P. 133, l. 2: *for* '(Rosenbaum, Ferguson, Davis & Rossmeisl,1952)'
read(for references see Rosenbaum, Ferguson, Davis & Rossmeisl, 1952)

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ACUTE POSTURAL CHANGES IN ALDOSTERONE AND ELECTROLYTE EXCRETION IN MAN

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The renal responses to change of posture have been ascribed to changes in renal haemodynamics (Rosenbaum, Ferguson, Davis & Rossmeisl, 1952); but Thomas (1957) has produced evidence that, at least in prolonged experiments, there is a change of tubular function such as could be produced by an increased secretion of aldosterone. There is evidence (Muller, Manning & Riondel, 1958) that aldosterone output over periods of twelve hours is higher in ambulant subjects than in those who remain in bed. Changes in output in relation to posture have not, however, been demonstrated over shorter periods such as those here reported. It has often been suggested that secretion of aldosterone is regulated by 'volume receptors' at some unknown situation (e.g. Bartter, Liddle, Duncan, Barber & Delea, 1956); if posture can be shown to affect aldosterone output, it seems likely that the same volume receptor mechanism is involved, and postural studies may therefore contribute towards the location of these receptors.

A preliminary account of this work has already appeared (Gowenlock, Mills & Thomas, 1958).

METHODS

Observations were made on healthy male subjects, 25 medical students and one of the authors (J.N.M.). For each experiment groups of 3–8 subjects were used; in order to investigate changes of aldosterone excretion over short periods, urine from the group of subjects was pooled, as only thus was it possible to obtain the 8–12 man-hours of urine required for accurate assay by the method used. Except in experiments 7 and 8 no two groups had the same composition; each subject was used in one to four experiments.

After a light breakfast without tea or coffee, subjects voided and discarded their urine at about 10.30 and then lay down for 2 or 2½ hr before voiding sample 1. They then either remained recumbent, for 5 hr in all, voiding sample 2 at the end; or they changed their posture to standing or sitting, voiding and discarding the urine after 1 hr and collecting the urine passed in the following 2 hr; or they remained recumbent and received an intravenous injection of 0·25 mg DL-aldosterone-21-monoacetate (Ciba), in 95% ethanol, diluted with isotonic NaCl, repeated 1½ hr later, and collected the urine passed during 3 hr from the first injection. They drank when thirsty.

Small samples of urine from each subject were removed for separate analysis of Na and K

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(flame photometer, EEL) and the residue from all subjects was pooled for steroid analysis (Ayres, Garrod, Simpson & Tait, 1957) on samples 1 and 2. Samples removed were so adjusted that each subject contributed for aldosterone assay the urine collected over an equal period of time, and the outputs were calculated per man-hour. The smallest amount used in any assay was 7·45 man-hours. A little thymol was added to each sample, which was stored in the refrigerator during the night before commencing assay next morning. One pair of samples was thus preserved until the next day but one. The analytical procedure was exactly that of Ayres, Garrod, Simpson & Tait (1957).

RESULTS

Our first comparison was made between 3 groups of three or four subjects who remained recumbent throughout, and another 3 groups of five to eight who changed from recumbent to standing; they were instructed to stand fairly still, but to move sufficiently to avoid feeling faint. Table 1 shows that

TABLE 1. Urinary aldosterone in all experiments

Expt. no.	No. of subjects	Aldosterone output (μg/man-hr) 1st period recumbent	Aldosterone output (μg/man-hr) 2nd period	Conditions during 2nd period
1	4	0·29	0·13	Recumbent
2	3	0·17	0·025	Recumbent
3	4	0·14*	0·02	Recumbent
4	5	0·14*	0·23	Standing in air
5	8	0·10	0·54	Standing in air
6	6	0·28	0·46	Standing in air
7	6	0·43	0·28	Sitting in air
8	6	0·21	0·22	Sitting in water
9	6	0·29	0·09	Standing in water
10	6	0·41	11·2	Recumbent, injected with aldosterone

* This figure is the result of an assay upon the pooled urine of both groups of subjects.

Experiments 7 and 8 were carried out on the same group of subjects. The code numbers of experiments do not represent the consecutive order in which they were performed.

the aldosterone output always declined in subjects who remained recumbent (Expts. 1–3), and rose in subjects who stood up (Expts. 4–6). In Expt. 5 free cortisol was also estimated in the pooled urine samples. The output changed very little, from 1·4 μg/man-hr during recumbency to 1·8 μg/man-hr during standing, despite a large increase in aldosterone output, so no further estimations were performed.

If the rise in aldosterone output on standing is due to a redistribution of body fluids, it should be prevented by standing in water instead of air. Table 1 shows (Expt. 9) that this was in fact the case: aldosterone output of a group of subjects standing in water immersed to between the nipples and the neck fell during the second period, just as if they had remained recumbent. The water in Expt. 9 was at 37° C; Expts. 7 and 8 test the possibility that immersion in water at this temperature might itself influence aldosterone secretion. In Expt. 7 the subjects sat in air in their ordinary clothes, and aldosterone excretion fell a little below that in the preliminary period of recumbency; in Expt. 8 the same subjects sat in water at 37° C and aldosterone secretion did

not change. Clearly, immersion in water did not itself cause any fall in aldosterone excretion, and the low values in the subjects in Expt. 9 who stood in water may be attributed to prevention of the circulatory changes of standing, rather than to any other effect of the immersion.

Since only one group of subjects has performed this experiment, we do not wish to emphasize the difference between sitting as contrasted with standing or lying, although the behaviour of the aldosterone output has in fact been intermediate between that in the other two postures.

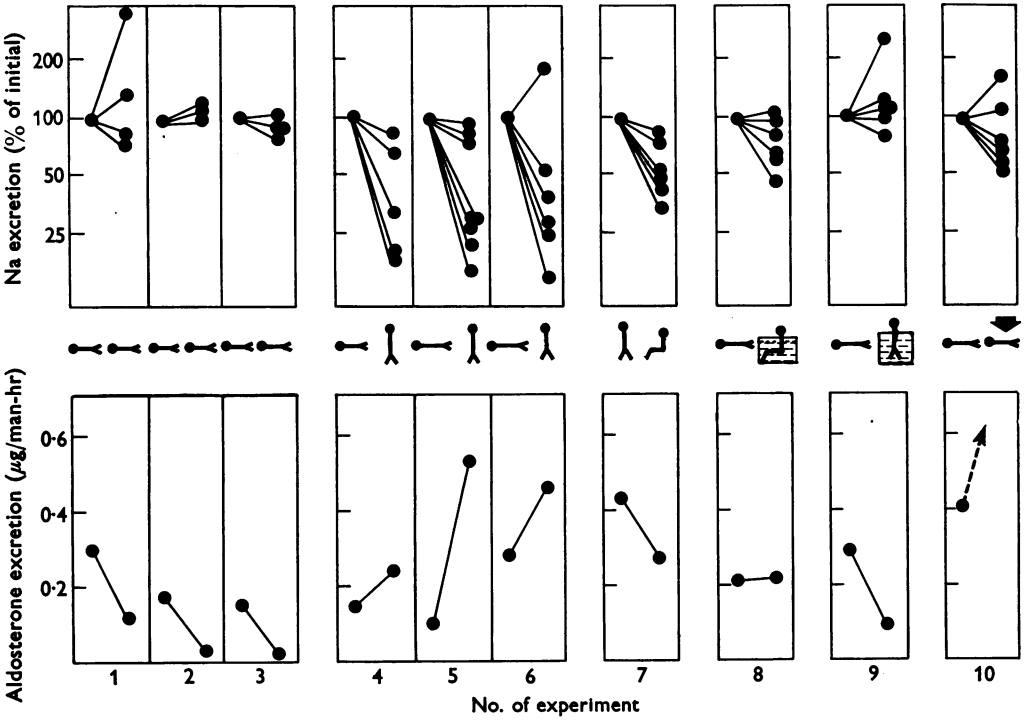

Fig. 1. Aldosterone outputs (μg/man-hr) in pooled urines, and individual sodium outputs as percentage of output in initial recumbent period. Second period: Expts. 1–3, continued recumbency; 4–6, standing in air; 7, sitting in air; 8, sitting in water; 9, standing in water; 10, recumbent, injected with aldosterone.

Sodium excretion and aldosterone output are shown in Fig. 1. Owing to the wide variation in initial values, 68–487 μ equiv/min, the sodium results are represented as a percentage of the output of the first period, on a logarithmic scale. The mean (geometric) values are shown in Table 2, with the statistical significance of the differences, calculated by the small-sample method of Fisher (1941).

In subjects who remained recumbent, sodium excretion was little altered in the second collection period of 3 hr. By contrast, excretion fell during standing,

in the majority of subjects, to below 40% of the previous level, although a wide scatter between individuals is apparent. Subjects standing in water, like those remaining recumbent, showed no change in sodium excretion; and in those sitting, whether in air or water, sodium excretion behaved in a manner intermediate between those who lay or stood. Aldosterone injection did not, however, reduce the sodium excretion to the levels commonly observed during standing.

The change in potassium excretion in the second period is shown in Fig. 2 and Table 2 in a manner similar to that used for sodium. The output in all

TABLE 2. The effect of posture on the excretion of sodium and potassium, and on the Na:K ratio. The ratio given in column 3 is that of the value in the second period to the value in the first period. Figures are geometric means, with probabilities calculated from the logarithms

	Condition	No. of observations	Ratio 2nd period: 1st period	Probability of identity with: Recumbency	Probability of identity with: Standing in air
Na	Recumbent	11	1·09	—	
	Standing in air	19	0·38	< 0·01	—
	Standing in water	6	1·21	0·7–0·6	< 0·01
	Sitting in air or water	12	0·63	< 0·01	0·05–0·02
	Recumbent, aldosterone injected	6	0·82	0·2	0·05–0·02
K	Recumbent	11	0·77	—	
	Standing in air	19	0·43	< 0·01	—
	Standing in water	6	0·48	0·3–0·2	0·7
	Sitting in air or water	12	0·57	0·2–0·1	0·2–0·1
	Recumbent, aldosterone injected	6	1·03	0·1–0·05	< 0·01
Na:K	Recumbent	11	1·49	—	
	Standing in air	19	0·99	< 0·01	—
	Standing in water	6	2·51	< 0·01	< 0·01
	Sitting in air or water	12	1·08	< 0·01	0·2–0·1
	Recumbent, aldosterone injected	6	0·70	< 0·01	0·3–0·2

postures almost always fell, presumably as part of the diurnal rhythm (Stanbury & Thomson, 1951; Mills & Stanbury, 1952, 1954), but the fall was significantly ($P < 0{\cdot}01$) greater on standing than in subjects who remained recumbent. On injection of aldosterone the usual fall in potassium excretion of recumbent subjects was apparently prevented, but the difference was not statistically significant ($0{\cdot}1 > P > 0{\cdot}05$). The difference from the behaviour of standing subjects was, however, significant ($P < 0{\cdot}01$).

The effect of these changes upon the ratio of sodium to potassium excretion, which has been regarded as an indication of the level of aldosterone production (Luetscher & Curtis, 1955), is shown in the same way in Fig. 2 and Table 2. This ratio rose in those experiments in which aldosterone output fell, that is, when subjects either remained recumbent or stood in water, and the rise differed significantly from the behaviour of all other groups. When subjects sat the Na:K ratio and aldosterone output both changed little; when they stood in air the ratio was again unaltered although aldosterone output rose. When as a result of injection aldosterone output rose greatly, the ratio fell.

In all these three conditions—sitting, standing, and aldosterone injection—behaviour of the Na:K ratio was significantly different from that in continued recumbency.

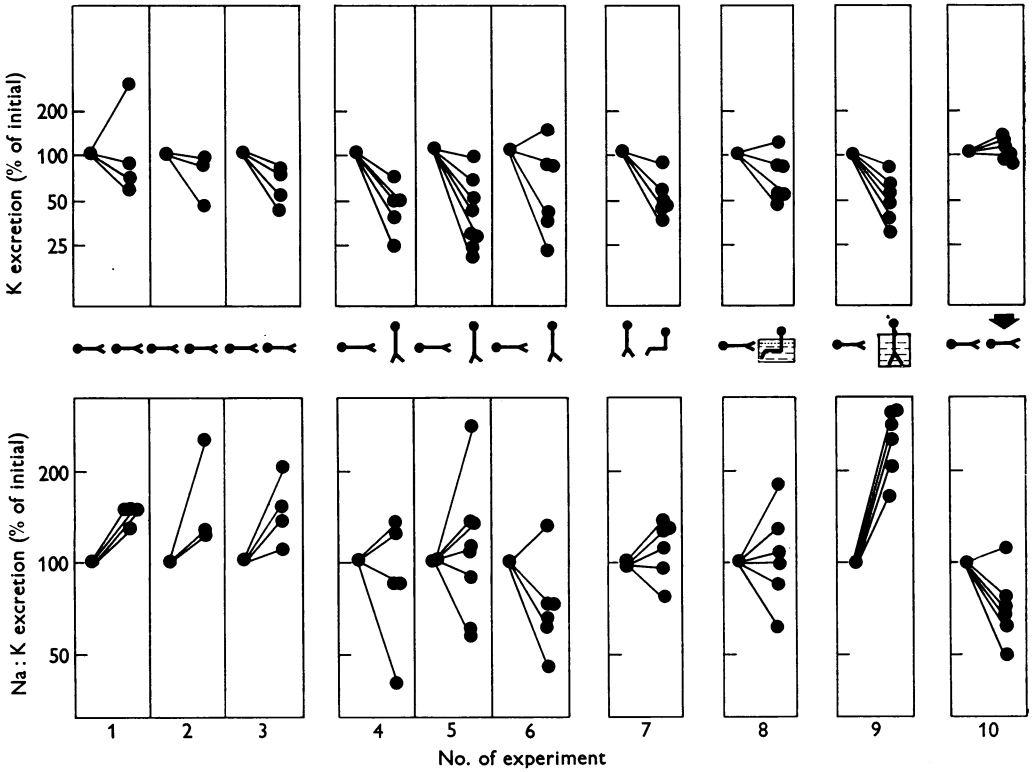

Fig. 2. Potassium outputs, and Na:K ratios, both expressed as percentage of values for initial recumbent period. Second period: Expts. 1–3, continued recumbency; 4–6, standing in air; 7, sitting in air; 8, sitting in water; 9, standing in water; 10, recumbent, injected with aldosterone.

DISCUSSION

The demonstration that aldosterone output is increased by standing in air is clear enough, and it is generally supposed that such an increase represents an increased secretion by the adrenal cortex. It must now be considered how far such an increase of secretion can account for the changes in urinary electrolytes.

The situation would be simple, were it possible to show that exogenous aldosterone, in amount similar to that produced on standing, could reproduce in a recumbent subject the renal consequences of standing. Such simplicity is not to be expected for two reasons: there is no reliable basis for comparing exogenous aldosterone dosage with endogenous production, and the urinary changes on standing are probably ascribable also to changes in renal circulation.

At first sight, the very high aldosterone excretion after injection (Expt. 10, Table 1) suggests that the dose of aldosterone given was quite out of proportion to natural production. This gross disproportion may be misleading for two reasons: the inclusion in our assay of the biologically inactive L-isomer after injection of racemic aldosterone, and the mode of administration.

Gross & Lichtlen (1958) produce evidence that activity is confined to the D-isomer; there is no evidence to suggest that both isomers are metabolized or excreted at the same rate, and as our method of assay does not discriminate between them the high excretion we observed might have been mainly of the inactive L-isomer.

Luetscher (1956) cites evidence that even slow intravenous infusion is a much less effective route of administration than intramuscular injection in an oily medium. Venning, Giroud, Dyrenfurth & Beck (1955) have shown that slow intravenous injection of the natural isomer leads to an unduly large urinary excretion. Of our discontinuous intravenous injection of the racemic monoacetate (Expt. 10, Table 1), 6·7% was excreted within 3 hr of the first dose. Slow intravenous infusion has resulted in excretion of 3·6% within 3 hr, and intramuscular administration in excretion of 2½–4½% over 24 hr (Gowenlock, unpublished observations). Intravenous administration of aldosterone seems to lead to a high urinary excretion and low physiological activity. It is further not known whether the monoacetate has the same activity as the free alcohol: it may well be in part inactivated before release from the ester.

Some basis of comparison between the standing and aldosterone injection experiments can be found by using the findings of Ayres, Garrod, Tait, Tait, Walker & Pearlman (1957). These workers gave small tracer doses of tritiated D-aldosterone, and calculated that daily excretion was, in two subjects, 8·2, 4·2 and 5·4% of daily production. On this basis our observed excretion of up to 0·54 μg/man-hr in standing subjects would indicate a production rate of up to 7–13 μg/hr. The dose we injected, if spread out over 3 hr, corresponds to 83 μg/hr of D-aldosterone monoacetate, or about 8 times the supposed secretion rate on standing. The work cited above suggests that this factor of 8 may be due, in part, to a less effect of brief peaks in aldosterone delivery to the kidney; but in addition, even slow intravenous administration appears to have less physiological effect than intramuscular. The reason for this is obscure.

It must next be considered how far the alterations of electrolyte excretion on standing may be ascribed to the additional aldosterone production rather than to altered renal haemodynamics, in particular to a reduced glomerular filtration rate (Smith, 1951; Thomas, 1956). It is probable that immersion in water prevents the renal haemodynamic changes of standing as well as the increase of aldosterone output, although only creatinine output appears to have been measured as evidence (Bazett, Thurlow, Crowell & Stewart, 1924).

It will be obvious from Figs. 1 and 2 that aldosterone injection did not exactly reproduce the effects of standing. Thus, aldosterone did not reduce the sodium output to the level produced by standing, although it reduced it below the level observed in continued recumbency.

Taken by themselves the changes in potassium output are difficult to interpret, since the effect of aldosterone was the reverse of that of standing; but a simple picture emerges if one considers the Na:K ratio. The increased ratio observed in continued recumbency or standing in water was often prevented, or even reversed, by standing in air or by injecting aldosterone, whereas sitting produced intermediate results. There thus seems to be an inverse correlation between Na:K ratio and aldosterone excretion.

One cannot necessarily infer an altered aldosterone production from an altered Na:K ratio. The following is, however, a reasonable interpretation of the electrolyte changes we observed. Reduction in G.F.R. on standing reduces the sodium presented to the distal tubule, and hence alike the sodium excreted and the sodium available for ionic exchange with potassium (Davidson, Levinsky & Berliner, 1958). Aldosterone may be supposed to promote potassium exchange for sodium, whether the amount of sodium available for this exchange is large, as in recumbent subjects injected with aldosterone, or small, as in subjects standing in air and provided by their own adrenals with more aldosterone. The sharp reduction in sodium presented to the ion exchange site may lead to an absolute reduction in potassium excretion on standing in air, despite the stimulus to ion exchange provided by increased endogenous aldosterone. This interpretation is supported by the observations of Thomas (1957) upon the progressive changes in successive half-hourly urine samples during 3 hr of standing, and more strongly by unpublished observations over more prolonged periods of standing; in these, Na:K ratio in the urine has progressively declined, often to considerably below unity, owing not only to fall in Na excretion but often also to an absolute rise in K excretion.

If it be presumed that the increased production of aldosterone on standing is mediated through volume receptors, the response is more rapid than has often been suggested. A sudden large change in volume of blood, as by haemorrhage or large intravenous infusion, leads to a notable fall or rise of G.F.R. (de Wardener & McSwiney, 1951; Robinson, 1954), which may account for the immediate changes in sodium excretion. Small or gradual changes in either blood or extracellular fluid volume usually, however, alter sodium output only after a considerable delay (Selkurt, 1954). Thus ingested isotonic saline is only excreted slowly; and Wrong (1956), who expanded body fluids by drinking water and injecting vasopressin, found an increased sodium output only after 10–15 hr. The idea has thus gained wide acceptance (e.g. Wrong, 1957) that the endocrine adjustment of sodium excretion, aroused by volume receptors, is comparatively slow. The delay might arise either in the secretion, or

cessation of secretion, of aldosterone—generally accepted as the effector hormone—or in the action of aldosterone at the target organ.

The present work contributes little information about the time course of renal response to aldosterone; it does, however, show that liberation of aldosterone may be rapid. Previous observations on aldosterone excretion in response to change of posture or blood volume have been confined to 12-hr periods (Muller *et al.* 1958; Wolff, 1958). The present procedure of pooling the urine from several subjects has permitted the demonstration of a more rapid increase in excretion—and presumably in production—within at most 3 hr. Even more rapid changes have been found in dogs by Holzbauer & Vogt (1958), by direct assay on adrenal vein blood collected over two periods of 30–45 min, with an intervening period during which the animal was bled or transfused.

The literature upon possible location of volume receptors is extensive (see Wrong, 1957; Smith, 1957). The present work merely adds evidence that they are in the upper half of the body, where volume depletion will result from fluid increase in the legs during standing.

SUMMARY

1. Aldosterone has been assayed in the pooled urine of groups of 3–8 subjects who either remained recumbent for 5 hr, or stood up for 3 hr after 2 hr recumbency.

2. In those who stood the aldosterone output rose; in those remaining recumbent, or standing in water, the output fell. Subjects sitting, whether in air or water, showed an aldosterone output intermediate between those of lying and standing subjects. Aldosterone injection into recumbent subjects led to very high outputs of aldosterone.

3. Sodium and potassium outputs were determined separately for each subject. Sodium fell on standing in air, and was unaltered by continued recumbency, with or without aldosterone injection, or by standing in water; sitting subjects showed an intermediate response.

4. Potassium output fell in all groups except those injected with aldosterone. The fall was greatest on standing in air, and least in recumbency.

5. The electrolyte behaviour is most simply explained by a drop in G.F.R. on standing in air, and an increased tubular secretion of potassium in exchange for sodium under the influence of aldosterone.

6. The changes in aldosterone excretion are discussed in relation to volume receptors in the upper half of the body.

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