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William H. Daughaday, Cyril M. MacBryde

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RENAL AND ADRENAL MECHANISMS OF SALT CONSERVATION: THE EXCRETION OF URINARY FORMALDEHYDOGENIC STEROIDS AND 17-KETOSTEROIDS DURING SALT DEPRIVATION AND DESOXYCORTICOSTERONE ADMINISTRATION ¹

BY WILLIAM H. DAUGHADAY AND CYRIL M. MACBRYDE

(From the Department of Internal Medicine, Washington University School of Medicine, and the Barnes Hospital, St. Louis)

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The preservation of normal sodium and potassium homeostasis requires the presence of the adrenal cortex. In man, as well as in many other animals, the removal or destruction of the adrenal cortices has resulted in a rapid depletion of the body sodium and an apparent retention of potassium, provided that replacement therapy either with additional sodium intake or with potent adrenal steroids is not instituted. The importance of the adrenals in electrolyte metabolism was further suggested by the isolation and identification of desoxycorticosterone from beef adrenals (1). The ability of this substance to correct many of the electrolyte disturbances of adrenal deficient animals and man has led to the hypothesis that it or similar steroids are secreted by the adrenal under conditions of sodium deficiency. Also, the demonstration of excessive retention of sodium following large doses of desoxycorticosterone has suggested that an adrenal hormone might be responsible for the retention of salt and water in pathologic conditions such as congestive heart failure and eclampsia (2).

Rather than study adrenal participation in disorders of sodium metabolism in disease states, we have attempted to determine whether the adrenal regulates the physiologic reabsorption of sodium by the renal tubule in a manner analagous to the control of water reabsorption by the pituitary antidiuretic hormone. In the latter case, it has been possible to detect increased amounts of antidiuretic hormone in body fluids following dehydration or experimental procedures increasing the osmotic pressure of the blood (3).

The mechanism and site of action of adrenal steroids on the renal tubule remains obscure. According to the concept of Wesson and Anslow (4), sodium is actively reabsorbed both in the proximal and distal tubules. In the proximal tubules, the reabsorption of approximately seven-eighths of the filtered sodium is accompanied by an equivalent amount of water, thus maintaining the osmotic equilibrium of the tubular urine. In the distal tubules the final and critical independent adjustments of sodium and water reabsorption take place. These authors believe that sodium excretion is largely determined by the quantity of sodium filtered and they minimize the importance of the adrenal cortex in determining tubular sodium reabsorption. Although there is general agreement concerning the existence of adrenal steroids promoting the retention of sodium by the tubule, the evidence is conflicting concerning the method of their liberation and their importance in total tubular activity. Considerable histologic evidence has been presented by Deane, Greep and their associates (5, 6) suggesting that there is a functional division of the rat adrenal; hormones active in carbohydrate and protein metabolism were ascribed to the zona fasciculata, while hormones active in sodium and potassium metabolism were ascribed to the zona glomerulosa. Evidence was presented that the latter zone is relatively immune to the atrophy following hypophysectomy and that stimulation of the adrenal with adrenocorticotrophic hormone (ACTH) is largely limited to the zona fasciculata. Restriction of sodium in the diet resulted in secretory changes in the cells of the zona glomerulosa while these changes were reversed by sodium chloride or desoxycorticosterone acetate (DCA) administration.

¹ This investigation was supported by a contract with the Office of Naval Research, Washington, D. C. Patients 1, 2 and 4 were hospitalized during a course of an investigation of hypertension supported by the U. S. Public Health Service.

This dualism of adrenal function has been challenged by a number of investigators who have studied electrolyte metabolism following the administration of ACTH to humans (7-9). Frequently such treatment has resulted in retention of sodium in addition to other evidence of adrenal These electrolyte changes have hyperactivity. been associated, when measured, with increased excretion of urinary corticoid and 17-ketosteroid substances. The response in the rat differs in that sodium is not retained (10).

Hypothetical mechanisms regulating the rate of sodium reabsorption are presented in Figure 1. According to the "unitarian" view, the physiologic stimulus for sodium conservation acts through the anterior lobe of the pituitary to promote increased liberation of ACTH, which in turn stimulates the adrenal to produce increased amounts of "salt" hormone in addition to other physiologically active principles indicated in the diagram by the terminology of Albright and Browne as "S" and "N" hormones.

Support for the unitarian theory of adrenal salt hormone liberation has appeared in the studies of Leaf and Couter (11). They have studied three normal subjects during sodium restriction and sodium administration. Associated with sodium restriction there appeared a negative nitrogen balance. Readministration of sodium resulted in a positive nitrogen balance. These changes occurred not only in urea but also in the excretion of uric acid, potassium and phosphorus. The au-

thors have suggested that these effects on protein metabolism represent alteration of adrenal cortical activity in respect to the "protein catabolic hormone" and the secretion of this hormone is linked to the secretion of the "salt-and-water" hormone. The authors do not present assays of adrenal metabolites in support of their contention that the observed negative nitrogen balance represented increased adrenal activity.

The "dualistic" view postulates an independent release of an adrenal "salt" hormone believed to arise from the zona glomerulosa in response to the stimulus of sodium deficiency.

Although it is agreed that normal tubular reabsorption of sodium requires the presence of adrenal steroids, it is possible that fluctuations of sodium reabsorption under most conditions are controlled by renal mechanisms and not by fluctuation of adrenal activity. This concept has been called an "idiorenal" mechanism. The rapid increase in sodium reabsorption following compression of the renal vein which occurs while renal filtration rate and blood flow are not markedly changed suggests such a mechanism (12). Also, Ingle (13) has observed that adrenalectomized rats maintained on a constant daily dose of adrenal cortical hormone possess kidneys capable of a wide range of activity in the excretion and conservation of sodium.

The studies to be presented have been designed, 1) to compare adrenal steroid excretion under conditions of extreme dietary sodium restriction

C. IDIO-RENAL



B. DUALISTIC

FIG. 1. DIAGRAM SHOWING THEORETICAL MECHANISMS OF SODIUM REABSORPTION

THEORIES OF SODIUM REABSORPTION

with that of liberal salt intake; 2) to determine the potency of desoxycorticosterone in depressing adrenal activity; and 3) to compare physiologic sodium conservation with the sodium retention produced by DCA and ACTH.

METHODS

All subjects were hospitalized on a metabolic ward permitting close supervision of fluid intake, diet, activity and the collection of the necessary specimens. They were ambulatory and took part in such mild activities as walking, making beds, carrying trays, etc. During an experimental period, the diets were as uniform as possible in caloric content and the proportion of fats, carbohydrate and protein. Caloric intakes were limited for therapeutic reasons: L.S. received 1,000 calories per day; M.L., 800 calories except for days 61 through 79 when her diet contained 1,600 calories daily; N.S. and F. B. received 1,200 calories. The sodium content of the diet was carefully calculated from standard tables (14). The preparation of low sodium diets was facilitated by the use of "Lanolac." The uniformity of potassium intake could not be maintained but analyses of the diets using standard tables showed that it was relatively constant.

Fluid intake was measured for each patient. Intake was maintained at a constant level for the studies of M.L., N.S. and C. M. The volumes of liquid foods and drinking water were only considered in the calculation of fluid intake. The patients were weighed daily before breakfast. Urine collections were made for 24 hour periods in carefully cleaned bottles with 10 cc of toluol as a preservative. After the collection was completed, they were kept at ice-box temperature until extractions were completed. Delay in extraction seldom exceeded three days.

Sodium and potassium determinations have been performed using a Weichselbaum and Varney (15) atomizersystem burner which has been adapted for use with a Beckman monochromator (model DU spectrophotometer). Analyses were done in duplicate or triplicate by bracketing the unknown between standard sodium and potassium solutions. The concentration of unknown was obtained by interpolation. Comparisons were made with standard gravimetric and volumetric estimations for sodium and potassium and these results were well within the limits reported by Weichselbaum and Varney. Blood for sodium and potassium determinations was drawn in heparinized syringes and the plasma separated promptly.

The determinations of urinary formaldehydogenic steroids, *i.e.*, "cortin," were performed by a method previously described (16). The method depends on the quantitative measurement of the formaldehyde liberated by the oxidation of partially purified urine extracts with periodic acid. The formaldehyde arises from the dihydroxy-acetone or ketol side chain characteristic of corticosteroids. Partitioning between benzene and water has been retained because it prevents artifacts which may be introduced from contaminants in redistilled chloroform and possibly from other sources. In most instances, the benzene fraction has also been measured and generally contained little formaldehydogenic substances. Results of assays on the benzene fraction will not be presented because of the questionable reliability of the data. The original method has been modified by the introduction of washes with 0.1 N sodium hydroxide to remove most of the pigments and other non-corticoid material present in the extract. The washes were all back-extracted. The concentration of periodic acid used in the oxidation step has been increased to 0.03 M and that of the sulfuric acid in this reagent to 0.3 M as suggested by Corcoran and Page (17). In place of the arbitrary "cortin" unit of the original publication results have been expressed in terms of the weight of desoxycorticosterone without the implication that such a substance is actually measured. Normal values generally range from 0.75 to 1.5 mg per day under basal conditions.

It is realized that this method is not specific for active adrenal steroids. Any compound with a free, terminal vicinal glycol or dihydroxy-acetone group which passes through the extraction procedure will liberate formaldehyde upon periodic acid oxidation. By comparison with biological assay (18) only a small portion of the material so measured represents active hormone. The remainder of the formaldehydogenic material consists of presumably inactive adrenal metabolites and some non-specific compounds. The justification for the use of this method depends upon the fact that it has given reasonable results in known adrenal disorders and that administration of ACTH greatly augments the excretion of formaldehydogenic steroids.

Measurements of 17-ketosteroids were made using the Holtorff and Koch modification of the Zimmermann reaction. We have used the simplified extraction and assay procedure used at the McGill University Clinic (19). Ketonic separation was not performed and only partial correction of non-ketonic chromogens was attempted using a KOH blank.

RESULTS

Case study 1, N.S.: This subject, a housewife, age 49, was selected for prolonged study because despite a questionable history of abdominal swelling and ankle edema, intensive tests of liver, cardiac and renal function were entirely normal. For this reason, she may be considered normal for the purpose of this study. The study was designed to compare adrenal steroid excretion during sodium restriction with that during high sodium intake. We also wished to assess the degree of competency of renal adjustment to the altered conditions. Finally, the effect of administration of DCA on the excretion of formaldehydogenic steroids and 17-ketosteroids and its ability to promote sodium retention in this subject were observed.



The solid horizontal line across the sodium excretion indicates the level of dietary intake.

The results of these studies are presented in graphic form in Figure 2. During the initial 12 days the diet contained 8 mEq of sodium and excretion of formaldehydogenic steroids and 17ketosteroids showed little fluctuation. The value for formaldehydogenic steroids is probably normal while that for 17-ketosteroids is below the usually accepted normal value. Renal excretion of sodium closely approximated intake early in the study because sodium had been restricted prior to the study. Following the increase of dietary sodium from 8 mEq to 205 mEq there was no appreciable change in the excretion of urinary formaldehydogenic steroids and 17-ketosteroids. The renal excretion of sodium rapidly rose, suggesting that there was little or no carry-over of a sodium conserving mechanism into the period of liberal

salt intake. DCA² administration did not depress the excretion of formaldehydogenic steroids although it did cause a great reduction in urinary sodium excretion.

Case study 2, M.L.: This subject, a housewife, age 46, had had hypertension for two and a half years. However, renal function and cardiac function were not impaired. She was selected for study because it had previously been demonstrated that her blood pressure fell significantly on sodium deprivation (20) and that there were physical signs and laboratory data suggesting a possible adrenal factor in her hypertension. Observations on this patient covered a period of three months. The plan of the experiment was to com-

² Kindly provided by Dr. Edward Henderson of the Schering Corporation, Bloomfield, N. J.

pare adrenal activity during sodium restriction with that during sodium abundance. Desoxycorticosterone acetate was administered both during the period of sodium deprivation and during sodium abundance. The results are presented graphically in Figure 3. For the first 14 days of the study, a diet containing 3 mEq of sodium was given. The virtual absence of sodium from the urine early in the study was again due to the fact that the patient had been on a low sodium diet before the study. The efficiency of the kidney in reducing the quantity of urinary sodium was excellent as evidenced by the fact that urine sodium during this period averaged less than 1 mEq per day. There was no evidence of increased adrenal activity judged by the steroid excretion during this period. The patient received DCA during days 15 through 29 to study the changes which might be produced by "exogenous salt hormone." The excretion of urinary formaldehydogenic steroids and 17-ketosteroids was not affected by DCA. Observations of sodium excretion, fluid balance and plasma sodium and potassium levels are presented in Figure 4. Urine sodium excretion remained negligible during DCA treatment and there was no increase in excretion after its cessation. A rise in plasma sodium occurred with little alteration of plasma potassium during DCA administration.

To demonstrate that the patient's adrenals could respond to normal stimulation, she was given ACTH⁸ for three days in daily doses of 22 mg, 16 mg, and 16 mg in divided doses expressed in terms of Armour's standard. A significant increase was observed in the excretion of both urinary formaldehydogenic steroids and 17-ketosteroids, Figure 3.

⁸ Kindly made available by Dr. John Mote of the Armour Laboratories, Chicago, Ill.



FIG. 3. OBSERVATIONS ON THE URINARY EXCRETION OF FORMALDEHYDOGENIC STEROIDS—"CORTIN," 17-KETOSTER-OIDS AND URINARY SODIUM IN CASE STUDY 2

The solid horizontal line across the sodium excretion indicates the level of dietary intake.

The sodium intake was suddenly increased from 3 mEq to 205 mEq per day on the 45th day of this study. As shown in Figure 4, there was a remarkable carry-over of sodium conservation which produced a sodium retention far in excess of any possible deficit accrued by sodium restriction. The concentration of serum sodium rose to 147 mEq per liter. The retention of sodium was accompanied by oliguria which partially protected the osmotic equilibrium of the body fluids. Clinically, during this period the patient complained of headache, lethargy and extreme thirst and despite peripheral edema the mucous membranes were dry. Ability to excrete sodium was regained after six to eight days and the patient entered a new electrolyte equilibrium. DCA was again administered from the 69th through the 78th day to determine whether depression of adrenal function by DCA required a liberal salt intake. Again, we were unable to detect significant changes in the urinary steroids measured despite the fact that there was a considerable retention of sodium.

Determinations of sweat sodium were performed on this patient by Dr. Dean F. Davies of the Hypertension Division. In comparison to figures published by Conn (21), the values given at the bottom of Figure 4 are low during the period of salt restriction, more normal during the high salt period and again low during DCA administration.

Case study 3, F.B.: This subject was a housewife, age 21, who had Cushing's syndrome manifested by obesity, mild hirsutism, purple striae, amenorrhea, plethoric appearance and mild hypertension for three years. She had been a subject



FIG. 4. CHANGES IN SERUM SODIUM, AND POTASSIUM LEVELS AND IN FLUID AND SODIUM BAL-ANCE FOLLOWING CHANGES IN SODIUM INTAKE AND ACTH AND DCA ADMINISTRATION IN CASE STUDY 2

The fluid and sodium intakes are plotted down from the zero level. Values for sweat sodium are at the bottom of the figure. The level of plasma sodium concentration is given by the solid line, of plasma potassium concentration by the broken line.



FIG. 5. CASE STUDY 3, CHANGES IN THE EXCRETION OF FORMALDEHYDOGENIC STEROIDS ("CORTIN") AND 17-KETOSTEROIDS, AND SODIUM INTAKE IN CUSHING'S SYNDROME WITH DCA ADMINISTRATION

for a previous investigation of salt metabolism from this laboratory (22). The present studies were designed to determine whether salt restriction would increase adrenal steroid excretion when there was already adrenal hyperactivity and whether DCA is a potent agent in lowering the elevated corticoid excretion in Cushing's syndrome. Results of hormone assays are presented in Figure 5. A normal salt intake of 154 mEq per day was provided from day 4 through day 10. Dietary sodium was then reduced to 8 mEq per day for the remainder of the study of 29 days. The excretion of formaldehydogenic steroids and 17-ketosteroids was elevated and showed a remarkable cyclic variation. These changes seemed To determine independent of dietary sodium. whether DCA was able to inhibit adrenal activity, the levels of formaldehydogenic steroids and 17ketosteroids without DCA have been averaged together and compared with the days under treatment. The mean of the formaldehydogenic steroids was 3.85 during the control period and 3.05 during the period of DCA treatment. The differences were not statistically significant. The mean excretion of 17-ketosteroids during the control period was 52.7 mg per day (SD 21) while during the DCA period it was 30.5 (SD 11). The difference in the means in this case appeared to be statistically significant despite the great variability of the data.

Case study 4, L.M.S.: This subject, a school teacher, age 47, had known of an elevated blood pressure for one and a half years. She had been very obese at this time and had lost 100 lbs during the course of about one year. She had responded well to sodium restriction with a drop in blood pressure and at the time of study her blood pressure averaged about 160 mm of Hg systolic and

100 mm diastolic. There was little measurable impairment of renal function. In addition, the patient had a severe mixed psychoneurosis with manifestations of anxiety and depression. She was selected for study of possible adrenal participation in her hypertension because of her response to sodium restriction (20). The patient was placed on a diet restricted in sodium, and urine sodium determinations varied from about 10 to 40 mEq per day. The excretion of urinary formaldehydogenic steroids by this patient was above normal in this laboratory. An attempt was made to inhibit adrenal activity by the administration of DCA. The results of these studies are presented in graphic form in Figure 6. Rather than a decrease, periods of greatly increased excretion of formaldehydogenic steroids resulted. It was believed that this was due to some extraneous factor and an attempt was made to correlate the peaks of excretion with the degree of anxiety and agitation which the patient exhibited but without great success. Possibly contributing to elevated excretion was the polyuria which this patient maintained. Increased excretion of corticoids has been produced in normal subjects by extreme water diuresis (23).

Case study 5, C.M.: This subject, a housewife, age 39, suffered from rheumatoid arthritis of moderate activity. Despite the brilliant therapeutic results of Hench and his colleagues (24), existing evidence indicates that adrenal function is probably normal in this disease. Observations on this patient are presented in Figure 7. After three days on an uncalculated ward diet, she was changed to a diet containing 8 mEq of sodium daily. On this diet there was a progressive decrease in urine sodium without change in the blood sodium concentration. Adrenal activity, as measured by steroid excretion, did not increase during this period. As in case study 2, we wished to



FIG. 6. CASE STUDY 4, EXCRETION OF FORMALDEHYDOGENIC STEROIDS ("CORTIN") AND 17-KETOSTEROIDS WITH DCA Administration



FIG. 7. CASE STUDY 5, CHANGES IN THE EXCRETION OF FORMALDEHYDOGENIC STEROIDS—"CORTIN," 17-KETOS-TEROIDS, SODIUM BALANCE AND BODY WEIGHT WITH SALT RESTRICTION AND ACTH THERAPY

Sodium intake is plotted down from the zero line.

demonstrate that here adrenals were responsive to small doses of ACTH. She was given 25 mg of ACTH daily in two divided doses for three days. There was a measurable increase in both types of steroids. Sodium excretion was actually increased on the first day of ACTH treatment. Dietary sodium intake was then raised to 205 mEq daily to which she accommodated rapidly, as evidenced by the rapid rise in urinary sodium excretion. On the fourth day of this diet, she was started on 100 mg of ACTH daily which was continued for five days. The dose of ACTH was then reduced to 50 mg daily and continued for five additional days. On this treatment there was a great rise in urinary formaldehydogenic steroids and 17-ketosteroids. Serum potassium fell as low as 2.7 mEq per liter. There was considerable retention of sodium during the treatment period

which was followed by a prompt sodium diuresis following the cessation of ACTH therapy.

DISCUSSION

From these studies, it appears that salt deficiency does not activate the ACTH-adrenal system as evidenced by the failure to observe an increased excretion of urinary formaldehydogenic steroids and 17-ketosteroids. While this criterion of adrenal activity may not be dependable, it has been demonstrated here and elsewhere that relatively small amounts of ACTH will provoke increase in the excretion of these steroids. It is possible that more drastic conditions of sodium depletion with or without potassium administration might be required to provoke increased excretion of these steroids.

Our studies throw little light on the possibility of the dualistic theory of adrenal function. The evidence for this view has been histologic and has been reviewed earlier in this paper. Fractionation studies of whole adrenal glands suggest that the amount of desoxycorticosterone contained must be very small. Most of the potency of the cortical extracts in the maintenance of electrolyte metabolism appears to reside in a fraction which is highly water soluble, *i.e.*, the amorphous fraction, and thus differs considerably from desoxycorticosterone. The remainder of the electrolyte activity probably can be explained by the amounts of corticosterone and dehydrocorticosterone present. From these data, there is little reason to suppose that we can recognize the excretion of an adrenal "salt" hormone by looking for compounds with the physical properties of desoxycorticosterone.

The method of assay used in this study of formaldehydogenic steroids measures only a fraction of total adrenal activity and is based on physical and chemical properties rather than upon biological potency of the substances secreted by the adrenal cortex. Recently Lloyd and Lobotsky (23) have demonstrated increased amounts of formaldehydogenic steroids in the benzene fraction of urinary residues subjected to benzene/water partition following DCA administration to patients with Addison's disease. Our failure to find increased amounts of formaldehydogenic steroids in this fraction following sodium restriction suggests that this will not prove to be a criterion of adrenal "salt" hormone activity. In view of the reports of a reduction in the size of the adrenal cortices in rats following DCA administration (25, 26) and its ability to prevent the release of ACTH when given in sizeable doses (27), the inability of DCA to depress the excretion of formaldehydogenic steroids and 17-ketosteroids is disappointing. In only one case (F.B.) was there any suggestion of decreased adrenal activity and that was only in the 17-ketosteroids. Larger doses probably must be given to affect adrenal steroid excretion and at the present time this is inconvenient. Our results are in agreement with those of Talbot (28) who could not lower the excretion of 11-oxysteroids with daily doses of 20 mg of DCA daily.

We have confirmed the reports of others that sodium retention follows large doses of ACTH (7-9). Smaller doses of ACTH may actually produce a sodium diuresis. It seems likely that the net effect of ACTH on sodium metabolism is a function of dose and salt load. It is difficult to assess the physiologic importance of sodium retention following activation of the ACTH-adrenal cortex system. The degree of adrenal stimulation with ACTH administration which has resulted in sodium retention probably occurs spontaneously only under conditions of severe stress. It is possible that some of the impairment of sodium excretion following severe trauma can be explained on this basis.

SUMMARY AND CONCLUSIONS

Adrenal function was measured by the excretion of formaldehydogenic steroids ("cortin") and by 17-ketosteroids in five patients over periods of from one to three months in order to determine the importance of the adrenal in the physiologic conservation of salt under conditions of salt deficiency. Our studies gave no indications of increased ACTH activity or of increased adrenocortical secretion during salt deprivation. This physiologic salt conservation has been compared to the increased sodium reabsorption which follows the administration of desoxycorticosterone acetate and large doses of adrenocorticotrophic hormone. The possibility exists that there is an adrenocortical "salt" hormone, the release of which is independent of ACTH and which is not measured by existing methods. Alternatively, an "idiorenal" mechanism can explain the existing data whereby the kidney provided with a requisite constant supply of adrenal hormone is able to regulate the excretion of sodium by purely intrinsic processes.

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