OBSERVATIONS UPON THE WITHDRAWAL OF SODIUM CHLORIDE FROM THE DIET IN HYPERTENSIVE AND NORMOTENSIVE INDIVIDUALS

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Luetscher & Johnson (1954) have recently shown that dietary restriction of salt in normal individuals results in an increase in the urine of a salt retaining compound which closely resembles aldosterone and which appears to be adrenocortical in origin. Until this discovery, only circumstantial evidence has supported the view that, with inadequate intake, the renal conservation of salt is accomplished by an increase in tubular reabsorption which is mediated by an adrenocortical hormone. This indirect evidence includes the histological studies of Deane, Shaw & Greep (1948) in rats and of Peschel & Race (1954) in human subjects besides observations upon the tubular reabsorption of salt by Black, Platt & Stanbury (1950) and upon the excretion of potassium by Leaf & Couter (1949) and Renwick, Robson & Stewart (1955).

Previous attempts to demonstrate an increase in urinary corticoids using nonspecific chemical methods under conditions of salt deprivation have not been successful. No significant change in the excretion of formaldehydogenic substances was noted by Daughaday & MacBryde (1950) or by Lloyd (1952) under these conditions. Both of these groups of authors used methods which determined only free steroids, or at best a small proportion of conjugates. More recently, using Archibald's chromatographic technique, Genest (1954) failed to find an increase in any of seven fractions after withdrawal of sodium chloride from the diet. It is now generally believed that the great potency of the natural salt retaining hormone and the fact that it is apparently present in urine in extremely small amounts are responsible for the failure of the crude methods of assay to detect any change in excretion (Simpson & Tait, 1953).

The development of Tompsett's (1953) method, however, which claimed to determine acid stable formaldehydogenic steroids in urine justified a reinvesti-
igation of the problem regarding the possibility of a general increase in adrenocortical activity following the withdrawal of salt, in contrast with and in addition to the increase in secretion of minute amounts of highly potent specific salt retaining hormone reported by Luetscher & Johnson (1954) to occur in such circumstances.

**PLAN OF EXPERIMENTS**

Observations are reported upon the urinary excretion of adrenocorticoids in normal and hypertensive adult subjects. The former included two subjects who were not suffering from any disease and four patients who had been admitted to the hospital for minor complaints and had recovered before the investigations began. The hypertensive subjects all had sustained diastolic pressure above 110 mm. Hg. but showed no signs of renal or cardiac failure and were not overweight.

Fuller details of the subjects and of the diets used are given by Renwick, Robson & Stewart (1955) and need not to be repeated here. It suffices to state that the diet provided each subject with a constant and adequate intake of calories, protein and potassium, the actual amounts varying, however, from subject to subject. This diet contained 8 m. eq. per day of sodium chloride and during the control period was supplemented by the addition of 3-6 gm. of sodium chloride per day. This control period was continued for 7-14 days after the urinary excretion of sodium and potassium became constant and during this time 24-hour collections of urine were analysed for sodium, potassium, nitrogen and adrenocorticoids. Thereafter administration of the extra sodium chloride ceased and the urine collection and analysis continued.

**Method for the determination of urinary corticosteroids and its significance.**

The recently published method of Tompsett (1953) with its slight modification (Tompsett & Smith, 1954) was used for the determination of urinary corticosteroids in twenty four hour collections of urine prior to and after salt withdrawal. This method involves the oxidation of an extract of acid-hydrolysed urine with periodic acid and subsequent estimation of the formaldehyde obtained by distillation of the extract. In case No. 2 of the normal group and case No. 3 of the hypertensive group (Figs. 1 and 2) the oxidation took place at boiling point, as described in Tompsett's (1953) original paper. In the later experiments the modified procedure was used (Tompsett & Smith, 1954), oxidation being affected at room temperature over a period of 12 to 18 hours and the reaction being terminated by the addition of 2 ml. 10% stannous chloride solution to the reaction flask. Both procedures give figures which change in the expected direction with known alterations of adrenocortical activity, but the modified procedure excludes
Normotensive subjects. Twenty-four hour urinary excretion of potassium and ASFS prior to and following withdrawal of salt from the diet. The horizontal lines represent the mean excretions during the control periods. In cases No. 2 ASFS was estimated using Tompsett's original method which involved oxidation by periodic acid at boiling point. The modified procedure in which oxidation by periodic acid is allowed to occur at room temperature was used in all other cases.

Hypertensive subjects. Twenty-four hour urinary excretion of potassium and ASFS prior to and following withdrawal of salt from the diet. The horizontal lines represent the mean excretions during the control periods. In cases No. 3 ASFS was estimated using Tompsett's original method which involved oxidation by periodic acid at boiling point. The modified procedure in which oxidation by periodic acid is allowed to occur at room temperature was used in all other cases.
certain artefacts — hydrolytic products of facts etc. — which the original was likely to include.

The application of this non-specific method to the problem under investigation requires preliminary comment. The method differs from others which also depend ultimately on the estimation of formaldehyde, in that the urine is subjected to a hydrolysis with strong acid prior to estimation. The method is to be regarded simply as determining acid stable formaldehydogenic steroids (ASFS) without specifying their chemical nature (Tompsett & Smith, 1954).

Tompsett (1953) himself has supplied evidence that, using his technique, the amount of ASFS excreted in the urine per twenty four hours does not vary greatly from one individual to another. The original procedure gave a mean daily excretion of 5.9 mg. per day with a range of 4.5 mg. to 7.5 mg. For seven normal persons with fifteen measurements of daily excretion we found an average of 6.4 mg. ASFS per day with a S. D. of ±1.8 mg. These standards, therefore, apply to case No. 2 of the normotensive group (Fig. 1) and to case No. 3 of the hypertensive group (Fig. 2). In the other subjects reported here, the cold oxidation method (Tompsett & Smith, 1954) was used and with this we have found in nine normal subjects (42 daily estimations) a mean excretion of 3.3 mg. ASFS per day with S. D. ± 0.95 mg. An estimate of the day to day variation to be expected in any one individual has been obtained by calculating the standard deviation of all the determinations prior to the withdrawal of salt expressed as a percentage of the mean values. Employing the cold oxidation method the standard deviation for four normal subjects is ±16% (17 determinations) and for the two hypertensive subjects is ±28% (20 determinations). Corresponding figures using the hot oxidation procedure are ±18% (1 normal case, 9 determinations) and ±23% (1 hypertensive case, 9 determinations). Obviously the day to day variation is the same whichever analytical procedure is employed.

The evidence that determination of ASFS provides an index of adrenocortical activity is inevitably circumstantial, as it must be with all such methods. Thus, the daily output of ASFS is decreased in Addison's disease and in panhypopituitarism. The excretion of ASFS is increased following administration of Corticotrophin to normal subjects and to patients with panhypopituitarism, in patients with adrenal hyperplasia, and also during the period following surgical operation when disturbances of water and electrolyte balance as well as the measurement of 17-hydroxy corticosteroids (Sandberg, Eik-Nes, Samuels & Tyler, 1954) suggest the existence of increased adrenal secretory activity. In one severe case of Addison's disease, Corticotrophin did not produce an increase in ASFS excretion although desoxycorticosterone did. We have made no observations which suggest an alteration in the ASFS output without changed adrenal activity (or the administration of certain steroids).

With proper reservations, therefore, it is justifiable to consider an increase in ASFS excretion as directing attention to a probable increase in adrenocortical activity.

**RESULTS**

A preliminary note of the results obtained by applying the method of Tompsett to the urine of two subjects from whose diets sodium chloride was suddenly withdrawn has been published (Stewart, Robson & Tompsett, 1952). These estimations have now been performed on consecutive 24 hour urine collections in three hypertensive subjects and in five normal individuals. The results of
all these determinations are shown in Figs. 1 and 2, and are expressed as mg. of desoxycorticosterone simply because this was used as the standard. The urinary excretion of potassium which has been previously described by the authors (Renwick, Robson & Stewart, 1955) is also shown in the figures for these subjects. Small but appreciable increases in the total amount of acid stable formaldehydogenic substances are seen to occur in two of the three hypertensive subjects (subjects No. 3 and 5) and in four of the five normal cases (subjects No. 2, 3, 4, 5) for some days following withdrawal of salt from the diet. The rise is detectable in from 1 to 6 days after restriction of salt and lasts for 3 to 15 days when the ASFS returns to control levels. Though there is some variability in the 24 hour determinations in the 4 to 10 day control period, the values obtained for a number of days in the period immediately following salt withdrawal are consistently above the mean control levels as represented by the horizontal line. The tendency was not observed in the remaining two subjects (hypertensive No. 6 and normotensive No. 6) though high values for single days were obtained in both of these in the salt restriction period.

In general, as may be seen from the figures, the temporary rise in the urinary excretion of ASFS corresponds in time to the temporary rise in the urinary excretion of potassium, both phenomena occurring while sodium in the urine is rapidly decreasing in amount.

**DISCUSSION**

It is now widely recognised that no existing chemical method is capable of determining accurately, in urine, individual adreno-steroids. This, however, is of little consequence when a given chemical procedure is employed merely to provide an index of adrenocortical activity, if it can be shown that known variations in secretory activity are paralleled by corresponding variations in the index. Demonstration of such parallelism is necessary even when determinations of individual known steroids are in question, for a change in the urinary output of a given cortical hormone (or one of its metabolic products) could be due (a) to altered production of the hormone, (b) altered metabolism of the hormone at some site other than the adrenal cortex or (c) altered renal excretion.

It cannot, of course, be claimed that an apparent absence of change in the amount of adrenocorticoids excreted (by whatever method they are determined) indicates an absence of change in adrenocortical activity. Hence nothing can be deduced from negative results. Indeed the danger of interpreting such results as showing constancy of adrenal activity in the face of changes in salt intake is emphasised by Luetscher & Johnson's (1954) demonstration, in these circumstances, of an altered rate of excretion of a salt retaining hormone resembling...
Aldosterone. Nevertheless it is not safe to assume that a small increase in the excretion of a salt-retaining hormone present only in minute amounts is the only result of salt restriction. The evidence given here suggests that there is a general response of the adrenal cortex to salt restriction. From the observations upon the excretion of potassium and ASFS this response appears to be temporary and to correspond in time to that period during which the body is correcting a large negative balance of sodium and chloride. There is no evidence of increased adrenal cortical activity after this adjustment has been accomplished when the body once more approximates to a balanced state in regard to sodium and chloride.

The published observations of Luetscher & Johnson (1954) do not provide data to compare the duration of the response of the increased urinary excretion of potassium and ASFS with the salt retaining compounds detected by bioassay. Observations providing such data would be a valuable means of determining the relationship, if any, between the appearance in the urine of increased amounts of ASFS and of the salt retaining hormone.

SUMMARY

(1) Observations upon the urinary excretion of adreno-corticoids, employing the method of Tompsett, have been made in normal and hypertensive subjects.

(2) The validity and significance of this method have been discussed in relation to a) other methods previously used in experiments involving dietary salt restriction, b) the nature of the acid stable formaldehydogenic steroids (ASFS) estimated and c) known changes in adrenocortical activity.

(3) A small but appreciable increase in the urinary excretion of acid stable formaldehydogenic steroids occurred in six out of eight subjects 1-6 days after salt restriction, corresponding in time to the increased urinary excretion of potassium previously reported by the authors.

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REFERENCES

ADDENDUM

A note may conveniently be added concerning criticisms by Marrian & Atherden (1953) of the method of Tompsett (1953) and, of the use of it by Stewart, Robson & Tompsett (1952). The latter criticism was based on a misconception—the adjective »specific« was taken as qualifying »hormone« whereas it was, as the present paper makes clear, intended to qualify »response«; it was claimed that withdrawal of sodium provided the actual (or specific) stimulus to increased adrenocortical activity but not that it stimulated the secretion of any one hormone.

The criticism of the method was founded on Tompsett’s (1953) claim, made as a deduction from his recovery experiments, that his method measured those steroids without hydroxyl substitution at C-17. This claim was later withdrawn by Tompsett & Smith (1954) when they merely termed the substances estimated »acid-stable formaldehydogenic steroids«.

However, the claim is not so completely erroneous as the criticisms of Marrian & Atherden (1953) would suggest. These authors point out, and we have confirmed, that when cortisone or desoxycorticosterone is added to urine, the increase in the ASFS figure corresponds to a recovery of about 40 % of the former and about 80 % of the latter. Recoveries from the urine of individuals given esters of these two compounds orally or parenterally are, however, very different. In six subjects given 300 mg. of cortisone acetate orally or parenterally an increase in the urine of ASFS equivalent to 2–5 % of the dose given was found. This figure agrees with that observed by Marrian & Atherden in one case of rheumatoid arthritis. When, however, 11-desoxycorticosterone was given either as acetate (10 mg. intramuscularly) or as gluconide (40 mg. intravenously) the increase in ASFS excretion during the subsequent 24 or 48 hours corresponded to 75–100 % of the steroid given. Quite clearly Tompsett’s method does not distinguish absolutely between the excretion products of these two steroids but the results may be interpreted as indicating that (a) the metabolic
products of administered cortisone esters excreted in the urine are predominantly such as to escape detection by Tomsett's procedure, (b) the excreted products of desoxycorticosterone ester metabolism are predominantly of the type included in the ASFS.

Further work is required to define the chemical identity of the urinary metabolites included in the ASFS but meanwhile it is fair with the reservations necessary with any non-specific method, to use ASFS determination as an index of adrenocortical activity.