BIOASSAY OF SODIUM-RETAINING ACTIVITY OF PURE CORTICOIDS AND OF EXTRACTS OF ADRENAL VEIN BLOOD

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THE mineralocortical activity of the cortical hormones is a complex function affecting both renal and extra-renal factors. The different methods of assay, of necessity, measure the effect of the cortical steroids only on some of the factors involved. The interpretation of the results is thus rendered difficult as the response measured varies from one method to the other.

Some of the available methods have a reasonable degree of accuracy and sensitivity in the hands of their authors. Because of the complexity of the technique, however, they do not prove easily reproducible in the hands of other workers. Some achieve their accuracy at the cost of a time-consuming procedure and either have a limited range of sensitivity[1] or the necessity for large quantities of the active material[2].

The use of the $^{24}Na/^{42}K$ ratio[3] produced an accurate and sensitive method. The technical difficulties involved, however, are considerable. In addition to the preparation and measurement of the isotopes, a special physical absorption technique is needed to estimate the $^{24}Na/^{42}K$ ratio. The method does not differentiate qualitatively between the sodium-retaining and sodium excreting corticoids.

A reproducible simple procedure that can achieve this differentiation may have a place in routine investigations.

METHODS

In all the following experiments on rats, adrenalectomy was performed under ether anæsthesia through a central dorsal skin incision. The adrenal glands with the surrounding fat as well as the upper third of the renal capsule were removed. The animals were maintained on a drinking solution of 1·0 per cent. sodium chloride and 5·0 per cent. glucose and on rat cubes. At the end of the experiment, the drinking solution was replaced by tap water, and if the animals did not die within eight days the results were discarded.

The temperature of the room was kept constant throughout the whole procedure at 22–24° C.

1. *Qualitative method for detecting mineralocortical activity on adult rats*

Male albino rats of 150–200 g. were adrenalectomised. On the third post-operative day the cubes were withdrawn at 5 p.m. and the drinking solution replaced by a 5 per cent. glucose solution. At 11 a.m. next morning the animals were weighed and given, by stomach tube, 5 ml. per 100 g. body weight of a solution of 14 per cent. ethanol containing 5 per

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cent. glucose. The rats were then kept in a quiet and darkened place at a constant temperature. They started to get drowsy in about ten minutes and were generally under light anæsthesia in thirty minutes. At 12 noon, 4·0 ml. of 0·9 per cent. sodium chloride solution was injected subcutaneously to each rat. A long fine hypodermic needle was used. The needle, introduced dorsally near the tail, was pushed under the skin to the shoulder region and the solution evenly distributed. Ten minutes later, 0·1 ml. of aqueous solution of deoxycortone glycoside, "Percorten" was injected intraperitoneally to the test animals while the control animals were given the corresponding amount of water.

Thirty minutes later, a catheter was introduced suprapubically, usually without the need for more anæsthetic. Occasionally, if the anæsthesia was light, a few whiffs of ether was used.

A suprapubic longitudinal incision 1·5 cm. long was made in the midline. The bladder was secured and, without pulling, and with the use of a pair of sharp fine scissors, a catheter was introduced through a small incision in the apex of the bladder and tied with a fine silk ligature. Kinking the bladder or damaging its wall were carefully avoided. The penis was ligated and a small amount of distilled water was then injected through the inner tube to test the patency of the catheter, minimum pressure being used. The catheter was a double walled polythene tube. The outer tube had an external diameter of 3·5 mm.; the inner tube of 1·5 mm. (Fig. 1); the thinner tube was connected to a blunt needle fitted in a syringe and fixed in a holder with a screw which could be rotated by hand so that 1–2 ml. was injected steadily in 30–45 seconds.

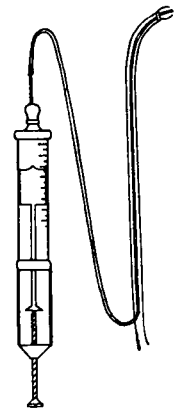

FIG. 1. Apparatus for washing the urinary bladder of the rat.

The rats were then put in a box which was kept warm by electric lamps and a thin thermometer was introduced into the rectum. The rectal temperature was kept as near to 37° C. as possible. Noises were avoided throughout the experiment.

Twelve noon, the time at which the sodium chloride had been injected, was considered zero hour. At 1.00 p.m. each bladder was washed out by injecting 2·0 ml. of distilled water through the inner tube. The washing was discarded. After this washing a graduated centrifuge tube was used to collect any fluid dripping from the catheter. Every 30 minutes the bladder was washed again by injection 2·0 ml. of distilled water. The washings were collected and the centrifuge tube changed. The collection was continued for at least one hour and a half.

The fluid in the centrifuge tubes was then made up to 10 ml. and the quantity of sodium estimated by the flame photometer.

2. *A quantitative method of assay on young rats*

Young female white rats 50–100 g. were used, with the same procedure for adrenalectomy and maintenance. Instead of single animals, about 40

rats generally in groups of ten were used. These were so arranged that animals with corresponding weights were evenly distributed.

On the fourth day after adrenalectomy, feeding was stopped at 11 p.m.; the saline-glucose drinking solution was available till 5 a.m. next morning, when it was replaced by 5 per cent. glucose only. At 10 a.m. the animals were injected with 4·0 ml. of Salt Load I (6·25 mg. sodium as chloride in 4 ml. 5 per cent. glucose).

Handling was kept to a minimum; for the injection, the animals were picked up by the tail and brought near a cone-shaped polythene container slit on its upper surface. They ran into the container which was then closed with a slit rubber stopper which allowed the tail to stay out. The thin needle (3 inches long) was introduced near the tail and pushed subcutaneously towards the shoulder region; then the injection was given slowly. One animal from each group was injected in turn.

At noon, each rat was caused to urinate by a gentle pull on the tail. One ml. of the Salt Load II (see below) and the corticoids were then administered to one rat from each group in turn, in the same order and taking the same precautions as for the first injection.

The Salt Load II contained 3·125 mg. sodium and 0·2 mg. potassium per ml. A tuberculin syringe with a fine needle was used to administer the corticoid in 0·1 ml. propylene glycol subcutaneously. The control group received the same amount of propylene glycol alone.

Once a rat was injected it was placed into a metabolism beaker. These were 1 litre pyrex glass beakers from the rim of which a tightly fitting wire mesh platform was suspended by wire hangers two-thirds of the way down the beaker. Before each platform was used it was waxed with hard paraffin wax and washed with cold distilled water. All the apparatus was kept thoroughly salt free. Each beaker had a perforated lid.

Six hours after the injection, the collection of urine was stopped from each rat successively in the same order as it had been started. The bladders were emptied by suprapubic massage after ether anæsthesia. Fæces lying on the wire mesh platform were removed with forceps, and the beaker and platform were washed four times with 20 ml. quantities of distilled water. The urine and washings were made up to 100 ml. The diluted urine was filtered through ashless acid-wash filter paper, and the amount of sodium estimated by the flame photometer.

Completeness of the operation was checked by keeping the rats on cubes and tap water only. They almost invariably died in eight days after the withdrawal of the saline drink.

Corticosterone (Compound B), 17-hydroxy-11-dehydrocorticosterone (cortisone, Compound E) and 17-hydroxy-corticosterone (hydrocortisone, Compound F) were dissolved in propylene glycol and given in 0·1 ml. of solution per rat. The effects were compared to those of deoxycortone acetate (DOCA) as a standard.

3. *Collection of adrenal venous blood*

(*a*) *Dog*

The operative technique described by Vogt[4] was used. Adult male dogs

of about 14 kg. body weight were anæsthetised with ether until a chloralose solution (70–80 mg. per kg. body weight) could be infused into the femoral vein.

Blood was collected, under heparin cover from the left adrenal gland into 50 ml. centrifuge tubes kept in an ice bath. The rate of flow was recorded. The blood was centrifuged and the plasma pipetted immediately after collection. Arterial blood from the carotid artery was collected before killing the dog and the left adrenal gland weighed. In dog C the splanchnic nerves were severed before blood collections started.

(*b*) *Rabbit*

Collection of adrenal plasma was carried out according to the method described by Vogt[4]. Rabbits of 2·5–3·0 kg. weight were anæsthetised with ether followed by chloralose infused into the jugular vein. Through a median abdominal incision the animal was eviscerated. Ligatures were then tied around all vessels draining into the inferior vena cava except the adrenal veins. Venules draining the fatty tissue surrounding the kidneys and those draining the vertebral column were tied.

A loose ligature was placed around the inferior vena cava above the entrance of the right adrenal veins. The aorta was ligated below the origin of the renal arteries and a cannula introduced into the cava below the level of the kidneys. Heparin was then administered through the jugular vein and a carotid cannula for blood pressure recording introduced. The ligature around the upper part of the vena cava was tied and the blood allowed to run into the cannula for the collection of samples. The blood pressure was maintained as constant as possible by infusing heparinized blood collected from another rabbit. The volume and time of each collection was recorded. At the end of the experiment the two adrenal glands were dissected out and weighed.

In the second experiment 10·0 units of ACTH dissolved in 40 ml. of saline were warmed to body temperature, and infused through the jugular cannula at the rate of 2·0 ml. per minute. A blood collection was carried out during the last phase of the infusion and for a short time afterwards.

4. *Chemical extraction and Assay*

(*a*) *Dog*

The method devised by Bush as reported by Bibile[5] was used. A mixture of ether and ethyl acetate 2 : 1 was prepared from freshly distilled stocks. Each volume of plasma was extracted by shaking it with its own volume of the mixture. After centrifuging, the non-aqueous phase was pipetted into a round-bottomed flask. The extraction process was repeated four times. The collected supernatants were evaporated *in vacuo*, at 40° C. until a thin film of fluid was left in the flask.

A volume of light petroleum corresponding to the initial volume of the plasma was added to the flask and its sides well scraped by a glass rod. A separating funnel was prepared containing 5/3 of the initial plasma volume of light petroleum, and the contents of the flask were added to the funnel. The evaporating flask was further washed and thoroughly

scraped with 70 per cent. redistilled ethanol in water, 1/7 of the initial plasma volume being used. The ethanolic washing was repeated, and the ethanol added to the funnel. The contents of the funnel were shaken thoroughly, and allowed to separate; the ethanol was then drained off. The whole process of ethanolic extraction was repeated twice. The combined ethanolic extracts were then evaporated *in vacuo* and the residue dissolved in propylene glycol.

When deoxycortone acetate was to be added to arterial plasma in recovery experiments, a concentrated solution of deoxycortone acetate in 40 per cent. ethanol was prepared and the solution added to the plasma before extraction started. The same volume of 40 per cent. ethanol was added to the control plasma.

In assaying adrenal samples, the control rats were injected with extracts of arterial plasma in propylene glycol. The volume of arterial plasma used was the same as the volume of adrenal plasma tested in the same experiment. Similarly, all standard doses of deoxycortone acetate were dissolved in extracts of the same volume of arterial plasma. In this way, the presence of active materials in the plasma not produced by the left adrenal and which might affect the assay in an unpredictable manner, was compensated.

Arterial plasma from control dogs was obtained and extracted in the same way. These extracts were assayed without the addition of any cortical steroids. The method of assay was used to test the mineralocortical activity of arterial plasma and adrenal venous plasma of dogs. 0·1 ml. of propylene glycol extract corresponded to between 2·0 and 2·25 ml. of adrenal plasma.

(*b*) *Rabbit*

It was not possible to collect sufficient arterial plasma from the animal supplying the adrenal blood to use its extract as a vehicle for the standard doses of deoxycortone acetate; these standard doses were thus given in 0·1 ml. propylene glycol alone. A sample of arterial plasma collected from two rabbits was extracted separately and assayed. Extracts of adrenal plasma were given to the rats in doses of 0·1 ml. propylene glycol per rat. Each 0·1 ml. corresponded to 1·7–3·5 ml. of the adrenal plasma.

Results

Preliminary experiments on mice, using the method of assay devised by Spencer[1], were abandoned because the day to day variations in the sodium excretion by the same mouse were large. In addition, repeated experiments on the same adrenalectomised mouse necessitated by the cross over technique of this method, were not well tolerated by the animals.

An attempt was made to study some of the variables involved in the use of the urinary excretion of sodium and potassium for the assay of mineralocortical activity. It was found that the urinary potassium excretion in adrenalectomised mice did not have a linear relation to the dose of deoxycortone acetate administered. Further, the urinary sodium excretion was

found to vary, for the same mouse, from day to day, over a considerable range, while the sodium excretion of groups of mice on the same post-operative day was reproducible.

Premedication with deoxycortone glycoside or with cortisone in the post operative period did not materially improve the results; neither did adrenaline-induced diuresis during the period of urine collection.

In the light of these observations the procedure described under the quantitative method of assay, using young adrenalectomised rats was followed. Another type of test used single anæsthetised rats and is in the nature of a screening test.

A qualitative biological method for detecting mineralocortical activity employing small groups of anæsthetised adult rats

As shown in Table I the amount of sodium excreted during 30 minutes, beginning 60 minutes after the injection of the sodium load, decreases with increases in the amount of "Percorten" administered.

TABLE I

EFFECT OF "PERCORTEN" ON SODIUM EXCRETION OF ADULT ANÆSTHETISED RATS

Experiment No.	Time of collection*	Na excretion in 30 minutes (μg. Na per minute)		
	Minute	Controls	3 μg. Percorten per rat	10 μg. Percorten per rat
1	90	3·51		2·21
	120	2·89		1·70
	150	3·50		1·50
2	90	5·00	1·70	0·56
	120	6·60	1·90	0·23
	150	3·70	3·10	0·20
3	90	7·80		2·90
	120	6·10		1·03
	150	8·30		0·90
4	90	3·13	3·93	2·27
	120	3·63	2·26	1·13
	150	2·36	2·90	1·13
5	90		2·36	2·63
	120		3·33	1·93
	150		2·53	0·90
Mean	90	4·89	2·66	2·12
	120	4·81	2·50	1·20
	150	4·24	2·84	0·93

* Zero time is taken as the time of the injection of the saline load.

Any damage to the bladder while introducing the catheter usually produced hæmaturia and partial or complete anuria, and these animals were discarded, as were also animals developing difficulty in breathing. Constant temperature and complete protection from environmental stimuli were found to be essential. The degree of anæsthesia was also found to play an important role. Animals with anæsthesia light enough to permit them to perform any degreee of spontaneous movements as well as animals with too deep a degree of anæsthesia were also discarded.

This method can be of some use as a screening test for mineralo-cortical activity. A group of 8 rats can be handled at one time and give a

result in a few hours. This method can also be of use in selecting the optimum dilution of the test material for quantitative estimation.

A quantitative method of assay employing larger groups of young conscious rats

Table II and Figure 2 show the relation between the dose of deoxycortone acetate and its effect on sodium retention. Table III contains the statistical analysis of the figures.

TABLE II

EFFECT OF DOCA ON SODIUM EXCRETION IN THE URINE OF YOUNG ADRENALECTOMISED RATS

Dose μg. DOCA	0·5	1·5	2·0	4·5	8·0
Number of rats	15	15	16	15	16
Mean Na excretion (per cent. of controls) ± S.D. of differences*.	88·1 ± 23	64·1 ± 20·7	51·3 ± 16·9	35·3 ± 17·4	25·0 ± 15

* Standard deviations of differences between test and control groups.

Tests of significance: Between control group and 2·0 μg., P 0·01
Between 1·5 μg. and 4·5 μg., P 0·05
Between 2·0 μg. and 8·0 μg., P 0·02

The effects on sodium excretion are expressed as per cent. of the mean control values. The deviations from linearity are small. The slope $b = 53{\cdot}3 \pm 10{\cdot}52$, giving a t value of 5·07 indicates that the response is dependent on the dose ($P < 0{\cdot}001$). The precision index however, is high:—

λ = (standard deviation of response)/slope = 0·714.

A dose of 2·0 μg. deoxycortone acetate gave a response significantly different from the control value ($P < 0{\cdot}01$).

TABLE III

ANALYSIS OF VARIANCE

Effects per cent. plotted against log dose of DOCA
Adjustment for means — 209,852·4

Nature of variance	Degrees of freedom	Sum of squares	Mean squares
Regression	1	37,268·3	37·263
Deviation from regression ..	3	418·7	139·57
Between doses	4	37,687·0	9421·7
Within doses	72	104,468·46	1450·95
Total	76	142,155·49	

Because of the flatness of the curve, the dose had to be increased by a relatively high factor to obtain responses differing significantly. In spite of attempts at standardising the conditions of the experiments, the variations between different groups of rats, necessitates the inclusion of a group of control animals in each assay and the expression of the responses of the test and standard animals as a percentage of the mean response of the control group. Because of variations in slope it is necessary to have two groups injected with known doses of the standard included in every assay.

The effect of pure cortical steroids on sodium excretion

As is shown in Table IV, the three cortical steroids with an oxygen at C(11) increase sodium urinary excretion under the conditions of the test. As a rule, sodium excretion increased with increasing dose of the steroid. Small doses of deoxycortone acetate (e.g., 2·0 and 4·0 μg. per rat) given to a group of animals concurrently, showed a marked sodium retention as usual.

When deoxycortone acetate was administered in conjunction with steroids causing increased sodium excretion, antagonism between the two steroids was observed in every case.

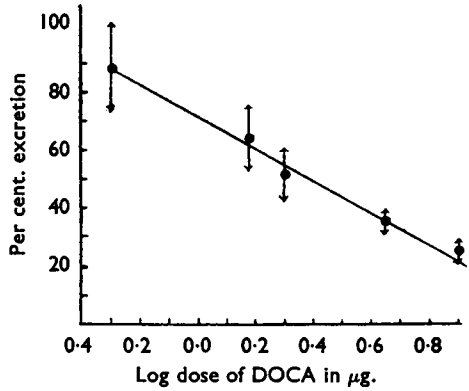

FIG. 2. The effect of deoxycortone acetate on urinary excretion of sodium by adrenalectomised rats (dose response curve). Ordinate: total sodium excretion expressed as percentage of the mean response of the control group. Abscissa: log dose of deoxycortone acetate per rat.

The potency of the steroids which increase the sodium excretion can be assessed roughly by their power to antagonise the sodium retaining effect of deoxycortone acetate. A dose of 10·0 μg. of each of these steroids was administered in conjunction with 4·0 μg. of deoxycortone acetate. The results obtained indicate that the potency of these compounds descends in the order; hydrocortisone, cortisone, corticosterone. To investigate further the quantitative nature of this antagonism several doses of deoxycortone acetate were administered in conjunction with two doses of cortisone. It was found that about 4·0 μg. deoxycortone acetate neutralised the effect of 10·0 μg. of cortisone in promoting sodium excretion. Eight μg. of deoxycortone acetate neutralised the effect of 31·25 μg. cortisone, while 2·0 μg. deoxycortone acetate produced no significant antagonism.

Corticosterone which is the weakest steroid of the group did on one occasion cause some degree of sodium retention when administered in a dose of 20·0 μg. per animal. In the other eight experiments carried out on this compound however, there was an increase in sodium excretion or an antagonism to the sodium retaining power of deoxycortone acetate.

Mineralocortical activity of adrenal venous blood of dog

Recovery. Extracts of arterial plasma did not cause any sodium retention (Table V). If anything, a tendency to enhance sodium excretion was observed, but this effect was not significant. When arterial plasma was extracted after the addition of 4·0 μg. deoxycortone acetate per 0·1 ml. of plasma this extract caused sodium retention to an extent slightly less

TABLE IV

PURE CORTICOIDS AND URINARY SODIUM EXCRETION

Steroid	Dose in μg.	DOCA μg.	No. of animals	Sodium excretion* per cent. ± S.E.
Hydrocortisone ..	5·0		15	219 ± 25
	20·0		14	474 ± 12
	10·0	+ 4·0	8	210 ± 31
Corticosterone ..	5·0		14	128 ± 24
	10·0†		7	135 ± 17
	20·0‡		13	240 ± 41
	40·0		7	189 ± 29
	10·0	+ 4·0	19	80 ± 13
Cortisone ..	31·25		8	187 ± 30
	125·0		7	203 ± 24
	500·0		8	290 ± 44
	10·0	+ 4·0	11	112 ± 14
	31·25§	+ 2·0	7	163 ± 21
	31·25	+ 8·0	6	103 ± 10

* Sodium excretion expressed as per cent. of mean excretion of control group.
† A group of 7 rats, run concurrently, each received 4·0 μg. DOCA. Their mean sodium excretion ± S.E. = 30 ± 9·7.
‡ A group of 8 rats, run concurrently, each received 4·0 μg. DOCA. Their mean sodium excretion ± S.E. = 34 ± 10·1.
§ A group of 6 rats, run concurrently, each received 2·0 μg. DOCA. Their mean sodium excretion ± S.E. = 25·8 ± 8·4.

TABLE V

DOG'S ARTERIAL PLASMA TESTED FOR MINERALOCORTICAL ACTIVITY

Experiment No.	No. of rats in each group	Mean sodium excretion of test group as per cent. of mean excretion of control group ± S.D. of differences		
		Extract of 0·75 ml. arterial plasma	4·0 μg. DOCA	Extract of 0·75 ml. arterial plasma containing 4·0 μg. DOCA†
1	7	103·0 ± 58*	61·02 ± 16	66·9 ± 16
2	7	115·0 ± 29·6	43·9 ± 20·9	64·0 ± 20·8

* Standard deviations of differences between test and control groups.
† The DOCA was added to the arterial plasma before extraction.

TABLE VI

MINERALOCORTICAL ACTIVITY OF ADRENAL VENOUS PLASMA IN THE DOG

Dog	A		B		C§
Assay	I	II	I	II	I
Volume of plasma per rat (ml.)	2·0*	2·0*	2·0†	2·0†	2·25‡
Mean systolic pressure during collection of sample (mm. Hg).	64		35		32
No. of rats per group	8	7	7	7	9
Mean Na excretion:					
Of test group as per cent.	78·98	64·2	102·4	96·3	75·06
Of mean excretion of control group .. ± S.D. of differences	± 20·14	± 21·93			± 22·32
DOCA equivalent { μg./ml. plasma	0·68	0·66	0	0	0·33
DOCA equivalent { μg. g./min.	0·95		0		0·44

* 2·0 ml. of the adrenal plasma collected over 1·98 min.
† 2·0 ml. of the adrenal plasma collected over 1·32 min.
‡ 2·25 ml. of the adrenal plasma collected over 3·06 min.
§ The splanchnic nerves were severed before the collection.

(differences non-significant) than that produced by 4·0 μg. deoxycortone acetate alone. In view of the fact that extracts of arterial blood usually increase sodium excretion, it is not quite clear whether recovery was incomplete or whether some of the effect of the added DOCA was masked.

Extracts of adrenal venous plasma. In order to obtain as high a concentration of corticoids in the adrenal effluent as possible, the blood obtained at higher blood pressures, and consequently at a more rapid rate of flow, was discarded. Only the later samples were used, when blood loss had diminished the pressure and blood flow. A sodium retaining activity of the samples examined could not be established with certainty (Table VI). The calculated activity (last column) being very near the threshold may not be real. Nevertheless, when the same sample was assayed a second time, the results agreed remarkably well (Dogs A and B, Table VI).

The results may either be interpreted as a sign of low release of sodium retaining hormones, or of high secretion of sodium diuretic hormones.

TABLE VII

MINERALOCORTICAL ACTIVITY OF RABBIT ADRENAL VENOUS PLASMA

Experiment No.		I	II		III
Sample assayed		Adrenal venous plasma	Adrenal venous plasma	Adrenal venous plasma after ACTH	Arterial plasma
No. of rats per group		11	16	16	8
Extract of plasma administered per rat	Volume (ml.)	3·0	1·7	3·5	3·0
	Collection time (min.)	1·62	2·85	3·05	
Mean sodium excretion of test and of standard groups as per cent. of mean of control group	Test group (plasma)	41·6	89·7	79·8	129·9
	Significance from control	$P < 0·001$	$P < 0·4$	$P < 0·2$	$P = 0·5$
	Standard group (2·0 μg. DOCA)	47·0	50·2	50·2	
	Significance from control	$P < 0·001$	$P < 0·001$	$P < 0·001$	
Estimated mineralocortical activity of plasma as μg. DOCA per ml. plasma		1·2	0·0	0·0	0·0

Mineralocortical activity of adrenal venous blood of the rabbit

Corticosterone being less active an antagonist of deoxycortone acetate than hydrocortisone, an animal species secreting predominantly corticosterone was also investigated. Adrenal venous plasma extract from one rabbit showed a significant sodium retaining power (Table VII), when extracts of 3 ml. plasma were injected into each rat of the test group. A second rabbit, however, showed no such activity. Arterial plasma on the other hand, had a slight sodium excreting effect.

DISCUSSION

The method reported here for the quantitative assay of mineralocortical activity offers certain advantages.

The procedure is simple. It has been the aim throughout this work to develop this method along lines which permit its adoption as a routine

method of investigation not requiring elaborate technical facilities or excessive labour.

It has a range of sensitivity. Significant sodium retention was achieved with 2·0 μg. doses of deoxycortone acetate in experiments where 10–15 rats per group were employed. In some experiments 1·0 and 1·5 μg. deoxycortone acetate gave significant sodium retention ($P < 0{\cdot}03$), but the results were not always reproducible. The highest dose used in the experiments reported here was 8·0 μg. deoxycortone acetate.

It is able to differentiate between mineralocorticoids and corticoids with an oxygen atom at C(11) (11-oxycorticoids).

The reported method, on the other hand, suffers from some serious handicaps. The index of precision is high — 0·714. As a result of this inaccuracy it is necessary to increase doses by a high factor (3–4) to achieve significant differences in response.

This method was used in a comparative study of the action of some of the known corticosteroids on electrolyte excretion. There was a demonstrable qualitative difference between deoxycortone acetate and the 11-oxycorticoids on the excretion of sodium by adrenalectomised animals. The 11-oxycorticoids caused an increase in sodium excretion instead of a decrease. Similar results were obtained by others[7–8].

Simpson and Tait's method, on the other hand, does not reveal such qualitative differences, the only difference being a quantitative one; the $\frac{Na}{K}$ ratio drops in all cases. An important factor which may account for this apparent discrepancy between the results obtained here and those obtained by Simpson and Tait is the duration of the experiment. Simpson and Tait found a decrease in the urinary $\frac{Na}{K}$ ratio after giving cortisone only when they used a two hours' collection period. Such a drop in the ratio was not obtainable with a six hours' collection.

Davis and Howell[9] pointed out that an important consideration which has often been neglected in studying water and electrolyte balances in response to cortisone is the role of glomerular filtration. This applied to dogs and rats. In normal dogs observed over two days, the authors found that cortisone and ACTH caused increased salt loss only in those dogs in which glomerular filtration rate was raised by the hormone. The rise in glomerular filtration and plasma flow was most profound with cortisone treatment and least with deoxycortone acetate treatment.

Roberts and Pitts[10], in their acute experiments on dogs demonstrating the sodium retaining power of cortisone, collected urine over a period of 2–3 hours only, after the administration of cortisone. The glomerular filtration rate and plasma flow were not significantly altered during this period. The changes in the subsequent period were not investigated.

Another factor which may be involved in a six hours' urinary collection and not in a shorter period has been indicated by Bloodworth[11]. In normal dogs, he found that administration of cortical extract for three to six hours caused a transfer of fluids from the intracellular to the

extra-cellular compartments. When deoxycortone acetate was used over the same period no such transfer was observed.

Deoxycortone acetate too, seems to have different actions when its effects are followed over a prolonged period. While its sodium retention effect is easily demonstrable a few hours after administration, its cold protection effect is demonstrable only twenty-four hours after administration[12]. The same holds for many of its effects on carbohydrate metabolism[13,14].

Spencer[1], in one experiment, estimated the sodium retaining activity of dog adrenal venous serum to be equivalent to 4·0 μg. deoxycortone acetate per ml. Simpson, Tait and Bush[15] demonstrated the presence of a highly active mineralocorticoid in the secretion of the adrenal gland of both dog and monkey. When the mineralocortical activities of all the fractions are added together the dog adrenal is estimated to secrete the equivalent of 20 μg. deoxycortone per minute.

The maximum effect of dog adrenal venous plasma in experiments recorded here, was equivalent to 0·95 μg. deoxycortone acetate per g. of gland per minute. This figure, however, was not significant (Table VI, Dog A). The reason why this figure is so low is, presumably, that the effect of the mineralocorticoids is masked by the action of 11-oxycorticoids in promoting sodium excretion.

Using the eosinophil test Bibile[5] estimated that the output from the dog's adrenal was equivalent to 11·3 μg. of cortisone or of hydrocortisone per g. per minute. The response utilised in this test is obtained with 11-oxycorticoids and not with deoxycortone. The results in Table IV show that the effect of 10 μg. hydrocortisone was not neutralized by 4 μg. deoxycortone acetate. The results therefore suggest that the mineralocorticoid activity of the dog's adrenal plasma was equivalent to more than 4 + 0·95 μg. per g. of gland per minute. This figure is compatible with the results of Simpson and others.

Bush[15] reported that in the rabbit the adrenal cortex secretes predominantly corticosterone. According to the work reported here, corticosterone is less effective than hydrocortisone in antagonising the sodium-retaining effect of deoxycortone acetate. From Bush's figures it also follows that the average concentration of corticosterone in the rabbit is low, namely 3·2 μg. per ml. of adrenal plasma. The rabbit thus seemed to be an animal where the neutralising effect of 11-oxycorticoids against the sodium-retaining action of DOCA-like substances, might cause less interference than in the dog. A significant mineralocortical activity of adrenal venous plasma was indeed found in one of two rabbits and was of the order of 2·7 μg. deoxycortone acetate equivalent per g. adrenal gland per minute.

Summary

1. A qualitative and a quantitative method of estimating mineralocortical activity are reported. Together, they supply a simple routine method of assay.

2. A comparative study of sodium-retaining activity of some pure

corticoids indicated the presence of an antagonism between 11-oxycorticoids and deoxycortone acetate. Quantitative data on this antagonism are reported.

3. Attempts to measure the mineralocortical activity of adrenal venous plasma in the dog and in the rabbit have been made.

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