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Investigations Into the Causes of the Rise in Aldosterone Secretion During Haemorrhage. Part I.

Margarethe Holzbauer and Marthe Vogt

Phil. Trans. R. Soc. Lond. B 1966 **250**, 243-276
doi: 10.1098/rstb.1966.0003

INVESTIGATIONS INTO THE CAUSES OF THE RISE IN ALDOSTERONE SECRETION DURING HAEMORRHAGE. PART I

By MARGARETHE HOLZBAUER* AND MARTHE VOGT, F.R.S.

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

(*Received* 31 *August* 1965)

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* Beit Memorial Research Fellow 1959–63.

Vol. 250. B. 767. (Price £1. 5*s*. 6*d*.; U.S. $3.80) 29 [Published 7 July 1966

The effect of haemorrhage on aldosterone secretion was studied in anaesthetized dogs with intact pituitary glands and kidneys subjected to the stress of adrenal vein cannulation. The following observations were made:

Acute haemorrhage was followed by a significant rise in aldosterone secretion in about one half of the animals studied.

In most of the remaining dogs, called non-reactors, premature stimulation of aldosterone secretion before the withdrawal of blood appeared to be the cause for the lack of response. This stimulation was traced in many instances to prolonged surgical 'stress', in others to incipient circulatory failure.

Another reason for a high initial secretion rate of aldosterone was low dietary sodium intake continued for a week or more.

Increase in aldosterone secretion after haemorrhage was unimpaired by sectioning the vagi or the splanchnic nerves, and by the absence of the proprioceptors of carotid sinus and thyro-carotid junction, or of liver, spleen and gastrointestinal tract.

During haemorrhage there is secretion of medullary amines and anoxia develops. The effect of these factors on aldosterone secretion was tested by infusing adrenaline and noradrenaline in the splanchnotomized animal, and by carrying out exchange transfusions with plasma till the dog had lost 50 % of its red cells. Provided the initial aldosterone secretion was low enough, these procedures caused small rises in output of aldosterone, but constituted less effective stimuli than blood loss.

Glucocorticoid secretion was in all animals maximal or near maximal and changed but little in the course of the experiments.

The findings suggest that, in the intact dog, aldosterone secretion is influenced by a variety of factors, most of which act indirectly by releasing *ACTH*, or renin, or both. The role of *ACTH* and of renin as mediators of the action of haemorrhage on secretion of aldosterone will be studied in part II.

INTRODUCTION

Acute haemorrhage stimulates aldosterone secretion in the dog (Farrell, Rosnagle & Rauschkolb 1956). This observation has been confirmed, extended to other species, and used as a standard stimulus in investigations on the control of aldosterone secretion in several laboratories. The present and the following paper deal with experiments which were designed to study the mechanism of this phenomenon.

Sudden withdrawal of substantial amounts of blood calls into action a great number of compensatory mechanisms, most of which are concerned with the maintenance of the arterial pressure at a level sufficient to insure adequate oxygen supply to the vital organs. The mechanism best studied is the increase in sympathetic activity (Cannon 1932) which results in vasoconstriction in the splanchnic bed and certain muscle areas and is triggered by baroreceptors of the carotid sinus and the aorta (Heymans & Neil 1958). Closely related are the effects caused by the catecholamines released from the adrenal medulla (Bedford & Jackson 1916). A different mechanism which contributes towards maintaining the blood

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pressure is the release of renin during bleeding (Huidobro & Braun-Menendez 1942; Scornik & Paladini 1964). Furthermore, haemorrhage causes release of *ACTH* (Sydnor & Sayers 1954) and of vasopressin (Ginsburg & Heller 1953; Ginsburg 1954; Weinstein, Berne & Sachs 1960). These factors have to be considered in an analysis of the processes involved in the rise in aldosterone secretion following haemorrhage.

All experiments described in this part of the work were carried out under conditions of operative stress in the presence of the pituitary gland and the kidneys, conditions under which both aldosterone and glucocorticoid secretion were already elevated prior to haemorrhage. Therefore it was necessary to determine how consistently, under these circumstances, a rise in aldosterone secretion would follow haemorrhage. Then experiments were designed to test whether receptors in any particular organ might be found to initiate the response, whether it disappeared in the absence of the vascular beds of liver and gastrointestinal tract, of the nerve supply to the baroreceptors and chemoreceptors of the carotid arteries, of the splanchnic nerves or of the vagi. Furthermore a possible participation of adrenal medullary hormones was studied. Finally, tissue oxygen tension was lowered in order to test whether chemoreceptor stimulation could lead to a rise in aldosterone secretion.

METHODS

Operative procedures

Mongrel dogs of both sexes were used and numbered in chronological order. Dogs 15 to 113 were used 1 to 4 days after admission so that their previous electrolyte intake was essentially unknown. The dogs of the subsequent experiments were often kept for long periods. Dogs 114 to 158 had a daily Na^+ intake in the range of only 30 m-equiv. (milli-equivalents). Later the food was supplemented by 70 m-equiv. Na^+/day in the form of NaCl so that the daily sodium intake was about 100 m-equiv. The potassium intake was about 65 m-equiv. throughout. Starting with dog 239, all dogs were vaccinated against distemper on admission.

The anaesthesia used was ether followed by chloralose (70 to 80 mg/kg, 0·7 to 1·0% solution in 0·9 % sodium chloride, i.v.). When required, artificial respiration was given through a tracheal cannula by a Starling pump, 20 rev/min, stroke 15 ml./kg body weight. One adreno-lumbar vein was approached either by an abdominal midline incision or an incision in one flank and prepared for collection of adrenal blood in such a way that the blood could be shunted into the femoral vein between periods of collection. Dogs 236 to 339 received an intravenous infusion of suxamethonium bromide B.P., 0·05 % solution in 0·9 % sodium chloride, about 0·3 ml./min, while the abdomen was opened and the adreno-lumbar vein dissected. Heparin, 350 to 450 u./kg, was injected before adrenal vein cannulation. Femoral arterial blood pressure was recorded with a mercury manometer. Blood losses incurred during the collection of adrenal venous blood and by spontaneous bleeding were replaced by infusions of donor blood. This was obtained from dogs which were either freshly admitted or had been kept on the same diet as the experimental dogs. The blood was preserved as described previously (Holzbauer 1964). Mepyramine maleate (5 mg/kg to dogs 49 to 217, 2 mg/kg to dogs 218 to 235, 1 mg/kg to all further dogs) was given before infusing donor blood in order to try and minimize sensitivity reactions which often result from intolerance to homologous blood (Bliss, Johns & Burgen 1959; Remington & Baker 1959). Starting with

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dog 272 an approximate estimate of the amount of blood lost by wound bleeding was obtained by covering the wounds with weighed pads of cotton wool. Adrenal blood flow as given in tables and figures represents all the blood collected per hour from the cannulated adreno-lumbar vein after ligation of accessible tributaries. Small blood specimens for the estimation of the haematocrit and the Na^+ and K^+ content of the plasma were taken from a femoral artery.

Variations in the operative procedures as they were necessitated by specific problems are discussed in the corresponding sections.

Chemical procedures

The methods used for the extraction of steroids from adrenal venous plasma or blood, the purification of the extracts, separation of the individual steroids by paper chromatography in three different systems and their quantitative estimation by a colour reaction with blue tetrazolium have been developed in the course of this investigation and differ slightly at different stages of the work. Detailed descriptions of the chemical methods as they were used at an early and at the last stage of the investigation have been given previously (Holzbauer & Vogt 1961; Holzbauer 1964).

Essential modifications introduced in the course of the work were as follows:

Extraction

The adrenal blood samples of dogs 15 to 54 were spun immediately after collection and only the plasma was extracted. In all further experiments whole blood was analysed.

Purification of extracts

Starting with the samples from dog 138, the ethanolic phase obtained after defatting the extracts, and the acetone remaining after precipitation of the phospholipids, were no longer evaporated *in vacuo*, but taken to dryness in a water bath of 40 to 50 °C by means of a stream of N_2. This was replaced later by a stream of filtered compressed air.

Chromatography

The width of paper used for chromatography was gradually decreased without loss of efficiency, the final sizes being those described by Holzbauer (1964).

Elution

The time allowed for the elution of larger strips of paper was extended from 2 h to 3 to 4 h starting with dog 236; with the shorter elution period there might have been occasional losses.

Colorimetry

All steroids were estimated by the colour formed with blue tetrazolium in an alkaline medium. The method described by Vogt (1955) was used for the estimation of cortisol and corticosterone throughout the work, with the exception of the experiments on hypophysectomized dogs, in which a micromethod (Holzbauer & Vogt 1961) was employed. This micromethod was used throughout for aldosterone starting with experiment 41.

'Paper blanks'

In order to remove non-steroidal impurities which are eluted from chromatography paper and which reduce blue tetrazolium, the papers used for the last chromatogram of aldosterone were washed. The procedure always included 48 h washing with a mixture of ethylacetate:methanol, 2:1. This was later found to be the only essential part of the washing procedure. It was also used for the chromatography of cortisol and corticosterone when small amounts of steroid only were available as in samples from hypophysectomized dogs. Eluates from one to three blank areas of each final chromatogram were reacted with blue tetrazolium and their photometer readings ('paper blank') subtracted from the reading of the eluate containing the steroid. At different periods of the work these paper blanks, expressed in μg aldosterone, varied; in any one group of estimations carried out on any one day a difference in aldosterone values between two extracts was only considered to differ from zero if it exceeded twice the highest difference between blanks. From dog 184 onwards redistilled benzene and petroleum ether were used for the last chromatogram. This reduced the blanks to a mean which was equivalent to 0·35 μg aldosterone, and the variation between blanks became very small.

Recoveries

The results of recovery experiments carried out by adding to peripheral blood non-radioactive aldosterone, cortisol and corticosterone in quantities likely to occur in the samples of adrenal venous blood have been described (Holzbauer & Vogt 1961). When radioactive aldosterone became available, it was possible to measure the losses of aldosterone sustained in each individual sample. Beginning with dog 232, a known quantity of 7-^{3}H-aldosterone was added to the blood immediately after collection, and the recovered radioactivity was measured after having carried out the colour reaction between aldosterone and blue tetrazolium. Originally, each coloured solution was transferred to a counting vial, evaporated to dryness and the scintillator was added. In this way no quenching occurred. It was, however, found that evaporation to dryness in the presence of the chemicals used for the reaction with blue tetrazolium sometimes led to losses in counts of up to 10 %, independent of the absolute amount of counts present. Starting with dog 284, the method was therefore changed to that already published (Holzbauer 1964) in which evaporation was avoided and the degree of quenching due to the ethanol estimated and taken into account. In all experiments in which radioactive aldosterone was used, the figures obtained from the reaction with blue tetrazolium were corrected for 100 % recovery. The validity of this calculation was investigated in experiments in which unlabelled and radioactive aldosterone were added to samples of peripheral blood and the samples were carried through the full extraction and estimation procedures. When the estimates for aldosterone obtained with the colour reaction were corrected for the losses estimated by counting the radioactivity, the mean amount of aldosterone found was 117 % $\pm$ 3·4 % (S.E.) of the amount added. It was, however, observed that small amounts of a substance reacting with blue tetrazolium are present in the aldosterone diacetate region of the last chromatogram of 'blank' samples of peripheral blood. If this quantity is subtracted from the result the figure for the mean corrected recovery of aldosterone becomes 103 % $\pm$ 3·4 % (S.E.).

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At a later stage of the work a decrease of the specific activity in the sample of 7-^{3}H-aldosterone was observed. Only about 65 % of the counts were found in the aldosterone region of paper chromatograms developed in Bush's system B_5. Most of the remaining activity had travelled with the solvent front. Allowance for this disintegration was made when losses were calculated.

From results obtained on 410 samples a mean recovery of 33 % (s.d. $\pm$ 2·4) of the aldosterone added at the beginning of the extraction procedure was calculated. The amount of radioactivity recovered was independent of the size of the original blood sample (30 to 200 ml.) and was the same in samples of whole blood, plasma or blood cells.

Estimation of the plasma concentration of Na^+ *and* K^+

Sodium and potassium were measured in the plasma of samples from arterial blood with an 'EEL' flame photometer.

RESULTS

I. Effect of haemorrhage on aldosterone secretion in the anaesthetized dog subjected to adrenal vein cannulation only

Procedures and observations

Variability of basal cortical secretion rates during consecutive periods of collection

In eight dogs the variability of corticoid secretion was studied by analysing two or three samples of adrenal blood collected at short intervals. The adrenal vein was approached through a midline incision or from the flank and collection was begun 30 to 60 min after completion of the dissection, collection periods being 20 to 25 min with intervals of 15 min.

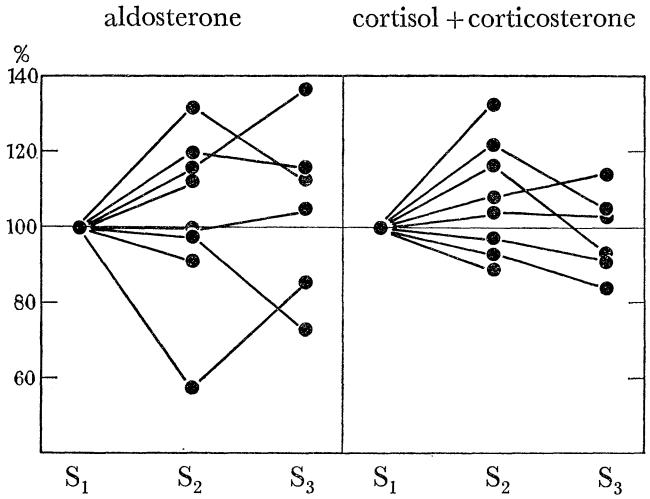

Figure 1. Spontaneous changes in adrenal steroid secretion during three consecutive collection periods of 20 to 25 min (S_1, S_2, S_3); 15 min intervals between collections. Results expressed as percentage of initial secretion rate.

The results are shown in figure 1. The largest rise in aldosterone secretion observed in two consecutive periods was 30 % in one dog. Changes in glucocorticoid secretion rates also reached 30 % only once. From these observations it was assumed that rises in aldosterone

secretion above 30 % do not occur under constant experimental conditions. All dogs which showed rises of more than 30 % in response to haemorrhage will therefore be referred to as 'reactors', those which failed to do so as 'non-reactors'.

Aldosterone secretion following expansion and subsequent reduction of the blood volume

These were early experiments in which corticoids were estimated in plasma. The adrenal vein was cannulated from a midline incision.

In a first series of dogs the blood volume was expanded by dog's plasma or by plasma substitutes (dextran or polyvinylpyrrolidine) and then reduced by bleeding. Significant changes in aldosterone secretion were only obtained after reduction of the blood volume, but interpretation was complicated because a number of dogs bled profusely as a result of the infusions; furthermore, the plasma substitutes were often poorly tolerated.

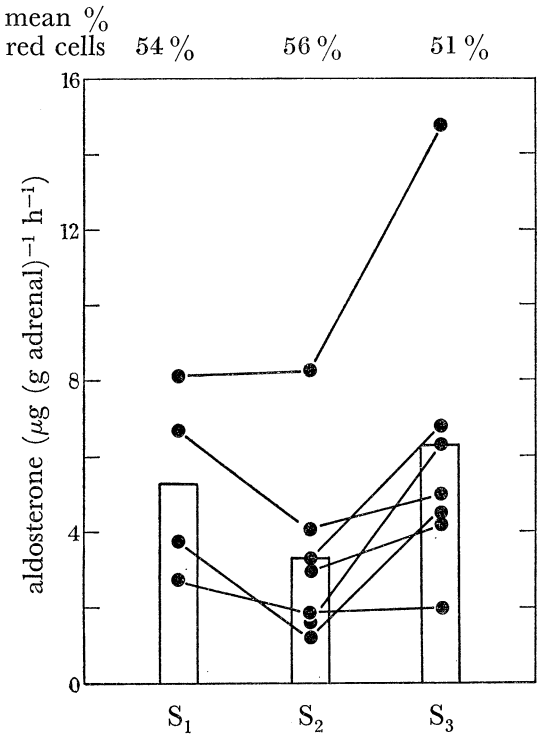

FIGURE 2. Effect of expanding and reducing the blood volume on the secretion rate of aldosterone (μg (g adrenal)$^{-1}$ h^{-1}). S_1, before blood infusion (mean and S.E. 5·3 ± 1·1); S_2, after infusion of blood, 20 ml./kg (mean 3·3 ± 1·0); S_3, after haemorrhage (mean 6·3 ± 1·6). Salt intake not controlled. First sample taken immediately after completion of operation. (Steroids extracted from plasma; aldosterone figures not corrected for losses; ●, individual figures; columns, means).

In a second series dog's blood was used to expand the blood volume before taking sample marked S_2 (figure 2), and also to replace the blood losses incurred during collection of adrenal blood. Variable and often very severe bleeding from the wounds followed hypervolaemia produced by homologous blood. Therefore, the amount of blood which could be safely withdrawn from the femoral artery before collection of sample 3 without causing shock was occasionally negligible (3 ml./kg) and sometimes as high as 30 ml./kg. Figure 2 shows that five of seven dogs reacted to blood loss with increases in aldosterone secretion

ranging from 40 to 318 %, some of these changes being larger than the falls following expansion. In view of the excessive bleeding caused by infusions of large volumes of homologous blood, experiments using expansion of the blood volume were discontinued and haemorrhage alone was used as the standard stimulus for aldosterone secretion.

Aldosterone secretion following haemorrhage; 30 min interval after surgery

In these and all subsequent experiments, corticoids were extracted from whole blood. In a first group of 15 dogs (tables 1 and 2), adrenal blood collection was started 30 min after completion of dissection.

TABLE 1. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION

First adrenal blood sample (S_1) collected 30 min after end of dissection; second sample (S_2) after haemorrhage. Dietary sodium intake not controlled, midline incision. (Steroids extracted from whole blood, aldosterone figures not corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol	corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	blood withdrawn between S_1 and S_2 (ml./kg)
			Group I. Reactors						
108, male	13·5	S_1	6·1	521	230	276	122	−	24
		S_2	9·2 +51	744 +43	230 0	161	74	+	
109, female	21·6	S_1	0·8	372	309	600	130	−	25
		S_2	3·0 +275	491 +32	261 −16	216	52	−	
111, male	17·1	S_1	4·7	972	605	336	138	−	28
		S_2	8·1 +72	1093 +12	460 −33	143	60	+	
mean ±s.e.		S_1	3·9 ± 1·6	622 ± 180	381 ± 114	404 ± 100	130 ± 4·6		26 ± 1·2
		S_2	6·8 ± 1·9	776 ± 175	317 ± 72	173 ± 22	62 ± 6·4		
			Group II. Non-reactors						
55, male	17·0	S_1	11·3	983	547	300	135	+	22
		S_2	9·2 −19	769 −22	275 −50	140	64	++	
99,* female	17·1	S_1	4·7	655	338	624	160	−	23
		S_2	5·8 +23	753 +15	305 −10	396	80	+	
102,* male	21·6	S_1	14·0	1299	558	272	160	−	14
		S_2	11·9 −15	1072 −18	346 −38	168	100	+++	
107,† male	16·6	S_1	8·3	747	362	459	128	−	22
		S_2	8·3 0	894 +20	272 −25	150	55	−	
mean ±s.e.		S_1	9·6 ± 2·0	921 ± 144	451 ± 59	414 ± 81	146 ± 8·4		20 ± 2·1
		S_2	8·8 ± 1·3	872 ± 74	299 ± 17	214 ± 61	75 ± 9·9		

* Some blood withdrawn before S_1. † Cheyne Stokes respiration.

In the seven dogs of table 1 salt supplements were not given, and the aldosterone figures were not corrected for losses.

Table 1 shows that the mean initial aldosterone secretion rate was much lower in the three dogs classified as reactors than in the remaining four animals. The raised initial secretion and failure to react might have been related to an impaired general condition of these animals. In dog 55 some abnormality was suggested by the occurrence, before the dog was bled, of regular blood pressure waves which were slower than the respiratory rhythm (Mayer waves); whereas such waves are the normal accompaniment of haemorrhage, their occurrence in the early phase of the experiment is indicative of cerebral anoxia or some other pathological condition. In keeping with this interpretation, dog 55 did not tolerate haemorrhage; after the blood loss glucocorticoid secretion fell by 30 %, a sign of impaired

adrenocortical function. Dogs 99 and 102 had been bled 5·5 ml./kg before adrenal vein cannulation in order to obtain 'donor-blood' from the same animal. This was done, in the obviously wrong assumption, that it was too little to interfere with the basal conditions of the animal. In dog 107 Cheyne Stokes respiration developed, which may have been due to cerebral damage.

Table 2 contains information on eight dogs in which an attempt was made at lowering the initial secretion rate of aldosterone by giving the dogs a salt supplement and by reducing the operative stress; this was done by approaching the adrenal from the flank. The aldosterone values are corrected for losses.

TABLE 2. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION

First adrenal blood sample (S_1) collected 30 min after end of dissection, second sample (S_2) after haemorrhage. Flank incision. (Steroids extracted from whole blood, aldosterone figures corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol	corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	Ht (% red cells)	blood withdrawn between S_1 and S_2 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
Group I. Reactors											
237, male	15·4	S_1	15·1	900	582	128	135	++	50	17	66
		S_2	21·7 + 44	782 − 13	455 − 22	124	95	++	53		
240, female	21·0	S_1	7·7	867	109	164	150	−	66	20	14
		S_2	18·1 + 135	971 + 12	235 + 116	168	126	−	62		.
241, male	12·6	S_1	14·5	.	.	161	172	−	51	19	14
		S_2	27·1 + 87	1138 .	836 .	101	92	+	54		.
243, male	12·5	S_1	14·1	717	463	172	164	−	.	20	17
		S_2	23·6 + 67	641 − 11	381 − 18	136	132	+	53		.
mean ± S.E.		S_1	12·9 ± 1·7	828 ± 56	385 ± 142	156 ± 9·7	155 ± 8			19 ± 0·7	
		S_2	22·6 ± 1·9	883 ± 109	477 ± 128	132 ± 14·0	111 ± 10	.	.		.
Group II. Non-reactors											
242, male	26·5	S_1	12·0	572	619	548	190	−	79	20	20
		S_2	11·5 − 4	605 + 6	504 − 19	528	144	−	71		.
244, male	12·5	S_1	8·3	839	375	355	160	−	61	20	21
		S_2	8·6 + 4	811 − 3	326 − 13	264	104	−	55		.
245, male	12·0	S_1	11·9	768	513	245	152	−	47	20	16
		S_2	14·0 + 18	702 − 9	561 + 9	178	112	+	49		.
246, female	17·0	S_1	14·0	757	810	594	182	−	50	20	8
		S_2	11·0 − 21	594 − 22	405 − 50	399	101	+	52		.
mean ± S.E.		S_1	11·6 ± 1·2	734 ± 57	579 ± 92	436 ± 82	171 ± 9			20 ± 0	
		S_2	11·3 ± 1·1	678 ± 51	449 ± 52	342 ± 77	115 ± 9·8	.	.		.
				cortisol + corticosterone							
combined means groups I and II		S_1	12·2 ± 1·0	1271 ± 75		296 ± 65	163 ± 6·0				
		S_2	17·0 ± 2·4	1256 ± 122		237 ± 54	113 ± 6·6	.	.	.	.

In this series the initial aldosterone secretion was low in all dogs, 12·2 μg (g adrenal)$^{-1}$ h^{-1} (corrected) being about equivalent to the amount of 3·9 (uncorrected) found in the reactors of table 1. Yet there was no response in four animals; an explanation can only be offered for dogs 242 and 246; dog 242 had a very high haematocrit and a blood pressure as high as 144 mmHg after haemorrhage. In this animal the withdrawal of blood might have constituted a therapeutic instead of a damaging measure. In dog 246 the fall of 36 % in glucocorticoid secretion after haemorrhage showed that there had been damage to the adrenal.

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Aldosterone secretion following haemorrhage; 180 *min 'rest' after surgery*

The next experiments were designed to try whether a longer interval between the surgery and the taking of the first blood sample would improve the general condition of the dogs and thus increase the number responding to haemorrhage. In 10 dogs (table 3) 150 min rest were allowed between the end of the dissection and the injection of heparin which

TABLE 3. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION

First adrenal blood sample (S_1) collected 180 min after end of dissection, second sample (S_2) after haemorrhage. Flank incision. (Steroids extracted from whole blood, aldosterone figures corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	drenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol	corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	blood withdrawn between S_1 and S_2 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
Group I. Reactors										
330, female	11·0	S_1	7·1	877	331	206	135	–	21	10
		S_2	15·5 +118	1108 +26	412 +24	150	130	–		.
335, male	11·8	S_1	8·9	877	351	166	155	–	28	3
		S_2	12·3 + 38	1038 +18	414 +18	130	115	+		.
337, female	12·7	S_1	19·5	1310	358	317	143	–	24	15
		S_2	27·1 + 39	1392 + 6	368 + 3	187	90	+		.
338, male	10·6	S_1	8·3	1094	302	237	125	–	25	16
		S_2	13·6 + 64	1484 +36	406 +34	188	100	+		.
339, male	12·9	S_1	5·9	1156	767	195	140	–	20	17
		S_2	9·0 + 53	1389 +20	661 −14	105	110	+		.
mean ±s.e.		S_1	9·9 ± 2·4	1063 ± 84	422 ± 87	224 ± 26	140 ± 5	.	25 ± 0·14	.
		S_2	15·5 ± 3·1	1282 ± 88	452 ± 53	152 ± 16	109 ± 7			
Group II. Non-reactors										
327, male	13·1	S_1	15·2	1722	954	240	175	–	21	42
		S_2	16·3 + 7	1638 − 5	720 −25	158	100	–		.
328, male	12·4	S_1	5·8	1046	571	424	170	–	21	32
		S_2	5·2 −10	1284 +23	659 +15	260	80	–		.
329, male	13·2	S_1	10·5	1258	1069	313	175	+	15	31
		S_2	4·2 −60	1160 − 8	545 −49	256	115	–		.
332, male	7·8	S_1	16·4	1304	535	150	140	–	23	9
		S_2	18·5 +13	1571 +20	545 + 2	113	95	+		.
333, male	14·0	S_1	13·3	1189	578	226	135	–	20	24
		S_2	14·1 + 6	1351 +14	611 + 6	267	115	+		.
mean ±s.e.		S_1	12·2 ± 1·9	1304 ± 113	741 ± 112	271 ± 46	159 ± 8·9	.	20 ± 1·3	.
		S_2	11·7 ± 2·9	1401 ± 89	616 ± 34	211 ± 32	101 ± 6·6	.		.
				cortisol + corticosterone						
combined means groups I and II		S_1	11·1 ± 1·5	1766 ± 147		247 ± 26	149 ± 5·8	.	.	.
		S_2	13·6 ± 2·1	1876 ± 87		181 ± 19	105 ± 4·7			

permitted the establishment of the shunt flow from the adrenal to the femoral vein. Another 30 min was allowed before collecting adrenal blood. As this group of experiments was also intended to serve as a control for experiments on nephrectomized dogs (see part II, p. 294), a minor intestinal operation was performed in order to simulate the surgical stress and manipulations of unilateral nephrectomy. A short piece of small intestine (dogs 327, 329, 330), a piece from the rectum (dog 328) or the tip of the caecum (dogs 332, 333, 335, 337, 338, 339) were removed.

The longer resting period did not consistently decrease the basal secretion rate of aldosterone. A rise in aldosterone secretion after haemorrhage was again present in only 50 % of the dogs. These dogs also showed rises in glucocorticoid secretion. With one exception in each group, the reactors had low and the non-reactors high initial aldosterone secretion rates. There were some anaemic dogs and some dogs with a high blood sedimentation rate in both groups; one of the non-reactors showed signs of circulatory collapse when bled only 15 ml./kg, whereas three of the reactors withstood the withdrawal of 24 to 28 ml./kg.

The cannulation of the adrenal vein is a severe stress and must lead to the release of *ACTH*. This *ACTH* release was not reduced, but enhanced, by the longer 'resting' period, as indicated by the higher secretion rate of glucocorticoids. From previous observations (Holzbauer 1964) it can be calculated that the *ACTH* secretion rate was more than doubled in the experiments with the longer rest. In spite of this, aldosterone secretion was not increased. This would suggest that the influence of some factor other than *ACTH* capable of stimulating aldosterone production was reduced during the longer resting period.

Sodium and potassium in plasma

Observations on the plasma concentrations of Na^+ and K^+ are summarized in table 4. There was no significant difference between the reactors and non-reactors. A slight rise in the plasma K^+ concentration (mean of 0·3 m-equiv./l.) was observed in all animals towards the end of the experiment. The mean plasma K^+ concentrations after a resting period of 180 min was 0·8 m-equiv./l. higher ($P < 0{\cdot}01$) than that found after a 30 min resting period.

TABLE 4. PLASMA CONCENTRATIONS OF Na^+ AND K^+ BEFORE AND AFTER HAEMORRHAGE IN DOGS UNDER CHLORALOSE ANAESTHESIA, SUBJECTED TO ADRENAL VEIN CANNULATION AND BLOOD COLLECTION

Plasma samples from femoral artery blood. A_1: immediately before first period of adrenal blood collection; A_2: after haemorrhage, and before collecting the second sample of adrenal blood; A_3: at the end of that collection. Means ± S.E.

group of dogs		arterial plasma sample no.	plasma concentration (m-equiv./l.) Na^+	K^+
dogs 237–246, table 2 (30 min after end of dissection)	'reactors'	A_1	142 ± 0·9	3·1 ± 0·20
		A_2	137 ± 0·6	3·4 ± 0·07
	'non-reactors'	A_1	143 ± 1·4	3·3 ± 0·23
		A_2	144 ± 1·7	3·5 ± 0·19
dogs 327–339, table 3 (180 min after end of dissection)	'reactors'	A_1	146 ± 1·3	4·0 ± 0·15
		A_2	144 ± 2·5	4·1 ± 0·15
		A_3	146 ± 1·2	4·3 ± 0·10
	'non-reactors'	A_1	152 ± 2·8	3·9 ± 0·10
		A_2	149 ± 1·6	4·2 ± 0·10
		A_3	156 ± 1·4	4·1 ± 0·20

Conclusions

Under our experimental conditions only 50 % of the dogs responded to haemorrhage with a rise in aldosterone secretion. Since these experiments were carried out, knowledge has accumulated on the stimulating effects of *ACTH* and angiotensin on the secretion of this hormone. The most likely explanation for the large number of non-reactors is a stimulation

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of the release of these agents during the surgical procedures. This may sometimes be large enough to cause maximal aldosterone secretion and so to obscure the effect of haemorrhage. The fact that many non-reactors showed high basal secretion rates confirms this view. In some animals the initial aldosterone secretion may have been high as a result of a sodium-deficient diet, as will be shown in the next section. In the few instances in which failure to respond to haemorrhage occurred in spite of a low initial aldosterone secretion, the secretory capacity of the gland or the sensitivity to stimuli may have been subnormal. In animals with an impaired state of the circulation, there might have been damage to extra-adrenal structures which form a link in the mechanism under investigation, or interference with steroid synthesis caused by lack of oxygen or essential precursors. However, interference with steroid synthesis would be expected to affect glucocorticoids and aldosterone alike, so that this factor is excluded in all experiments in which glucocorticoid secretion showed no abrupt fall after haemorrhage.

II. Basal aldosterone secretion: effect of dietary sodium intake and of various surgical procedures

Table 5 illustrates the basal rates of aldosterone secretion obtained after surgical procedures of varying severity in dogs on low and high dietary sodium. The dogs are divided into twenty groups according to sodium intake and surgery performed. The daily potassium consumption was approximately 65 m-equiv. in all groups. The dietary changes did not affect plasma sodium concentrations, as estimated in femoral arterial blood before collection of the first sample of adrenal blood. In fact, the plasma sodium was lowest in the sodium-loaded dogs, but remained within the normal range. Laragh (1960) observed that in man, too, dietary sodium lack sufficient to cause an increase in aldosterone secretion was not associated with a low plasma sodium.

Table 5 confirms the dependence of aldosterone secretion on sodium intake. Among the dogs in which the only surgical procedure was adrenal vein cannulation through an abdominal midline incision (table 5, groups I to VI), the highest secretion rates of aldosterone were found in group I, the dogs which were kept for the longest time on the lowest sodium intake, and the lowest secretion rates in group VI, the dogs fed longest on the high sodium diet. The differences in the mean secretion rates are significant between groups I and II, I and IV and IV and VI (P between 0·05 and 0·01).

The type of operative procedures before adrenal blood collection also influenced aldosterone secretion rates. The mean aldosterone secretion rate of dogs in which the adrenal vein was approached from the midline (group IV) was significantly higher than that of dogs on the same sodium intake in which the incision was in the flank (groups VII and VIII), a procedure which greatly reduces traction on the viscera. After evisceration (groups XIV to XVI) and splanchnotomy (groups XVII to XX) there was also a general tendency for a raised initial aldosterone production. However, some of the groups are too small to allow any conclusions to be drawn.

Sodium deficient dogs in which both kidneys had been removed (group IX) did not show the elevated secretion rates of aldosterone found in dogs with intact kidneys (group I).

Variations in the sodium intake and different surgical procedures did not significantly affect the secretion rates of glucocorticoids.

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TABLE 5. INFLUENCE OF SODIUM INTAKE ON BASAL SECRETION RATES OF CORTICOSTEROIDS (μg (g ADRENAL)$^{-1}$ h^{-1}, MEAN $\pm$ S.E.) IN DOGS SUBJECTED TO ADRENAL VEIN CANNULATION ALONE OR TO CANNULATION PRECEDED BY NEPHRECTOMY, EVISCERATION OR SPLANCHNOTOMY

(Steroids extracted from whole blood, aldosterone figures not corrected for losses.) n = no. of dogs. $F+B$ = cortisol + corticosterone.

dietary Na^+ intake/day (m-equiv. Na^+)	no. of days		operative procedure: no additional operation: midline incision	no additional operation: right flank incision	no additional operation: left flank incision	midline incision: bilateral nephrectomy	midline incision: right nephrectomy	midline incision: evisceration	midline incision: bilateral splanchnotomy
30*	6–84 (mean: 26)	group no.	I	.	.	IX	.	XIV	XVII
		n	13			4		1	1
		aldosterone	10·8 ± 1·0			6·3 ± 2·2		10·9	10·8
		$F+B$	1627 ± 82			1754 ± 134		1816	1422
30	1–5 (mean: 2)	group no.	II	.	.	X	XIII	.	XVIII
		n	4			5	4		7
		aldosterone	6·9 ± 0·7			7·1 ± 1·3	8·8 ± 1·1		9·9 ± 2
		$F+B$	1361 ± 65			1487 ± 75	1932 ± 133		1532 ± 72
100	1–5 (mean: 4)	group no.	III	.	.	XI	.	.	.
		n	3			1			
		aldosterone	7·1 ± 1·8			6·4			
		$F+B$	1705 ± 152			1565			
100	7–31 (mean: 20)	group no.	IV	VII	VIII	XII	.	XV	XIX
		n	15	6	8	5		12	3
		aldosterone	7·4 ± 1·0	4·8 ± 0·8	3·8 ± 0·5	7·1 ± 1·2		9·8 ± 1·0	12·4 ± 0·4
		$F+B$	1637 ± 115	1518 ± 97	1467 ± 109	1579 ± 176		1640 ± 136	2084 ± 180
170 followed by 100	5–22 (mean: 12) 24–38 (mean: 29)	group no.	V	.	.	.	.	XVI	.
		n	5					2	
		aldosterone	5·8 ± 0·7					8·6, 12·7	
		$F+B$	1616 ± 112					1709, 1208	
170	4–9 (mean: 7)	group no.	VI	.	.	.	.	.	XX
		n	4						2
		aldosterone	3·9 ± 1·0						4·1, 4·4
		$F+B$	1370 ± 128						1457, 1091

* 60 m-equiv. is considered to be a normal daily sodium intake for an average size dog.

III. THE EFFECT OF EVISCERATION ON THE RESPONSE OF ALDOSTERONE SECRETION TO HAEMORRHAGE

These experiments were carried out in order to see whether the removal of viscera interferes with the rise in aldosterone secretion after haemorrhage.

Procedures and observations

The intestinal tract, the spleen and the pancreas were removed and the blood supply to the liver (from portal vein and hepatic arteries) interrupted before the adrenal vein was cannulated. Collection of a control sample of adrenal venous blood was started 40 to 120 min after evisceration (20 to 100 min after injection of heparin and adrenal vein cannulation); then the dogs were bled and a second adrenal blood sample was taken.

Preliminary experiments were carried out in three dogs at a time when the steroids were still being extracted from plasma. The amount of aldosterone and of glucocorticoids found in the adrenal venous plasma was very low. Aldosterone secretion rates were 3·5, $<0·2$ and 2·2 μg (g adrenal)$^{-1}$ h^{-1} before haemorrhage and rose after blood loss to 4·9, 1·3 and 5·1 μg respectively.

The results obtained by analysing whole blood samples from 14 eviscerated dogs are summarized in table 6. Only two of 14 dogs responded to haemorrhage with a rise in aldosterone secretion of more than 30 %. As discussed earlier, eviscerated animals showed a tendency to higher initial secretion rates of aldosterone than controls on the same sodium intake, irrespective of the time of rest allowed after completion of surgery. Accordingly, among the 12 non-reactors, 10 had initial secretion rates higher than the mean of the non-eviscerated groups on the same diet, in one (192) the rate was equal, in one (203) it was less. In the two reactors the rate was equal to that of the non-eviscerated controls.

TABLE 6. THE EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION IN EVISCERATED DOGS

Collection of first adrenal blood sample (S_1) started between 40 and 120 min after evisceration, second sample (S_2) after haemorrhage. (Steroids extracted from whole blood, aldosterone figures not corrected for losses.)

dog no.	body weight (kg)	blood sample no.	blood sample time after evisceration (min)	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	blood withdrawn between S_1 and S_2 (ml./kg)	Na^+ intake (m-equiv./day)
Group I. Reactors										
182, male	7·6	S_1	75	7·6	1955	276	142	·	12	100 for more than 7 days (as group IV, table 5)
		S_2		14·8 +95	2293 +17	139	78	·		
196, male	8·5	S_1	75	7·2	1156	254	180	–	19	
		S_2		9·7 +35	854 −26	247	105	–		
Group II. Non-reactors										
159, male	9·1	S_1	78	10·9	1816	144	114	+	10	30 m-equiv. for 28 days (as group I, table 5)
		S_2		11·4 + 5	1438 −21	76	60	+ +		
176, male	13·1	S_1	72	8·6	1709	338	123	–	12	170 for 18 days followed by 100 for 31 and 38 days (as group V, table 5)
		S_2		8·4 − 2	1533 −10	199	62	–		
179, male	16·8	S_1	84	12·7	1208	190	92	–	14	
		S_2		12·3 − 3	1697 +40	180	64	+		
160, female	7·6	S_1	44	10·7	1856	341	171	–	18	
		S_2		8·3 −22	1395 −25	151	74	–		
161, female	8·1	S_1	41	14·1	2533	336	140	–	21	
		S_2		12·4 −12	2489 − 2	154	78	–		
184, female	7·0	S_1	93	11·9	1450	228	164	–	8	
		S_2		12·9 + 8	1670 +15	106	90	–		
187, female	10·4	S_1	67	8·4	1328	302	165	–	19	
		S_2		8·5 + 1	1221 − 8	295	113	–		
192, male	15·6	S_1	72	7·2	1130	197	176	+	14	100 for more than 7 days (as group IV, table 5)
		S_2		7·2 0	1063 − 6	211	94	+		
203, female	12·5	S_1	120	3·4	1268	192	144	–	16	
		S_2		3·5 + 3	1150 − 9	116	80	+		
206, female	11·3	S_1	120	10·0	1620	411	146	–	20	
		S_2		10·0 0	1577 − 3	174	88	–		
209, female	7·3	S_1	117	9·7	1660	245	170	–	14	
		S_2		12·2 +26	1591 − 4	178	85	–		
210, male	12·4	S_1	113	15·4	2418	160	159	+	13	
		S_2		19·9 +29	1958 −19	82	70	+		

Table 7 summarizes some circulatory data and the plasma electrolyte changes in the eviscerated dogs. The mean blood loss tolerated was only 15 ml./kg as compared with 20 or more when no evisceration had been performed. The plasma potassium concentration tended to be higher than in the non-eviscerated dogs with similar resting periods (compare table 4).

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Conclusions

In eviscerated dogs, the failure of aldosterone secretion to respond with a rise to the stimulus of haemorrhage appears to be linked with high basal secretion rates of the hormone. The most likely explanation is an increase of the release of aldosterone-stimulating factors by the stress of the evisceration. Interference with steroid anabolism by excluding the liver (Ayers, Davis, Lieberman, Carpenter & Berman 1962) is another possibility. However, this should not prevent further rises, in view of the fact that there is no evidence for an aldosterone feedback mechanism within the adrenal gland (Blair-West, Coghlan & Denton 1962). Hypothetical receptors in the abdominal vascular bed forming part of a reflex pathway which inhibits aldosterone secretion and which evisceration would remove would not be incompatible with the results.

TABLE 7. MEAN VALUES AND STANDARD ERRORS OF ARTERIAL BLOOD PRESSURES, HAEMATOCRITS AND PLASMA CONCENTRATIONS OF SODIUM AND POTASSIUM BEFORE AND AFTER HAEMORRHAGE IN EVISCERATED DOGS

adrenal venous blood sample	mean b.p. (mmHg)	adrenal blood flow (ml./h)	arterial blood sample taken	haematocrit (% red cells)	plasma concentration (m-equiv./l.)	
					Na^+	K^+
S_1 control	$149 \pm 6{\cdot}8$	258 ± 21	before S_1	$58 \pm 1{\cdot}9$	145 ± 1	$3{\cdot}9 \pm 0{\cdot}1$
S_2 after haemorrhage	$82 \pm 4{\cdot}2$	165 ± 16	at end of haemorrhage	$54 \pm 1{\cdot}4$	145 ± 1	$4{\cdot}5 \pm 0{\cdot}2$
.	.	.	at end of experiment	$53 \pm 1{\cdot}3$	147 ± 2	$4{\cdot}6 \pm 0{\cdot}2$

IV. PROPRIOCEPTORS IN THE CAROTID VASCULAR BED AND ALDOSTERONE SECRETION

The suggestion that the carotid sinus may contain receptors for a reflex arc which is involved in the control of aldosterone secretion was first made by Barger, Muldowney & Liebowitz (1959). In the same year, Bartter, Mills, Biglieri & Delea (1959) reported that in nine dogs, which were anaesthetized and under the stress of adrenal vein cannulation, compression of the carotid artery low in the neck caused a significant rise in aldosterone secretion. This effect was no longer present when the carotid and thyroid arteries had been denervated 3 to 11 days previously. Biglieri & Ganong (1961) attributed the rise to a release of *ACTH* since it disappeared after hypophysectomy.

Bartter, Mills & Gann (1960) reported that the rise in aldosterone secretion which follows constriction of the thoracic vena cava is prevented by denervation of the carotid sinuses along with denervation of the origin of the thyroid arteries, a region which they found to contain additional pressure sensitive structures in the dog (Gann & Bartter 1959). In fact, in their hands, denervation of this region alone was sufficient to suppress the response. In a detailed study Carpenter, Davis & Ayers (1961 *a*) were unable to confirm these findings. They came to the conclusion that the carotid or aortic baroreceptors are not involved in the mechanism which leads to a rise in aldosterone secretion after constriction of the thoracic inferior vena cava. In this section the response of aldosterone secretion to acute haemorrhage is studied after denervation of carotid sinuses and thyroid arteries.

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Procedures and observations

In a preliminary aseptic operation both sinus nerves were cut, the region of both carotid sinuses stripped of nerve plexuses and adventitial tissue, and the two thyro-carotid junctions carefully stripped in a similar way. The adrenal blood collection was carried out 14 to 27 days later. All dogs were hypertensive. A control sample of adrenal venous blood was collected for 20, 25 or 30 min half an hour after the dissection had been completed, then 14 to 27 ml./kg of blood were withdrawn from a femoral artery and a second sample of blood was taken 5 min later. When artificial respiration was used, the stroke of the pump was increased by about 10 % during this period. At the end of each experiment the blood withdrawn during the haemorrhage was re-infused and the effect of carotid occlusion on the blood pressure was checked before and after vagotomy.

The results obtained are shown in tables 8A and B. Experiments were done on 14 dogs. In nine of these (table 8A) the Hering reflex was completely absent at the end of the experiments. In the remaining dogs vestiges of this reflex were observed after bilateral vagotomy, but the high initial blood pressures indicated that denervation must have been quite extensive.

Out of nine dogs, in which denervation was complete, five dogs responded to haemorrhage with rises in aldosterone secretion varying from 40 to 124 % whereas their glucocorticoid secretion remained unchanged (−12 to +16 %). In the remaining four dogs aldosterone secretion did not change but glucocorticoid secretion was slightly decreased, and once (dog 297) sufficiently to suggest failure of steroid synthesis. Out of five dogs in which denervation might have been incomplete, dog 295 responded to haemorrhage with a rise of aldosterone secretion by 73 %; its glucocorticoid secretion was unchanged (−7 %). The other four did not show significant changes in aldosterone secretion but there was some rise in glucocorticoid secretion. Reactors and non-reactors did not differ by the height of their blood pressure either before or after haemorrhage, they tolerated withdrawal of blood equally well (see means of table 8B), and they showed no difference in haematocrit or plasma electrolyte concentration. There was, however, a clear difference between the mean initial secretion rate of aldosterone, which was 11·7 in the reactors and 18·4 in the non-reactors. Owing, however, to one exception in each group, the difference between the means is not statistically significant. The exception among the reactors, dog 305, had the unusually high initial secretion rate of aldosterone of 27·2 μg (g adrenal)$^{-1}$ h^{-1}, and yet secretion increased further after bleeding. Dog 305 was also the only one which had Mayer waves before haemorrhage. The exception among the non-reactors, dog 283, stands out, together with 305, by an exceptionally high initial secretion of glucocorticoids; this may indicate prolonged pre-operative stimulation of the pituitary and suggest some chronic abnormal state. It is interesting that only one of the two dogs (305) showed both the glucocorticoids and aldosterone raised in parallel. Table 8B also indicates that complete sinus denervation (groups I and II) is accompanied by a tendency to high glucocorticoid secretion, a fact which is not unexpected since the dogs must be in a state of chronic stress.

Conclusions

Denervation of the baroreceptors and chemoreceptors in the carotid region did not abolish the rise in aldosterone secretion following haemorrhage, the proportion of reacting

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TABLE 8. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION 14 TO 27 DAYS AFTER BILATERAL DENERVATION OF THE CAROTID SINUSES

First adrenal blood sample (S_1) collected 30 min after end of dissection, second sample (S_2) after haemorrhage. Flank incision. (Steroids extracted from whole blood, aldosterone figures corrected for losses.)

dog no.	body weight (kg)	sample no.	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change): aldosterone	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change): cortisol + corticosterone	mean b.p. (mmHg)	Mayer waves	blood withdrawn between S_1 and S_2 (ml./kg)
A. DOGS IN WHICH DENERVATION WAS FOUND TO BE COMPLETE							
Group I. Reactors							
279, male	15·4	S_1	10·6	1591	220	–	21
		S_2	23·7 +124	1853 +16	135	–	
282, female	9·1	S_1	11·0	1703	135	–	23
		S_2	16·5 + 50	1506 −12	88	+	
294, male	13·1	S_1	5·5	820*	187	–	14
		S_2	11·8 +115	869* +6	107	+	
298, female	18·0	S_1	7·5	1024	165	–	21
		S_2	13·3 + 77	1186 +16	80	+	
305, female	10·6	S_1	27·2	2167	170	+	27
		S_2	38·2 + 40	2175 0	95	+	
Group II. Non-reactors							
280, male	15·8	S_1	18·8	1287	162	–	19
		S_2	17·5 −7	1109 −14	80	+	
281, male	12·4	S_1	31·0	1945	180	–	24
		S_2	32·2 +4	1398 −28	143	–	
283, male	11·3	S_1	9·0	2564	203	–	20
		S_2	9·2 +2	2000 −22	112	–	
297, male	21·0	S_1	12·9	1940	190	–	21
		S_2	12·1 −6	1039 −46	80	+	
B. DOGS IN WHICH DENERVATION WAS POSSIBLY INCOMPLETE							
Group III. Reactor							
295, female	13·4	S_1	8·2	1525	176	–	20
		S_2	14·2 +73	1420 −7	104	+	
Group IV. Non-reactors							
292, female	14·3	S_1	13·4	1290	197	–	20
		S_2	16·1 +20	1420 +10	90	+	
293, male	17·8	S_1	16·2	818*	178	–	20
		S_2	16·9 + 4	1386* +69	100	+	
296, female	13·8	S_1	26·0	1865	182	–	18
		S_2	26·3 + 1	2352 +26	80	+	
306, male	14·3	S_1	19·9	1060	165	–	26
		S_2	24·2 +22	1279 +21	135	+	
means of groups I+III ($n = 6$)		S_1	11·7 ± 3·2	1602 ± 183	176 ± 11	·	21 ± 1·7
		S_2	19·6 ± 4·1	1628 ± 174	102 ± 8		
means of groups II+IV ($n = 8$)		S_1	18·4 ± 2·6	1707 ± 198	182 ± 5	·	21 ± 0·9
		S_2	19·3 ± 2·7	1514 ± 183	103 ± 9		
means of all groups ($n = 14$)		S_1	15·5 ± 2·1	1663 ± 133	179 ± 6	·	21 ± 0·9
		S_2	19·4 ± 2·2	1561 ± 125	102 ± 6		

* Cortisol only.

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dogs (6 out of 14) being about normal. Therefore, in animals with intact pituitary glands and kidneys, the part played in this phenomenon by a postulated aldosterone regulating system with receptor sites in the carotid arteries, cannot be a crucial one.

V. Effect of splanchnotomy on the response to haemorrhage

These experiments were designed to test whether haemodynamic or other changes taking place in the splanchnic bed during haemorrhage might elicit a reflex which could accelerate aldosterone secretion and which would use the splanchnic nerves as its pathway.

Since the work by Gammon & Bronk (1935) it is known that there are receptors in the splanchnic area which respond to changes in blood flow. Reflexes elicited by their activity seem to be of little importance in the regulation of the systemic blood pressure (Heymans, de Schaepdryver & de Vleeschhouwer 1960) but may be concerned with local distribution of blood in abdominal viscera (Heymans & Neil 1958). The experiments tried to establish whether unilateral or bilateral splanchnotomy alter either basal aldosterone secretion or its rise elicited by haemorrhage. Adrenocortical secretion rates were also measured before, during and after electrical stimulation of the central ends of the sectioned splanchnic nerves.

Procedures and observations

In the four groups of experiments the abdomen was opened by a midline incision, the greater and lesser splanchnic nerves were dissected out at their emergence from the diaphragm and ligatures placed under the nerves. Then the left adreno-lumbar vein was dissected for cannulation. Adrenal blood samples were usually collected for 30 min.

Unilateral splanchnotomy and haemorrhage

In four dogs, one of which was eviscerated, the left splanchnic nerves were sectioned before the collection of a control sample from the left adrenal. Then the dogs were bled and a second sample of adrenal venous blood was taken. Steroids were extracted from plasma in one, from whole blood in three experiments.

With the exception of one dog, in which secretion was already high before haemorrhage, bleeding raised the rate of aldosterone secretion. Glucocorticoids were not affected.

Acute bilateral splanchnotomy and haemorrhage

In a preliminary group of five dogs (table 9), four of which were eviscerated, a control sample of adrenal venous blood was taken 41 to 70 min after bilateral splanchnotomy. Then the blood volume was expanded by an infusion of donor blood (20 to 23 ml./kg over 10 min) and a second blood sample collected. This was followed by withdrawal of blood (5 to 23 ml./kg over 10 min) and collection of a last blood sample. In the non-eviscerated dog the initial expansion of the blood volume was omitted. Steroids were extracted from plasma alone, no correction for losses were made.

In three dogs expansion of the blood volume caused a fall in aldosterone secretion of 25, 32 and 15 %, whereas in the fourth dog which had an initial secretion rate of only 1·7 μg (g adrenal)$^{-1}$ h^{-1} the value rose to 2·9 μg. In no instance was haemorrhage followed by a significant rise in aldosterone secretion. Glucocorticoid secretion rates tended to fall during

the course of the experiments. Adrenal blood flow after haemorrhage was nearly halved. The initial blood pressure was lower than usual and the amount of blood withdrawn was less than control dogs tolerated.

The second series of experiments was carried out on five dogs in which the dietary salt intake was controlled. None of these dogs was eviscerated. A control sample of adrenal blood was collected 55 to 112 min after sectioning the splanchnic nerves on both sides. Then the dogs were bled (8 to 18 ml./kg) and a second sample was taken. Steroids were estimated in extracts from whole blood. The aldosterone figures were not corrected for losses.

TABLE 9. EFFECT OF CHANGES IN BLOOD VOLUME ON ALDOSTERONE SECRETION IN DOGS AFTER ACUTE BILATERAL SPLANCHNOTOMY

(Steroids extracted from plasma only, aldosterone figures not corrected for losses.)

dog no.	adrenal blood sample	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1}) aldosterone	cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	blood infused (ml./kg)	blood withdrawn (ml./kg)
46–49, mean ± s.e. (1 female, 3 males) eviscerated	44–70 min after bilateral splanchnotomy	6·0 ± 1·8	600 ± 62	305 ± 35	116 ± 15	21 ± 0·7	
	immediately after infusion of donor blood	4·8 ± 0·9	529 ± 104	359 ± 77	122 ± 13		14 ± 1·1
	5 min after haemorrhage	4·7 ± 0·8	434 ± 85	191 ± 34	70 ± 14		
54, male, not eviscerated	41 min after bilateral splanchnotomy	1·5	550	136	110		9
	5 min after haemorrhage	1·7	513	71	65		

The results are seen in table 10. The group includes animals with low and high Na^+ intake. None of the dogs responded to haemorrhage with a significant rise in aldosterone secretion. With the exception of dog 143 the initial secretion rates of aldosterone were higher than the mean of the corresponding control groups on the same diet (see table 5). The dogs showed some fall in glucocorticoid secretion. Initial blood pressures were low. The amount of blood which could be withdrawn during haemorrhage without causing a circulatory collapse averaged only 12·6 ml./kg. During collection of the second adrenal blood sample the mean arterial pressure was below 60 mmHg in three of the five dogs. The adrenal blood flow was about halved. The mean plasma K^+ concentration was slightly elevated, and rose by a further 1·6 m-equiv./l. in the course of the experiment.

These experiments might be interpreted in two ways. Either severing of the splanchnic nerves interfered with a mechanism which leads to a rise in aldosterone secretion following haemorrhage, or the failure to observe a rise was due to the poor state of the acutely splanchnotomized animal: table 10 shows that the circulation was impaired, haemorrhage was badly tolerated, and initial secretion rate of aldosterone was high. The first alternative

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would imply that under normal circulatory conditions the splanchnic nerves conduct impulses from abdominal proprioceptors which may either inhibit an 'aldosterone stimulating' or stimulate an 'aldosterone inhibiting' mechanism. If stretch receptors were responsible, bilateral splanchnotomy should raise, and stimulation of the central end of the nerve should lower aldosterone secretion. If chemoreceptors were involved, which started to fire after blood loss, section of the nerves should not affect secretion rate but stimulation of the central ends should raise it.

These different possibilities are tested in the next sections.

TABLE 10. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION IN ACUTELY SPLANCHNOTOMIZED DOGS

Collection of first adrenal blood sample (S_1) started 55 to 112 min after splanchnotomy and 10 to 60 min after end of dissection; second sample (S_2) taken after haemorrhage. Arterial blood samples taken at start of S_1 (A_1), end of haemorrhage (A_2), and end of S_2 (A_3). (Steroids extracted from whole blood, aldosterone figures not corrected for losses.)

dog no.	body weight (kg)	adrenal blood sample no.	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	blood withdrawn (ml./kg)	arterial blood sample no.	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	K^+	Na^+ intake
164, female	16·7	S_1	12·7	2357	180	122	–	9	A_1	59	142	4·7	100 m-equiv. Na^+/day for more than 7 days
		S_2	12·4 − 2	1981 − 16	96	57	–		A_2	59	137	6·5	
									A_3	57	135	6·6	
165, female	19·1	S_1	13·0	2151	312	124	–	15	A_1	52	144	3·8	
		S_2	15·1 + 16	2091 − 3	174	82	–		A_2	52	132	4·7	
									A_3	48	144	5·0	
143, male	17·4	S_1	10·8	1422	240	153	–	13	A_1	65	.	.	30 m-equiv. Na^+/day for 26 days
		S_2	10·6 − 2	1372 − 4	86	70	–		A_2	58	.	.	
									A_3	.	.	.	
169, male	12·6	S_1	4·1	1457	158	100	+	8	A_1	54	138	4·1	170 m-equiv. Na^+/day for 5 days
		S_2	5·3 + 29	1159 − 20	64	52	+		A_2	52	137	4·6	
									A_3	48	139	5·0	
170, male	8·4	S_1	4·4	1091	234	79	–	18	A_1	50	139	4·1	
		S_2	4·8 + 9	1024 − 6	110	44	+		A_2	49	139	4·7	
									A_3	48	143	5·6	

Bilateral splanchnotomy and basal steroid secretion

In seven dogs, one of which was eviscerated, a control sample of adrenal venous blood was collected first, then the splanchnic nerves were severed and a few minutes later a second sample was taken. Steroids were extracted from whole blood, no correction was made for losses.

Table 11 shows that there was no difference in the rates of secretion of aldosterone or glucocorticoids before and after sectioning of the nerves. In five out of seven dogs the initial secretion rate of aldosterone was higher than the mean observed in control dogs which were studied at the same time. This might have obscured rises which could be expected if the splanchnic nerves were transmitting baroreceptor activity. An attempt at imitating the effect of afferent impulses was made in the experiments of the next section.

Electrical stimulation of splanchnic nerves

Six dogs were eviscerated and the splanchnic nerves sectioned bilaterally between ligatures. In two dogs, sectioning of the splanchnic nerves was carried out before evisceration,

in an attempt at obtaining lower initial aldosterone secretion by reducing the stressful afferent impulses elicited by ligation of the abdominal arteries. Though secretion rate was indeed low in these experiments, it was also low in three instances in which the order of the operative procedures had not been reversed.

The central stumps of the nerves were placed on shielded platinum electrodes and a first blood sample collected. Stimulation of the nerves was started 2 to 10 min before, and continued throughout the collection of the second blood sample. It was carried out for 30 s in every minute with rectangular pulses of 10 ms duration at a frequency of 33/s and a voltage ranging from 5 to 9 V.

TABLE 11. EFFECT OF ACUTE BILATERAL SPLANCHNOTOMY ON ADRENAL STEROID SECRETION

Means ± S.E. of observations made on dogs 60 to 67. Sample 1 (S_1) before, sample 2 (S_2) 2 to 15 min after splanchnotomy. (Steroids extracted from whole blood, aldosterone figures not corrected for losses.)

	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1})		mean b.p.		adrenal blood flow	
adrenal blood sample no.	aldosterone	cortisol + corticosterone	(mmHg)	% change	(ml./h)	% change
S_1	11·9 ± 1·9	1145 ± 94	128 ± 6	−17	221 ± 29	−46
S_2	10·9 ± 2·9	1075 ± 86	106 ± 6		119 ± 14	

Electrical stimulation of the central ends of the sectioned splanchnic nerves twice produced rises, twice falls and 4 times no change in aldosterone secretion. The rises, which were quite large, were associated with a reduction by 50 % in adrenal blood flow, due to a fall in mean blood pressure in the first, and to high-amplitude Mayer waves in the second dog. The other dogs showed but small changes in adrenal blood flow during the period of stimulation. It is obviously not justifiable to attribute the rises in aldosterone production in two dogs to the stimulation of the splanchnic nerves when impairment of the circulation would easily account for the increases.

In all experiments, glucocorticoid secretion was little affected. The blood pressure records became very irregular during stimulation. Sometimes sharp rises occurred during the 30 s when stimulation was on, sometimes during the intervals. Occasionally stimulation of the nerves on one side seemed to influence the systemic pressure more than stimulation of the contralateral nerves. Onset of stimulation often caused muscular jerks or respiratory arrest. In conclusion, the results of the experiments in which aldosterone secretion was measured before and after acutely performed splanchnotomy or during electrical stimulation of the central end of the severed nerves did not lend support to the theory that splanchnic activity exerted a specific control over aldosterone secretion.

Aldosterone secretion after haemorrhage in dogs subjected to chronic bilateral splanchnotomy

It was obvious that the failure to obtain a rise in aldosterone secretion by bleeding acutely splanchnotomized dogs would defy interpretation as long as the circulatory embarrassment resulting from the procedure complicated the picture. The splanchnic nerves were therefore sectioned in a preliminary aseptic operation and the response to haemorrhage was tested 9 to 20 days later when the dogs had completely recovered.

Bilateral splanchnotomy was performed in 10 dogs under pentobarbitone sodium anaesthesia. After an interval of at least 9 days the left adreno-lumbar vein was cannulated

via a flank incision. A control sample was collected 30 min later, the dog was bled (9 to 20 ml./kg), and a second sample was taken. Steroids were extracted from whole blood and the aldosterone figures were corrected for 100 % recovery.

TABLE 12. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION IN DOGS SPLANCHNOTOMIZED BILATERALLY 9 TO 20 DAYS BEFORE THE EXPERIMENT

All dogs received 100 m-equiv. Na^+/day for more than 16 days. First adrenal blood sample (S_1) collected 30 min after end of dissection, second sample (S_2) after haemorrhage. Arterial blood samples taken from femoral artery: A_1, end of dissection; A_2, end of haemorrhage; A_3, end of experiment. Flank incision. (Steroids extracted from whole blood, aldosterone figures corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	blood withdrawn (ml./kg)	arterial blood sample no.	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	K^+
Group I. Reactors												
267, male	9·8	S_1	10·6	1496	240	120	–	18	A_1	37	143	3·6
		S_2	21·0 + 98	·	112	80	+		A_2	41	147	3·5
									A_3	38	146	3·7
268, male	13·0	S_1	14·4	1773	262	157	–	9	A_1	51	146	3·4
		S_2	35·2 + 144	2524 + 42	173	80	–		A_2	52	141	3·4
									A_3	48	146	4·3
272, female	9·1	S_1	8·7	1437	254	150	–	20	A_1	50	145	3·5
		S_2	12·4 + 43	1374 − 4	103	90	+		A_2	50	140	3·8
									A_3	50	143	3·1
273, female	7·2	S_1	10·8	1092	384	140	–	20	A_1	57	143	3·3
		S_2	18·7 + 73	·	192	70	–		A_2	55	140	3·6
									A_3	51	143	3·8
274, male	13·0	S_1	9·5	1445	285	150	–	20	A_1	42	148	3·3
		S_2	12·6 + 33	1588 + 10	165	85	+		A_2	45	146	3·1
									A_3	41	143	3·5
276, female	8·9	S_1	10·5	1374	214	150	–	16	A_1	59	158	3·1
		S_2	22·1 + 110	1499 + 9	126	80	+		A_2	56	152	3·9
									A_3	53	152	3·7
277, male	11·6	S_1	7·1	1814	166	140	–	17	A_1	48	150	3·8
		S_2	10·8 + 52	1514 − 17	96	80	+		A_2	47	138	3·5
									A_3	48	137	3·3
mean ± S.E.		S_1	10·2 ± 0·9	1569 ± 93	258 ± 26	144 ± 4·6	·	17 ± 0·2	A_1	49 ± 2·9	148 ± 2·0	3·4 ± 0·1
		S_2	19·0 ± 3·2	1584 ± 168	138 ± 14	81 ± 2·3			A_2	49 ± 2·1	143 ± 1·9	3·5 ± 0·1
									A_3	47 ± 2·1	144 ± 1·7	3·6 ± 0·1
Group II. Non-reactors												
266, female	8·1	S_1	34·4	1622	202	130	–	20	A_1	38	143	3·6
		S_2	21·4 − 38	·	101	85	+		A_2	45	141	2·6
									A_3	44	145	4·0
270, female	13·4	S_1	15·5	1629	295	140	–	20	A_1	45	148	3·4
		S_2	14·8 − 5	1666 + 2	180	100	–		A_2	50	148	3·5
									A_3	45	145	3·7
271, male	9·5	S_1	16·4	1160	112	130	–	16	A_1	53	143	3·2
		S_2	20·2 + 23	1306 + 13	46	80	–		A_2	43	139	4·6
									A_3	44	145	3·7
mean ± S.E.		S_1	22·1 ± 6·2	1629, 1160	203 ± 53	133 ± 3·3	·	19 ± 1·4	A_1	45 ± 4·3	145 ± 1·7	3·4 ± 0·1
		S_2	18·8 ± 2·0	1531 ± 113	109 ± 39	88 ± 6·0			A_2	46 ± 2·1	143 ± 2·7	3·6 ± 0·2
									A_3	44 ± 0·4	145 ± 0	3·8 ± 0·1
combined results group I + II mean ± S.E.		S_1	13·8 ± 2·5	1519 ± 88	241 ± 24	141 ± 3·6	·	18 ± 1·1	A_1	48 ± 2·4	147 ± 1·5	3·4 ± 0·07
		S_2	18·9 ± 2·2	1568 ± 119	129 ± 15	83 ± 2·4			A_2	48 ± 1·6	143 ± 1·5	3·6 ± 0·16
									A_3	46 ± 1·5	145 ± 1·1	3·7 ± 0·1

As can be seen from table 12, satisfactory circulatory conditions were maintained throughout the experiment in all but one dog. Out of the 10 splanchnotomized dogs seven responded to haemorrhage with an increase in aldosterone secretion of more than 30 %. The initial aldosterone secretion rates of the reactors were below 11 μg (g adrenal)$^{-1}$ h^{-1} with the exception of dog 268 in which it was 14·4 μg. In the non-reactors it was higher with a mean of 22·1. In both groups of dogs the secretion rates of the glucocorticoids changed very little after haemorrhage. The initial blood pressure was not lower than usual, and the lowest figure after haemorrhage was 70 mmHg (dog 273). The decrease in adrenal blood flow after bleeding was of the same order of magnitude as in dogs with intact splanchnic nerves. The concentration of K^+ in peripheral plasma at the end of the dissection was similar to that in normal dogs. There was a mean rise in K^+ of 0·3 m-equiv./l. in the course of the experiment. All reactors had an uneventful recovery period after the operation, although dog 267 was anaemic at the time of the experiment (Ht 37 %), and dog 272 had a high blood sedimentation rate. Among the non-reactors dog 266 was anaemic (Ht 38 %). Dog 270 had been suffering from diarrhoea for 2 days after the operation. Dog 271 bled profusely from a spleen injury during the dissection and lost, in spite of infusion of donor blood, 7·8 ml./kg before the intentional haemorrhage.

One dog (275) has not been included in table 12 but deserves mention because it showed an extremely low initial secretion rate of aldosterone (2·5 μg (g adrenal)$^{-1}$ h^{-1}). This was probably the result of its being kept on the high sodium intake for a period of over 3 months. Among other dogs kept for similar periods on the same diet some, but not all, showed extreme depression of aldosterone secretion (see section on anoxia). It appears that dogs which respond in this way have also lost the capacity of increasing aldosterone production to normal levels during acute demands. Dog 275 apparently accelerated its aldosterone secretion by 30 % after bleeding, but since this represented an absolute increase of only 0·15 μg (in 25 min), the readings could not be relied on and the results have been disregarded.

The difference between the acute and chronic effect of splanchnotomy on secretion of aldosterone is not related to adrenal blood flow. This is shown in figure 3 in which adrenal effluent (which, of course, may include some extra-adrenal blood) is plotted against the time elapsed after splanchnotomy. Flow was smallest during collection periods starting 2 to 15 min after section of the nerves, but was back to normal 60 to 120 min after splanchnotomy. The blood pressure was hardly changed during the recovery of flow.

Conclusions

No evidence was obtained for a specific link between splanchnic nerve activity and aldosterone secretion. The possibility of such a relation had been suggested by the failure of acutely splanchnotomized dogs to respond to haemorrhage with a rise in aldosterone secretion. However, this effect was found to be unspecific and connected with the deterioration in the condition of the circulation after acute splanchnotomy. The factor responsible was not the reduction of adrenal blood flow, since this was present for the first 10 to 40 min only after severing the nerves, and reverted to normal at about 1 h. During the period of reduced blood flow aldosterone secretion was high and glucocorticoid secretion normal. Intolerance to haemorrhage and high basal aldosterone secretion rates were the main

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reasons for the failure of response seen in the acutely splanchnotomized dog. The failure to modify basal aldosterone secretion rate by severing the splanchnic nerves or by stimulating their central ends, and the return of the response to haemorrhage when the dogs were allowed to recover from the acute effects of splanchnotomy, demonstrated that neither afferent nor efferent activity of the splanchnic nerves were essential for the effect of haemorrhage on aldosterone secretion.

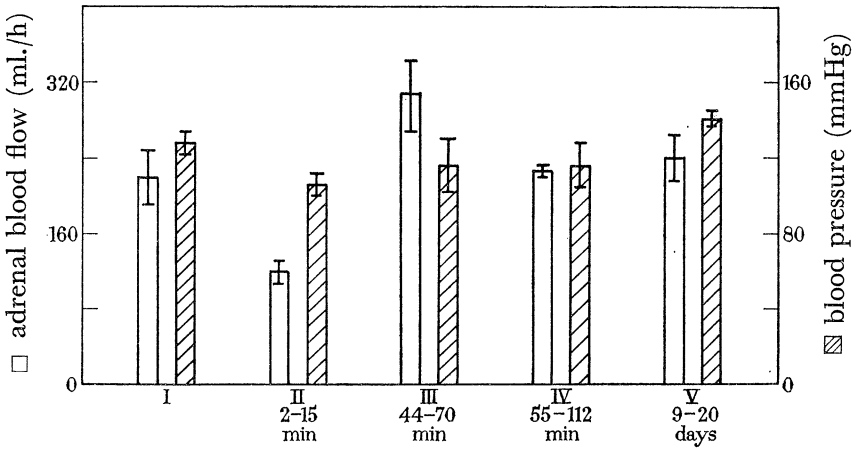

FIGURE 3. Effect of bilateral splanchnotomy on adrenal blood flow (□) and arterial pressure (▨). I and II: observations on 8 dogs, I, before, and II, after splanchnotomy (see table 11). III, IV and V: observations, after splanchnotomy, on three different groups of dogs (see tables 9, 10 and 12). Figures beneath columns indicate time after splanchnotomy at which adrenal blood collection was started.

VI. EFFECT OF VAGOTOMY ON THE RESPONSE TO HAEMORRHAGE

Farrell (1959) found that sectioning the vagus nerves in non-hypophysectomized, anaesthetized dogs caused wide fluctuations in the secretion rates of aldosterone and he assumed that vagal impulses affect aldosterone output. Mills, Casper & Bartter (1958) did not find significant changes in basal aldosterone secretion after vagotomy. However, the decrease in aldosterone secretion which follows the release of constriction of the inferior vena cava was not seen in vagotomized dogs. They concluded that the vagi form part of a reflex arc involved in decreasing aldosterone output.

In the course of the present work the effect of haemorrhage on aldosterone secretion was studied in vagotomized dogs.

Procedures and observations

In eight dogs both vagi were divided in the neck. In one group of six dogs the blood volume was increased initially by an infusion of about 18 ml./kg body weight of dog's blood or of 'Dextraven' (dextran, 6 g/100 ml. in 0·9 % NaCl). Adrenal blood was collected, then the dogs were bled (7 to 22 ml./kg body weight), and a second sample of blood was taken. Steroids were extracted from plasma only.

The mean aldosterone secretion (±S.E.) after volume expansion was $3{\cdot}6 \pm 1{\cdot}0\,\mu g$ $(g\,adrenal)^{-1}\,h^{-1}$. It rose after haemorrhage in five dogs by 31 to 189 %. However, the haematocrit was slightly lower after haemorrhage and the proportion of the total blood

aldosterone estimated in the plasma higher in the second sample than in the first, thus exaggerating the actual rise in secretion. The experiments were, therefore, repeated in two dogs in which the steroids were extracted from whole blood. No initial expansion of the blood volume was carried out. Table 13 shows that both dogs responded to haemorrhage with a rise in aldosterone secretion.

TABLE 13. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION IN VAGOTOMIZED DOGS

First adrenal blood sample (S_1) collected immediately after end of dissection, second sample (S_2) after haemorrhage. (Steroids extracted from whole blood, aldosterone figures not corrected for losses.)

dog no.	body weight (kg)	adrenal blood sample no.	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	blood withdrawn (ml./kg)	30 m-equiv. Na^+/day (no. of days)
135, male	11·0	S_1	7·8	2840	367	110	.	9
		S_2	11·7 +50	2793 −2	156	55	24	.
136, male	9·5	S_1	12·1	2651	112	130	.	2
		S_2	17·9 +48	2495 −6	64	78	18	.

Conclusions

There was no evidence to show that vagal activity directly influenced aldosterone secretion in dogs subjected to adrenal vein cannulation; the increase after haemorrhage was maintained, and so, apparently, was the fall after expansion of the blood volume, when secretion was $3{\cdot}6 \pm 1{\cdot}0 \mu$g (g adrenal)$^{-1}$ h^{-1}. This was similar to the figure of $3{\cdot}3 \pm 1{\cdot}0$ observed in a comparable group of dogs in which the vagi were intact (figure 2), and lower than the figures of $5{\cdot}3 \pm 1{\cdot}1$ found in the same group before expansion of the blood volume.

VII. ADRENAL MEDULLA AND ALDOSTERONE SECRETION

Some experiments were performed in order to find out whether the release of adrenaline and noradrenaline occurring during haemorrhage might participate in the mechanism which causes a rise in aldosterone secretion after haemorrhage.

Procedures and observations

In seven dogs the left and right splanchnic nerves were sectioned 53 to 137 min before collection of a control sample of adrenal blood. Intravenous infusion of L-adrenaline or L-noradrenaline-bitartrate (1μg min^{-1} (kg body wt)$^{-1}$) was then started 10 to 15 min before, and continued during, the collection of a second sample. A third blood sample was taken after an interval of 30 to 60 min.

The results are summarized in table 14. Of the three dogs given adrenaline, the one with the lowest initial secretion showed a significant rise in aldosterone production (dog 70), whereas no such rise occurred when 'resting' secretion was high (dog 71). The corresponding figures for dog 72 fell between the two extremes. Precisely the same happened when noradrenaline was infused: provided the secretion rate before the infusion was not over 6μg (g adrenal)$^{-1}$ h^{-1} (uncorrected), noradrenaline increased aldosterone production

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(dogs 72, 74), but no rise was seen whenever initial secretion was higher (dogs 76, 77, 89). Glucocorticoid secretion was raised only once. Blood pressure changes were of the order of 20 %. These results show that medullary amines are able to accelerate aldosterone secretion in the splanchnotomized dog. It is probable that adrenomedullary secretion during haemorrhage contributes to the increase in aldosterone secretion, but such contribution is obviously not essential, as shown by the experiments in which the effect of haemorrhage persisted in splanchnotomized dogs.

TABLE 14. EFFECT OF INTRAVENOUS INFUSIONS OF ADRENALINE AND NORADRENALINE ON CORTICOSTEROID SECRETION IN ACUTELY SPLANCHNOTOMIZED DOGS

First sample (S_1) collected immediately after the dissection, second (S_2) or fourth (S_4) sample started 10 to 15 min after the beginning of an infusion of L-adrenaline bitartrate (A) or L-noradrenaline bitartrate (N) which was continued during the whole collection period. Dose 1 μg base min^{-1} (kg body weight)$^{-1}$. (Steroids extracted from whole blood, aldosterone figures not corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)
70, male	12·6	S_1	0·9	917	89	138
		S_2 A	2·4 +167	1003 + 9	103	150
		S_3 32 min after S_2	1·4 − 42	852 −15	70	112
71, male	9·0	S_1	11·7	1075	175	95
		S_2 A	13·2 + 13	1288 +20	160	115
		S_3 30 min after S_2	14·3 + 8	1217 − 6	128	110
72, female	17·0	S_1	3·7	1070	194	104
		S_2 A	4·8 + 30	1207 +13	211	128
		S_3 30 min after S_2	5·5 + 15	1121 − 7	137	78
		S_4 N	8·1 + 47	1145 + 2	123	90
74, male	11·3	S_1	6·0	1424	192	112
		S_2 N	8·8 + 47	1500 + 5	179	140
		S_3 60 min after S_2	8·1 − 8	1565 + 4	108	100
76, female	17·5	S_1	8·4	1279	219	90
		S_2 N	9·0 + 7	1034 −19	120	77
77, male	17·5	S_1	7·5	1409	175	115
		S_2 N	7·6 + 1	1330 − 6	189	145
		S_3 35 min after S_2	7·8 + 3	1224 − 8	154	90
89, male	15·8	S_1	8·0	1151	172	135
		S_2 N	9·6 + 20	1545 +34	116	170
		S_3 61 min after S_2	11·2 + 17	1391 −10	92	129

VIII. OXYGEN LACK AND ALDOSTERONE SECRETION

Two sets of experiments were carried out in order to see whether the decrease in tissue oxygen tension participated in causing the rise in aldosterone secretion following acute haemorrhage.

Procedures and observations

In all animals a midline incision was made and the left adrenal vein cannulated. Steroids were extracted from whole blood and the aldosterone figures not corrected for losses.

Decrease in the oxygen content of the inspired air

In the first group of dogs anoxia was produced by decreasing the oxygen supply to the lungs. Once, mechanical obstruction of the trachea was used; in the other dogs artificial respiration was applied throughout the experiment, and the composition of the inspired air changed; a control sample of adrenal blood was taken while the dog was breathing air, then the inlet tube of the respiration pump was connected to a Douglas bag filled with a mixture of nitrogen and oxygen. The percentage of O_2 was varied between 10 and 17 %. The dogs inhaled this mixture for 4 or 5 min before, and during the entire collection period of a second blood sample. Then breathing was returned to room air and a few minutes later a third blood sample collected.

The results are listed in table 15. In most animals aldosterone secretion changed but little during the period of anoxia; there was, however, a large fall in both aldosterone and glucocorticoid secretion in dog 91 in which asphyxia was more severe than in the other dogs. In this experiment the oxygen supply to the adrenal must have been the limiting factor for hormone synthesis.

When breathing of normal air was resumed after anoxia, aldosterone secretion rose in eight of the nine dogs, 5 times significantly. Perhaps this rise represented an overshoot resulting from a hormone 'debt' incurred during the preceding period when hormone synthesis was restricted by the oxygen supply. There was little change in glucocorticoid secretion except for the one instance when it was much reduced during the anoxic period; neither were there large effects on adrenal blood flow or blood pressure. Mayer waves did not appear as a result of anoxia; in two of the experiments they were present throughout.

Though the nature of the influence of anoxia on secretion of aldosterone is not clear from these experiments, the results suggest that there are such influences, which become manifest at the time when a period of oxygen lack is superseded by normal oxygen supply.

Reduction of the number of circulating erythrocytes

In a second set of experiments tissue anoxia was produced by removal of red cells without changing the blood volume. This was done by exchange-transfusion with dextran ('Dextraven', as before) or dog's plasma (kept for 1 to 2 days at +4 °C, filtered and warmed immediately before use). After the collection of a control sample of adrenal blood, 40 ml./kg body wt. of either fluid was infused into a femoral vein and an equal volume of blood simultaneously withdrawn from a femoral artery. The exchange was completed in 10 to 15 min and was followed by a second collection of adrenal vein blood. The blood taken from the adrenal vein was replaced by blood with low haematocrit in order to keep the proportion of red cells low. In seven dogs the splanchnic nerves of both sides were sectioned 1 h before collecting the first sample; this was done in order to block hypothetical chemoreceptor impulses which might ascend in the splanchnic nerves.

The results are summarized in figures 4 to 6 and table 16. Figure 4*a* shows aldosterone secretion rates observed on a group of dogs in which the daily Na^+ intake was 30 m-equiv. The five dogs (left panel, reactors) which had been on the low Na^+ diet for a very short period had low initial aldosterone figures and responded to transfusion with plasma by a rise in secretion. None of the 15 dogs which had been left on this diet for a longer period

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responded. Many, but not all, animals had a high initial rate of aldosterone secretion (figure 4*a*, right panel).

Figure 5 shows the observations obtained on six dogs in which dextran was exchanged against blood. The dogs had been newly admitted. Only one dog responded with a rise, and this was the animal with the lowest initial secretion rate.

The picture (figure 6) for a group of seven dogs which were splanchnotomized immediately before the experiment is not very different. The dogs were on the low Na^+ diet

TABLE 15. EFFECT OF REDUCING THE OXYGEN CONTENT OF THE INSPIRED AIR ON ALDOSTERONE SECRETION

Respiratory rate kept at 20/min. Dietary sodium intake unknown. Midline incision. (Steroids extracted from whole blood, aldosterone figures not corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	adrenal blood sample conditions	duration of collection periods (min)	volume delivered per stroke/kg body weight (ml.)	% O_2 in mixture inhaled	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves
88, female	10	S_1	control				5·4	934	255	153	–
		S_2	partial occlusion of trachea started 9 min before and continued during collection	40	.	.	6·7 + 24	725 − 22	237	150	–
							+ 25	+26			
		S_3	started immed. after airways freed				8·4	911	186	143	–
90, male	10·8	S_1	control				5·7	1550	511	124	–
		S_2	during anoxia	25	18·5	10	5·2 − 9	1494 − 4	684	130	–
		S_3	started 5 min after return to room air				6·3 + 21	1714 + 15	432	126	–
91, male	19·5	S_1	control				7·6	1808	264	167	–
		S_2	during anoxia	30	15·4	10	1·9 − 75	1188 − 34	306	135	–
		S_3	started 7 min after return to room air				8·0 + 321	1860 + 57	256	150	–
92, female	18·0	S_1	control				7·1	1090	552	120	–
		S_2	during anoxia	20	16·7	15	7·7 + 8	1096 0	651	127	–
		S_3	started 10 min after return to room air				10·8 + 40	1120 + 2	561	118	–
93, male	10·4	S_1	control				11·0	1128	154	158	–
		S_2	during anoxia	30	14·4	15	7·8 − 29	1148 + 2	148	172	–
		S_3	started 7 min after return to room air				14·6 + 87	1121 − 2	120	185	–
94, male	12·6	S_1	control				5·1	1221	248	165	–
		S_2	during anoxia	30	23·4	17	7·1 + 39	1192 − 2	276	152	–
		S_3	started 10 min after return to room air				5·9 − 17	1133 − 5	226	96	–
95, male	9·0	S_1	control				6·9	1653	126	103	+
		S_2	during anoxia	40	22·2	17	5·6 − 19	1647 0	126	110	+
		S_3	started 10 min after return to room air				8·4 + 50	1882 + 14	93	135	+
96, male	13·5	S_1	control				9·7	1423	372	159	+
		S_2	during anoxia	25	14·8	16	8·2 − 15	1492 + 5	372	155	+
		S_3	started 10 min after return to room air				10·0 + 22	1319 − 12	293	152	+
97, female	6·8	S_1	control				5·0	994	144	148	–
		S_2	during anoxia	35	18·8	16	5·5 + 10	1091 + 10	144	175	–
		S_3	started 10 min after return to room air				9·3 + 69	1104 + 1	114	168	–
mean ± s.e.		S_1	control				7·1 ± 0·7	1311 ± 103	292 ± 52	144 ± 8	
		S_2	during anoxia				6·2 ± 0·6	1230 ± 92	327 ± 70	145 ± 7	
		S_3	after anoxia				9·1 ± 0·9	1352 ± 123	253 ± 52	141 ± 9	

for 1 or 2 days only, too short a period to cause sodium deficiency and increase aldosterone production, except for one non-reactor which was given this diet for 6 days. The high initial aldosterone secretion seen in four of the five non-reactors is most probably due to the stressing effect of the operation (see section on acute splanchnotomies). In fact, it is remarkable that two dogs 'reacted', and that one of them did so in spite of a high initial output of aldosterone.

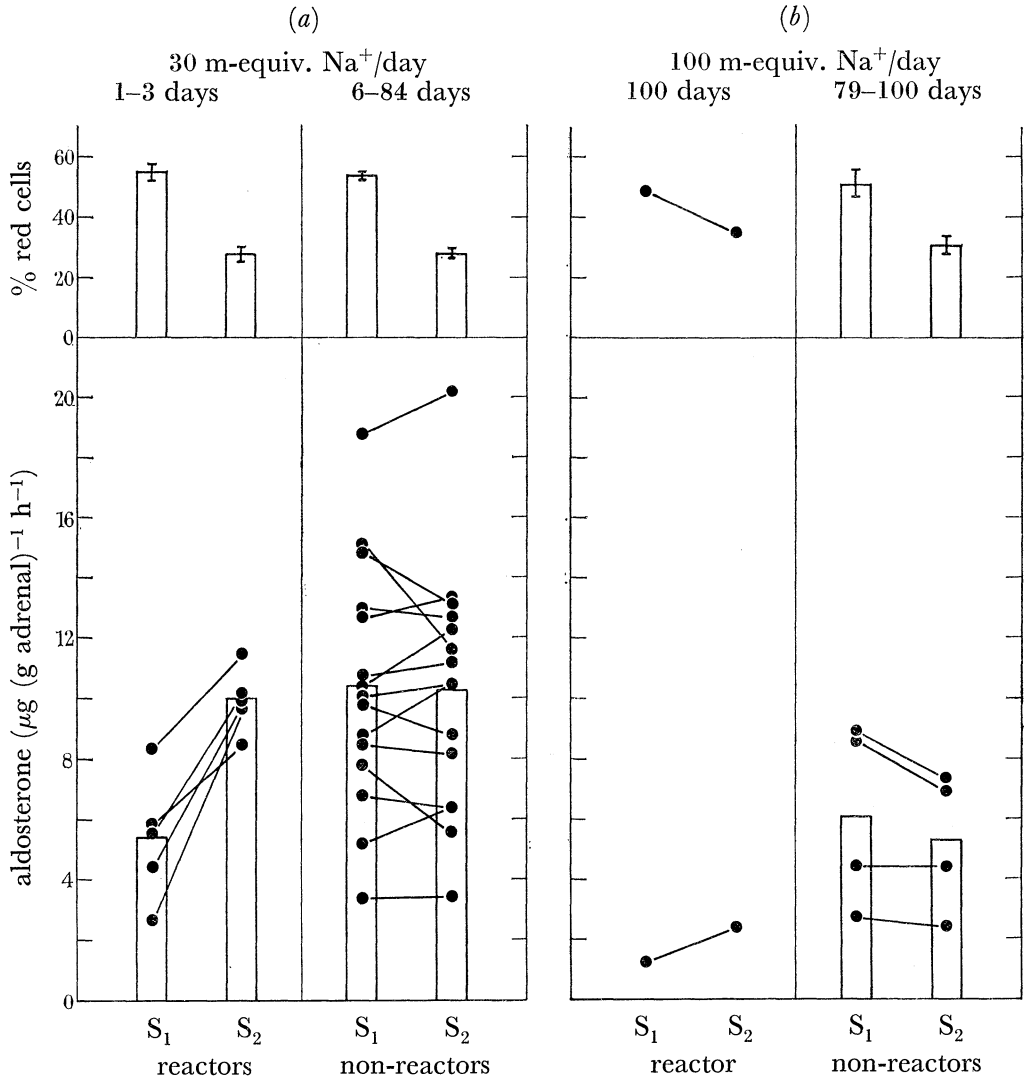

FIGURE 4. (*a*) dogs on low, (*b*) dogs on high dietary sodium intake. Aldosterone secretion before (S_1) and after (S_2) decreasing the haematocrit by an exchange transfusion with dog's plasma ($39 \pm 0{\cdot}8$ ml./kg). (Steroids extracted from whole blood, aldosterone figures not corrected for losses.) ●, individual experiments; Columns, means ± S.E.

Figure 4*b* represents the results in five dogs which had been on a high sodium intake for an excessively long period. This procedure considerably reduced the initial rate of aldosterone secretion, yet a response to the loss of red cells occurred only once, and, precisely as in the group represented in figure 5, the reactor was the dog with the smallest resting secretion. Even so, the response was much smaller than in the dogs of figure 4*a*, which had

not been given extra salt, and one cannot escape the suspicion that the chronic depression of aldosterone secretion by sodium loading had also reduced the reactivity of the aldosterone synthesizing mechanism. The findings on dog 275 in the series of chronically splanchnotomized dogs had previously suggested a similar conclusion.

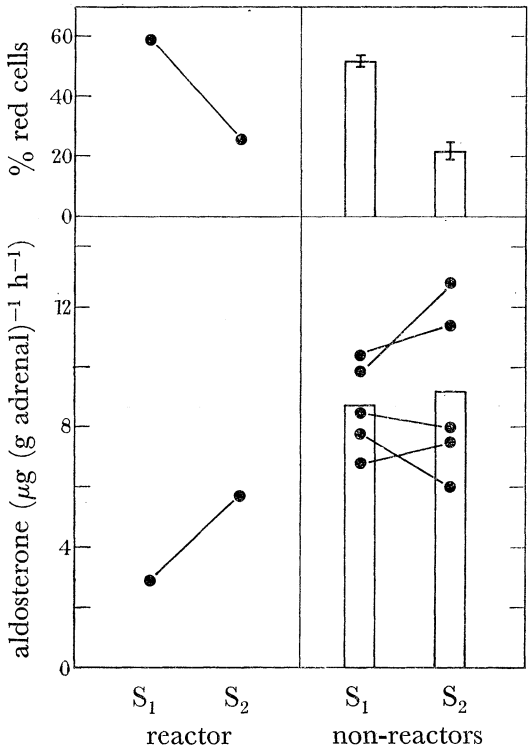

FIGURE 5. Aldosterone secretion before (S_1) and after (S_2) decreasing the haematocrit by exchange transfusion with dextran ($46 \pm 3{\cdot}1$ ml./kg). Dogs newly admitted and dietary sodium intake therefore unknown. (Steroids extracted from whole blood, aldosterone figures not corrected for losses.) ●, individual experiments; Columns, means ± S.E.

The means of the glucocorticoid secretion rates, of adrenal blood flow and arterial blood pressure are given in table 16. Glucocorticoid secretion was not affected by the removal of red cells. The largest amounts of glucocorticoids were secreted by the dogs which had been on a high sodium intake for a prolonged period. The plasma was usually well tolerated; at the beginning of the exchange there was frequently a slight fall in blood pressure followed as a rule by a rise of 20 to 40 mmHg above the original value and then by a gradual return to normal. Mayer waves occurred only in two dogs during collection of the second sample. In three dogs Mayer waves were present throughout the experiment.

Conclusions

The results suggest that a fall in the oxygen tension in the tissues can contribute to the rise in aldosterone secretion which follows upon haemorrhage. However, in the experiments on exchange-transfusion the proportion of 'reacting' dogs was small when compared with the percentage normally seen after haemorrhage. This is not surprising when the experiments on acutely splanchnotomized dogs and on dogs kept for an excessively long time on a high Na^+ intake (figures 6 and 4*b*) are considered; these experiments were car-

ried out before it was known that these procedures create adverse conditions for a further rise in aldosterone output—splanchnotomy, by raising initial aldosterone secretion rate, excessive sodium intake by apparently depressing the reactivity of the aldosterone producing cells. When, however, the large number of non-reactors in figure 4*a* is considered, it is

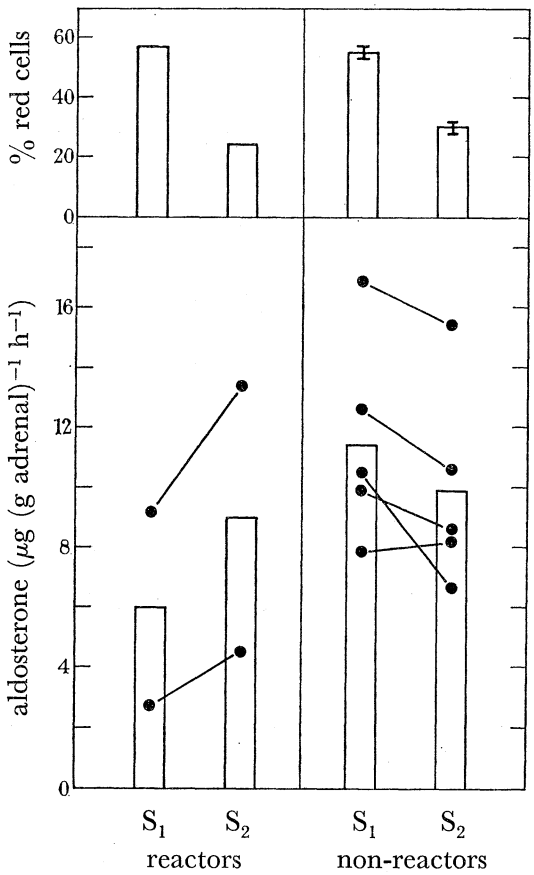

FIGURE 6. Aldosterone secretion of acutely splanchnotomized dogs before (S_1) and after (S_2) decreasing the haematocrit by an exchange transfusion with dog's plasma ($39 \pm 1{\cdot}7$ ml./kg). Daily sodium intake 30 m-equiv. (reactors for 1 day, non-reactors for 1 to 6 days). (Steroids extracted from whole blood, aldosterone figures not corrected for losses.) ●, individual experiments; Columns, means ± S.E.

difficult to escape the conclusion that loss of red cells is a less potent stimulus of aldosterone production than haemorrhage and can therefore play only a minor role in eliciting the response to haemorrhage. On the assumption that the increase in aldosterone secretion seen after exchange transfusion is elicited by a reflex, involving chemoreceptor activity, the results on splanchnotomized dogs suggest that such activity is not transmitted to the centres by the splanchnic nerves.

DISCUSSION

The experiments described in this paper were done on dogs in which pituitary and kidneys were intact. It is obvious from the results that aldosterone secretion is as subject to being increased by a multitude of noxious conditions or 'stresses' as is glucocorticoid secretion. Theconcept held 10 years ago that *ACTH* affects production of aldosterone but little

TABLE 16. OBSERVATIONS ON GLUCOCORTICOID SECRETION, ADRENAL BLOOD FLOW AND MEAN ARTERIAL PRESSURE

First sample (S_1) before, second sample (S_2) after an exchange transfusion with dextran or dogs' plasma (40 ml./kg). Means ± standard errors. A_1: arterial blood sample taken before S_1; A_2: arterial blood sample taken after S_2.

dog nos.	adrenal blood sample	cortisol + corticosterone (μg (g adrenal)$^{-1}$ h^{-1})	adrenal blood flow (ml./h)	mean b.p. (mmHg)	arterial blood sample	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	plasma conc. (m-equiv./l.) K^+
GROUP I. BLOOD-PLASMA EXCHANGE, SPLANCHNIC NERVES INTACT (ALDOSTERONE SEE FIGS. 4(*a*) AND 4(*b*))								
A. Reactors (30 m-equiv. Na^+/day for 1 to 3 days)								
112, 113, 114, 122, 123	S_1	1411 ± 190	207 ± 36	132 ± 9	A_1	55 ± 2·1	.	.
	S_2	1483 ± 181	299 ± 54	126 ± 11	A_2	28 ± 1·9	.	.
B. Non-reactors (30 m-equiv. Na^+/day for 6–84 days)								
110, 115, 117, 119, 126, 141, 144, 145, 147, 148, 149, 150, 151, 152, 154	S_1	1568 ± 82	256 ± 24	125 ± 7	A_1	54 ± 1·0	148 ± 2	3·7 ± 0·1
	S_2	1535 ± 81	289 ± 25	121 ± 8	A_2	28 ± 0·8	148 ± 1	4·1 ± 0·1
C. Reactor (100 m-equiv. Na^+/day for 100 days)								
230	S_1	1893	278	168	A_1	49	156	3·0
	S_2	1899	276	164	A_2	35	145	3·2
D. Non-reactors (100 m-equiv. Na^+/day for 79–100 days)								
221, 227, 228, 231	S_1	1827 ± 133	215 ± 26	146 ± 2	A_1	51 ± 5	148 ± 2	3·6 ± 0·2
	S_2	1944 ± 337	216 ± 42	145 ± 3	A_2	31 ± 3	144 ± 2	3·4 ± 0·1
GROUP II. BLOOD-DEXTRAN EXCHANGE, SPLANCHNIC NERVES INTACT, NEWLY ADMITTED (ALDOSTERONE SEE FIGURE 5)								
A. Reactor								
98	S_1	1017	154	148	A_1	59	.	.
	S_2	1057	185	130	A_2	26	.	.
B. Non-reactors								
100, 101, 103, 105, 106	S_1	1040 ± 70	308 ± 34	142 ± 12	A_1	52 ± 1·2	.	.
	S_2	1371 ± 104	306 ± 42	121 ± 15	A_2	22 ± 2·3	.	.
GROUP III. ACUTELY SPLANCHNOTOMIZED DOGS; BLOOD-PLASMA EXCHANGE (ALDOSTERONE SEE FIGURE 6)								
A. Reactors (30 m-equiv. Na^+/day for 1 day)								
118, 128	S_1	1523, 1200	81, 123	95, 114	A_1	52, 62	.	.
	S_2	1408, 1098	80, 158	63, 104	A_2	25, 24	.	.
B. Non-reactors (30 m-equiv. Na^+/day for 1–6 days)								
116, 121, 124, 127, 129	S_1	1600 ± 66	146 ± 31	100 ± 10	A_1	55 ± 2	.	.
	S_2	1578 ± 58	148 ± 42	92 ± 14	A_2	30 ± 2	.	.

has since been revised (Davis, Carpenter, Ayers & Bahn 1960; Mulrow, Ganong, Kuljian & Boryczka 1961; Holzbauer 1964), and the ready rise in aldosterone secretion during stress should, in part, be due to the stimulating effect of *ACTH*. In the meantime detailed knowledge has also accumulated on the effect of angiotensin (Carpenter, Davis & Ayers 1961*b*; Mulrow *et al.* 1961; Slater, Barbour, Henderson, Casper & Bartter 1963) on aldosterone secretion in dogs. Renin, too, is probably released during surgery so that the 'basal'

secretion rate of aldosterone will also depend on the amount of the angiotensin formed by it in the circulating blood.

In spite of the fact that aldosterone secretion was elevated as a result of adrenal vein cannulation, it was possible to elicit further rises in aldosterone secretion by haemorrhage in about one half of the animals not subjected to further surgery. This relatively small percentage of 'reactors' necessitated the use of large groups of dogs in order to establish the importance of any factor in the acceleration of aldosterone secretion after haemorrhage. The greatest difficulty was encountered when, in order to exclude certain structures of organs as participants in the effect, procedures were required which so raised aldosterone production before haemorrhage that no further rise could be expected. Some of the difficulties were avoided by preliminary aseptic operations from which the dog was allowed to recover before the experiment was carried out. In some experiments, incipient circulatory failure was associated with high initial secretion rate of aldosterone. In such dogs, the circulation sometimes deteriorated to the point of shock when the dog was bled; in this condition aldosterone secretion usually fell to low levels.

The experiments have established that the presence of the following structures was not required for the response to haemorrhage to occur: the vagi, the splanchnic nerves, the baroreceptors of carotid sinus and thyro-carotid junctions, the chemoreceptors of the carotid sinus, the liver, spleen and gastrointestinal tract. Provided the initial secretion was low enough, small rises in aldosterone secretion were seen after infusion of adrenaline and noradrenaline in the splanchnotomized animal, after exchange transfusions involving a loss of 50 % of the dog's red cells, and as an after-effect of a period of breathing a mixture of low oxygen content. It is likely that these rises were brought about by a release of *ACTH*. The fact that the effects were small shows that anoxia alone is a less potent stimulus of aldosterone secretion than is blood loss, and suggests that the circulatory effect of haemorrhage plays an important role in eliciting the response, the mediating agents presumably including both *ACTH* and renin.

Two obvious questions are posed by this work: are we to assume that, in the anaesthetized animal subjected to surgical stress, haemorrhage releases enough additional *ACTH* and renin to account for the further rise in aldosterone secretion, or is there yet another 'aldosterone-releasing factor' involved? Is the release of *ACTH* or that of renin more important when a dog is bled under the conditions of these experiments?

An attempt at answering these questions will be made in the next paper dealing mainly with experiments on hypophysectomized and nephrectomized dogs.

Our thanks are due to Benger Laboratories for a gift of Dextraven, to Dr C. D. Falconer (Ciba Laboratories) for a sample of aldosterone and to the Endocrinology Study Section, U.S. Department of Health for a gift of 7-^{3}H-aldosterone. To the U.S. Department of Health, Education and Welfare we would like to express our gratitude for a grant to buy a Tri-Carb Scintillation Spectrometer.

Miss Helen Newport's unfailing help in these investigations is greatly appreciated. The sodium and potassium estimations were carried out by Mr R. J. Hall.

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