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

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

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The role of *ACTH* and of renin as mediators of the stimulating effect of haemorrhage on aldosterone secretion was investigated. The following experiments showed that release of *ACTH* is not indispensible for the effect: in non-hypophysectomized dogs with intact kidneys, in which the blood *ACTH* concentration was artificially raised by infusing *ACTH*, there was still a rise in aldosterone production after blood loss. Hypophysectomy did not abolish or reduce the response, in fact, it increased its frequency of occurrence in dogs in which steroid synthesis was maintained at a submaximal level by a constant infusion of *ACTH*.

Another group of studies demonstrated that the release of renin is also not a necessary condition for the rise in aldosterone production after bleeding; dogs in which both kidneys had been removed, but the pituitary left intact, responded to bleeding by a rise in aldosterone secretion of the same magnitude as normal dogs. However, in the simultaneous absence of kidneys and pituitary gland aldosterone production did not rise after bleeding although the basic conditions for synthesis of steroids were provided by a constant infusion of *ACTH*. On the contrary, severe falls in steroid secretion rates were the rule.

These falls were attributed to the fact that hypophysectomized-nephrectomized dogs often reacted to blood loss with collapse of the circulation, and it was possible to argue that this collapse, and not the absence of kidney and pituitary, might have prevented the rise in aldosterone secretion. Attempts were therefore made to improve the circulation by supplying pressor substances known to be released in haemorrhage: of these noradrenaline did not improve the tolerance to haemorrhage, angiotensin improved it only very slightly, but prolonged infusions of extracts of posterior lobe restored it nearly to normal. In some of these experiments, the post-haemorrhage fall in aldosterone secretion was also prevented, but a rise was never seen.

Aldosterone secretion of the hypophysectomized-nephrectomized dog was stimulated by infusion of large volumes of donor blood obtained from dogs with intact kidneys which presumably contains renin, but not of blood from nephrectomized donors.

No evidence was obtained for the existence of agents other than *ACTH* and angiotensin as mediators of the stimulating effect of haemorrhage on aldosterone secretion. Furthermore, these two agents could fully replace each other, as shown by the finding that the effect of haemorrhage was not diminished by either nephrectomy or hypophysectomy alone.

INTRODUCTION

This paper examines the role played by the pituitary gland and the kidneys in the sequence of events which leads to a rise in aldosterone secretion following haemorrhage. Effects of *ACTH* and of angiotensin on aldosterone secretion are now well established. The questions dealt with are whether the two substances can replace each other or whether one of them can be shown to be more important than the other in the adrenal response to haemorrhage; furthermore, whether a response can still be obtained when pituitary and kidneys are removed.

METHODS

Most operative procedures and the chemical methods used in these experiments have been outlined in the preceding paper. Steroids were extracted from whole blood in all experiments. Two batches of *ACTH* were used: for the experiments in chapter I 'Cortrophin' Organon, (corticotrophin B.P.) for intravenous use, carboxy-cellulose-purified; for all remaining experiments, 'Cortrophin' Organon, of porcine origin, for intramuscular use; this batch was the same as that used by Holzbauer (1964). The *ACTH* was administered by a constant-infusion pump through a fine needle inserted into an unobstructed jugular vein. All solutions were acidified with HCl.

The pituitary gland was removed through the roof of the mouth at least 2 h before starting the collection of samples of adrenal blood. Completeness of the operation was checked by inspection of the sella turcica and the base of the brain at the end of the experiment.

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During extirpation the kidneys were handled as little as possible and, from dog 301 onwards, the renal arteries and veins were ligated simultaneously in order to minimize the escape of renin into the circulation.

RESULTS

I. Aldosterone secretion before and after haemorrhage in dogs with intact pituitary given infusions of *ACTH*

The object of these experiments was to try and cancel out the effect of any *ACTH* released as a result of bleeding by artificially producing a high constant background of blood *ACTH*. If release of *ACTH* was indispensable for the rise in aldosterone secretion after haemorrhage, this procedure should reduce or abolish the response. Infusion was made into the jugular vein and either started before adrenal blood was taken or after collection of control samples. Unless otherwise stated it was continued to the end of the experiment.

Procedures and observations

Dogs bled once

One or two control samples of adrenal venous blood were collected before, and one after the start of a continuous infusion of *ACTH*; the dogs were then bled and a further sample taken. In preliminary experiments (table 1) a wide range of doses of *ACTH* was explored; in the remaining dogs the mean amount infused (0·35 m-u. min^{-1} (kg body wt.)$^{-1}$) was slightly higher than the lower concentrations used before. Only the aldosterone figures of this last group are corrected for losses and the data are therefore listed separately (table 2).

The results were the same for all doses of *ACTH*. None of the 15 dogs responded to the *ACTH* infusion with a significant rise in the secretion rates of cortisol and corticosterone, but in three dogs (204, 208, 251) aldosterone secretion rose by 40, 32 and 73 % respectively; five dogs (201, 204, 205, 207, 253) responded to haemorrhage with rises in aldosterone. Three of the four non-reactors of table 1 (dogs 198, 208, 229) had a high aldosterone secretion immediately before the haemorrhage; lack of response in the fourth dog (202) is probably accounted for by adrenal damage (suggested by the simultaneous fall, after bleeding, in secretion of aldosterone and glucocorticoids). Among the seven dogs of table 2, there was only one, 253, in which aldosterone secretion rate was low before bleeding, and this was the only dog that responded.

Dogs bled twice

The preceding experiments showed that corticosterone and cortisol only rarely rose in dogs when they were given infusions of *ACTH* ranging from 0·09 to 1·7 m-u. min^{-1} kg^{-1}. This suggested that the conditions of the experiment released enough *ACTH* to produce maximal secretion of glucocorticoids, though not necessarily of aldosterone. Among the infused dogs reactors were fewer than normal. To try and overcome the difficulty caused by individual variations in the response, experiments were carried out in which the effect of haemorrhage was examined in the same dog before and during infusion of *ACTH*, the blood volume being restored between the two halves of the experiment.

Samples of adrenal blood were collected before and after bleeding, the shed blood was re-infused, and either 10 or 60 min later another sample was taken. Then an infusion of *ACTH* (0·2 to 0·35 m-u. min^{-1} kg^{-1} body weight) was started, and adrenal blood collected

TABLE 1. EFFECT OF *ACTH*-INFUSION IN DOGS WITH INTACT PITUITARY GLAND ON CORTICOSTEROID SECRETION BEFORE AND AFTER HAEMORRHAGE

First adrenal blood sample collected 30 min after end of dissection. Collection time usually 25 to 30 min, but 15 min in dog 205. One or two samples (S_0 and S_1) before, the remaining samples during constant infusion of ACTH. S_2 before, S_3 after haemorrhage. Na^+ intake 100 m-equiv./day for 9 to 31 days. (Aldosterone figures not corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	dose of *ACTH* (m-u. $min^{-1}\,kg^{-1}$)	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 before S_3 (ml./kg)
Group I (midline incision)										
198,* male	11·7	S_1	0	5·1	999	91	155	+	61	.
		S_2	1·6	5·8 +14	1019 + 2	120	155	+	62	.
		S_3	1·6	4·5 −22	954 − 6	96	80	+	58	10
201, male	10·4	S_1	0	3·3	2050	166	125	−	52	.
		S_2	1·6	3·8 +15	2035 − 1	149	142	−	50	.
		S_3	1·6	6·5 +71	2190 + 8	115	70	+	49	20
202, male	10·8	S_1	0	4·2	1660	144	156	−	56	.
		S_2	1·7	4·5 + 7	1718 + 3	156	162	+	55	.
		S_3	1·7	2·3 −49	1307 −24	101	120	+	55	20
mean ± S.E.		S_1	0	4·2 ± 0·5	1570 ± 307	134 ± 22	145 ± 10		56 ± 2·6	.
		S_2	1·63	4·7 ± 0·6	1591 ± 300	142 ± 11	153 ± 6		56 ± 3·4	.
		S_3	1·63	4·4 ± 1·2	1484 ± 368	104 ± 6	90 ± 15		54 ± 2·6	17 ± 3
Group II (right flank incision)										
204, female	9·0	S_1	0	1·5	1335	92	137	−	43	.
		S_2	0·2	2·1 +40	1371 + 3	88	137	−	43	.
		S_3	0·2	3·1 +48	1264 − 9	48	120	+	46	20
205, female	19·3	S_1	0	4·1	1527	324	170	−	43	.
		S_2	0·09	3·3 −20	1420 − 7	244	170	−	41	.
		S_3	0·09	6·4 +94	1869 +22	192	166	−	43	20
207, male	11·6	S_1	0	7·3	1807	187	192	+	57	.
		S_2	0·3	4·9 −33	1975 + 9	175	180	−	55	.
		S_3	0·3	7·0 +43	1789 − 9	156	80	+	50	21
208, female	11·2	S_1	0	3·8	1473	314	175	−	60	.
		S_2	0·3	5·0 +32	1581 + 7	343	180	+	58	.
		S_3	0·3	5·6 +12	1808 +14	295	145	+	58	20
229, male	13·5	S_0	0	6·1	2237	262	162	−	49	.
		S_1	0	6·4 + 5	2230 0	235	165	−	49	.
		S_2	0·3	8·3 +30	2435 + 9	240	168	−	51	.
		S_3	0·3	8·5 + 2	2090 −14	144	141	+	48	18
mean ± S.E.		S_1	0	4·6 ± 1·0	1674 ± 159	230 ± 43	168 ± 9	.	50 ± 3	.
		S_2	0·24	4·7 ± 1·0	1756 ± 200	218 ± 42	167 ± 8	.	50 ± 3	.
		S_3	0·24	6·1 ± 0·9	1764 ± 136	167 ± 40	130 ± 15	.	49 ± 3	20 ± 0·5

* Enteritis.

before and after bleeding. Figure 1 shows the results. Three of the seven dogs reacted to the first bleeding by increases in aldosterone secretion ranging from 44 to 135 %, but re-infusion of the lost blood did not restore the initial low aldosterone secretion. The subsequent infusion of *ACTH* did not raise either aldosterone or glucocorticoid secretion.

TABLE 2. EFFECT OF *ACTH*-INFUSIONS IN DOGS WITH INTACT PITUITARY GLAND ON CORTICOSTEROID SECRETION BEFORE AND AFTER HAEMORRHAGE

First adrenal blood sample collected 30 min after end of dissection. Collection time 25 to 30 min. One or two samples (S_0 and S_1) before, the remaining samples during constant infusion of *ACTH*. S_2 before, S_3 after haemorrhage. Na^+ intake 100 m-equiv./day for 7 to 42 days. (Flank incision, aldosterone figures corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	dose of *ACTH* (m-u. min^{-1} kg^{-1})	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 before S_3 (ml./kg)
234, male	15·4	S_0	0	13·0	1551	226	150	+	54	.
		S_1	0	15·9 +22	1522 − 2	197	156	+	50	.
		S_2	0·3	19·3 +21	1160 −24	173	158	−	49	.
		S_3	0·3	22·3 +16	1351 +16	82	78	+	50	18
235, male	15·3	S_0	0	22·6	1832	118	164	−	50	.
		S_1	0	23·4 + 4	1761 − 4	118	165	−	50	.
		S_2	0·3	24·0 + 3	1638 − 7	115	160	−	49	.
		S_3	0·3	28·4 +18	1426 −13	67	80	+	51	16
236, male	14·4	S_0	0	20·3	2144	246	133	−	53	.
		S_1	0	18·4 − 9	1646 −23	230	140	−	.	.
		S_2	0·3	21·0 +14	1214 −26	177	145	−	53	.
		S_3	0·3	21·7 + 3	1712 +41	99	75	+	50	18
250, male	11·7	S_1	0	9·7	1520	240	173	−	53	.
		S_2	0·4	10·4 + 7	1731 +14	193	170	−	51	.
		S_3	0·4	11·1 + 7	1996 +15	156	114	+	53	20
251, male	11·6	S_1	0	7·1	2079	286	155	−	40	.
		S_2	0·4	12·3 +73	2090 0	274	160	−	43	.
		S_3	0·4	13·3 + 8	1525 −27	143	82	+	44	16
252, male	14·4	S_1	0	8·2	1221	264	182	−	67	.
		S_2	0·3	9·8 +15	1500 +23	240	182	−	65	.
		S_3	0·3	8·9 − 5	1116 −26	274	136	+	61	20
253, female	9·4	S_1	0	5·7	1673	98	150	−	47	.
		S_2	0·5	4·9 −14	1317 −21	84	140	−	49	.
		S_3	0·5	10·5 +114	1495 +14	126	95	+	44	20
mean ± S.E.		S_0	0	18·6 ± 2·9	1842 ± 171	197 ± 40	149 ± 9	.	52 ± 1·2	.
		S_1	0	12·6 ± 2·5	1632 ± 99	205 ± 27	160 ± 5	.	51 ± 3·6	.
		S_2	0·35	14·5 ± 2·6	1521 ± 124	179 ± 25	159 ± 5	.	51 ± 2·6	.
		S_3	0·35	16·6 ± 2·8	1517 ± 105	135 ± 26	94 ± 9	.	50 ± 2·2	18 ± 0·7

Of the six dogs in which adrenal blood was obtained after a second haemorrhage, three responded with a rise in aldosterone secretion, which was significant in two animals. The reactors were not the ones which had reacted initially, but those with the lowest secretion rate just before haemorrhage (see figure 1). The need for considering individual experiments on their own merits is illustrated by dog 242 (figure 1) in which the failure to respond to the first haemorrhage may have been due to an elevated initial secretion rate, but may equally have been the result of an unusually high haematocrit, which was 79 % at the time of the first bleeding. Under these circumstances loss of blood must have had a beneficial effect on circulation and failed to elicit compensatory mechanisms. Before the second haemorrhage the haematocrit was 66 %, and now a response was obtained.

The dogs which were bled twice were difficult to keep in good condition to the end of the

experiments. In order to have conditions optimal while *ACTH* was being infused, the procedure was therefore reversed in three dogs: *ACTH* was infused before and during the first haemorrhage and discontinued before restoration of the blood volume. An hour later a second pair of samples was taken, one before and one after bleeding. The result was clear-cut (figure 2). All dogs responded to the first bleeding, in spite of the fact that *ACTH* was

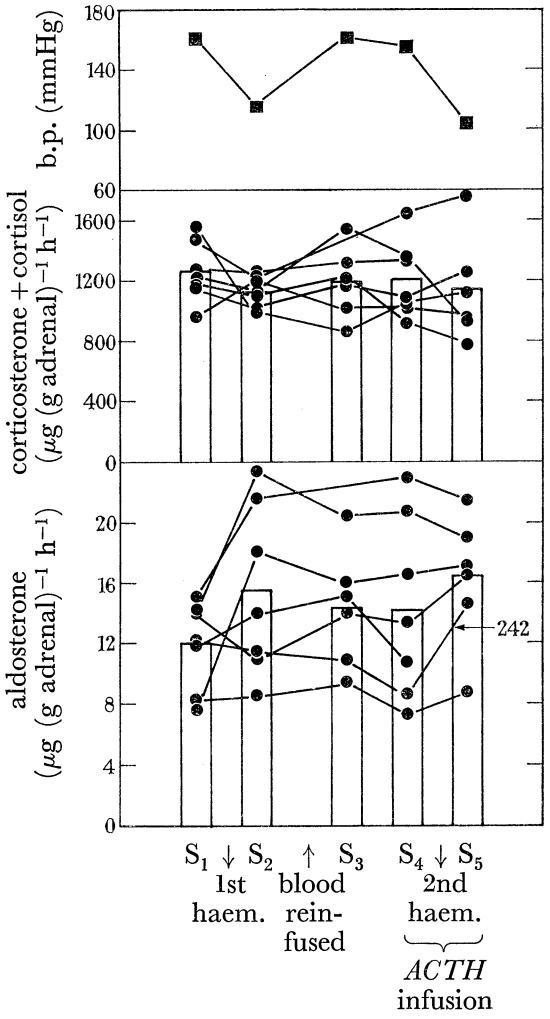

FIGURE 1

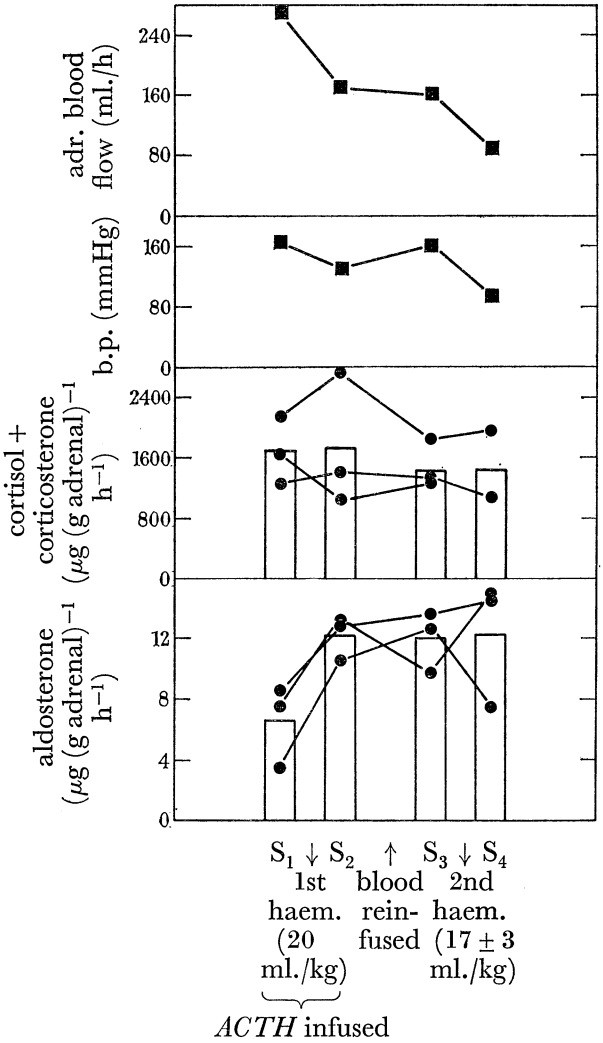

FIGURE 2

FIGURE 1. Effect of *ACTH* infusion on the aldosterone response to blood loss in dogs with intact pituitary. Adrenal blood samples collected for 15 to 30 min: S_1 before, S_2 after first haemorrhage (20 ± 0 ml./kg); S_3 10 to 60 min after restoration of the initial blood volume; S_4 5 min after the start of an *ACTH* infusion (0·2 to 0·35 m-u. min^{-1} (body kg wt)$^{-1}$); S_5 after second haemorrhage (18 ± 1 ml./kg). *ACTH* infusion stopped 5 min before end of S_5. Daily sodium intake 100 m-equiv. for more than 7 days. Flank incision, aldosterone figures corrected for losses. ●, Individual observations; columns and ■, means.

FIGURE 2. Effect of *ACTH* infusion on the aldosterone response to blood loss in dogs with intact pituitary. Experiments similar to those in figure 1, but *ACTH* infusion (0·4 to 0·5 m-u. min^{-1} kg^{-1} body weight) during first haemorrhage, started 5 min before S_1 and stopped 5 min before end of S_2. Daily sodium intake 100 m-equiv. for 2 to 14 days. Flank incision, aldosterone figures corrected for losses. ●, Individual observations; columns and ■, means.

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being infused, but only one dog showed an increased output of aldosterone at the second haemorrhage. Characteristically, it was the dog with the lowest secretion during the second control period.

Comparison of infusion of freshly shed and day-old donor blood

In the experiments of the two previous groups (figures 1, 2) it was found nearly impossible to depress aldosterone secretion to pre-haemorrhage rates by re-infusing the dog's own blood. This raised the question whether freshly drawn blood contained some active 'glomerulotrophin'. In donor-blood which had been standing for 24 h or longer, such substances might have been destroyed, as would be the fate of any *ACTH* present (Nelson & Hume 1955). Experiments were therefore carried out in order to see whether infusion of 'stale' blood would be better suited than that of fresh blood to decrease aldosterone secretion which had been raised by blood loss. With the intention of starting from a high rate of aldosterone secretion, the animal was bled 20 ml./kg while *ACTH* (0·2 m-u. min^{-1} kg^{-1}) was being infused. Shortly after the haemorrhage, a first adrenal blood sample was collected, while the infusion of *ACTH* was continued. Then the initial blood volume was restored by infusing 1-day-old donor blood. About 5 or 45 min later, a second adrenal blood sample was taken, the dog then bled a second time, and a third sample collected. Details and results are found in table 3. In two dogs (258, 259) aldosterone secretion fell after re-infusion of the lost blood; the effect was rapid, since the intervals between end of re-infusion and beginning of sampling of adrenal blood were only 5 and 9 min. Both dogs were in good condition, and a second haemorrhage of 20 ml./kg was well tolerated and increased aldosterone secretion. In the other two dogs (256, 257) restoration of the initial blood volume did not inhibit secretion of aldosterone, in dog 256 secretion even rose. In these dogs, a longer interval had been allowed between restoration of the blood volume and sampling of adrenal vein blood. This proved to be a highly damaging procedure because the dogs bled heavily from all wounds during the interval. As a result, the second haemorrhage was poorly tolerated and did not constitute blood withdrawal from a starting point of normal blood volume but of already greatly reduced blood volume; damage to the adrenals is suggested by the fall in glucocorticoid secretion during the last collecting period.

These experiments do not support the idea that freshly shed blood contains any glomerulotrophins disappearing on storage and detectable under the conditions of these experiments. The observation that infusion of shed blood, fresh or stale, may fail to lower aldosterone secretion is best explained by the heavy wound bleeding frequently elicited by these infusions, particularly if long intervals are allowed between collection periods. Another possibility will be dealt with at the end of this paper.

Conclusions

When these experiments were carried out information on secretion rates of *ACTH* in stressed dogs was not available, so that the infusion rates had to be chosen arbitrarily. An investigation into the rate at which *ACTH* is secreted by the dog under the conditions of these experiments (Holzbauer 1964) has since shown it to lie between 0·03 and 0·3 m-u. min^{-1} kg^{-1}. Glucocorticoid secretion was stimulated maximally by 0·3 m-u. min^{-1} kg^{-1},

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but aldosterone secretion could be further increased by *ACTH* infusions of 3 m-u. min^{-1} kg^{-1}.

In some of the foregoing experiments the amount of hormone infused must have exceeded, in others it will have equalled the endogenous production. The infusions were found to increase aldosterone secretion in four out of 22 dogs; glucocorticoid secretion

TABLE 3. CORTICOSTEROID SECRETION IN DOGS WITH INTACT PITUITARY GLANDS SUBJECTED TO TWO HAEMORRHAGES SEPARATED BY RESTORATION OF THE INITIAL BLOOD VOLUME WITH ONE-DAY-OLD BLOOD

Constant infusion of *ACTH* (0·2 m-u. min^{-1} kg^{-1}) started shortly before the initial haemorrhage. Adrenal blood collected for periods of 20 to 25 min. (Flank incision, aldosterone figures corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	adrenal blood sample conditions	*ACTH* infusion	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)	plasma conc. (m-equiv./l.) Na^+	K^+	blood withdrawn (ml./kg)
256, male	13·1	S_1	after first haemorrhage	till end of S_2	16·5	1032	307	94	+	59	143	3·3	1st haem: 20
					+78	+52							
		S_2	started 45 min after restoration of blood volume		29·4	1572	194	136	–	49	141	4·0	.
										50	139	4·3	.
					+3	–29							
		S_3	after second haemorrhage		30·4	1117	206	73	+	48	138	3·8	2nd haem: 12
257, male	13·4	S_1	after first haemorrhage	till start of S_2	14·6	1449	456	160	–	48	141	3·7	1st haem: 20
					+7	+1							
		S_2	started 45 min after restoration of blood volume		15·6	1467	327	174	–	52	141	3·5	.
					–37	–20							
		S_3	after second haemorrhage		9·8	1167	201	90	+	53	139	3·7	2nd haem: 16
258, male	12·3	S_1	after first haemorrhage	throughout	10·9	1680	390	90	–	57	137	2·9	1st haem: 20
					–22	–39				55	135	2·9	
		S_2	started 5 min after restoration of blood volume		8·5	1022	276	160	–	54	132	3·0	.
										51	139	3·4	.
					+88	+31							
		S_3	after second haemorrhage		16·0	1339	264	130	–	51	139	3·7	2nd haem: 20
259, male	13·4	S_1	after first haemorrhage	throughout	38·3	1008	173	148	–	52	133	3·6	1st haem: 22
					–27	+2							
		S_2	started 9 min after restoration of blood volume		27·8	1032	173	160	–	52	134	3·6	.
										54	137	4·0	.
					+25	–14							
		S_3	after second haemorrhage		34·8	888	144	135	–	54	137	3·5	2nd haem: 21

never rose. The mean secretion rates of both types of corticosteroids in response to infusion of *ACTH* were often found to be lower in the present experiments on non-hypophysectomized dogs than in acutely hypophysectomized dogs; work on this problem will be published separately.

Haemorrhage, superimposed on a constant infusion of *ACTH*, stimulated aldosterone output in 13 out of 28 dogs. These results could have two reasons: either bleeding caused a sudden release of much larger quantities of *ACTH* than expected, or the rise in aldosterone was caused by a factor different from *ACTH*. This conclusion is much more likely and agrees with results on hypophysectomized dogs.

II. Aldosterone secretion after haemorrhage in hypophysectomized dogs maintained with *ACTH*

Ganong & Mulrow (1961), and Davis, Carpenter, Ayers, Holman & Bahn (1961) observed that hypophysectomy does not abolish the increase in aldosterone secretion after haemorrhage. The interpretation of results obtained on hypophysectomized dogs is complicated by the very low secretion rate of all adrenal steroids and the fact that the raised secretion rate obtained after haemorrhage remains far below the pre-haemorrhage figure of the non-hypophysectomized, stressed dog. Experiments were therefore carried out in order to investigate whether increases in aldosterone of the order of magnitude observed in our experiments can be obtained after haemorrhage in the absence of the pituitary gland. For this purpose *ACTH* was infused at a rate of less than one third of that expected to be secreted during operative stress. Infusion at a constant speed was started 10 or 40 min before the adrenal vein was cannulated and continued to the end of the experiment.

Procedures and observations

Shorter periods of ACTH *infusion*

In six dogs *ACTH* infusion (0·01 m-u. min^{-1} kg^{-1}) was started 10 min before the collection of a control sample and continued throughout the experiment. After the first collection period the dogs were bled (9 to 22 ml./kg) and a second sample was collected.

Table 4 shows that haemorrhage increased aldosterone secretion in four out of six dogs by amounts ranging from 52 to 149 %. The two dogs which failed to react had a high initial aldosterone secretion rate, and it is interesting that this was of the same order as that often found in 'non-reactors' with intact pituitaries.

During the control periods, aldosterone and glucocorticoid secretion rate (figure 3, open circles) lay approximately on the extrapolated dose-response curve obtained earlier (Holzbauer 1964) for the relationship between infusion rates of *ACTH* (applied for shorter periods) and secretion of corticoids. After haemorrhage, however, two of the 'reactors' showed considerable increases in glucocorticoid secretion (table 4). This suggested the possibility that the period of *ACTH* infusion before haemorrhage might not have been long enough to obtain a steady adrenal response to *ACTH*.

Longer periods of ACTH *infusion*

In order to ensure that adrenal secretion had reached a steady state before haemorrhage, longer infusion periods were used in a group of six dogs. Before the collection of the initial sample, *ACTH* was infused for 40 min and the speed of the infusion doubled for the first 10 min (0·02 m-u. min^{-1} kg^{-1}); furthermore, two consecutive control samples (S_1 and S_2, table 5) were taken before bleeding. The amount of *ACTH* infused was checked after each collection period.

As shown in table 5 and figure 3 (points marked ●), the glucocorticoid secretion rates during the two control periods fitted the original dose-response curve well and there was no increase during the second samples. The mean aldosterone secretion, however, though equal in the two control samples, was higher than after the brief infusions and corresponded to values obtained previously when *ACTH* was infused for a short time at 3 times the rate.

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TABLE 4. EFFECT OF HAEMORRHAGE ON CORTICOSTEROID SECRETION IN HYPOPHYSECTOMIZED DOGS, MAINTAINED ON A CONTINUOUS I.V. INFUSION OF 0·01 m-u. *ACTH* min^{-1} (kg body wt)$^{-1}$

Hypophysectomy 2 h, start of *ACTH* infusion 10 min before collection of first adrenal blood sample (S_1); second sample (S_2) after haemorrhage. (Flank incision, adrenal blood collected for periods of 30 min, aldosterone figures corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	adrenocortical secretion rates adrenal (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol + corticosterone	adrenal blood flow (ml./h and % change)	mean b.p. (mmHg)	Mayer waves	Ht (% red cells)	blood withdrawn before S_2 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
Group I. Reactors										
345, female	8·5	S_1	6·7	1069	186	110	–	47	14	16
		S_2	16·7 + 149	1222 + 14	180 − 3	70	–	49		.
347, female	6·8	S_1	5·3	294	114	140	–	48	22	17
		S_2	12·2 + 129	569 + 94	144 + 26	100	+	51		.
350, female	7·0	S_1	6·9	147	160	85	–	47	13	24
		S_2	10·5 + 52	141 − 4	98 − 40	58	–	52		.
351, male	12·4	S_1	8·7	1092	444	135	–	56	22	3
		S_2	14·1 + 62	1643 + 50	314 − 29	120	+	58		.
mean ± s.e.		S_1	6·9 ± 0·7	651 ± 250	226 ± 74	118 ± 13	.	.	17·8 ± 2·5	.
		S_2	13·4 ± 1·3	894 ± 334	184 ± 46	87 ± 14	.	.		.
Group II. Non-reactors										
346, male	9·8	S_1	14·7	1132	389	135	–	51	16	17
		S_2	14·4 − 2	1024 − 9	206 − 47	75	–	47		.
348, female	10·3	S_1	9·7	1027	288	105	–	58	9	16
		S_2	12·1 + 25	1170 + 14	224 − 22	70	–	53		.
mean		S_1	12·2	1080	339	120	.	.	12·5	.
		S_2	13·3	1097	215	73	.	.		.
combined mean ± s.e. of all dogs		S_1	8·7 ± 1·4	794 ± 183	264 ± 54	118 ± 8·9	.	51 ± 2·0	16 ± 2·1	.
		S_2	13·3 ± 0·9	962 ± 217	194 ± 30	82 ± 9·5	.	52 ± 1·5		.

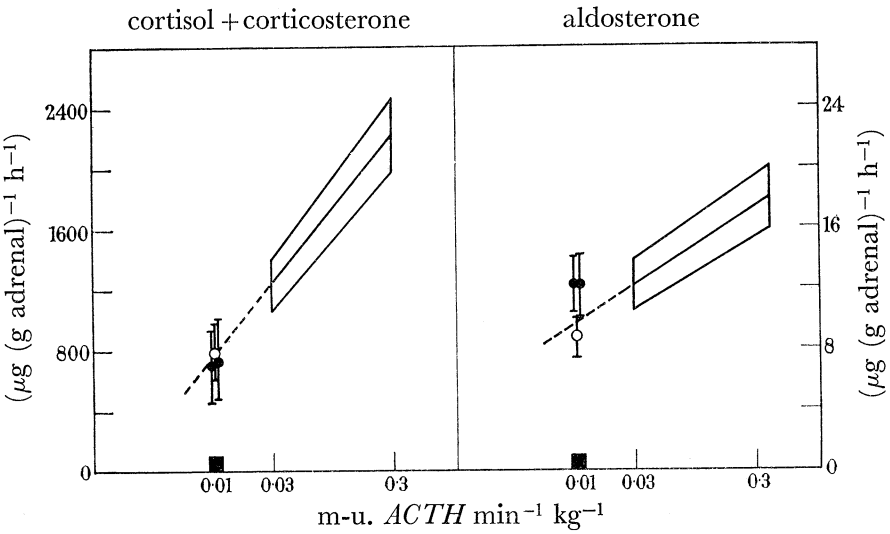

FIGURE 3. Steroid secretion in response to *ACTH* infusions in hypophysectomized dogs. (Same batch of *ACTH* used on all occasions.) Enclosed areas: mean responses (± s.e.) obtained in a previous set of experiments (Holzbauer 1964) when *ACTH* was infused for 20 to 30 min, starting 5 min before the start and ending 5 min before the end of each collection period. ○, ●, Present experiments: *ACTH* infusion started 10 (○) or 40 (●) min before the first blood collection and continued throughout experiment; during the first 10 min of the 40 min infusion period *ACTH* was administered at twice the speed of that used subsequently. The paired black dots correspond to samples collected consecutively (S_1 and S_2 of table 5). All samples were taken before bleeding.

In spite of the high initial values, haemorrhage increased aldosterone secretion further in five of six dogs (table 5). The two largest increases (64 and 174 %, experiments 354 and 357) were, as in the previous group, associated with large rises in cortisol or glucocorticoid secretion.

TABLE 5. EFFECT OF HAEMORRHAGE ON CORTICOSTEROID SECRETION IN HYPOPHYSECTOMIZED DOGS MAINTAINED ON A CONTINUOUS INFUSION OF 0·01 m-u. *ACTH* $min^{-1} kg^{-1}$ (0·02 FOR THE FIRST 10 min)

Hypophysectomy 2 h, start of *ACTH* infusion 40 min before collection of first adrenal blood sample (S_1); second sample (S_2) immediately following S_1, third sample (S_3) after haemorrhage. (Flank incision, adrenal blood collected for periods of 30 min, 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	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) cortisol + corticosterone	adrenal blood flow (ml./h and % change)	mean b.p. (mmHg)	Mayer waves	Ht (% red cells)	blood withdrawn before S_3 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
			Group I. Reactors							
354, male	13·4	S_1	12·3	506*	172	165	−	.	.	55
		S_2	14·7 + 20	114 − 78*	192	170	−	.	.	.
		S_3	24·1 + 64	931 +717	146 −24	80	+	.	19	.
355, male	10·5	S_1	8·0	921*	154	155	−	45	.	3
		S_2	7·3 − 9	927 0*	174	145	−	48	.	.
		S_3	10·1 + 38	1149 + 24	144 −17	80	+	45	15	.
356, female	9·3	S_1	20·4	594*	250	135	+	55	.	3
		S_2	20·6 + 1	325 − 45*	240	105	+	50	.	.
		S_3	31·1 + 51	364 + 12	170 −29	90	+	48	23	.
357, male	10·1	S_1	8·6	294	222	120	−	46	.	8
		S_2	8·0 − 7	190 − 35	288	115	−	47	.	.
		S_3	21·9 +174	834 +339	146 −49	80	−	46	19	.
359, female	11·4	S_1	10·1	1147	184	120	−	31	.	10
		S_2	8·8 − 13	1083 − 6	150	110	+	51	.	.
		S_3	13·7 + 56	724 − 33	128 −15	70	−	52	11	.
mean ± s.e.		S_1	11·9 ± 2·3	721	196 ± 17	139 ± 9	.	.	.	.
		S_2	11·9 ± 2·5	637	209 ± 25	129 ± 12	.	.	.	.
		S_3	20·2 ± 3·7	779	147 ± 7	80 ± 3	.	.	17·4 ± 2	.
			Group II. Non-reactor							
358, female	10·4	S_1	13·5	629	118	130	−	38	.	9
		S_2	13·5 0	928 + 48	188	115	−	43	.	.
		S_3	16·9 + 25	1793 + 93	110 −41	85	−	42	19	.
combined mean ± s.e. of all dogs		S_1	12·2 ± 1·9	690 ± 248	183 ± 19	138 ± 7·6	.	.	.	.
		S_2	12·2 ± 2·1	734 ± 275	205 ± 21	127 ± 10·4	.	.	.	.
		S_3	19·6 ± 3·1	1117 ± 339	141 ± 8	81 ± 2·7	.	.	18 ± 1·7	.

* Cortisol only; results not included in the mean.

Though the circulatory conditions were somewhat better in the second group of dogs, in both groups the mean arterial pressure was lower than in intact dogs. Mayer waves were less frequent after haemorrhage or of smaller amplitude. Haematocrits and plasma electrolytes showed the same small changes in the course of the experiment which have been reported for dogs with intact pituitary. One reactor (354) and the non-reactor (358) showed signs of disease: the first dog had tape worms and diarrhoea, the second was anaemic and its blood sedimentation rate was high.

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Conclusions

Out of 12 hypophysectomized dogs, in which adrenal steroid synthesis was maintained at a submaximal rate by infusions of *ACTH*, nine responded to haemorrhage by increased aldosterone output. Initial levels and increments in aldosterone secreted were similar to those observed in dogs with intact pituitaries, but the number of reactors, 75 % instead of 50 %, was greater after hypophysectomy. This is probably a consequence of being able, after hypophysectomy, to control the *ACTH* available to the adrenal gland and thus keep initial aldosterone secretion sufficiently low to allow an increase after haemorrhage. The large increase in cortisol secretion observed in four dogs after haemorrhage cannot be accounted for by an increase in blood *ACTH* concentration due to a reduction in blood volume; this would amount to 25 % at most, and changes in *ACTH* concentration of 25 % would not lead to measurable changes in steroid output (see figure 3). Since in these experiments basal glucocorticoid secretion was kept lower than the value found in stressed dogs with intact pituitaries, it is conceivable that the action on glucocorticoids of an aldosterone releasing factor became noticeable, while it previously escaped detection.

Whereas the results confirm the view that release of *ACTH* is not essential for the occurrence of a rise in aldosterone secretion after bleeding, there is one other factor which could account for both the larger percentage of reactors in the hypophysectomized dog and the occasional rise in glucocorticoids after bleeding. It is feasible that haemorrhage interferes with the distribution and fate of the infused *ACTH*. An effect of haemorrhage on the fate or action of an injected substance was observed by Scornik & Paladini (1964). These authors injected equal doses of renin into nephrectomized dogs before and after bleeding and measured the blood angiotensin concentration. It was found to be much higher after haemorrhage than before.

III. Aldosterone secretion before and after haemorrhage in nephrectomized dogs

The investigations of Davis and his colleagues have provided evidence for a part played by the renin-angiotensin system in the initiation of high aldosterone secretion rates in hypophysectomized dogs suffering from malignant hypertension (Davis, Carpenter & Ayers 1962) or from chronic heart failure caused either by constriction of the inferior vena cava (Davis, Ayers & Carpenter 1961) or by a large arteriovenous shunt (Davis, Urquhart, Higgins, Rubin & Hartroft 1964). Rises in aldosterone secretion after caval constriction were also seen in hypophysectomized dogs with kidneys deprived of their nerve supply by transplantation (Carpenter, Davis, Holman, Ayers & Bahn 1961). Furthermore, a rise in the secretion rates of aldosterone and also of glucocorticoids was produced by infusions of angiotensin II in dogs (Mulrow & Ganong 1961; Carpenter, Davis & Ayers 1961; Bartter, Casper, Delea & Slater, 1961), in man (Biron, Koiw, Nowaczynski, Brouillet & Genest 1961; Laragh, Angers, Kelly & Lieberman 1960), and in the sheep (Blair-West, Coghlan, Denton, Goding, Munro, Peterson & Wintour 1962).

It is uncertain whether angiotensin exerts a significant control over the adrenal cortex under more physiological conditions. In dogs, the rise in aldosterone secretion caused by sodium depletion seems to depend on the presence of the kidneys (Davis, Ayers &

Carpenter 1961), but the pituitary is also involved: the increase in aldosterone secretion during sodium depletion in conscious dogs is smaller when the pituitary gland is absent (Binnion, Davis, Brown & Olichney 1965). In the sheep the kidney does not seem to be indispensable for the response to sodium loss (Blair-West, Coghlan, Denton, Goding, Wintour & Wright 1963), and the same appears to hold for the rat (Cade & Perenich 1965). The effect of potassium on aldosterone secretion is independent of the kidneys in both species (Davis, Urquhart & Higgins 1963; Blair-West *et al.* 1963).

From experiments on hypophysectomized-nephrectomized dogs, it has also been concluded that the kidneys mediate the rise in aldosterone secretion evoked by haemorrhage (Ganong & Mulrow 1961, 1962; Davis, Carpenter, Ayers, Holman & Bahn 1961). This possibility is supported by the fact that haemorrhage releases renin from the kidneys (Huidobro & Braun-Menendez 1942; Scornik & Paladini 1964; Regoli & Vane 1964).

In the following experiments the effect of haemorrhage on the adrenal cortex was studied in the absence of the kidneys but in the presence of the pituitary gland.

Procedures and observations

Effect of acute bilateral nephrectomy

The object of the first experiments was to see whether nephrectomy would affect basal aldosterone secretion. A control sample of adrenal blood was taken in four dogs. Both kidneys were then removed and the collection of a second sample was started 37 to 44 min, that of a third sample 55 to 94 min later. Forty minutes after nephrectomy, rises in aldosterone secretion of 55 and 48 % were observed in two dogs; these were not maintained; no changes occurred in the remaining two dogs. In another two experiments adrenal blood was collected only after nephrectomy, four consecutive samples being taken in one and two in the other dog. There was no change in aldosterone secretion when up to four consecutive samples were examined between 0·5 and 3 h after nephrectomy. Glucocorticoid secretion remained constant. Rises in the plasma potassium concentration in the course of the experiments were of the same order of magnitude as those observed in non-nephrectomized dogs. The two increases in aldosterone secretion seen after nephrectomy were probably a consequence of the surgical trauma and due to increased release of *ACTH* and renin; when the immediate effect of the surgery had passed, aldosterone secretion remained steady in the nephrectomized dog.

Dogs bled once

In seven experiments control samples were collected before and after bilateral nephrectomy. The dogs were then bled ($22 \pm 1{\cdot}0$ ml./kg) and a post-haemorrhage sample was taken. The results are shown in table 6. In six dogs aldosterone secretion was slightly elevated 26 to 38 min after nephrectomy, but three dogs responded to haemorrhage with a further rise in aldosterone secretion of 31, 40 and 149 %. In the four dogs which failed to respond, the mean aldosterone secretion before bleeding was higher than in the reactors. Circulatory conditions remained satisfactory after haemorrhage. Glucocorticoid secretion rates and blood pressure remained unchanged in all dogs, but adrenal blood flow decreased. There were no abnormalities in the plasma concentrations of Na^+ and K^+.

In another group of dogs the experiments were shortened by removing both kidneys at

the beginning of the experiment and collecting only one control sample 26 to 88 min later. Then the dogs were bled and a second sample taken. The results are given in tables 7 and 8. In table 8 aldosterone figures are corrected for losses.

TABLE 6. THE EFFECT OF ACUTE BILATERAL NEPHRECTOMY ON CORTICOSTEROID SECRETION AND ON THE RESPONSE OF ALDOSTERONE SECRETION TO HAEMORRHAGE

First adrenal blood sample (S_1) collected 30 min after end of adrenal vein cannulation, second sample (S_2) started 26 to 38 min after nephrectomy, third sample (S_3) after bleeding. Arterial blood samples: A_1 at start of S_1, A_2 at end of haemorrhage, A_3 at end of S_3. (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	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change): cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	plasma conc. (m-equiv./l.) K^+	blood withdrawn before S_3 (ml./kg)	Na^+ intake/day: m-equiv.	Na^+ intake/day: no. of days
Group I. Reactors														
172, male	12·3	S_1	7·9	1793	158	166	–	A_1	59	141	3·5	.	170	12
		S_2	10·2 + 29	1811 + 1	132	170	–	A_2	58	136	5·0	.	100	11
		S_3	13·4 + 31	1819 0	96	125	–	A_3	56	136	7·0	23	.	.
174, male	12·6	S_1	4·3	1761	180	136	–	A_1	49	138	3·7	.	170	5
		S_2	4·5 + 5	1808 + 3	144	142	–	A_2	49	138	4·8	.	100	25
		S_3	11·2 + 149	1675 − 7	55	90	+	A_3	55	138	5·2	20	.	.
175, female	10·8	S_1	6·9	1389	202	126	–	A_1	50	138	3·9	.	170	5
		S_2	8·0 + 16	1295 − 7	96	138	–	A_2	41	134	5·3	.	100	31
		S_3	11·2 + 40	1234 − 5	125	115	+	A_3	43	136	5·4	25	.	.
mean ± S.E.		S_1	6·4 ± 1·1	1648 ± 130	180 ± 13	143 ± 12	.	A_1	53 ± 3·2	139 ± 1·0	3·7 ± 0·1	.	.	.
		S_2	7·6 ± 1·7	1638 ± 172	124 ± 14	150 ± 10	.	A_2	49 ± 4·9	136 ± 1·1	5·0 ± 0·1	.	.	.
		S_3	11·9 ± 0·7	1576 ± 176	92 ± 20	110 ± 10	.	A_3	51 ± 4·2	137 ± 0·7	5·9 ± 0·6	23 ± 1	.	.
Group II. Non-reactors														
180, female	15·4	S_1	8·4	1968	305	150	–	A_1	68	145	3·3	.	100	10
		S_2	9·6 + 14	1860 − 5	298	150	–	A_2	66	153	4·2	.	.	.
		S_3	10·6 + 10	1776 − 5	293	110	+	A_3	61	148	4·5	26	.	.
183, male	15·0	S_1	8·6	2465	269	162	–	A_1	53	139	4·4	.	100	10
		S_2	9·7 + 13	2361 − 4	228	168	–	A_2	51	136	5·6	.	.	.
		S_3	10·2 + 5	2312 − 2	174	95	+	A_3	52	147	5·3	20	.	.
186, female	17·2	S_1	10·5	1875	168	138	–	A_1	56	145	3·5	.	100	1
		S_2	10·5 0	1942 + 4	108	152	–	A_2	53	142	5·4	.	.	.
		S_3	10·6 + 1	1868 − 4	91	120	–	A_3	51	145	5·8	23	.	.
189, male	12·0	S_1	6·4	1611	240	148	–	A_1	60	144	3·4	.	100	4
		S_2	7·0 + 9	1456 − 10	112	145	–	A_2	65	142	4·7	.	.	.
		S_3	8·1 + 16	. .	168	100	+	A_3	62	147	4·0	20	.	.
mean ± S.E.		S_1	8·5 ± 0·8	1980 ± 179	246 ± 29	150 ± 4·8	.	A_1	59 ± 3·3	143 ± 1·4	3·7 ± 0·3	.	.	.
		S_2	9·2 ± 0·8	1905 ± 186	187 ± 46	154 ± 5·0	.	A_2	59 ± 3·9	143 ± 3·5	5·0 ± 0·3	.	.	.
		S_3	9·9 ± 0·6	1985 ± 165	182 ± 42	106 ± 5·5	.	A_3	57 ± 2·9	147 ± 0·6	4·9 ± 0·4	22 ± 1·4	.	.
groups I and II combined mean ± S.E.		S_1	7·6 ± 0·7	1837 ± 127	217 ± 21	147 ± 5·0	.	A_1	56 ± 2·5	141 ± 1·2	3·7 ± 0·1	.	.	.
		S_2	8·5 ± 0·8	1813 ± 122	160 ± 28	152 ± 4·6	.	A_2	55 ± 3·4	140 ± 2·4	5·0 ± 0·2	.	.	.
		S_3	10·8 ± 0·6	1781 ± 142	143 ± 30	108 ± 5·0	.	A_3	54 ± 2·4	142 ± 2·1	5·3 ± 0·3	22 ± 1·0	.	.

Out of 11 nephrectomized dogs shown in table 7, five responded to haemorrhage by an increase in aldosterone secretion ranging from 31 to 62 %, whereas there was no change in the glucocorticoids. Dog 139 produced the rise in aldosterone in spite of a low blood pressure, a haematocrit of 75 % and a high initial secretion rate. However, in this animal plasma potassium increased by 2·7 m-equiv./l. in the course of the experiment. This increase was unusually large and may have contributed to the rise in aldosterone secretion.

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Table 7. Effect of acute bilateral nephrectomy on the response of aldosterone secretion to haemorrhage

Collection of first adrenal blood sample (S_1) started 26 to 84 min after nephrectomy, second sample (S_2) after bleeding. Arterial blood samples for haematocrit and plasma K^+ and Na^+ estimation taken at beginning of S_1 (A_1) and end of S_2 (A_2). (Aldosterone figures not corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	time between the removal of the 2nd kidney and S_1 (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	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	plasma conc. (m-equiv./l.) K^+	blood withdrawn before S_2 (ml./kg)	Na^+ intake/day m-equiv.	Na^+ intake/day no. of days
Group I. Reactors															
134, male	8·8	S_1	32	2·1	1879	290	114	−	A_1	.	.	.	.	30	9
		S_2	.	3·4 +62	1384 −26	129	70	+	A_2	.	.	.	20	.	.
139, male	7·8	S_1	28	10·6	1367	154	77	−	A_1	75	160	3·8	.	30	1
		S_2	.	15·3 +42	1233 −10	90	48	+	A_2	67	168	6·5	21	.	.
194, male	8·9	S_1	84	3·0	1284	233	155	−	A_1	61	148	3·9	.	100	16
		S_2	.	4·8 +60	1300 + 1	154	98	+	A_2	59	157	4·1	19	.	.
195, female	11·0	S_1	67	3·7	1195	136	140	−	A_1	58	157	3·0	.	100	16
		S_2	.	5·5 +49	1328 +11	92	120	+	A_2	51	152	4·9	21	.	.
197, female	18·3	S_1	70	6·4	1565	230	166	+	A_1	58	157	3·2	.	100	3
		S_2	.	8·4 +31	1565 0	168	80	+	A_2	51	153	3·4	13	.	.
mean ±s.e.		S_1	.	5·2 ± 1·5	1458 ± 122	209 ± 28	130 ± 16	.	A_1	63 ± 4·1	156 ± 2·6	3·5 ± 0·2	.	.	.
		S_2	.	7·5 ± 2·1	1362 ± 56	127 ± 16	83 ± 12	.	A_2	57 ± 3·8	158 ± 3·7	4·7 ± 0·7	19 ± 1·5	.	.
Group II. Non-reactors															
137, male	8·5	S_1	37	3·2	1636	213	100	+	A_1	52	149	2·9	.	30	2
		S_2	.	2·8 −13	1529 − 7	144	65	−	A_2	49	139	4·0	19	.	.
138, male	13·7	S_1	26	8·7	1415	169	153	+	A_1	56	165	4·0	.	30	1
		S_2	.	10·4 +20	1213 −14	84	65	−	A_2	56	150	6·1	11	.	.
153, male	11·9	S_1	30	10·5	1515	214	133	+	A_1	60	150	3·9	.	30	42
		S_2	.	11·9 +13	1518 0	108	65	−	A_2	58	144	5·1	17	.	.
190, male	12·3	S_1	75	7·4	2073	254	118	−	A_1	62	150	3·4	.	100	16
		S_2	.	8·3 +12	2245 + 8	152	64	+	A_2	57	147	3·7	17	.	.
191, female	9·9	S_1	61	7·4	1483	180	155	−	A_1	55	150	4·7	.	100	16
		S_2	.	9·6 +30	1745 +18	199	95	+	A_2	49	143	5·3	20	.	.
193, female	15·5	S_1	80	10·7	1898	274	132	−	A_1	55	143	4·8	.	100	10
		S_2	.	10·2 − 5	2078 + 9	240	90	+	A_2	52	138	5·9	16	.	.
mean ±s.e.		S_1	.	7·6 ± 1·0	1673 ± 90	215 ± 14	130 ± 7·4	.	A_1	57 ± 1·1	152 ± 3·0	4·1 ± 0·2	.	.	.
		S_2	.	8·5 ± 1·2	1694 ± 135	153 ± 20	72 ± 5·5	.	A_2	53 ± 1·4	145 ± 3·0	5·0 ± 0·3	16 ± 1·1	.	.
groups I and II combined. mean ±s.e.		S_1	.	6·6 ± 0·9	1584 ± 76	213 ± 14	130 ± 7·5	.	A_1	59 ± 1·8	154 ± 2·0	3·9 ± 0·2	.	.	.
		S_2	.	8·1 ± 1·1	1556 ± 94	142 ± 13	77 ± 6·0	.	A_2	55 ± 1·6	149 ± 2·9	4·9 ± 0·3	17 ± 1·0	.	.

In four of the six non-reactors circulation was unsatisfactory. The fifth, 191, which showed a rise of 30 %, was pregnant; all these dogs except 137 had a high initial aldosterone secretion rate.

The four dogs listed in table 8 follow the same pattern, two showing increases in aldosterone secretion after haemorrhage, and two failing to respond; one of the non-reactors (dog 261) had poorly arterialized blood and a high initial aldosterone secretion; the poor response of dog 262 had no obvious reason.

TABLE 8. EFFECT OF ACUTE BILATERAL NEPHRECTOMY ON THE RESPONSE OF ALDOSTERONE SECRETION TO HAEMORRHAGE

Collection of first adrenal blood sample (S_1) started 70 to 88 min after nephrectomy, second sample (S_2) after haemorrhage. Arterial blood samples for haematocrit and plasma K^+ and Na^+ estimation taken at the beginning of S_1 and at the end of S_2. (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)	plasma conc. (m-equiv./l.) Na^+	K^+	blood withdrawn before S_2 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
				Group I. Reactors								
263, male	10·8	S_1	13·3	1252	228	153	(+)	65	138	3·2	.	24
		S_2	19·9 +50	1367 + 9	132	96	+	55	143	4·6	20	.
264, male	17·0	S_1	13·8	1192	360	122	+	67	145	2·6	.	24
		S_2	19·8 +43	1184 − 1	139	70	+	63	145	3·2	19	.
				Group II. Non-reactors								
261, male	11·0	S_1	18·9	1232	252	146	+	58	138	4·5	.	26
		S_2	20·1 + 6	1008 −18	202	90	+	59	145	4·1	20	.
262, male	14·2	S_1	10·2	1253	426	164	−	65	148	3·2	.	26
		S_2	13·1 +28	1175 − 6	270	118	+	.	.	.	23	.
mean ± S.E.		S_1	14·1 ± 1·8	1232 ± 14	317 ± 46	146 ± 8·9	A_1	64 ± 2·0	142 ± 2·5	3·4 ± 0·4	.	.
		S_2	18·2 ± 1·7	1184 ± 73	186 ± 32	94 ± 9·9	A_2	59 ± 2·3	144 ± 0·7	4·0 ± 0·4	21 ± 0·9	.

Dogs bled twice

The difficulty experienced in using a response which, even with the kidneys present, may only be seen in 50 % of the animals, prompted attempts at bleeding the same dog twice, once before and once after nephrectomy.

In three dogs (table 9) the kidneys were mobilized from their surroundings and ligatures placed loosely underneath the main vessels. After collection of a control sample of adrenal blood, the dogs were bled and a second sample was taken. Then the shed blood was re-infused, and after an interval of 45 to 60 min both kidneys were removed; 30 to 36 min later a third blood sample was collected, the dogs were bled again and a final sample was taken. In the first experiment (dog 162), re-infusion of blood followed by nephrectomy reduced aldosterone secretion below the initial level, and the second haemorrhage produced a significant rise, output of glucocorticoids remaining steady throughout. In the next dog (166), circulatory conditions did not remain satisfactory to the end, and the amount of blood loss tolerated after nephrectomy was less than half the previous amount. No significant increase in aldosterone production was found after the second haemorrhage. The third dog (171) bled heavily throughout the experiment, and did not tolerate withdrawal of the

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TABLE 9. COMPARISON OF THE EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION IN THE SAME DOG BEFORE AND AFTER BILATERAL NEPHRECTOMY

First arterial blood sample (A_1) taken at beginning of S_1, second (A_2) after first haemorrhage, third (A_3) after second haemorrhage, fourth (A_4) at the end of S_4. (Aldosterone figures not corrected for losses.)

dog no.	body wt. (kg)	adrenal blood sample no.	conditions	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	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	K^+	blood withdrawn (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
162, male	7·5	S_1	control	7·2	1368	140	108	–	A_1	52	140	3·9	.	8
				+28	+24									
		S_2	after 1st haemorrhage	9·2	1695	98	75	+	A_2	50	141	4·1	1st: 13	.
				−37	−19									
		S_3	80 min after re-infusion of blood, 30 min after bilateral nephrectomy	5·8	1376	127	110	–	A_3	55	141	4·7	.	.
				+48	+ 4									
		S_4	after 2nd haemorrhage	8·6	1428	90	86	+	A_4	52	140	5·2	2nd: 15	.
166, male	15·1	S_1	control	3·7	1554	315	145	–	A_1	64	147	3·6	.	5
				+65	− 6									
		S_2	after 1st haemorrhage	6·1	1454	234	80	+	A_2	51	146	5·3	1st: 19	.
				−25	− 1									
		S_3	75 min after re-infusion of blood, 30 min after bilateral nephrectomy	4·6	1438	282	124	–	A_3	58	146	5·2	.	.
				+13	− 8									
		S_4	after 2nd haemorrhage	5·2	1323	126	60	–	A_4	55	145	5·6	2nd: 9	.
171, female	16·4	S_1	control	2·9	996	300	155	+	A_1	58	136	4·5	.	10
				−21	− 7									
		S_2	after 1st haemorrhage	2·3	924	180	67	+	A_2	56	137	5·0	1st: 11	.
				+30	− 2									
		S_3	96 min after re-infusion of blood, 36 min after bilateral nephrectomy	3·0	912	237	134	+	A_3	49	141	4·9	.	.
				−40	−20									
		S_4	after 2nd haemorrhage	1·8	732	99	48	–	A_4	46	139	4·4	2nd: 8	.
mean ± S.E.		S_1		4·6 ± 1·3	1306 ± 164	252 ± 56	136 ± 14·0	.	A_1	58 ± 3·5	141 ± 3·2	4·0 ± 2·6	1st:	.
		S_2		5·9 ± 2·0	1358 ± 228	171 ± 40	74 ± 3·7	.	A_2	52 ± 1·9	141 ± 2·6	4·8 ± 3·6	14 ± 2·4	
		S_3		4·5 ± 0·8	1242 ± 166	215 ± 46	123 ± 7·0	.	A_3	54 ± 2·6	143 ± 1·6	4·9 ± 1·5	2nd:	.
		S_4		5·2 ± 2·0	1161 ± 217	105 ± 11	65 ± 11·0	.	A_4	51 ± 2·6	141 ± 1·9	5·1 ± 3·5	11 ± 2·2	

usual amount of blood even at the first bleeding period; there was a fall in aldosterone secretion after both haemorrhages.

Another modification of the same experiment, in which the right kidney was extirpated before taking the first control sample and a shorter interval allowed after restoration of the initial blood volume, was no more successful in producing a sufficiently low secretion rate of aldosterone before the second haemorrhage and creating conditions which would have allowed a significant rise afterwards. It was therefore decided to try and reduce the operative stress by removing the first kidney at a preliminary aseptic operation. This not only shortened the operation, but avoided the stress of a midline incision.

Effect of extirpation of left kidney after preliminary right nephrectomy

The right kidney was removed in an aseptic operation under pentobarbitone sodium anaesthesia 2 to 3 weeks before the experiment, at which left nephrectomy and adrenal cannulation were performed through a flank incision. In four dogs blood collection was started 1 h after removal of the left kidney. It can be seen (table 10) that in these animals the circulatory conditions remained satisfactory, but in two dogs (303, 304) initial aldosterone secretion was very high. After bleeding, no rise in aldosterone secretion was observed. The reason might have been that not enough time had been allowed for renin, released during nephrectomy, to disappear from the circulation.

TABLE 10. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION IN NEPHRECTOMIZED DOGS

Right kidney removed 13 to 15 days before the experiment, left kidney 1 h before the start of the collection of the first adrenal blood mple (S_1); second sample (S_2) after haemorrhage. Arterial blood samples taken at the start of S_1 (A_1) and at the end of S_2 (A_2). (Aldoerone figures corrected for losses.)

og 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	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	K^+	blood withdrawn before S_2 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
)1, nale	10·5	S_1	11·3	1576	504	130	–	A_1	51	156	2·4	.	21
		S_2	8·9 −21	1766 +12	327	80	–	A_2	48	162	3·3	19	.
)2, nale	10·0	S_1	12·6	1596	264	165	–	A_1	50	152	3·0	.	15
		S_2	13·0 + 3	1636 + 3	168	110	–	A_2	44	161	4·0	23	.
)3, nale	24·8	S_1	17·5	1387	692	160	+	A_1	58	156	3·1	.	21
		S_2	15·1 −14	952 −31	495	135	+	A_2	53	156	3·3	25	.
)4, emale	11·9	S_1	22·2	2778	366	182	–	A_1	46	185	3·6	.	38
		S_2	23·8 + 7	3097 +12	117	95	+	A_2	39	163	3·6	16	.
ean ±s.E.		S_1	15·9 ± 2·5	1834 ± 318	457 ± 93	159 ± 11	.	A_1	51 ± 2·5	162 ± 7·6	3·0 ± 0·3	.	.
		S_2	15·2 ± 3·1	1863 ± 448	277 ± 85	105 ± 12	.	A_2	46 ± 3·0	161 ± 1·6	3·6 ± 0·2	21 ± 2·0	.

Thus, in the remaining 13 dogs an interval of 3 h was allowed between removal of the second kidney and collection of the control sample. As can be seen from table 11, this procedure reduced the initial aldosterone secretion in most instances, and now six dogs responded to haemorrhage with rises ranging from 32 to 133 %. Glucocorticoid secretion was hardly affected. A control group of 10 non-nephrectomized dogs was run at the same time. In these animals a small piece of intestine was removed 3 h before the first collection of adrenal blood to simulate the stress caused by the manipulation during left nephrectomy.

A positive response to haemorrhage was observed in five dogs (for details see part I, table 3). The percentage of responding dogs was, thus, the same in control and in nephrectomized animals.

TABLE 11. EFFECT OF HAEMORRHAGE ON ALDOSTERONE SECRETION IN NEPHRECTOMIZED DOGS

Right kidney removed 21 to 30 days before the experiment, left kidney 3 h before start of collection of the first adrenal blood sample (S_1), second sample (S_2) collected after haemorrhage. Haematocrit and plasma electrolyte concentrations estimated before S_1 (A_1) and at the end of S_2 (A_2). (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	adrenocortical secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change): cortisol + corticosterone	adrenal blood flow (ml./h)	mean b.p. (mmHg)	Mayer waves	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	plasma conc. (m-equiv./l.) K^+	blood withdrawn before S_2 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
Group I. Reactors													
310, male	12·4	S_1	9·5	1804	240	140	–	A_1	49	169	4·2	.	31
		S_2	18·5 + 95	2319 + 29	240	90	+	A_2	51	176	4·3	27	.
311, male	12·8	S_1	6·5	1766	139	170	–	A_1	56	171	3·3	.	32
		S_2	9·8 + 51	1738 − 2	89	100	+	A_2	53	162	4·0	20	.
316, female	8·7	S_1	7·6	1592	144	160	–	A_1	47	144	4·1	.	61
		S_2	10·0 + 32	963 − 40	67	120	+	A_2	45	146	5·6	23	.
317, female	9·8	S_1	4·8	1281	434	170	–	A_1	46	149	4·1	.	32
		S_2	7·9 + 65	1641 + 28	240	90	–	A_2	46	144	5·0	20	.
318, male	8·7	S_1	6·5	1375	132	155	–	A_1	50	150	4·7	.	32
		S_2	11·3 + 74	859 − 38	122	90	+	A_2	54	152	4·9	24	.
319, female	14·5	S_1	5·7	2162	323	180	–	A_1	56	146	4·8	.	34
		S_2	13·3 + 133	2229 + 3	216	100	+	A_2	53	152	5·0	21	.
mean ± s.e.		S_1	6·8 ± 0·7	1663 ± 131	235 ± 50	163 ± 5·7	.	.	51 ± 1·7	155 ± 4·9	4·2 ± 0·2	.	.
		S_2	11·8 ± 1·5	1625 ± 251	162 ± 32	98 ± 4·8	.	.	50 ± 1·6	155 ± 4·9	4·8 ± 0·2	23 ± 1·1	.
Group II. Non-reactors													
307, male	14·1	S_1	20·3	2239	259	175	–	A_1	55	175	4·5	.	49
		S_2	16·8 − 17	1950 − 13	209	115	+	A_2	52	162	5·1	22	.
308, female	15·7	S_1	11·0	2070	330	180	–	A_1	65	169	3·8	.	29
		S_2	11·1 + 1	2422 + 17	330	150	+	A_2	63	174	4·4	24	.
313, male	17·0	S_1	8·0	2028	264	155	–	A_1	49	151	4·6	.	64
		S_2	10·4 + 30	1428 − 30	249	100	+	A_2	55	143	4·7	21	.
314, male	12·8	S_1	6·1	1411	264	170	–	A_1	62	143	3·3	.	29
		S_2	5·0 − 18	1190 − 16	147	120	–	A_2	50	145	3·7	27	.
315, female	11·5	S_1	6·5	1171	462	170	–	A_1	58	150	3·3	.	29
		S_2	6·4 − 2	1206 + 3	351	125	–	A_2	48	145	3·7	22	.
320, male	8·1	S_1	2·7	1668	163	195	–	A_1	61	151	4·2	.	24
		S_2	3·4 + 26	1125 − 33	51	100	+	A_2	58	163	5·2	16	.
321, male	17·0	S_1	8·5	1542	257	165	–	A_1	46	152	4·2	.	29
		S_2	10·8 + 27	1481 − 4	259	140	+	A_2	49	143	5·7	21	.
mean ± s.e.		S_1	9·0 ± 2·1	1733 ± 148	286 ± 35	173 ± 4·7	.	A_1	57 ± 2·6	156 ± 4·4	4·0 ± 0·2	.	.
		S_2	9·1 ± 1·7	1543 ± 180	228 ± 39	121 ± 7·1	.	A_2	54 ± 2·1	154 ± 4·8	4·6 ± 0·3	22 ± 1·3	.
combined mean ± s.e. of all dogs		S_1	8·0 ± 1·2	1701 ± 96	262 ± 29	168 ± 3·8	.	A_1	54 ± 1·8	155 ± 3·1	4·1 ± 0·1	.	.
		S_2	10·4 ± 1·2	1581 ± 145	198 ± 27	111 ± 5·4	.	A_2	52 ± 1·4	154 ± 3·3	4·7 ± 0·2	22 ± 0·1	.

There was, however, an interesting difference between nephrectomized and control dogs (figure 4). Whereas in the controls, prolongation of the interval between adrenal vein cannulation and collection of adrenal blood from 30 min to about 3 h did not affect aldosterone secretion, it increased glucocorticoid secretion. In contrast, aldosterone secretion of nephrectomized dogs was high soon after the nephrectomy, and fell during the waiting

period. These results indicate that the long 'rest' after surgery is, in fact, a stress, as judged from the high glucocorticoid secretion; furthermore, that the rise in aldosterone secretion shortly after nephrectomy might indeed be due to a release of renin during excision of the kidney; as the circulating renin is gradually disposed of, its effect on aldosterone secretion disappears during the long waiting period.

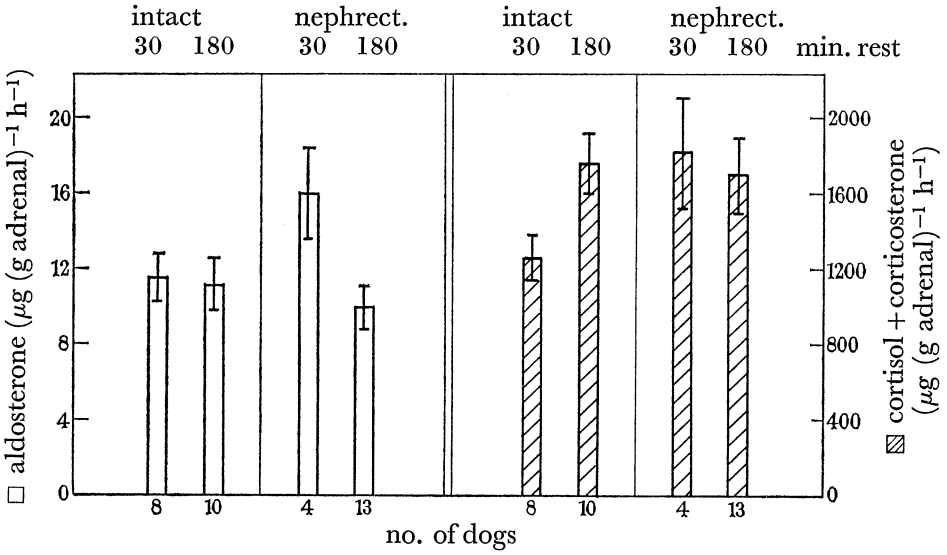

FIGURE 4. The influence of the duration of the resting period after completion of surgery on the secretion rates of corticosteroids (means ±S.E.) in control dogs and nephrectomized dogs. (Aldosterone figures corrected for losses.)

Conclusions

The experiments on nephrectomized dogs have shown that the kidneys are not essential for the response of aldosterone secretion to haemorrhage in dogs with intact pituitary glands. Assuming that renin and *ACTH* are the most potent stimuli involved in this mechanism, the results on hypophysectomized and on nephrectomized dogs provide evidence that the two agents can replace each other. If this assumption is correct, the rise in aldosterone secretion observed in nephrectomized dogs indicates that the pituitary gland is able to increase its *ACTH* output under conditions under which glucocorticoid secretion is already maximal.

IV. ALDOSTERONE SECRETION AFTER HAEMORRHAGE IN HYPOPHYSECTOMIZED-NEPHRECTOMIZED DOGS

The results of the previous sections could also have been obtained if renin and *ACTH* were not the only stimuli for aldosterone secretion called into action by haemorrhage. The actions of these two substances could be supplementary to that of other factors. As discussed on p. 289, this possibility was tested by Ganong & Mulrow (1961, 1962) and by Davis, Carpenter, Ayers, Holman & Bahn (1961) who found no evidence for it. Their experiments were conducted on hypophysectomized-nephrectomized dogs in which secretion rates of all adrenal steroids were extremely low. Thus the question arose, whether in the

absence of these organs, particularly through lack of *ACTH*, steroid synthesis was inhibited to a degree that it could not be accelerated by any stimulus. Experiments were therefore carried out on hypophysectomized-nephrectomized dogs in which steroid synthesis was maintained by a continuous infusion of *ACTH*. The rate was kept below that at which *ACTH* would be expected to be released during operative stress.

Procedures and observations

Dogs infused with ACTH *alone or with noradrenaline and* ACTH

In all animals the right kidney was removed under sodium pentobarbitone anaesthesia and aseptic conditions 10 to 61 days before the acute experiment. On the day of the experiment the pituitary was extirpated first, and then the left kidney. Adrenal blood collection was begun 3 h after nephrectomy and 40 min after starting an infusion of *ACTH* (0·01 or 0·04 m-u. min^{-1} kg^{-1}) which was continued throughout the experiment. For the first 10 min infusion rate was double that indicated. In some dogs L-noradrenaline bitartrate was infused into the right jugular vein. Table 12 summarizes the results of experiments in which the infusion rate of *ACTH* min^{-1} kg^{-1} was 0·01 m-u.; tables 13 and 14 those in which it was 0·04 m-u. Noradrenaline was infused in the experiments of table 14.

TABLE 12. EFFECT OF HAEMORRHAGE ON CORTICOSTEROID SECRETION IN HYPOPHYSECTOMIZED-NEPHRECTOMIZED DOGS, MAINTAINED ON A CONTINUOUS i.v. INFUSION OF 0·01 m-u. *ACTH* min^{-1} kg^{-1} (0·02 FOR THE FIRST 10 min)

at nephrectomy 12 to 19 days, hypophysectomy 3½ h, left nephrectomy 3 h, start of *ACTH* infusion 40 min before collection of first adrenal blood sample (S_1); second sample (S_2) immediately after S_1, third sample (S_3) after haemorrhage. Arterial blood samples taken at start of S_1 (A_1), at end of haemorrhage (A_2) and at end of S_3 (A_3). Left flank incision, adrenal blood collection periods 30 to 40 min. (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	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	K^+	blood withdrawn before S_3 (ml./kg)	100 m-equiv. Na^+/day for (no. of days)
Circulation satisfactory															
364,* male	20·0	S_1	(2·2)*	(−5)*	(424)*	(−78)*	190	140	−	A_1	58	164	3·7	.	135
		S_2	2·3	9	92	+ 7	176	145	−	A_2	55	161	4·1	.	.
		S_3	2·1		98		130	95	+	A_3	56	156	4·3	17	.
Circulation unsatisfactory															
361,† female	12·5	S_1	14·5	0	475	−45	128	125	−	A_1	47	155	4·3	.	99
		S_2	14·5	−37	264	+16	152	140	−	A_2	47	159	4·8	.	.
		S_3	9·2		307		106	85	+	A_3	45	148	5·1	4·1	.
362, female	9·4	S_1	12·6	+20	137	+51	138	118	−	A_1	41	155	3·7	.	100
		S_2	15·1	−77	206	−28	128	125	−	A_2	50	153	5·0	.	.
		S_3	3·5		148		93	63	−	A_3	47	145	4·9	9·6	.

* *ACTH* infusion for 30 min before S_1 erroneously 0·05 m-u. min^{-1} kg^{-1}.
† Had suffered from diarrhoea after first nephrectomy.

With one exception only (dog 392, table 14), aldosterone secretion after haemorrhage fell, or, at most, remained unchanged. Similarly, in 14 of the 21 dogs, glucocorticoid secretion fell by amounts ranging from 28 to 67 %. Signs of circulatory failure during withdrawal of blood were frequent, and a glance at the tables shows that removal of the usual

TABLE 13. EFFECT OF HAEMORRHAGE ON CORTICOSTEROID SECRETION IN HYPOPHYSECTOMIZED-NEPHRECTOMIZED DOGS, MAINTAINED ON A CONTINUOUS INTRAVENOUS INFUSION OF 0·04 m-u. *ACTH* min^{-1} (kg BODY WEIGHT)$^{-1}$ (0·08 FOR THE FIRST 10 min)

Right nephrectomy 10 to 61 days, hypophysectomy 3½ h, left nephrectomy 3 h, start of *ACTH* infusion 40 min before collection of first adrenal blood sample (S_1). Second sample (S_2) immediately after S_1, third sample (S_3) after haemorrhage. Arterial blood samples taken at start of S_1 (A_1), at end of haemorrhage (A_2) and at end of S_3 (A_3). Left flank incision, adrenal blood collection periods 25 to 40 min. (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 and % change)	mean b.p. (mmHg)	Mayer waves	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	K^+	blood withdrawn before S_3 (ml./kg)	100 m-equiv. Na^+/day (no. of days)
Group I. Circulation satisfactory													
3,	19·3	S_1	9·2	1802	184 .	140	–	A_1	52	150	3·9	.	30
male		S_2	13·4 + 46	2052 + 14	159	135	–	A_2	53	150	3·6	.	.
		S_3	7·3 − 45	1687 − 18	112 − 30	90	–	A_3	51	148	4·1	15·1	.
4,	13·0	S_1	10·3	1058	425 .	165	–	A_1	58	152	2·9	.	38
male		S_2	13·1 + 27	1261 + 19	360	175	–	A_2	54	146	2·7	.	.
		S_3	7·8 − 40	781 − 38	218 − 39	90	–	A_3	49	148	2·8	24·6	.
7,	15·8	S_1	9·2	1759	248 .	140	–	A_1	47	138	4·0	.	30
male		S_2	9·5 + 3	2267 + 29	336	130	–	A_2	50	130	4·0	.	.
		S_3	7·0 − 26	1610 − 29	196 − 42	80	+	A_3	53	133	3·7	15·5	.
0,	10·0	S_1	12·3	2417	254 .	143	–	A_1	60	152	4·0	.	29
male		S_2	7·0 − 43	1917 − 21	264	155	–	A_2	55	140	3·7	.	.
		S_3	3·4 − 51	742 − 61	168 − 36	78	–	A_3	51	140	4·0	16	.
ean ± s.e.		S_1	10·3 ± 1·0	1759 ± 364	278 ± 67	147 ± 8	.	.	.	.	.	.	32 ± 2·
		S_2	10·8 ± 2·0	1874 ± 284	266 ± 51	149 ± 14	.	.	.	.	.	.	.
		S_3	6·4 ± 1·3	1205 ± 336	174 ± 30	85 ± 4	.	.	.	.	.	17·8 ± 3	.
Group II. Circulation unsatisfactory													
6,*	12·5	S_1	27·3	1492	110 .	115	+	A_1	51	149	5·3	.	64
male		S_2	18·0 − 34	1367 − 8	100	130	+	A_2	52	146	4·8	.	.
		S_3	9·7 − 46	733 − 46	76 − 24	78	–	A_3	53	152	5·2	12·8	.
69,	11·5	S_1	8·9	902	140 .	140	–	A_1	50	148	2·4	.	43
male		S_2	7·6 − 15	1016 + 13	188	150	–	A_2	51	147	2·3	.	.
		S_3	6·6 − 13	857 − 16	90 − 62	75	+	A_3	52	150	2·2	10·4	.
72,*	16·0	S_1	5·8	1374	312 .	120	–	A_1	60	152	3·4	.	36
male		S_2	8·6 + 48	1336 − 3	348	105	–	A_2	57	.	.	.	.
		S_3	4·6 − 47	713 − 47	149 − 57	70	–	A_3	54	143	3·0	14	.
5,*	12·5	S_1	13·4	1373	154 .	100	–	A_1	53	127	3·8	.	45
male		S_2	12·2 − 9	1380 + 0·5	102	110	–	A_2	53	130	4·2	.	.
		S_3	11·3 − 7	973 − 30	102 0	60	–	A_3	49	131	3·8	5·0	.
3,	13·8	S_1	2·9	269	114 .	125	+	A_1	43	151	2·9	.	91
male		S_2	3·6 + 24	367 + 36	172	120	–	A_2	43	150	3·4	.	.
		S_3	1·6 − 56	246 − 32	64 − 63	60	–	A_3	38	151	3·3	5·4	.
4,	14·0	S_1	12·3	531	188 .	100	–	A_1	52	159	3·4	.	156
male		S_2	10·8 − 12	523 − 2	188	130	–	A_2	54	151	4·1	.	.
		S_3	10·0 − 7	249 − 53	98 − 48	50	–	A_3	51	151	4·1	0	.
5,	15·3	S_1	18·9	1346	269 .	130	–	A_1	55	163	3·4	.	155
male		S_2	12·6 − 33	944 − 31	283	115	–	A_2	54	156	3·3	.	.
		S_3	4·7 − 63	362 − 62	77 − 73	50	–	A_3	49	159	3·2	8·2	.
ean ± s.e.		S_1	12·8 ± 3·1	1041 ± 182	184 ± 30	119 ± 5·6	.	.	.	.	.	.	84 + 19·6
		S_2	10·5 ± 1·7	990 ± 156	197 ± 34	123 ± 5·7	.	.	.	.	.	.	.
		S_3	6·9 ± 1·3	590 ± 113	94 ± 11	63 ± 4·2	.	.	.	.	.	8·0 ± 1·9	.
oups I and		S_1	11·9 ± 2·0	1302 ± 182	218 ± 29	129 ± 5·9	.	A_1	53 ± 1·6	149 ± 2·9	3·6 ± 0·2	.	65 ± 14·5
I combined.		S_2	10·6 ± 1·2	1312 ± 180	227 ± 29	132 ± 6·3	.	A_2	52 ± 1·1	145 ± 2·8	3·6 ± 0·2	.	.
ean ± s.e.		S_3	6·7 ± 0·9	814 ± 144	123 ± 16	71 ± 4·3	.	A_3	50 ± 1·3	145 ± 2·6	3·6 ± 0·2	11·5 ± 2·0	.

* Suffered from enteritis after the first nephrectomy.

amount of blood was tolerated only once, and that the mean blood pressure after even small haemorrhages was often very low. In the tables, the dogs are grouped according to whether or not their circulatory conditions could be regarded as 'satisfactory'; the criterion was whether an amount of blood exceeding 15 ml./kg could be withdrawn without producing signs of haemorrhagic shock or a fall in blood pressure below 70 mmHg. In both categories, haemorrhage caused similar falls in aldosterone and glucocorticoid secretion and in adrenal blood flow.

Table 14. Use of vasoconstrictors I: noradrenaline

Procedure as in experiments of table 13, but an infusion of L-noradrenaline (0·13 to 0·49 μg min^{-1} (kg body weight)$^{-1}$ begun 1 h before the start of the infusion of *ACTH* and continued throughout the experiment.

g 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 and % change)	mean b.p. (mmHg)	Mayer waves	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.): Na^+	K^+	blood withdrawn before S_3 (ml./kg)	100 m-equiv. Na^+/day (no. of days)
					Group I. Circulation satisfactory								
', ale	12·3	S_1	6·9	733	112 .	155	–	A_1	44	153	3·8	.	72
		S_2	6·7 − 3	1333 + 82	120 + 12	155	–	A_2	48	151	3·6	.	.
		S_3	5·3 − 21	1163 − 13	134	100	–	A_3	47	153	3·2	17·9	.
', ale	18·9	S_1	13·6	1308	264 .	143	–	A_1	45	164	3·7	.	92
		S_2	13·4 − 1	1891 + 45	240 + 8	148	–	A_2	46	159	4·1	.	.
		S_3	14·2 + 6	1662 − 12	259	70	+	A_3	46	164	3·8	16·1	.
					Group II. Circulation unsatisfactory								
', male	15·0	S_1			.	.	–	A_1	55	141	3·8	.	132
		S_2	18·9	1620	348 − 17	140	–	A_2	56	128	3·1	.	.
		S_3	11·0 − 42	1734 + 7	288	70	–	A_3	52	114	2·9	13	.
', male	11·0	S_1	5·0	622	96 .	130	–	A_1	53	144	2·9	.	162
		S_2	3·8 − 24	604 − 3	90 − 67	140	–	A_2	55	153	3·2	.	.
		S_3	3·5 − 5	201 − 67	30	55	–	A_3	53	155	3·1	13·7	.
', ale	12·3	S_1	8·6	1451	302 .	187	–	A_1	57	159	2·4	.	23
		S_2	8·4 − 2	1457 0	360	185	–	A_2	54	154	2·5	.	.
		S_3	5·2 − 38	971 − 33	149 − 59	60	–	A_3	52	155	2·4	12·2	.
', male	14·5	S_1	6·3	1145	204 .	135	–	A_1	53	145	3·0	.	18
		S_2	9·1 + 44	960 − 16	165	115	–	A_2	53	150	3·5	.	.
		S_3	4·7 − 48	646 − 33	150 − 9	85	+	A_3	52	142	3·7	10·7	.
', male	20·0	S_1	2·4	443	216 .	100	–	A_1	50	153	3·2	.	16
		S_2	2·7 + 13	643 + 45	246	90	+	A_2	52	159	3·0	.	.
		S_3	4·1 + 52	429 − 33	132 − 46	50	–	A_3	47	153	3·2	2·6	.
an ± S.E.		S_1	5·6 ± 1·3	915 ± 233	205 ± 42 .	138 ± 18	.	.	.	.	.	.	70 ± 32
		S_2	8·6 ± 2·9	1057 ± 657	242 ± 52	134 ± 16	.	.	.	.	.	.	.
		S_3	5·7 ± 1·4	796 ± 267	150 ± 41 − 38	64 ± 6	.	.	.	.	.	10·4 ± 2·2	.
mbined roups I and I. mean ± S.E.		S_1	7·1 ± 1·5	950 ± 166	199 ± 33 .	142 ± 12	.	A_1	51 ± 1·9	151 ± 3·2	3·3 ± 0·2	.	74 ± 22
		S_2	9·0 ± 2·1	1215 ± 186	224 ± 40	139 ± 11	.	A_2	52 ± 1·4	151 ± 3·9	3·3 ± 0·2	.	.
		S_3	6·9 ± 1·5	972 ± 223	163 ± 33 − 27	70 ± 7	.	A_3	50 ± 1·1	148 ± 6·1	3·2 ± 0·2	12·3 ± 1·9	.

Table 14 illustrates the effect of giving a constant infusion of noradrenaline throughout the greater part of the experiment. The proportion of dogs in which the circulation collapsed was not decreased, but in the two dogs in which it remained satisfactory the fall in adrenal blood flow was prevented. Perhaps as a result of this, secretion of aldosterone and glucocorticoids did not fall appreciably after haemorrhage, but it did not rise either. One dog (392) tolerated a very small blood loss only and yet appeared to secrete 52 % more

aldosterone after 'haemorrhage'; the absolute figures in this instance were, however, too near the threshold of the method of estimation to be trusted.

The poor circulatory conditions prevailing in the majority of these dogs make it difficult to decide whether the failure of aldosterone secretion to respond to haemorrhage was specifically linked with the absence of pituitary and kidneys or whether it was due to the circulatory collapse, as had been observed in recently splanchnotomized dogs (see part I, table 10, p. 262).

It also seemed important to clarify how far the circulatory collapse was due to the absence from the blood stream of substances known to be released during haemorrhage by the missing organs—renin or posterior lobe hormones—and whether administration of these substances would prevent the collapse.

Effect of angiotensin on tolerance to haemorrhage

In the first experiments an attempt was made to replace the missing effect of the kidneys on the circulation by a constant infusion of angiotensin. The general plan was the same as for the dogs of tables 12 to 14. One hour before starting the infusion of *ACTH* (0·01 m-u. min^{-1} kg^{-1}) a constant infusion of angiotensin was begun (0·01 μg min^{-1} kg^{-1}) which lasted to the end of the experiment. Table 15 shows that in two of the six dogs circulatory conditions were, in fact, very good: initial blood pressure was high and remained at 80 or 90 mmHg after blood loss of 20 ml./kg. Circulation in the remaining experiments, however, was not in the least improved. Reduction in adrenal blood flow after haemorrhage was not consistently lessened by the infusion.

One effect of angiotensin seen in all dogs was the absence of any severe falls in aldosterone secretion after haemorrhage, whereas glucocorticoid secretion was seriously reduced in five dogs. The fact that glucocorticoid secretion was not protected, even in the two dogs in which circulation was good throughout, indicates that the aldosterone secretion was kept high not as a result of improvement in the circulation but by the direct stimulatory action of angiotensin on aldosterone production.

The good circulatory condition of only two out of six similarly treated dogs need not mean that release of renin does not, under natural conditions, help the circulation during haemorrhage. It may be that a constant infusion of angiotensin does not achieve the support of a collapsing circulation which may be achieved by a sudden change in angiotensin concentration, such as one would expect to be produced by an escape of renin from the kidney during haemorrhage.

Effect of posterior lobe hormones on tolerance to haemorrhage

In these experiments infusion of hormones from the posterior lobe of the pituitary was substituted for the release of these hormones during haemorrhage and the effect on the state of the circulation observed. Whereas there is a direct effect of angiotensin on aldosterone secretion, no such effect is established for small doses of vasopressin and oxytocin. Therefore these hormones offer the advantage that their infusion rate can be increased during haemorrhage, as would happen normally, without producing direct effects on synthesis of corticoids.

The experimental plan was similar to that of the previous section, but in place of

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angiotensin, infundin ('Infundin', Burroughs Wellcome and Co., a posterior lobe extract containing 10 u./ml.) was infused at rates which are reported (Lauson 1960) to produce nearly maximal antidiuresis in hydrated dogs. This extract was preferred to pure vasopressin for two reasons: it has been shown (Brooks & Pickford 1958) that vasopressin has very different

TABLE 15. USE OF VASOCONSTRICTORS II: ANGIOTENSIN

Procedure as in experiments of table 13, but *ACTH* infusion reduced to 0·01 m-u. min^{-1} kg^{-1} (0·02 m-u. during first 10 min) and intravenous infusion of angiotensin II, 0·01 μg min^{-1} kg^{-1}, begun 1 h before the start of the infusion of *ACTH* and continued throughout the experiment.

dog no.	body wt. (kg)	adrenal blood 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	adrenal blood flow (ml./h and % change)	mean b.p. (mmHg)	Mayer waves	arterial blood samples	Ht (% red cells)	plasma conc. (m-equiv./l.) Na^+	plasma conc. (m-equiv./l.) K^+	blood withdrawn before S_3 (ml./kg)	100 m-equiv. Na^+/day (no. of days)
Group I. Circulation satisfactory													
390, female	8·8	S_1	17·7	247	81	160	+	A_1	48	131	3·3	.	45
		S_2	15·7 − 11	108 − 56	56 − 31	170	+	A_2	50	130	4·2	.	.
		S_3	12·3 − 22	57 − 47	30 − 46	80	+	A_3	34	138	4·4	20	.
400, female	12·0	S_1	10·3	244	102	150	+	A_1	48	154	3·7	.	22
		S_2	9·2 − 11	183 − 25	60 − 41	155	−	A_2	49	157	4·6	.	.
		S_3	7·9 − 14	107 − 42	92 + 53	90	+	A_3	47	152	3·8	20	.
mean		S_1	14·0	246	92	155	.	A_1	48	143	3·5	.	.
		S_2	12·5	146	58	163	.	A_2	50	144	4·4	.	.
		S_3	10·1	82	61	85	.	A_3	41	145	4·1	20	.
Group II. Circulation unsatisfactory													
394, male	11·8	S_1	12·7	217	132 − 17	95	+	A_1	44	142	3·4	.	43
		S_2	11·5 − 9	443 + 104	110	110	+	A_2	45	145	5·0	.	.
		S_3	11·1 − 3	202 − 54	124 + 13	60	+	A_3	54	151	4·6	8† (19)	.
396, female	11·3	S_1	9·8	150	116 − 14	115	+	A_1	46	153	3·6	.	42
		S_2	15·3 + 56	127 − 15	100 + 30	125	+	A_2	48	145	4·3	.	.
		S_3	17·5 + 14	155 + 22	130	65	+	A_3	47	148	4·1	5† (20)	.
397,* female	12·3	S_1	30·8	236	139 − 1	115	+	A_1	48	153	3·6	.	36
		S_2	19·8 − 36	258 + 9	137 − 11	115	+	A_2	55	151	4·0	.	.
		S_3	23·8 + 20	191 − 26	122	55	+	A_3	55	145	4·3	0† (8)	.
398, female	14·3	S_1	14·6	679	144 − 4	145	−	A_1	56	147	3·0	.	13
		S_2	13·9 − 5	428 − 37	138 − 30	150	−	A_2	48	152	3·5	.	.
		S_3	13·0 − 6	240 − 44	96	65	+	A_3	52	147	3·5	5† (12)	.
mean of group II ± s.e.		S_1	17·0 ± 4·7	321 ± 121	133 ± 6·0	118 ± 10·0	.	A_1	49 ± 3	149 ± 3	3·4 ± 0·1	.	.
		S_2	15·1 ± 1·7	314 ± 75	121 ± 9·5	125 ± 8·8	.	A_2	49 ± 2	148 ± 2	4·2 ± 0·3	.	.
		S_3	16·4 ± 2·8	197 ± 17	118 ± 7·4	61 ± 2·4	.	A_3	52 ± 2	148 ± 1	4·1 ± 0·2	.	.
groups I and II combined, mean ± s.e.		S_1	16·0 ± 3·2	296 ± 78	119 ± 9·9	130 ± 10·0	.	A_1	48 ± 2	147 ± 4	3·4 ± 0·1	.	.
		S_2	14·2 ± 1·5	258 ± 60	100 ± 14·7	138 ± 9·8	.	A_2	49 ± 1	147 ± 4	4·3 ± 0·1	.	.
		S_3	14·3 ± 2·3	159 ± 27	99 ± 15·1	69 ± 5·3	.	A_3	48 ± 3	147 ± 2	4·1 ± 0·2	.	.

* Enteritis and sinusitis.

† Amount of blood initially withdrawn in parentheses; abrupt falls in blood pressure necessitated re-infusion, so that final blood removed amounted to the figures shown outside the parentheses.

effects in the presence of oxytocin than when administered on its own, and that most physiological stimuli appear to release both hormones simultaneously (Harris 1955; Baird & Pickford 1958; Pickford 1960). There is, however, no evidence that, during haemorrhage, oxytocin is in fact released along with vasopressin.

In the experiments listed in table 16, a control sample was taken before infundin infusion was started at a rate of 0·036 m-u. min^{-1} kg^{-1}. After 10 min the amount was decreased to

0·009 m-u. min^{-1} kg^{-1} and kept at this speed during the collection of a second adrenal blood sample. During the next 10 min blood was withdrawn and simultaneously the infusion of infundin speeded up fourfold. After the bleeding the slow infusion rate was resumed. In the experiments of table 17 infundin infusion (0·009 m-u. min^{-1} kg^{-1}) was much more prolonged. It was started 1 h before collection of two consecutive samples. Then the dog was bled and during this brief period infusion of infundin was increased fourfold, to be reduced to the original level for the remainder of the experiment.

TABLE 16. USE OF VASOCONSTRICTORS III: POSTERIOR LOBE EXTRACT, SHORT INFUSIONS

Effect of posterior lobe extract ('Infundin', Burroughs Wellcome and Co.) and of haemorrhage on corticosteroid secretion in hypophysectomized-nephrectomized dogs infused with *ACTH*, 0·01 m-u./min/kg body weight (0·02 for the first 10 min). Right nephrectomy 18 days, left nephrectomy 3 h, hypophysectomy 2½ h and start of *ACTH* infusion 40 min before start of collection of first adrenal blood sample. Daily sodium intake 100 m-equiv. for 3 weeks. (Aldosterone figures corrected for losses.)

dog no.	body wt. (kg)	time (min)	adrenal blood sample no.	infusion of infundin (m-u. min^{-1} kg^{-1})	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 and % change)	mean b.p. (mmHg)	Mayer waves	blood withdrawn (ml./kg)
422, male	9·5	0– 40	S_1	0	4·6	449	171	150	–	.
		40– 50	none	0·036	· + 4	· −24	· −33	150	–	.
		50– 55	none	0·009	.	.	.	155	–	.
		55– 97	S_2	0·009	4·8	343	114	160	–	.
		99–109	haemorrhage	0·036	· −33	· −50	· −34	140	+	20
		109–114	none	0·009	.	.	.	140	+	.
		114–154	S_3	0·009	3·2	170	87	90	+	.
423, male	14·0	0– 30	S_1	0	3·3	142	190	150	–	.
		30– 40	none	0·036	· 0	· −45	· −16	155	–	.
		40– 45	none	0·009	.	.	.	160	–	.
		45– 75	S_2	0·009	3·3	78	160	170	–	.
		75– 88	haemorrhage	0·036	· −55	· −23	· −27	160	+	19·3
		88– 91	none	0·009	.	.	.	160	+	.
		91–121	S_3	0·009	1·5	61	116	105	+	.
mean			S_1		4·0	296	181	150	.	.
					.	.	.	152	.	.
					.	.	.	158	.	.
			S_2		4·1	211	137	165	.	.
					.	.	.	150	.	19·7
					.	.	.	150	.	.
			S_3		2·4	116	102	98	.	.

From tables 16 and 17 it can be seen that infundin maintained satisfactory circulatory conditions in four out of five dogs, but adrenal blood flow decreased after haemorrhage. No pressor effect of infundin was noticeable at the concentrations used, and the blood pressure fell somewhat during bleeding in spite of the accelerated rate of infusion. However, the falls were less abrupt, and there is no doubt that the infusions exerted a blood pressure maintaining influence.

The short infusion periods of infundin (table 16) did not alter aldosterone secretion during the control period (S_2), and failed to prevent the fall induced by bleeding. In contrast, prolonged infusion (table 17) stimulated aldosterone secretion before, and prevented any appreciable fall after haemorrhage. Production of glucocorticoids was less influenced than that of aldosterone: there was one rise during a pre-haemorrhage period, but no check to the severe falls after bleeding.

In one experiment, vasopressin (0·016 m-u. min^{-1} kg^{-1}) was used instead of infundin. The infusion was started 40 min before taking a control sample and was not speeded up

during the bleeding period. There was circulatory collapse, so that the object of the experiment was not achieved, but an interesting feature was that after 'haemorrhage' (amount of blood withdrawn was 6 ml./kg only) the adrenal blood flow rose and the glucocorticoid secretion did not fall. This observation confirms the striking dependence on blood flow of glucocorticoid secretion in nephrectomized-hypophysectomized dogs, a relation which does not exist in the normal dog except in extreme conditions.

TABLE 17. USE OF VASOCONSTRICTORS III: POSTERIOR LOBE EXTRACT, PROLONGED INFUSIONS

Experimental procedures as in table 16, but Infundin infusion (0·009 m-u. min^{-1} kg^{-1}) started 1 h before the first adrenal blood collection. Rate of infusion quadrupled during the time of blood withdrawal (10 min). Right nephrectomy 5 months previously. Daily sodium intake 100 m-equiv. for 3 months followed by 30 m-equiv. for 6 weeks, and 100 m-equiv./day for the last 2 weeks. Dogs are litter-mates.

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 and % change)	mean b.p. (mmHg)	Mayer waves	blood withdrawn before last sample (ml./kg)
416, female	20·0	S_1	3·4	105	132	145	+	.
		S_2	4·5 +32	159 +51	188 +42	150	+	.
		S_3	4·6 + 2	37 −77	108 −43	72	−	10(14)*
417, male	15·6	S_1	3·5	129	116	140	−	.
		S_2	5·9 +69	93 −28	81 −30	145	−	.
		S_3	5·7 − 3	52 −44	76 − 6	120	+	19
421, female	24·0	S_1	3·8	259	209	177	−	.
		S_2	4·2 +11	200 −23	249 +19	180	−	.
		S_3	3·0 −29	86 −57	158 −37	80	−	17
mean ± S.E.		S_1	3·6 ± 0·12	164 ± 48	152 ± 29	154 ± 12	.	.
		S_2	4·9 ± 0·52	151 ± 31	173 ± 49	158 ± 11	.	.
		S_3	4·4 ± 0·80	58 ± 14	114 ± 24	91 ± 15	.	15 ± 3

* Explanation see table 15†.

Effect of blood obtained from dogs with intact kidneys on aldosterone secretion

One unexpected feature of the experiments on hypophysectomized-nephrectomized dogs receiving constant *ACTH* infusions was the great variation between individual dogs of the aldosterone secretion during the control periods. One reason for these variations could be that the response to *ACTH* is influenced by the degree to which renin has previously participated in keeping the rate of aldosterone secretion high. It may be significant in this connexion that two dogs (366, 397, tables 13 and 15) which were suffering from diarrhoea prior to the experiment, had the very high initial aldosterone secretion rates of 27 and 31 μg (g adrenal)$^{-1}$ h^{-1}.

Another factor which could contribute to variations is the replacement of lost blood by donor blood obtained from animals with intact kidneys and pituitary glands. It is well established, that *ACTH* is destroyed quickly in dog's blood on standing (Nelson & Hume 1955), but renin and angiotensin are probably more stable. It is possible that the concentration of these substances in blood taken from dogs with intact kidneys remains high enough, even after storage, to stimulate aldosterone secretion in nephrectomized dogs. However, of two consecutive control samples of adrenal blood collected during constant infusion of

ACTH in nephrectomized-hypophysectomized dogs, the second sample only rarely contained more aldosterone than the first in spite of the increasing amounts of donor blood infused; for example, in table 14, there was only one significant increase in dog 389, and this dog had, in fact, received per kg body weight less donor blood by the end of the second sample than most dogs of the same group.

In order to obtain conditions favourable for the demonstration of an influence on aldosterone secretion of any renin which might be present in donor blood, experiments were carried out in which, in contrast to previous procedures, very large amounts of donor

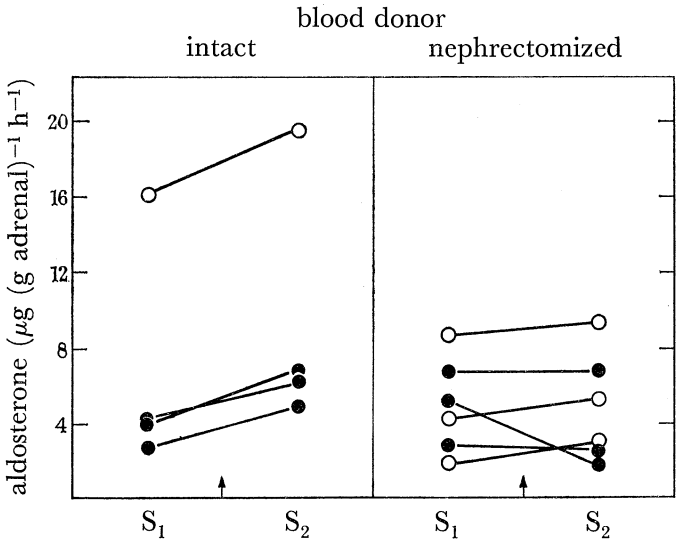

FIGURE 5. Aldosterone secretion of nephrectomized-hypophysectomized dogs given large volumes (20 ml./kg body wt.) of donor blood. Comparison of infusion of donor blood from intact or from nephrectomized dogs. The donor blood was given rapidly between the collection of a first (S_1) and a second (S_2) adrenal blood sample. *ACTH* was infused at rates of 0·01 (●) or 0·02 (○) m-u. min^{-1} kg^{-1} body wt. throughout the experiment (see also tables 18 and 19).

blood were infused during a short period. Blood taken from dogs with intact kidneys or from dogs nephrectomized under chloralose 3 h before bleeding was infused into nephrectomized-hypophysectomized dogs given a constant infusion of *ACTH*. A control sample of adrenal blood was collected, then donor blood 20 ml./kg infused within 10 min and a second adrenal blood sample taken. The blood lost during the collection periods was also replaced by the appropriate donor blood. The results are summarized in figure 5. The dogs given blood from dogs with intact kidneys responded with rises in aldosterone secretion. Among the dogs receiving blood from nephrectomized donors significant rises did not occur. These experiments provide evidence for the presence, in blood stored at 4 °C for 1 to 4 days, of a substance which stimulates aldosterone secretion and which is not evident in blood taken 3 h after removal of both kidneys. In all probability this substance is renin released during haemorrhage. Its stimulant effect is not evident unless large amounts of donor blood are rapidly infused, but its lack might have contributed to the severe falls in aldosterone secretion after bleeding seen in tables 12 to 17 and coinciding with cessation of infusion of donor blood.

Effect of expansion and subsequent reduction of the circulating blood volume on aldosterone secretion

In the previous sections it was shown that the circulation of nephrectomized-hypophysectomized dogs maintained on *ACTH* infusions was so labile that bleeding was not tolerated unless vasoconstrictor substances were infused. It was hoped that haemorrhage might be tolerated without the need of supporting therapy if the blood was withdrawn after a preliminary expansion of the blood volume. This was tried on the dogs used for the experiments illustrated in figure 5 by bleeding them after the collection of the second sample of

TABLE 18. EFFECT OF EXPANSION AND REDUCTION OF THE BLOOD VOLUME ON CORTICOSTEROID SECRETION IN HYPOPHYSECTOMIZED-NEPHRECTOMIZED DOGS MAINTAINED ON *ACTH*. DONOR BLOOD OBTAINED 1 TO 4 DAYS PREVIOUSLY FROM DOGS WITH INTACT KIDNEYS

Experimental details: right nephrectomy 3 to 5 months, left nephrectomy 3 h, hypophysectomy 2½ h, start of *ACTH* infusion 40 min before collection of first adrenal blood sample (S_1), S_2 after expansion, S_3 after reduction of blood volume. Left flank incision, aldosterone figures corrected for losses, all dogs litter-mates of dogs from table 17.

dog no.	body wt. (kg)	adrenal blood 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	adrenal blood flow (ml./h and % change)	mean b.p. (mmHg)	Mayer waves	change in blood volume (ml./kg)	donor blood infused during collection periods (ml./kg)	*ACTH* infusion (m-u. min^{-1} kg^{-1})	daily Na$^+$ intake (m-equiv.)
418, male	16·5	S_1	2·6	167	180	170–80*	+		7·9		
		S_2	4·9 +88	95 −43	174 − 3	50–90	−	+20	10·3		
		S_3	3·6 −27	43 −55	72 −59	60–80	−	−21	.	0·01 (0·02 for the first 10 min)	100 for 100 days, then 30 for 31 days, then 100 for 8–18 days
419, female	17·5	S_1	4·0	423	240	140	−		7·4		
		S_2	6·2 +55	612 +45	260 + 8	160	−	+20	11·7		
		S_3	1·9 −69	243 −60	212 −18	95	−	−17	.		
420, male	15·3	S_1	3·9	404	290	130	−		9·9		
		S_2	6·8 +74	486 +20	290 0	160	+	+20	9·3		
		S_3	4·0 −41	247 −49	250 −14	80	+	−20	.		
mean ± S.E.		S_1	3·5 ± 0·4	331 ± 82	237 ± 32	135†	.				
		S_2	6·0 ± 0·6	398 ± 156	241 ± 35	160	.	+20 ± 0			
		S_3	3·2 ± 0·6	178 ± 67	178 ± 54	88	.	−19 ± 1·2			
415, male	20·0	S_1	16·1	1004	345	164	−		6·3	0·02 (0·04 for first 10 min)	100 for 100 days
		S_2	19·7 +22	822 −18	432 +25	165	−	+20	7·3		
		S_3	10·6 −46	505 −39	276 −36	147	−	−20	.		

* Donor blood badly tolerated; blood pressure values at beginning and end of respective sample.
† Means of dog 419 and 420.

adrenal blood. At an earlier stage of this work (see figure 2, part I, p. 249) rises in aldosterone secretion had been observed under similar conditions in dogs possessing pituitaries and kidneys. At that time this type of experiment had been abandoned because of the frequent occurrence of incompatibility reactions to foreign blood, no antihistamine drug having been administered. However, in all subsequent work mepyramine was given and this greatly reduded the incidence of reactions to moderate volumes of donor blood. The plan of the experiments was, thus, to take a control sample of adrenal blood, then to infuse donor blood, 20 ml./kg, over a period of 10 min and to start collecting a second sample 5 min later. After this the dog was bled to remove the surplus blood volume and a third sample was taken. Table 18 shows the results when donor blood from dogs with intact kidneys was used, and table 19 those obtained with nephrectomized donors. The dogs of table 18 all responded to infusion of, presumably, renin-containing blood by an increase in

aldosterone secretion, but after withdrawal of the excess volume, secretion fell to or below the original level. The haemodynamic effect of the withdrawal of blood was as severe as the effect of true haemorrhage in normal dogs.

TABLE 19. PROCEDURE AS IN TABLE 18, BUT DONOR BLOOD OBTAINED FROM DOGS 3 h AFTER NEPHRECTOMY, AND STORED AT 4 °C FOR 1 OR 2 DAYS

100 m-equiv. Na^+ daily for 23 to 43 days, right nephrectomy 20 to 38 days before experiment.

dog no.	body wt. (kg)	adrenal blood sample no.	adrenocorticol secretion rates (μg (g adrenal)$^{-1}$ h^{-1} and % change) aldosterone	cortisol + corticosterone	adrenal blood flow (ml./h and % change)	mean b.p. (mmHg)	Mayer waves	change in blood volume (ml./kg)	donor blood infused during collection periods (ml./kg)	*ACTH* infusion (m-u. min^{-1} kg^{-1})
424,* male	15·3	S_1	5·1	122	105	100	–		6·5	
		S_2	1·8 −65	115 −6	65 −38	160	–	+20	19·6	
		S_3	2·5 +39	92 −20	43 −34	70	–	−16	.	
425, male	16·5	S_1	2·8	226	152	135	–		4·5	
		S_2	2·5 −11	112 −50	152 0	100	–	+20	8·2	
		S_3	3·6 +44	101 −10	132 −13	70	–	−19	.	0·01 (0·02 for the first 10 min)
426,† male	15·5	S_1	.	142	172	120	–		6·5	
		S_2	4·5	130 −8	140 −19	140	+	+20	6·8	
		S_3	1·3 −71	34 −74	69 −51	80	+	−21‡	.	
427, male	15·2	S_1	6·7	146	192	95	–		8·2	
		S_2	6·7 0	111 −24	128 −33	110	–	+20	9·2	
		S_3	8·0 +19	91 −18	102 −20	110	–	−20	.	
mean ± s.e.		S_1	4·9 ± 1·1	159 ± 23	155 ± 19	113 ± 9				
		S_2	3·9 ± 1·1	117 ± 4	121 ± 19	128 ± 14		+20 ± 0		
		S_3	3·9 ± 1·5	80 ± 15	87 ± 19	83 ± 9		−19 ± 1·1		
429, male	11·5	S_1	8·5	431	240	120	–		10·9	
		S_2	9·4 +11	557 +29	184 −23	160	–	+20	13·0	
		S_3	12·4 +32	658 +18	152 −17	95	–	−25	.	
432,§ male	13·4	S_1	1·9	341	226	102	–		9·0	
		S_2	3·0 +58	389 +14	238 +5	150	–	+20	11·2	0·02 (0·04 for the first 10 min)
		S_3	1·5 −50	177 −54	126 −47	75	–	−17	.	
433, female	10·8	S_1	4·2	408	176	110	–		9·3	
		S_2	5·3 +26	369 −10	150 −15	140	–	+20	11·1	
		S_3	4·7 −11	631 +71	180 +20	120	+	−23	.	
mean ± s.e.		S_1	4·9 ± 1·9	393 ± 27	214 ± 19	111 ± 5	.	.	.	.
		S_2	5·9 ± 1·8	438 ± 60	191 ± 26	150 ± 6	.	+20 ± 0	.	.
		S_3	6·2 ± 3·2	489 ± 156	153 ± 16	97 ± 13	.	−22 ± 2·4	.	.

* Heavy wound bleeding during S_2 only (all blood lost simultaneously replaced by donor blood).
† Ht = 70%.
‡ Blood withdrawn between S_2 and S_3 9 ml./kg when b.p. fell to 60 mmHg; 5 min after start of S_3 b.p. recovered and bleeding was resumed. (S_1 and S_2 collected for 30 min, S_3 for 45 min.)
§ No mepyramine given. Dog developed severe urticaria after donor blood infusion.

Of the dogs infused with blood from nephrectomized donors only one (424, table 19) responded to the expansion of the blood volume by a fall in aldosterone secretion such as one would expect to see in a dog possessing kidneys and a pituitary gland. Since no other dog responded in this way no explanation of the result can be attempted. None of the dogs tolerated subsequent withdrawal of blood exceeding the surplus previously infused; restoration of the original blood volume was followed in three animals by small increases in aldosterone secretion and this was significantly above the initial secretion rate only once (dog 429). All aldosterone values were small in absolute terms and small differences therefore unreliable.

Two dogs (426, 432) showed sharp falls in aldosterone secretion after withdrawal of blood; in these animals glucocorticoid secretion and adrenal blood flow were also greatly

diminished, and the reduced secretion of aldosterone can be interpreted as damage due to impaired circulation.

It would, therefore, appear that in those dogs in which adrenal circulation remained satisfactory, withdrawal of surplus blood following initial expansion led to small increments in aldosterone secretion. Yet even with this technique haemorrhage was not tolerated, and the infusion of the large quantities of donor blood needed for the initial expansion of the circulatory volume frequently caused excessive wound bleeding or falls in blood pressure in spite of the antihistamine drug.

Conclusions

Maintenance of the capacity for steroid synthesis in hypophysectomized-nephrectomized dogs by a constant infusion of *ACTH* did not restore the ability of these animals to respond to haemorrhage by a rise in aldosterone secretion. The result was not changed by administering a constant infusion of angiotensin. These observations are compatible with the view that the sudden increase in the blood concentration of *ACTH* and renin, as elicited by blood loss, is the essential stimulus for aldosterone secretion after haemorrhage.

The interpretation of experiments on acutely hypophysectomized dogs without kidneys was complicated by the extreme lability of the circulation. Many experiments had to be carried out in order to obtain a sufficient number in which blood loss was tolerated, so that circulatory breakdown could be excluded as cause for the failure of aldosterone secretion to rise after blood loss. Whereas haemorrhage was well tolerated by intact and by nephrectomized dogs, circulatory collapse occurred in 40 % of the hypophysectomized dogs, and in more than 60 % of the dogs in which the kidneys were absent as well. Acute hypophysectomy may cause some damage to hypothalamic centres involved in vasomotor control. More important probably, is the loss of the posterior lobe which is known to release vasopressin in severe haemorrhage.

A blood pressure supporting effect of the posterior pituitary hormones was demonstrated in the experiments with infundin. The doses used were those known to exert a full antidiuretic effect; they were much lower than the peak secretion rate found in dogs after blood withdrawal of approximately 30 ml./kg by Weinstein, Berne & Sachs (1960): for a few minutes, these authors observed a release of vasopressin of the order of 15 m-u. min^{-1} kg^{-1}. However, even the small doses caused a direct stimulation of corticoid, particularly of aldosterone secretion, so that the use of larger doses would have interfered with the object of the experiments. The observation that glucocorticoid secretion was only once enhanced by these low concentrations agrees with the observation (Hilton 1960) that at least 1 m-u./min of vasopressin is required by arterial infusion into the dog adrenal gland to produce acceleration of cortisol secretion.

Severe falls in the secretion rates of all corticosteroids after bleeding was another feature in which hypophysectomized-nephrectomized dogs differed from any other group. Part of the reduction in aldosterone secretion may have been caused by the infusion schedule of donor blood: during the collection of pre-haemorrhage samples, moderate quantities of donor blood which must have contained a little renin were infused to replace the blood lost from the adrenal, whereas no such replacement was made of blood collected after haemorrhage. Though it is doubtful whether the amount of donor blood infused was sufficient to act as a stimulus to aldosterone production, larger quantities were shown to act in this way

(figure 5). Such an explanation can hardly be valid for the fall in glucocorticoids since there was no evidence that infusion of angiotensin enhances glucocorticoid secretion (table 15). Yet, circulatory failure is not the sole explanation of reduced corticoid production after haemorrhage. This follows from the fact that it also occurred when blood pressure and adrenal blood flow remained higher than the critical level at which steroid secretion would be inhibited in intact dogs. Simultaneous absence of the pituitary gland and the kidneys appears to impair the reserve power of the adrenal to supply corticosteroids in adverse conditions.

DISCUSSION

After acute blood loss a release of aldosterone might exert a beneficial effect by preserving salt and water, and its increased secretion under these circumstances can be regarded as a homeostatic mechanism. In the intact organism stimulation of aldosterone secretion after haemorrhage is safeguarded by at least two mechanisms, an increased secretion of *ACTH* and a release of renin. These two factors can replace each other so that neither factor can rightly be regarded as 'the' glomerulotrophic hormone. Thus, in the presence of the pituitary, post-haemorrhage increase in aldosterone secretion was of the same frequency and size whether or not the kidneys were there. This indicated that under our experimental conditions, the influence of *ACTH* on the zona glomerulosa was just as important as that of angiotensin.

In hypophysectomized dogs with intact kidneys, in which a submaximal secretion rate of adrenal steroids was maintained by a constant infusion of *ACTH*, rises in aldosterone and glucocorticoid secretion after bleeding were presumably caused by angiotensin. This interpretation does not seem quite compatible with the observation of Scornik & Paladini (1964) that after bleeding a significant rise in the concentration of angiotensin in arterial blood occurs only after 1 h, much later than the release of aldosterone. However, it is possible that effective amounts of angiotensin reach the adrenal long before any increase in peripheral blood concentration becomes measurable.

In nephrectomized dogs with intact pituitaries the *ACTH* released by bleeding was probably the main stimulus for the adrenal cortex, though a contributory role of vasopressin is possible (see table 17). Glucocorticoid secretion was usually near maximal beforehand, so that the effect was confined to an increase in aldosterone secretion.

Rises of aldosterone secretion after haemorrhage were very rarely seen under circumstances in which aldosterone secretion was already high before bleeding, e.g. in sodium depletion, after severe surgery, or during an attack of enteritis. Dogs in which the circulation collapsed after haemorrhage also frequently failed to show the increase in aldosterone. This may be due to circulatory failure at many sites, possibly to reduced hormone synthesis in the adrenal itself. Furthermore, certain observations suggested that prolonged sodium loading can render the zona glomerulosa insensitive to stimuli. Too little is yet known about the suggested inhibitory effect of extracts from a diencephalic-pineal complex (Farrell 1964) on aldosterone secretion to assess its possible role in certain types of failure.

Although the present experiments have shown that the rise in aldosterone secretion in response to a massive haemorrhage does not depend on angiotensin as the sole stimulus, there is little doubt that the increased aldosterone production observed when the renal circulation is impeded by constriction of the inferior vena cava, or by large aortic-caval

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fistulae, or by narrowing of the renal arteries, is caused by excessive renin and disappears when the kidneys are removed. Under these conditions, stimulation of aldosterone production cannot be regarded as a homeostatic mechanism. On the contrary, aldosterone adds to the damage done to the organism by increasing salt and water retention.

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References

Baird, S. & Pickford, M. 1958 *J. Physiol.* **144**, 80–91.
Bartter, C. F., Casper, A. G. T., Delea, C. S. & Slater, J. D. H. 1961 *Metabolism*, **10**, 1006–1020.
Binnion, P. F., Davis, J. O., Brown, T. C. & Olichney, M. J. 1965 *Amer. J. Physiol.* **208**, 655–661.
Biron, P., Koiw, E., Nowaczynski, W., Brouillet, J. & Genest, J. 1961 *J. Clin. Invest.* **40**, 338–347.
Blair-West, J. R., Coghlan, J. P., Denton, D. A., Goding, J. R., Munro, J. A., Peterson, R. E. & Wintour, M. 1962 *J. Clin. Invest.* **41**, 1606–1627.
Blair-West, J. R., Coghlan, J. P., Denton, D. A., Goding, J. R., Wintour, M. & Wright, R. D. 1963 *Recent Progr. Hormone Res.* **19**, 311–383.
Brooks, F. P. & Pickford, M. 1958 *J. Physiol.* **142**, 468–493.
Cade, R. & Perenich, T. 1965 *Amer. J. Physiol.* **208**, 1026–1030.
Carpenter, C. C. J., Davis, J. O. & Ayers, C. R. 1961 *J. Clin. Invest.* **40**, 2026–2042.
Carpenter, C. C. J., Davis, J. O., Holman, J. E., Ayers, C. R. & Bahn, R. C. 1961 *J. Clin. Invest.* **40**, 196–204.
Davis, J. O., Ayers, C. R. & Carpenter, C. C. J. 1961 *J. Clin. Invest.* **40**, 1466–1474.
Davis, J. O., Carpenter, C. C. J., Ayers, C. R., Holman, J. E. & Bahn, R. C. 1961 *J. Clin. Invest.* **40**, 684–696.
Davis, J. O., Carpenter, C. C. J. & Ayers, C. R. 1962 *Circulation Res.* **11**, 171–184.
Davis, J. O., Urquhart, J. & Higgins, J. T. 1963 *J. Clin. Invest.* **42**, 597–609.
Davis, J. O., Urquhart, J., Higgins, J. T., Jr., Rubin, E. C. & Hartroft, P. M. 1964 *Circulation Res.* **14**, 471–485.
Farrell, G. 1964 *Aldosterone*, pp. 243–249. Blackwell Scient. Publications, Oxford. (Edited by E. E. Baulieu and P. Robel.)
Ganong, W. F. & Mulrow, P. J. 1961 *Nature, Lond.*, **190**, 1115–1116.
Ganong, W. F. & Mulrow, P. J. 1962 *Endocrinology*, **70**, 182–188.
Harris, G. W. 1955 *Neural control of the pituitary gland.* London: Arnold.
Hilton, J. G. 1960 *Circulation*, **21**, 1038–1046.
Holzbauer, M. 1964 *J. Physiol.* **172**, 138–149.
Huidobro, F. & Braun-Menendez, E. 1942 *Amer. J. Physiol.* **137**, 47–55.
Laragh, J. H., Angers, M., Kelly, W. G. & Lieberman, S. 1960 *J. Amer. Med. Ass.* **174**, 234–240.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Lauson, H. D. 1960 *Hormones in human plasma*, pp. 225–293. Boston, Mass.: Little, Brown and Co.
Mulrow, J. P. & Ganong, W. F. 1961 *Yale J. Biol. Med.* **33**, 386–395.
Nelson, D. H. & Hume, D. M. 1955 *Research Report, Naval Medical Research Institute*, **13**, 481–490.
Pickford, M. 1960 *J. Physiol.* **152**, 515–526.
Regoli, D. & Vane, J. R. 1964 *J. Physiol.* **172**, 34*P*.
Scornik, O. A. & Paladini, A. C. 1964 *Canad. Med. Ass. J.* **90**, 269–271.
Weinstein, H., Berne, R. M. & Sachs, H. 1960 *Endocrinology*, **66**, 712–718.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES