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THE ACUTE EFFECT OF HYDROCORTISONE, DEOXYCORTICOSTERONE AND ALDOSTERONE UPON THE EXCRETION OF SODIUM, POTASSIUM AND ACID BY THE HUMAN KIDNEY

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It is now generally agreed that an important action of adrenal steroids is to stimulate the reabsorption of sodium by the renal tubules. The mechanism is more debatable; but it is often tacitly assumed that the mineralocorticoid action of different steroids is the same, if the dose is suitably adjusted.

The direct renal effects are best studied in acute experiments extending over a few hours, as secondary effects, due to disturbance of total body supplies of electrolytes, are thus minimized. Since cation excretion varies markedly at different times of day, any effect of steroids upon renal cation excretion must be assessed by comparison with control experiments, in order to take account of this diurnal rhythm.

In a series of observations in the early afternoon, which have already been briefly reported (Mills, Thomas & Williamson, 1959*a*), it appeared that hydrocortisone (cortisol) promoted tubular reabsorption of sodium in exchange for potassium, without any increase in output of hydrion, whereas aldosterone and deoxycorticosterone (DOC) increased the excretion of both hydrion and potassium in exchange for reabsorbed sodium. These observations have now been extended, by measuring the effect of these steroids under three other conditions: in the morning, when excretion of potassium is normally high and of acid low; at night, when the reverse condition prevails (briefly reported by Mills, Thomas & Williamson, 1959*b*); and in the afternoon after administration of acetazoleamide, which by inhibiting carbonic anhydrase blocks a large fraction of renal tubular acid secretion.

METHODS

Plan of experiments. The subjects were healthy males aged 21–44. Where subjects have participated in our earlier experiments (Longson, Mills, Thomas & Yates, 1956; Mills & Thomas, 1957, 1958) the same initial letters are used to refer to them. Four series of experiments have been performed: afternoon, morning, night and afternoon with acetazoleamide. Each subject participating in any series performed at least one control experi-

ment without injection; circumstances—timing, posture, etc—were closely similar in all experiments of one series upon any subject. Except at night, the subject usually remained seated, apart from rising to void urine. A light breakfast was eaten on the morning of an experiment, but no food thereafter. No caffeine was consumed, nor was it for 4 hr before bedtime in night experiments. Subjects drank freely.

Steroids used have been hydrocortisone (free alcohol) (Upjohn), 100 mg, supplied dissolved in 20 ml. 50% ethyl alcohol, and added to 0·5 l. isotonic lactose before intravenous injection; hydrocortisone hemisuccinate (Upjohn or Glaxo), 133·7 mg, representing 100 mg hydrocortisone, dissolved in 2–4 ml. water and given intravenously; deoxycorticosterone glucoside (DOCG) (Ciba), 20 or 30 mg given intravenously; DL-aldosterone (Ciba) 0·5 mg, either the 21-monoacetate in 95% ethanol, diluted with isotonic NaCl, or the free alcohol in aqueous solution. DOCG was used in earlier experiments, before aldosterone was available. Its action appears identical with that of aldosterone and its use was discontinued. Where mean results of aldosterone injection have been calculated, experiments with DOCG have been included with them.

In afternoon experiments, subjects sat down usually at 11.00 hr, and received injections at about 13.00 hr. The dose of aldosterone was given to F and T in a single injection, and to A, K and M in two equal portions separated by 1½ hr. Urine was usually voided every half hour, except in control experiments, when it was voided hourly. A few of these experiments have already been briefly described (Mills & Thomas, 1958).

Morning experiments were similar, except that the injection was given about 9.30, when the subject arrived and sat down in the laboratory.

In night experiments the whole urine produced during a night's sleep of about 8 hr was collected. Injections were given immediately before going to bed, and in most experiments aldosterone was given intramuscularly to prolong its action. In a few further experiments the night urine was collected in two consecutive portions.

Acetazoleamide experiments were similar to the afternoon series, but in addition 250 mg acetazoleamide (Diamox, Lederle) was given, orally at 11.00 hr to M and intravenously at 12.30 hr to A and W. Rapidity of absorption after oral administration seems to vary between individuals: alkalinity of the urine indicated that M absorbed the drug rapidly, whilst when W took it by mouth the rise of urinary pH was inconveniently slow. Steroids, when given, were always injected at 12.30 hr.

Analytical methods. The following procedures were used: pH, glass electrode at 37° C; titratable acid, potentiometric titration with N/5-NaOH to pH 7·4 at 37° C; ammonium, aeration and titration (Hawk, Oser & Summerson, 1947); total CO_2, Van Slyke & Neill (1924) bicarbonate was calculated from total CO_2, pH and the pK_1 of $6{\cdot}32-0{\cdot}5\sqrt{B}$ (Hastings & Sendroy, 1925); B was taken as $[Na+K+NH_4]$; these analyses were almost always completed on the day of the experiment; sodium and potassium, flame photometer, EEL (Collins & Polkinhorne, 1952); inorganic phosphate, Fiske & Subbarow (1925); creatinine, Bonsnes & Taussky (1945).

Hydrion output (H) was calculated as ammonium + titratable acid − bicarbonate. In many of the earlier experiments titratable acid was not determined but that fraction due to phosphate was calculated (Longson & Mills, 1953). Hydrion thus calculated is designated H′.

In considering cation pattern in urine, ammonium + titratable acid is referred to as h, ammonium + $H_2PO_4^-$ as h′, and Na + K + h or h′ as B.

RESULTS

Afternoon experiments

Complete series of experiments, including at least one control and one each with injection of cortisol, aldosterone, and DOCG, were performed on

subjects A, F, K, M, and T. In the control experiments, when no injection was given, progressive changes were observed in electrolyte excretion between 10.30 and 17.00 hr. Potassium excretion (Fig. 1) always declined, as did sodium excretion (Fig. 2) in all subjects except F, in whom it was low at the start. Na:K ratio (Fig. 2) always rose, although in M the rise was slight. Sodium and hydrion always formed an increasing, and potassium

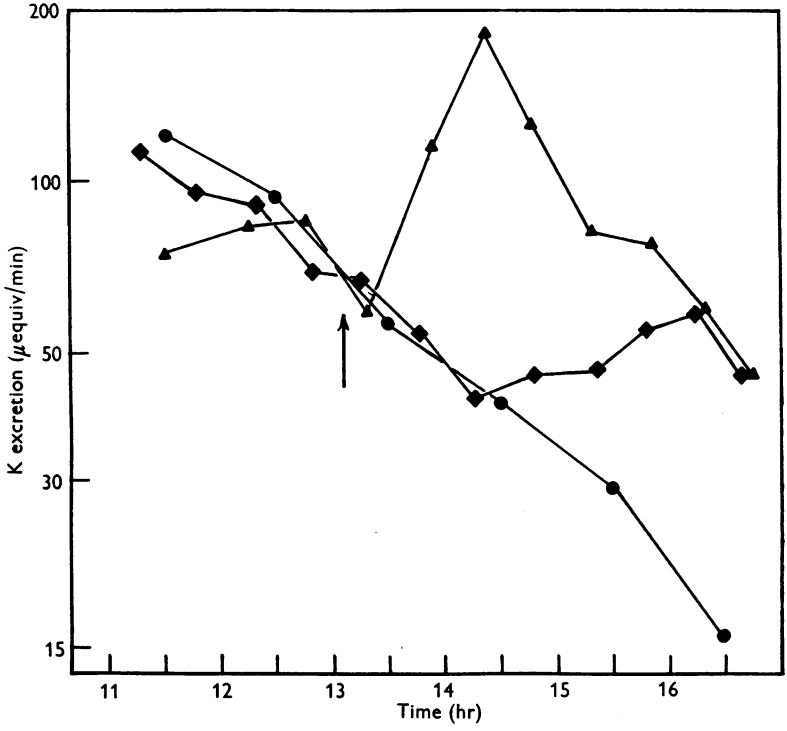

Fig. 1. Afternoon expts., subject T. Potassium output in urine, log. scale: ●, control. ◆, aldosterone. ▲, cortisol. Arrow indicates time of steroid injection. In this, as in all other plots of urinary variables against time, the time is the mid point of the period of collection.

a decreasing, proportion of the total urinary cations. After every steroid injection, in every subject, sodium excretion fell further than in control experiments, and the Na:K ratio (Fig. 2), instead of rising, fell sharply. Potassium excretion, both absolute (Fig. 1) and as a proportion of total cations, rose after cortisol injection; after aldosterone or DOCG K:*B* remained unaltered or rose, but only in subject M was the rise as great as after cortisol.

Differences in hydrion excretion manifest themselves as differences in output of ammonium, titratable acid and bicarbonate, and in urinary pH.

The effects of the various steroids upon urinary pH, which is closely correlated with bicarbonate concentration, are shown in Table 1. The period between 2 and 2½ hr after steroid injection has been chosen to assess the differences, as at this period all the steroids seem to be active. It will be seen that in all subjects except M the pH after cortisol was higher than in any other experiment. In this subject the action of cortisol was slow, and higher values of pH were observed later.

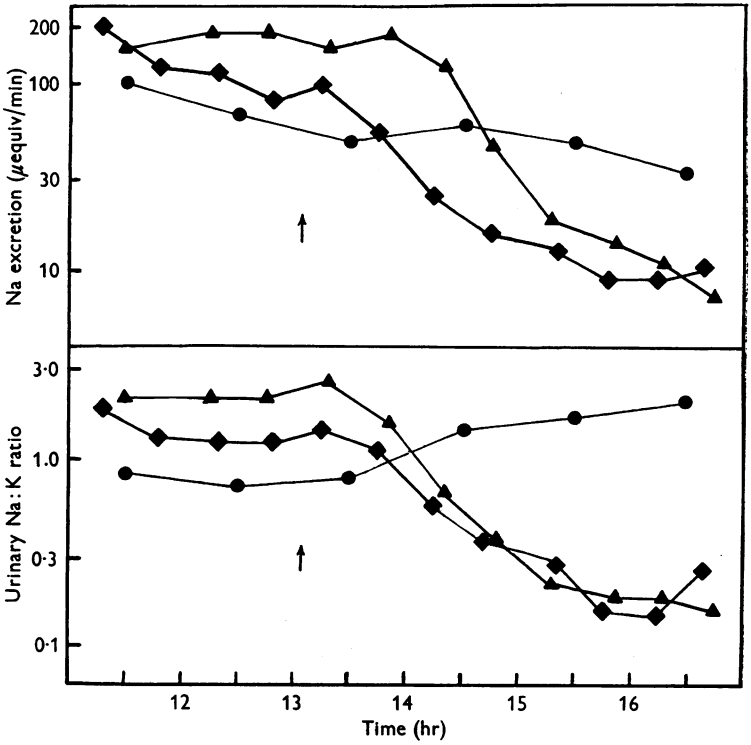

Fig. 2. Afternoon expts., subject T. Sodium output, and Na:K ratio in urine, log. scale. Symbols as in Fig. 1.

The effect of cortisol on pH is even clearer if one considers the change in pH after injection, since in the afternoon urinary pH usually fell steadily and more so after aldosterone and DOCG. After cortisol pH always rose, and only in one experiment with aldosterone was a comparable rise seen. This pH change will be discussed further when changes in phosphate excretion are described.

Owing to considerable changes in total cation excretion, hydrion outputs are best considered as a proportion of the total cations. This proportion $h' : B$, is shown in Table 1 for the period 2–2½ hr after steroid

administration. After aldosterone it was usually increased but sometimes unaltered: after cortisol it was unaltered or reduced. By contrast K:*B* was highest after cortisol, and Na:*B* was very low after all steroids.

There thus appears to be a difference between the action of cortisol and that of aldosterone, in that cortisol seems to promote sodium reabsorption in exchange for secreted potassium, with perhaps a drop in hydrion secretion, whilst aldosterone promotes sodium reabsorption in exchange for an

TABLE 1. pH of urine, and outputs of Na, K and h′ expressed as a percentage of total cations, 2–2½ hr after injection of hormone, or at a corresponding time for control expts. Afternoon expts.

Subject		Control	Aldosterone	Cortisol
A	Na	47	11	19
	K	31	52	71
	h′	22	37	10
	pH	6·24	6·11	6·47
F	Na	62 48	30	31
	K	23 19	53	64
	h′	15 33	17	5
	pH	5·64 5·85	5·92	6·83
K	Na	60	22	27
	K	22	32	52
	h′	18	46	21
	pH	5·52	5·21	6·08
M	Na	48	14	21
	K	25	37	47
	h′	27	49	32
	pH	6·30	5·95	5·84
T	Na	45	16	16
	K	31	57	70
	h′	24	27	14
	pH	6·15	5·48	6·60

increased secretion of both potassium and hydrion The urinary h′:*K* ratio discriminates sharply between the action of these steroids. This ratio rose in all control experiments and all those after injection of aldosterone. Cortisol always reduced or even reversed this change. At midday potassium excretion was as much as ten times as high as hydrion excretion, whilst by 16.00 hr hydrion excretion roughly equalled that of potassium except when cortisol had been injected. The h′:*K* ratios at 12.00 and at 16.00 are shown in Table 2, and the full time course of h′:K for experiments on subject T in Fig. 3. Despite the fact that the times chosen were not in every experiment optimal for demonstrating the action of the hormones, and that more convincing figures could be presented by choosing a time apparently optimal for each experiment, the difference between the actions of the steroids is clear. It appears that in the experiment of Fig. 3 this effect of cortisol was wearing off in the last periods, covering the fourth hour after injection.

It has already been briefly reported (Mills & Thomas, 1958) that phosphate excretion falls after cortisol injection, probably as a result of the fall

of plasma concentration (Mills & Thomas, 1957, 1959). The same fall in phosphate excretion has been observed in the present experiments, as is seen in Fig. 4. All subjects at some stage after cortisol injection were excreting under 10 μmole/min of phosphate. The timing of this fall was somewhat variable in different subjects, but was roughly synchronized with the rise of pH which has already been mentioned. In subjects K and M both changes were slow and pH in later periods rose beyond the values recorded for cortisol in Table 1.

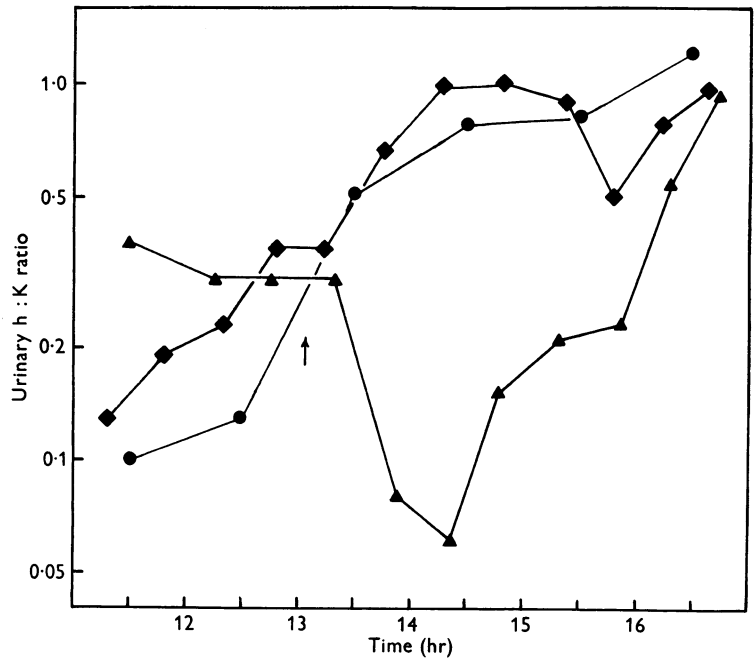

Fig. 3. Afternoon expts., subject T. Urinary h′:K ratio, log. scale. Symbols as in Fig. 1.

TABLE 2. Urinary hydrion:potassium ratio (h′:K) at 12.00 and at 16.00 hr

Subject	Control	Aldosterone	Cortisol
A 12·00	0·27	0·46	0·24
16·00	1·32	0·88	0·24
F 12·00	0·15 0·23	0·12	0·15
16·00	0·76 1·30	0·35	0·11
K 12·00	0·36	0·30	0·23
16·00	2·39	2·12	0·41
M 12·00	0·13	0·16	0·22
16·00	1·08	0·87	0·47
T 12·00	0·10	0·19	0·38
16·00	1·20	0·50	0·23

Cortisol or DOCG has been injected into four further subjects upon whom control observations have not been made. The results support our previous conclusions. In particular, potassium excretion has always risen after cortisol injection, at a time of day when a falling excretion is regularly observed under control conditions.

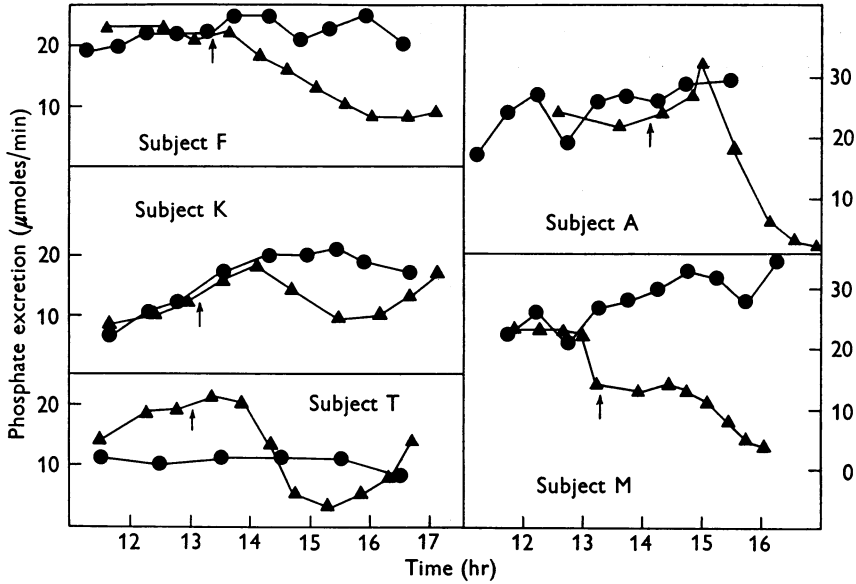

Fig. 4. Afternoon expts., subjects F, K, T, A and M. Urinary phosphate output in control expts. and after cortisol injection. Symbols as in Fig. 1.

Creatinine excretion was measured in all experiments, both as a check upon bladder emptying and as an indication of any changes in glomerular filtration rate (G.F.R.). The mean output before injection varied between 1·05 and 1·36 mg/min in different subjects. The mean changes in each successive hour after steroid injection are shown in Fig. 5. These were barely significant, but they suggest a small rise after cortisol, and perhaps a small fall after aldosterone and DOCG.

Acetazoleamide experiments

Acetazoleamide, in inhibiting carbonic anhydrase, drastically reduces (Hanley, Jowett, Kilpatrick & Platts, 1959) or even abolishes (Schwartz & Relman, 1954) the renal tubular secretion of hydrion. A series of control cortisol and aldosterone experiments was therefore performed upon subjects A, M and W under the influence of acetazoleamide. In these experiments, urinary pH usually rose above 7·4, and ammonium excretion fell to a very low level of 2–14 μequiv/min and remained there throughout

all experiments, whether or not hormones were injected. Bicarbonate excretion rose to a high level of 300–700 μequiv/min, which is nevertheless far below the likely filtered load. Thereafter it fell steadily, reaching levels of 50–300 μequiv/min 4 hr after injection; the peak level of bicarbonate output was usually in the first half hour after intravenous injection, or in the second half hour after oral administration to subject M. It is uncertain how far the falling excretion of bicarbonate reflects a wearing off of the inhibition of carbonic anhydrase, or a diminishing filtered load. Since we measured neither plasma bicarbonate concentration nor glomerular filtration rate, it is impossible to calculate the rate of tubular reabsorption of

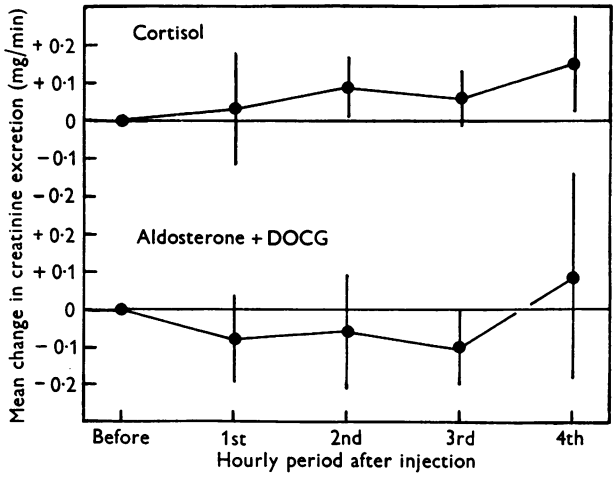

Fig. 5. Afternoon expts., subjects A, F, K, M and T. Mean change in creatinine output in each successive hour after steroid injection, with reference to the hour before injection. Above, after cortisol; below, after aldosterone and DOCG. Vertical lines indicate 95% confidence limits of the mean change.

bicarbonate; and since in addition it is uncertain whether all bicarbonate reabsorption is dependent upon hydrion secretion, it is impossible to make any calculations of hydrion secretion in these experiments, although it was certainly low. Neither steroid had any consistent effect upon bicarbonate excretion.

The high urinary bicarbonate was associated with a copious excretion of sodium and potassium. All subjects at some period were excreting sodium at a rate in excess of 380, and potassium in excess of 140, μequiv/min, and the highest rates were 801 μequiv/min of Na and 373 μequiv/min of K. It is upon the relative contributions made by these two cations that the steroids appear to have exerted their effect. The Na:K ratio (Fig. 6) remained high in all control experiments, with usually a tendency to a progressive rise. After injection of aldosterone or cortisol this ratio fell

abruptly and was still low at the end of the observations. No discrimination between the action of the two hormones was thus possible. Neither steroid caused any consistent change in creatinine excretion.

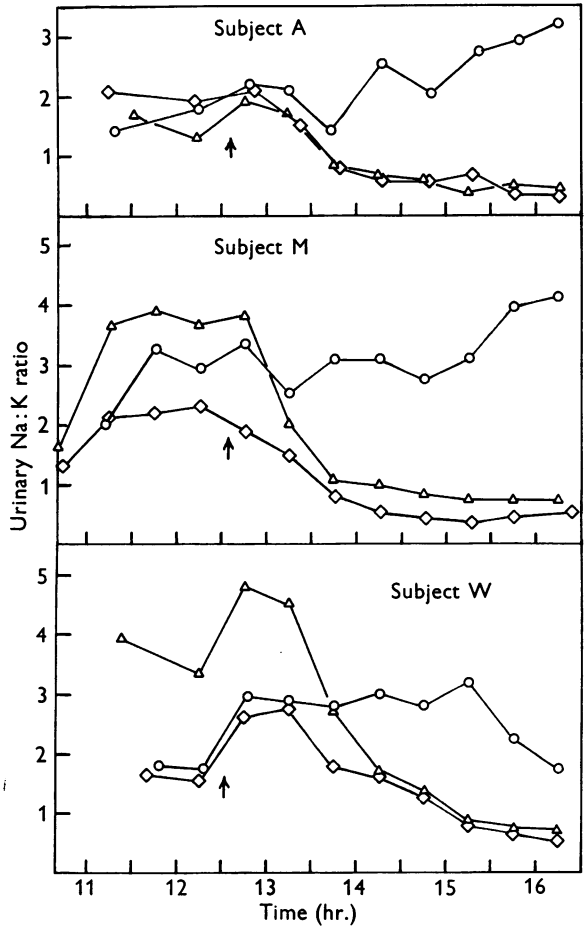

Fig. 6. Acetazoleamide expts., subjects A, M and W. Urinary Na:K ratio: ○, control; ◇, aldosterone; △, cortisol. Arrow indicates time of injection of acetazoleamide, with or without steroid, except that in subject M acetazoleamide was taken by mouth at 11.00 hr, and arrow indicates time of injection of steroids only.

Phosphate excretion was raised by acetazoleamide to values 15–20 μmole/min above those observed in control experiments. Cortisol depressed this output sharply, but not to such low values as were found when cortisol was given without acetazoleamide.

Time course of steroid action

An attempt has been made to assess, by visual inspection of plots of urinary variables against time, the delay between intravenous injection of steroids and onset of change in renal cation handling. In afternoon and acetazoleamide experiments, the ratio Na:K was the most sensitive index of hormone activity, but in the morning this ratio was rather variable, and Na:*B* provided a better criterion. The delay was assessed in all experiments where controls were available for comparison: sixteen afternoon, seven morning and six acetazoleamide. The values ranged from $\frac{1}{2}$ to $1\frac{3}{4}$ hr with a mean of 54 min, probable limits ($P = 0{\cdot}05$) 45–63 min. The scatter of values is probably due, in part, to the difficulty of accurate assessment of the delay. No evidence was found that the delay was affected by the steroid used, or by the dose of aldosterone (0·25 or 0·125 mg of the D-isomer).

Little can be said about the duration of action of the steroids, since there was seldom any sign of diminution of action when observations ended, 4 hr after injection. In one experiment, after a single dose of 0·25 mg D-aldosterone, activity was clearly diminishing after 3 or 4 hr.

There were certain indications that the action of cortisol might change qualitatively after some hours, so that different criteria might suggest a different duration of action. Thus, despite a continued sodium retention, the potassium excretion after cortisol injection did not always remain high, (Subject T, Figs. 1, 2 and 3), and when it fell, hydrion output rose. The time course of the diminished phosphate excretion after cortisol injection is much briefer than that of the changes in cation excretion. Mills & Thomas (1958) made the same comment about the changes in plasma phosphate concentration, and these changes in phosphate metabolism are apparently extrarenal (Mills & Thomas, 1959).

Night experiments

During the night the excretion of potassium is usually very low, and that of hydrion high, with sodium output more variable but usually also low (Black & Mills, 1954). Fairly consistent levels of excretion are observed, at least for some subjects, on a series of nights (compare Figs. 3, 2 and 1 of Black & Mills, 1954, with Fig. 7 of this paper). The action of steroids has therefore been studied against this background, which is very different from that during the afternoon. Five subjects were used. Figure 7 shows the outputs of Na, K and hydrion (H′), and the mean effects of the steroids upon each subject are shown in Table 3. In order to assess, on the five subjects collectively, the significance of the effects ascribed to steroid administration, analysis of variance (Rao, 1952) has been applied to the

data for Na, K, H′ and creatinine outputs; the P values in the next paragraphs are those derived from this analysis.

Both aldosterone and cortisol produced a decreased excretion of sodium of about 20 μequiv/min ($0{\cdot}01 > P > 0{\cdot}001$ and $0{\cdot}05 > P > 0{\cdot}025$, respectively); the difference between the effects of aldosterone and cortisol was

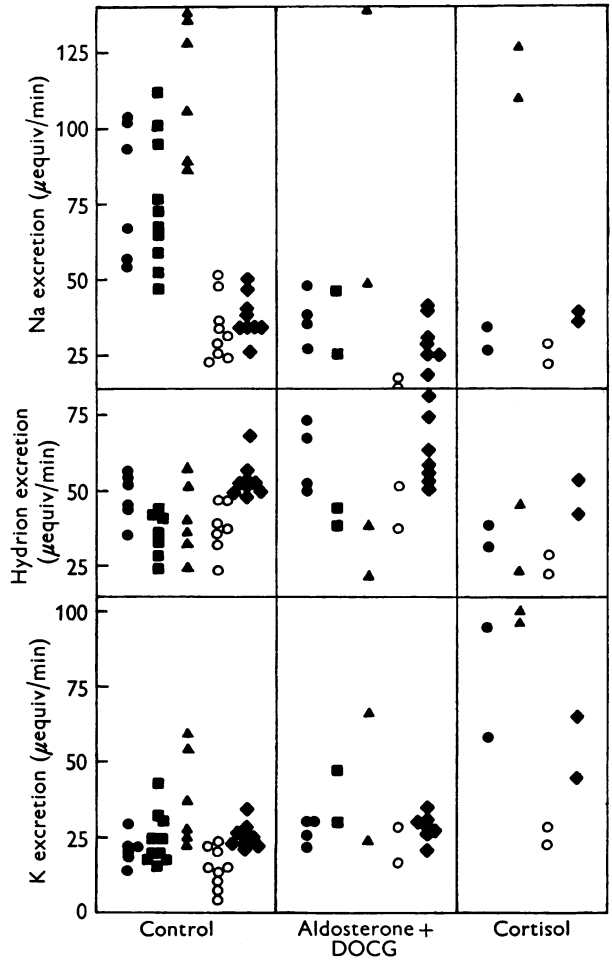

Fig. 7. Night expts., urinary sodium, potassium and hydrion (H′) outputs: ●, subject H; ■, subject F; ▲, subject I; ○, subject T; ◆, subject W.

not significant ($P > 0{\cdot}05$). Potassium output was only slightly increased by aldosterone (mean 6 μequiv/min, $0{\cdot}025 > P > 0{\cdot}01$), in contrast with a large increase following cortisol (mean 32 μequiv/min, $P < 0{\cdot}001$). The difference in effect of the two steroids was significant ($P < 0{\cdot}001$). The fall in Na and rise in K output after all steroids depressed the Na:K ratio

(Fig. 8). The depression tends to be somewhat greater after cortisol, presumably due to the greater elevation of K output.

The mean change in hydrion output (H′) was a rise of 6 μequiv/min after aldosterone ($0{\cdot}05 > P > 0{\cdot}025$), and a fall of 10 μequiv/min after cortisol ($0{\cdot}01 > P > 0{\cdot}001$). Titratable acidity was measured in all steroid experiments, though not in all controls; in calculating the difference between the steroids the more adequate expression of hydrion output (H) can be used. Thus calculated, hydrion output after aldosterone exceeded that after cortisol injection by a mean of 19 ± 5 μequiv/min.

TABLE 3. Mean increase in urinary night outputs after different steroids. Where the variable is a ratio (Na:K, h′:K) the figure given is the ratio of the geometric means of the two series of experiments

	Subject	Na	K	H*	Creatinine	Na:K	h′:K	Phosphate
Aldosterone and DOCG–control	F	−39	+14	+ 6	+0·21	0·30	1·09	+1
	H	−42	+ 6	+14	+0·05	0·35	0·92	+2
	I	−20	+ 8	−14	+0·04	0·66	0·67	−2
	T	−18	+ 8	+ 6	−0·01	0·32	0·70	+2
	W	− 8	+ 3	+10	+0·05	0·69	1·03	+2
	W^1	+ 3	+11	+17	—	0·71	—	—
	W^2	−13	+ 3	+18	—	0·55	—	—
Weighted mean		-19 ± 4	$+ 6 \pm 2$	$+ 6 \pm 3$	—	—	0·95	—
Cortisol–Control	H	−49	+56	−15	+0·03	0·10	0·17	−6
	I	0	+60	+ 1	+0·08	0·29	0·34	−8
	T	−10	+12	−13	+0·02	0·39	0·32	−7
	W	0	+29	− 6	−0·04	0·48	0·43	−15
Weighted mean	—	-18 ± 9	$+32 \pm 3$	-10 ± 3	—	—	0·353	—
Aldosterone and DOCG–cortisol	H	—	−50	+29	—	—	4·22	—
	I	—	−53	−15	—	—	2·00	—
	T	—	− 4	+24	—	—	2·19	—
	W	—	−26	+16	—	—	2·40	—
Weighted mean	—	—	-26 ± 4	$+19 \pm 5$	—	—	—	—

W^1 and W^2 refer to the first and the last half of a night in which the urine collection was divided.

* Owing to the lack of data on titratable acidity of the control nights on subjects F and T, titratable acid phosphate has been used in all calculations involving controls on these subjects.

In Table 3 it may be observed that after aldosterone or cortisol the changes in Na, K and hydrion add together to nearly zero. This suggests that the effect of both steroids was upon cation exchange in the renal tubule; the total cation exchanging was, however, considerably greater under the influence of cortisol. The pattern of the exchange produced by the different steroids was dissimilar; cortisol produced a much greater increase in K output and depressed, instead of elevating, hydrion output. This difference is clearly demonstrated, as in the afternoon experiments, by the hydrion:K ratios shown in Table 3 and Fig. 8. As with the Na:K ratio, the statistical significance of the differences and the weighted mean effect have been calculated from the logarithms. h′:K ratio was in no subject significantly altered by aldosterone but it was grossly reduced by cortisol, exactly as in the afternoon experiments.

The effect of a single intravenous injection of aldosterone may decrease within 4 hr (see above). In all except two experiments aldosterone was injected intramuscularly at bedtime, to prolong its action, but the two intravenous injections had much the same effect. As a check upon the time course, further experiments, three controls and three with aldosterone

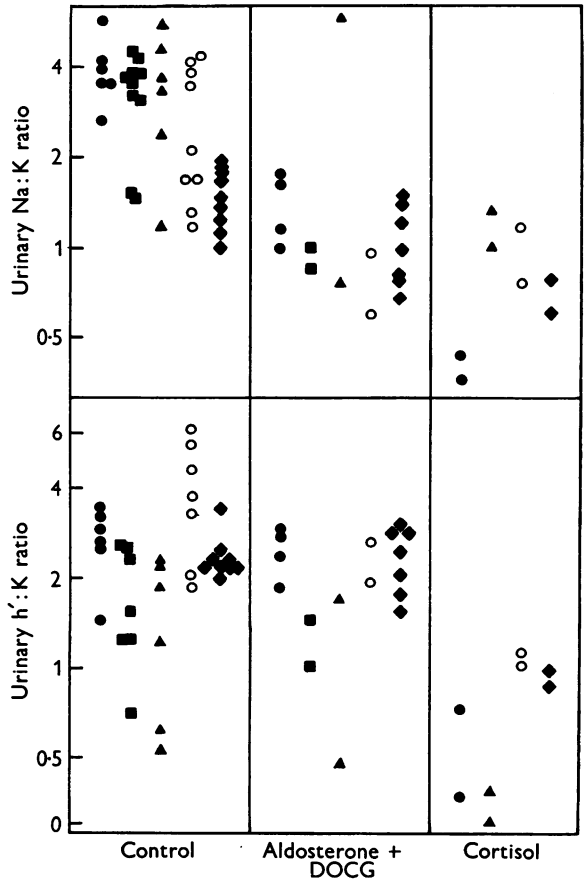

Fig. 8. Night expts., urinary Na:K and h′:K ratios, log. scale. Symbols as in Fig. 7.

injection, were performed upon subject W, in whom the effect of aldosterone appeared minimal. He was awakened by an alarm clock after 4 hr of sleep and passed urine, thus dividing the night's collection into 2 portions. The mean differences between aldosterone and controls are included in Table 3. The only significant effect was a rise in K output in the earlier half of the night, but this was much smaller than that produced by cortisol over the whole night. There is little suggestion that useful information is lost by

pooling the urine of a whole night. The outputs of the whole night covered by these experiments have, therefore, been calculated and included with the figures for W during a continuous night's sleep.

As by day, phosphate excretion was unaffected by aldosterone (Table 3). Cortisol depressed phosphate excretion; the fall was less notable than by day, but this is probably due to the pooling of 8 hr urine, since the time course of the lowered phosphate excretion after cortisol is brief (Fig. 4).

Creatinine outputs were measured in nearly all night experiments, as an indication of the G.F.R. The means of control nights were 1·09, 1·34, 1·28, 1·01 and 1·04 mg/min in subjects F, H, I, T and W, respectively. The changes due to steroids are shown in Table 3; all were trifling and analysis of variance shows them to be not significant.

Morning experiments

A series of control cortisol and aldosterone experiments in the morning was performed upon subjects C, P and W. The pattern of electrolyte excretion immediately before steroid injection, at 9.30 hr, was exceedingly variable; sodium outputs were as low as 46 and as high as 253 μequiv/min on different occasions on a single subject, and potassium outputs covered nearly as wide a range. There was a suggestion of a difference between the action of cortisol and aldosterone, similar to that observed in the afternoon, but the results are difficult to assess, owing to the wide variability of the starting point. For this reason, it appeared that no firm conclusions could be drawn without considerable multiplication of morning experiments.

DISCUSSION

Vander, Malvin, Wilde, Lapides, Sullivan & McMurray (1958) have shown by the stopped flow technique that, at least in the dog, one site of action of aldosterone is the distal renal tubule. We suggest that cortisol differs in its action upon cation exchange at this site. Three other possible mediators of altered cation excretion must, however, first be excluded: changes in glomerular filtration or in buffer excretion, and extrarenal actions. Different steroids might mediate their effects in any of these three ways.

Adrenal steroids can alter Na output by changing G.F.R. (Green, Johnson, Bridges, Lehmann & Gray, 1950). Creatinine output, which we have used as a rough indication of G.F.R., rose slightly after cortisol in the afternoon and perhaps fell after aldosterone, but the effects on sodium excretion were indistinguishable. By night creatinine excretion was not affected by steroids though sodium excretion fell. Mills & Thomas (1958) observed consistent effects of cortisol upon Na retention despite very variable effects

upon G.F.R. and renal plasma flow. Na output after adrenal steroids does not thus appear to be mainly determined by changes in G.F.R.

Cortisol depresses phosphate excretion by lowering plasma concentration, probably mainly through diversion of phosphate into muscle (Mills & Thomas, 1959). Since phosphate is a major urinary buffer, accounting for about half the titratable acidity of acid urines (Wrong & Davies, 1959; our own unpublished observations), decreased phosphate excretion may well lead to reduced excretion of hydrion. Schiess, Ayer, Lotspeich & Pitts (1948) have shown that in man excretion of titratable acid increases linearly with output of any particular buffer; they did not, however, measure any other component of hydrion output, and there is no evidence that total urinary hydrion excretion is dependent on the amount of urinary buffer, particularly when this is normal or diminished. It may well be that a fall in the amount of urinary buffer will merely result in the diversion of more secreted hydrion into other channels, namely, combination with ammonia or with bicarbonate ions.

If cortisol stimulates hydrion secretion to the same extent as do aldosterone and DOCG, but the response is limited by deficiency of buffer, then one would expect the pH of the urine to remain unaltered or more probably to fall. Since, however, in afternoon experiments cortisol elevated the urinary pH, the reduced hydrion excretion after its injection is not determined by a low buffer excretion. In night experiments the drop in phosphate excretion after cortisol injection was small and could hardly account for a difference of 19 μequiv/min in hydrion output, nor could lack of available buffer easily explain the greater over-all increase in cation exchange observed after cortisol injection.

Bartter & Fourman (1957), whose observations resembled our own, ascribed the kaliuresis after cortisol to a rise in plasma potassium concentration, due perhaps to increased tissue break-down; Knight, Kornfeld, Glaser & Bondy (1955) also found a rise of plasma potassium concentration after giving cortisol, but other workers have found the concentration to remain unchanged or to fall (Frawley, 1955; Raisz, McNeely, Saxon & Rosenbaum, 1957; Mills & Thomas, 1957). Figure 9 of Mills & Thomas (1957) includes plasma potassium concentrations for subjects M and F in the cortisol experiments here recorded, so that rising excretion and falling plasma concentration have been observed simultaneously. There is thus no support for an extra-renal origin of the kaliuresis. One might expect that an extrarenal action would still be observed in subjects given acetazoleamide.

Reports in the literature of acute steroid effects in man, fully controlled, are few. The somewhat erratic results of Huffman, Wilson, Clark & Smyth (1956) and of Knight *et al.* (1955), with intravenous doses of cortisol

similar to ours, may be due to insufficient allowance for the large spontaneous variability of Na output, particularly in the morning. There is, however, sufficient evidence in the literature for acute sodium retention under the influence of all steroids here considered.

Many workers have observed a rise in potassium excretion after giving cortisol to human subjects (Fourman, Bartter, Albright, Dempsey, Carroll & Alexander, 1950; Knight *et al.* 1955; Bartter & Fourman, 1957; Jefferies, Kelly, Sydnor, Levy & Cooper, 1957; Dingman, Finkenstaedt, Laidlaw, Renold, Jenkins, Merrill & Thorn, 1958). Raisz *et al.* (1957) described the change of potassium output as modest, but they made their observations over a time of day when potassium excretion was falling steeply in control experiments; inspection of their data shows that after cortisol injection potassium excretion rose to a level two or three times as great as in control experiments, and that the increase in potassium output was at least as great as the sodium retention. By contrast, an acute rise of potassium excretion has been less regularly observed after DOC or aldosterone, and has usually been smaller than the fall in sodium excretion (Fourman, Reifenstein, Kepler, Dempsey, Bartter & Albright, 1952; Sheppard, Morse, Renold & Thorn, 1955; Thorn, Sheppard, Morse, Reddy, Beigelman & Renold, 1955; Liddle, 1958).

Berliner (1950) first suggested that adrenal steroids might stimulate the ion exchange mechanism whereby the distal tubule is supposed to secrete potassium in exchange for reabsorbed sodium (Berliner, Kennedy & Hilton, 1950). This suggestion has been widely accepted, though usually only by implication. The possibility that adrenal steroids stimulate sodium reabsorption in exchange for secreted hydrion, the mechanism proposed by Pitts & Alexander (1945), has been considered by Pitts (1952), Knight *et al.* (1955), Bartter (1956), Dingman *et al.* (1958), Perlmutt & Olewine (1958), and Liddle (1958). Only Bartter & Fourman (1957), however, mention the possibility that different steroids may differ in their action upon these two cation exchange mechanisms; and we have found no report in which all the separate indications of hydrion exchange—urinary pH, ammonium output, titratable acid and bicarbonate—have been measured in human subjects after intravenous administration of steroids. No detailed comparison with the findings of other workers is thus possible.

Numerous observations over more prolonged periods, such as days or weeks, suggest differences between the action of different steroids on electrolyte balance. These are, however, outside the scope of the present discussion, since over long periods many secondary effects may obscure the direct renal actions.

Findings similar to our own have been reported on other species. Desaulles (1958), working with adrenalectomized rats, found that for a

similar sodium retention corticosterone and cortisol caused much greater excretion of potassium than did aldosterone or DOC. Perlmutt & Olewine (1958), also using rats, found that DOCG depressed Na output without increasing excretion of potassium; after acetazoleamide and DOCG, however, the fall in Na excretion was paralleled by a rise in potassium excretion.

A quite distinct method of investigating the renal effects of aldosterone is to observe the immediate effects of a blocking agent such as SC–8109 (Searle), which is without direct effect on renal function but prevents the action of aldosterone (Kagawa, Cella & Van Arman, 1957; Liddle, 1957; Ross & Bethune, 1959). In circumstances where aldosterone secretion is presumed to be considerable, this drug has been found to diminish excretion of titratable acid and ammonium (Liddle, 1958; McCrory & Eberlein, 1958; Wiggins, Hutchin, Carbone & Doolan, 1959), as well as increasing sodium and decreasing potassium excretion. Confirmation is thus provided for our observation that aldosterone can stimulate acid as well as K excretion by the kidney.

The findings can all be explained by supposing that the direct action of cortisol is to stimulate potassium secretion in exchange for sodium. This usually leads to a substantial fall in sodium excretion, but if sodium reabsorption is already nearly complete, as in some subjects at night, then the potassium secretion is at the expense of hydrion secretion (Berliner, Kennedy & Orloff, 1951). Aldosterone and DOC, on this hypothesis, stimulate sodium reabsorption, in exchange for whatever cation is available; both in the afternoon and at night, excretion of hydrion and of K increase in the same proportion. When, however, hydrion secretion is blocked by acetazoleamide, reabsorption is largely or entirely in exchange for potassium. Under other circumstances it is still not known what determines the partition of secretion between potassium and hydrion, but it varies spontaneously over the twenty-four hours (Mills & Stanbury, 1954). A part of this 24 hr cycle is shown in the afternoon control experiments; the behaviour of subject T (Fig. 3) is typical. A further portion is represented by the night experiments. It will be seen that, although aldosterone and deoxycorticosterone increase the total potassium and hydrion secretion, the proportional distribution between the two continues to follow the habitual diurnal rhythm.

SUMMARY

1. Urine was collected from healthy men after injection of cortisol, aldosterone, or deoxycorticosterone, and compared with collections from the same subjects without injection. The doses of steroid used produced similar Na retention.

2. In the afternoon cortisol increased K output by an amount similar to the Na retention, and hardly altered hydrion output. Hydrion:K excretory ratio was thus grossly reduced. Aldosterone and deoxycorticosterone increased excretion of both hydrion and K, and the hydrion:K ratio followed a similar course to that observed in control experiments.

3. When steroids were given at bedtime and the night's urine collected, cortisol caused a rise of K excretion much greater than the Na retention, balanced by a fall in hydrion excretion. Aldosterone and deoxycorticosterone, as in the afternoon, increased outputs of hydrion and K but left the hydrion:K ratio unaltered.

4. In subjects with hydrion secretion blocked by acetazoleamide, the difference between the steroids disappeared: all steroids reduced Na and increased K excretion.

5. In morning experiments random variations in outputs were too great to permit discrimination between the effects of the steroids.

6. Changes in creatinine excretion were trifling after all steroids.

7. It is suggested that cortisol stimulates K excretion, resulting in either Na retention or diminished hydrion $\rightleftharpoons$ Na exchange, whilst aldosterone stimulates Na reabsorption in exchange for K and hydrion in whatever proportions are available.

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REFERENCES

BARTTER, F. C. (1956). The role of aldosterone in normal homeostasis and in certain disease states. *Metabolism*, **5**, 369–383.

BARTTER, F. C. & FOURMAN, P. (1957). The non-renal effect of adrenal cortical steroids upon potassium metabolism. *J. clin. Invest.* **36**, 872–3.

BERLINER, R. W. (1950). Renal regulation of water and electrolytes. *Amer. J. Med.* **9**, 541–559.

BERLINER, R. W., KENNEDY, T. J., Jr. & HILTON, J. G. (1950). Renal mechanisms for excretion of potassium. *Amer. J. Physiol.* **162**, 348–367.

BERLINER, R. W., KENNEDY, T. J., Jr. & ORLOFF, J. (1951). Relationship between acidification of the urine and potassium metabolism. *Amer. J. Med.* **11**, 274–282.

BLACK, D. A. K. & MILLS, J. N. (1954). Nocturnal electrolyte excretion after oral administration of sodium and potassium chloride and bicarbonate. *Clin. Sci.* **13**, 211–219.

BONSNES, R. W. & TAUSSKY, H. H. (1945). On the colorimetric determination of creatinine by the Jaffe reaction. *J. biol. Chem.* **158**, 581–591.

COLLINS, G. C. & POLKINHORNE, H. (1952). An investigation of anionic interference in the determination of small quantities of potassium and sodium with a flame photometer. *Analyst*, **77**, 430–436.

DESAULLES, P. (1958). Comparison of the effects of aldosterone, cortexone and cortisol on adrenalectomized rats under various salt loads. In MULLER, A. F. & O'CONNOR, C. M. *An International Symposium on Aldosterone*, pp. 29–38. London: Churchill.

DINGHAM, J. F., FINKENSTAEDT, J. T., LAIDLAW, J. C., RENOLD, A. E., JENKINS, D., MERRILL, J. P. & THORN, G. W. (1958). Influence of intravenously administered adrenal steroids on sodium and water excretion in normal and Addisonian subjects. *Metabolism*, **7**, 608–623.

FISKE, C. H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *J. biol. Chem.* **66**, 375–400.

FOURMAN, P., BARTTER, F. C., ALBRIGHT, F., DEMPSEY, E., CARROLL, E. & ALEXANDER, J. (1950). Effects of 17-hydroxy-corticosterone (Compound F) in man. *J. clin. Invest.* **29**, 1462–1473.

FOURMAN, P., REIFENSTEIN, E. C., KEPLER, E. J., DEMPSEY, E., BARTTER, F. C. & ALBRIGHT, F. (1952). Effect of deoxycorticosterone acetate on electrolyte metabolism in a normal man. *Metabolism*, **1**, 242–253.

FRAWLEY, T. F. (1955). The role of the adrenal cortex in glucose and pyruvic acid metabolism in man, including the use of intravenous hydrocortisone in acute hypoglycaemia. *Ann. N.Y. Acad. Sci.* **61**, 464–493.

GREEN, D. M., JOHNSON, A. D., BRIDGES, W. E., LEHMANN, J. H. & GRAY, F. (1950). Mechanisms of deoxycorticosterone action: effects of the water-soluble glycoside on human circulatory and renal functions. *Endocrinology*, 47, 102–107.

HANLEY, T., JOWETT, G. H., KILPATRICK, R. & PLATTS, M. M. (1959). The role of carbonic anhydrase in renal reabsorption of bicarbonate. *J. Physiol.* **145**, 277–288.

HASTINGS, A. B. & SENDROY, J. (1925). The effect of variation in ionic strength on the apparent first and second dissociation constants of carbonic acid. *J. biol. Chem.* **65**, 445–455.

HAWK, P. B., OSER, B. L. & SUMMERSON, W. H. (1947). *Practical Physiological Chemistry*, 12th ed., p. 828. London: Churchill.

HUFFMAN, E. R., WILSON, G. M., CLARK, G. M. & SMYTH, C. J. (1956). Acute renal effects of 9-α-fluoro-hydrocortisone in human subjects with intact adrenals. *J. Lab. clin. Med.* **47**, 747–751.

JEFFERIES, W. McK., KELLY, L. W., SYDNOR, K. L., LEVY, R. P. & COOPER, G. (1957). Metabolic effects of a single intravenous infusion of hydrocortisone related to plasma levels in a normal versus an adrenally insufficient subject. *J. clin. Endocrin.* **17**, 186–200.

KAGAWA, C. M., CELLA, J. A. & VAN ARMAN, C. G. (1957). Action of new steroids in blocking effects of aldosterone and deoxycorticosterone on salt. *Science*, **126**, 1015–1016.

KNIGHT, R. P., KORNFELD, D. S., GLASER, G. H. & BONDY, P. K. (1955). Effects of intravenous hydrocortisone on electrolytes of serum and urine in man. *J. clin. Endocrin.* **15**, 176–181.

LIDDLE, G. W. (1957). Sodium diuresis induced by steroidal antagonists of aldosterone. *Science*, **126**, 1016–1018.

LIDDLE, G. W. (1958). Aldosterone antagonists. *Arch. intern. Med.* **102**, 998–1004.

LONGSON, D. & MILLS, J. N. (1953). The failure of the kidney to respond to respiratory acidosis. *J. Physiol.* **122**, 81–92.

LONGSON, D., MILLS, J. N., THOMAS, S. & YATES, P. A. (1956). Handling of phosphate by the human kidney at high plasma concentrations. *J. Physiol.* **131**, 555–571.

MCCRORY, W. W. & EBERLEIN, W. R. (1958). The nature of the sodium diuresis produced by an 'Aldosterone Antagonist', Spirolactone. *J. clin. Invest.* **37**, 917.

MILLS, J. N. & STANBURY, S. W. (1954). A reciprocal relationship between K^+ and H^+ excretion in the diurnal excretory rhythm in man. *Clin. Sci.* **13**, 177–185.

MILLS, J. N. & THOMAS, S. (1957). The acute effect of adrenal hormones and carbohydrate metabolism upon plasma phosphate and potassium concentrations in man. *J. Endocrin.* **16**, 164–179.

MILLS, J. N. & THOMAS, S. (1958). The acute effects of cortisone and cortisol upon renal function in man. *J. Endocrin.* **17**, 41–53.

MILLS, J. N. & THOMAS, S. (1959). The influence of adrenal corticoids on phosphate and glucose exchange in muscle and liver in man. *J. Physiol.* **148**, 227–239.

MILLS, J. N. THOMAS, S. & WILLIAMSON, K. S. (1959*a*). The acute effect of different adrenal corticoids upon the excretion of acid and potassium by the human kidney. *J. Physiol.* **146**, 34–35*P*.

MILLS, J. N., THOMAS, S. & WILLIAMSON, K. S. (1959*b*). Effect of adrenal steroids upon cation excretion at night. *J. Physiol.* **149**, 21*P*.

PERLMUTT, J. H. & OLEWINE, D. A. (1958). Alteration of renal response to carbonic anhydrase inhibitor by synthetic adrenal steroids. *Amer. J. Physiol.* **195**, 142–146.

PITTS, R. F. (1952). Effects of adrenal cortical hormones on renal function. In RALLI, E. P., *Adrenal Cortex*, Trans. of the Third Conference, pp. 11–53. New York: Josiah Macy Foundation.

PITTS, R. F. & ALEXANDER, R. S. (1945). The nature of the renal tubular mechanism for acidifying the urine. *Amer. J. Physiol.* **144**, 239–254.

RAISZ, L. G., MCNEELY, W. F., SAXON, L. & ROSENBAUM, J. D. (1957). The effects of cortisone and hydrocortisone on water diuresis and renal function in man. *J. clin. Invest.* **36**, 767–779.

RAO, C. R. (1952). *Advanced Statistical Methods in Biometric Research*, pp. 94–102. New York: Wiley.

ROSS, E. J. & BETHUNE, J. E. (1959). Antagonism between the effect of aldosterone and a synthetic steroid lactone on the renal excretion of sodium and potassium in man. *Lancet*, **276**, 127–129.

SCHIESS, W. A., AYER, J. L., LOTSPEICH, W. D. & PITTS, R. F. (1948). The renal regulation of acid-base balance in man. II. Factors affecting the excretion of titratable acid by the normal human subject. *J. clin. Invest.* **27**, 57–64.

SCHWARTZ, W. B. & RELMAN, A. S. (1954). The dependence of renal sodium reabsorption on hydrogen exchange. *J. clin. Invest.* **33**, 965.

SHEPPARD, R. H., MORSE, W. I., RENOLD, A. E. & THORN, G. W. (1955). Studies on aldosterone in man. *J. clin. Endocrin.* **15**, 853–854.

THORN, G. W., SHEPPARD, R. H., MORSE, W. I., REDDY, W. J., BEIGELMAN, P. M. & RENOLD, A. E. (1955). Comparative action of aldosterone and 9-alpha-fluorohydrocortisone in man. *Ann. N.Y. Acad. Sci.* **61**, 609–619.

VANDER, A. J., MALVIN, R. L., WILDE, W. S., LAPIDES, J., SULLIVAN, L. P. & MCMURRAY, V. M. (1958). Effects of adrenalectomy and aldosterone on proximal and distal tubular sodium reabsorption. *Proc. Soc. exp. Biol., N.Y.*, **99**, 323–325.

VAN SLYKE, D. D. & NEILL, J. M. (1924). The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. *J. biol. Chem.* **61**, 523–573.

WIGGINS, R. A., HUTCHIN, M. E., CARBONE, J. V. & DOOLAN, P. D. (1959). Effect of spirolactone SC 8109 on renal function in normal human subjects. *Proc. Soc. exp. Biol., N.Y.*, **100**, 625–627.

WRONG, O. & DAVIES, H. E. F. (1959). The excretion of acid in renal disease. *Quart. J. Med.* **28**, 259–313.