary pressure must be similar in all the preparations after haemorrhage. This effect is probably achieved by different mechanisms in the various groups: by selective vasoconstriction of a number of vascular beds in animals with intact orthosympathetic pathways (Mellander, 1960; Öberg, 1964; Haddy, Scott & Molnar, 1965), and more uniformly in animals without sympatho-adrenal control as a result of

TABLE 5. Mean respiratory minute volume ($\dot{V}_E$) and rate (RR) in different groups at various times after start of haemorrhage. C = control period. Number in brackets after each group is the number of animals. Standard error is standard error of mean of a single time interval based on within-animal comparisons. Animals given atropine are included with each group

		Control	Time after bleeding			
		C	15 min	30 min	4 hr	S.E.
Normal (6)	$\dot{V}_E$	1·15	1·11	1·15	1·36	±0·09
	RR	70	69	81	136	±19·3
Adrenalectomy (6)	$\dot{V}_E$	1·14	1·29	1·34	1·34	±0·12
	RR	84	99	138	162	±23·6
Guanethidine (7)	$\dot{V}_E$	0·96	1·01	1·22	1·16	±0·09
	RR	82	104	132	131	±15·4
Guanethidine + adrenalectomy (4)	$\dot{V}_E$	1·35	1·40	1·75	1·34	±0·14
	RR	110	110	215	109	±25·6
Carotid sinus + aortic nerve section (2)	$\dot{V}_E$	0·95	1·51	2·18	2·03	±0·07
	RR	106	238	317	280	±6·1

TABLE 6. Changes in arterial P_{O_2}, P_{CO_2} and pH following haemorrhage. C = initial control value; times refer to time after haemorrhage. S.E. of mean determined by analysis of variance for within-animal comparisons

		Arterial P_{O_2} (mm Hg)					Arterial P_{CO_2} (mm Hg)					Arterial pH				
		C	15 min	30 min	4 hr	S.E.	C	15 min	30 min	4 hr	S.E.	C	15 min	30 min	4 hr	S.E.
Normal	(2)	95	104	105	99	±1·8	30	27	26	33	±0·7	7·47	7·50	7·50	7·48	±0·022
Adrenalectomy	(3)	94	102	108	106	±3·2	34	28	28	32	±1·7	7·46	7·48	7·51	7·50	±0·022
Guanethidine	(3)	95	101	99	101	±1·9	33	31	34	37	±1·4	7·46	7·47	7·46	7·45	±0·008
Guanethidine + adrenalectomy	(1)	88	98	96	90	—	36	38	35	35	—	7·49	7·49	7·50	7·49	—

the greater lowering of the arterial perfusion pressure. The rapid restoration of the blood volume is probably an important factor in the recovery of all preparations, since it helps restoration of the right atrial filling pressure and cardiac output.

'Passive' effects of haemorrhage. The results in completely 'de-efferented' animals indicate the magnitude of the passive mechanical and local effects of removing blood in the absence of any reflex control through the autonomic nervous system of the circulation. In view of the relative distensibility ($\Delta V/\Delta P$) of low to high pressure sections of the circulation of about 150/1 (Gauer & Henry, 1963), the blood removed will come almost exclusively from the low pressure vessels. This in turn will result in an immediate lowering of central venous pressure, poor cardiac filling, reduction in cardiac output and a fall in the arterial pressure without marked alteration in total peripheral resistance. The fall of about 2·1 mm Hg observed in the 'de-efferented' rabbit during the first 40 min after the start of haemorrhage is similar to the passive pressure changes calculated for man for an equivalent change in blood volume (Henry, Gauer & Sieker, 1956). The 'passive' effects resulting from bleeding are most clearly evident during and immediately after haemorrhage. In the 'de-efferented' animals the usual tachycardia observed during bleeding is absent, and instead there is bradycardia about 10–15 min after the start of bleeding, coinciding with the period of maximum arterial hypotension and reduction in cardiac output. It seems probable that the local effects of prolonged poor coronary perfusion on the cardiac pace-maker (Schaefer, 1960; James & Nadeau, 1963) contribute to the slowing of the heart rate.

After 4 hr, when blood volume and right atrial pressure had been restored, the arterial pressure and, to a lesser extent, the cardiac output were still considerably below the values reached in normal animals. In animals without autonomic control there is thus considerable circulatory depression after 4 hr when compared to normal, a difference no longer evident after 24 hr. This depression could involve both the heart and peripheral vessels, but the present experiments provide no information concerning the nature of the depressant factors or their site of action (Crowell & Guyton, 1962; Lundgren, Lundwall & Mellander, 1964; Mellander & Lewis, 1963; Fine, 1965).

Role of the autonomic effectors. Probably most of the blood removed from normal animals with reflexes intact also comes from the low pressure regions of the circulation. The magnitude of the reflex effects has been assessed by comparison of the circulatory findings of normal and 'de-efferented' animals following removal of a similar fraction of the blood volume from each. In the normal group the fall in right atrial pressure and cardiac output is significantly smaller than in 'de-efferented' animals;

there is less pronounced arterial hypotension, an immediate and sustained increase in total peripheral resistance, and cardiac acceleration. In normal rabbits the initial reflex adjustments thus include peripheral vasoconstriction and myocardial stimulation. The smaller reduction in central venous pressure does not depend on the differences in initial blood volumes between normal and 'de-efferented' animals (Table 4), or on changes in the heart rate during haemorrhage (cf. response of guanethidine-treated adrenalectomized groups with and without atropine), but reflects changes in systemic venous capacity as a result of reflex activity. Such changes could result from: (1) reflex emptying of blood depots such as the spleen; this is probably of greater importance in some species such as the dog, than in the rabbit (Wiggers, 1950); (2) selective (e.g. Price, Deutsch, Marshall, Stephen, Behar & Neufeld, 1966) or uniform increase in venomotor tone; (3) a passive diminution in volume of, for example, the highly distensible intestinal veins following splanchnic vasoconstriction. These factors could all help to minimize the fall in central venous pressure in normal animals during and after haemorrhage, and would thus in turn contribute towards a more adequate cardiac output than found in 'de-efferented' animals.

After 4 hr the blood volume, right atrial pressure and cardiac output had virtually recovered in normal animals. However there was still significant arterial hypotension suggesting some circulatory depression even in this group. This was much less evident than in 'de-efferented' animals and suggests that increased reflex activity is present for at least 4 hr after haemorrhage.

In animals with only one orthosympathetic effector pathway intact, the circulation is less effectively maintained than normal, but better than in 'de-efferented' animals, both during the acute phase of blood loss and for 4 hr after haemorrhage. The present results indicate that under normal circumstances the reflex increase in sympathetic nerve activity and in adrenal medullary secretion are both of almost equal importance in the control of the circulation after haemorrhage. This contrasts with the results obtained during arterial hypoxia where an increase in sympathetic nerve activity appears to be quantitatively more important than the reflex release of adrenal catecholamines (Korner & White, 1966).

The results in adrenalectomized animals with only sympathetic nerves intact indicate that the increased reflex activity after bleeding is as effective as in normal animals in minimizing the fall in central venous pressure and, to a slightly lesser extent, in cardiac output. The degree of arterial hypotension is, however, greater than normal for at least 4 hr after haemorrhage. The blood pressure falls to a value about halfway between the response of normal and 'de-efferented' animals, and the rise in peripheral resistance is only about half as great as normal.

In the guanethidine-treated rabbit orthosympathetic effector control occurs only through reflex release of adrenal medullary hormones (e.g. Korner & White, 1966) acting on hypersensitive effector structures. In these animals there is a large fall in right atrial pressure immediately after the start of bleeding, similar in extent to the 'passive' effects observed in 'de-efferented' animals. However, the reduction in cardiac output is smaller and is similar to that of normal rabbits. The magnitude of the arterial hypotension is about half way between the response of normal and 'de-efferented' rabbits and is thus the same as in adrenalectomized animals. The somewhat greater tachycardia observed in guanethidine-treated animals compared to adrenalectomized rabbits, and the maintenance of a similar cardiac output at lower right atrial (and presumably left atrial) filling pressure against the same arterial pressure load, may be taken as evidence of myocardial hypersensitivity in the guanethidine-treated animals, with both chronotropic and inotropic manifestations. It is likely that hypersensitivity to the effects of adrenal catecholamines is also present at sympathetic nerve endings on the peripheral blood vessels, but the amount of these hormones reflexly released due to haemorrhage is insufficient to raise the arterial pressure to normal values, and the maximum rise in total peripheral resistance is only about half normal. The haemodynamic pattern after reflex adrenal medullary hormone liberation following haemorrhage differs mainly from normal with respect to the effects on central venous pressure. The present findings suggest that increased sympathetic nerve activity is necessary to produce the changes in venous capacity referred to above, which minimize the fall in right atrial pressure after haemorrhage.

The present findings indicate that some reflex effects after haemorrhage are also mediated through the efferent vagus nerves. This is most clearly seen by comparing the responses of the two groups of rabbits with no functioning orthosympathetic effectors with and without vagal block with atropine. In animals with intact vagus the initial tachycardia after bleeding is nearly as great as in normal rabbits, and probably accounts partly for the smaller initial reduction in cardiac output and arterial pressure compared to completely 'de-efferented' animals. In addition the possibility of ionotropic effects exerted as a result of withdrawal of vagal activity (De Geest, Levy, Zieske & Lipman, 1965) cannot be excluded. In animals with one or more orthosympathetic pathway intact, vagal blockade resulted in less tachycardia throughout the post-haemorrhage period and a more marked reduction in cardiac output during the period immediately following haemorrhage. This suggests that reflex diminution of vagal activity to the heart plays some part in the normal circulatory response to haemorrhage. In rabbits with intact reflexes the marked

bradycardia associated with human vaso-vagal attacks following bleeding (Shepherd, 1963) was never observed.

Role of carotid and aortic receptor zones. In animals with chronic section of the carotid sinus and aortic nerves there is some resting increase in tonic sympathetic activity (Heymans & Neil, 1958; Korner, 1965). In these animals the fall in right atrial pressure after haemorrhage was small as in normal animals. Despite the relatively slight change in atrial filling pressure, there was a much more extensive fall in arterial pressure and cardiac output soon after the start of bleeding, and the rise in total peripheral resistance seen in normal animals was abolished. In addition, the tachycardia normally observed after haemorrhage was not present in rabbits with section of the carotid sinus and aortic nerves. The present experiments demonstrate that the carotid and aortic reflexes are of particular importance in the maintenance of the arterial pressure after haemorrhage, and also account for the increase in heart rate. In their absence the arterial pressure was not well maintained even 4 hr after bleeding when the blood volume had been restored.

The arterial baroreceptors probably play the dominant part in the above response. Landgren & Neil (1951) have shown in anaesthetized animals that after severe haemorrhage there is a considerable increase in respiratory minute volume due to increased chemoreceptor activity. Even a small increase in arterial chemoreceptor activity could be important, in the unanaesthetized animal in potentiating the circulatory effects of the baroreceptor reflexes (Euler & Schmiterlöw, 1944; Heymans & Neil, 1958).

The small magnitude of the changes in right atrial pressure after bleeding in animals with section of the carotid sinus and aortic nerves requires comment. The results indicate maintenance of central venous pressure after bleeding above the 'passive' levels of the de-efferented animals. The high background tonic sympathetic activity in these animals might contribute to this, or it might be due to evoked reflex activity after haemorrhage in these animals as suggested by the transient rise in arterial pressure observed for about 1 hr after haemorrhage. The present experiments give no information regarding possible sources of additional reflex activity, or indeed whether this has physiological significance in the normal response to haemorrhage. Receptors from the heart and pulmonary circulation could contribute to the response (Langrehr & Kramer, 1960; Schaefer, 1960; Ross, Frahm & Braunwald, 1961; Sharpey-Schaefer, 1961; Coleridge & Kidd, 1963; Duke, Green, Heffron & Stubbens, 1963; Paintal, 1963; Coleridge, Coleridge & Kidd, 1964), possibly in conjunction with the arterial baroreceptor reflexes (cf. Share & Levy, 1962). Central excitation of the vasomotor centres owing to cerebral ischaemia (Sagawa, Ross & Guyton, 1961) could also produce stimulation of the autonomic nervous

system. The demonstration of increased ventilation both in animals with section of the carotid sinus and aortic nerves and in the 'de-efferented' animals at the time of maximum hypotension suggests excitation of the respiratory centres in the central nervous system. It seems probable that similar excitation of the vasomotor centres may have been present in animals with section of the carotid sinus and aortic nerves. It is unlikely that cerebral ischaemia plays a part in the response of the normal animal with intact carotid and aortic reflexes, since the presence of these reflexes prevents the arterial pressure from falling below 75–80 mm Hg.

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