Aldosterone Excretion

PHYSIOLOGICAL VARIATIONS IN MAN MEASURED BY RADIOIMMUNOASSAY OR DOUBLE-ISOTOPE DILUTION

By J. E. Sealey, F. R. Bühler, J. H. Laragh, E. L. Manning, and H. R. Brunner

ABSTRACT

A reliable, simple radioimmunoassay for measurement of the urinary excretion rate of the acid-labile conjugate of aldosterone is described, in which aldosterone, reconstituted by acid hydrolysis of a urinary metabolite, is quantified. The results were compared with those obtained by double-isotope dilution. Using both methods, aldosterone excretion rates were characterized in normal subjects over a wide range of dietary sodium intakes. Identity of results from the two methods was demonstrated. Aldosterone excretion ranged from 18 to 85 μg/day at sodium excretion rates of less than 25 mEq/day, from 5 to 26 μg/day at sodium excretion rates between 75 and 125 mEq/day, and from 1.5 to 12.5 μg/day at excretion rates above 200 mEq/day. However, a more precise index was derived with limits inscribed by the whole curve used as a nomogram. During sodium depletion of normal subjects, aldosterone excretion reacted slowly and inadequately until a critical level of sodium or volume depletion was reached. During maintained sodium loading for up to 11 days, initial sodium retention was followed by natriuresis and then by slight sodium retention. Mean urinary aldosterone during these three periods was 23.4, 3.6, and 4.5 μg/day, respectively. Urinary sodium excretion exhibited a more consistent relationship to aldosterone activity than did either sodium intake or cumulative sodium balance, even when constant dietary regimens were used. These data suggest a dynamic role for aldosterone in governing renal sodium excretion, even at very high levels of sodium excretion.

KEY WORDS antibodies to steroids sodium depletion natriuresis comparison of methods for measuring aldosterone sodium excretion sodium balance aldosterone 3-oxo-conjugate

Determinations of aldosterone secretion and excretion rates and of plasma aldosterone levels were made for a number of years by double-isotope dilution (1–6), involving a time-consuming, tedious, expensive set of procedures which permitted the measurement of relatively few samples. The development of antibodies to steroids conjugated with albumin (7) offered a new, inherently simple approach to measurement of aldosterone which used radioimmunoassay. Several such methods for the measurement of plasma aldosterone have been published (8–11), and two reports considered the application of this technique to the measurement of urinary excretion or secretion rates (12, 13).

The present study was designed to evaluate the reliability and precision of a radioimmunoassay procedure as compared with the more established technique of double-isotope dilution. To this end, a radioimmunoassay method based on slight modifications of the method of Wahlen and co-workers (13) was developed and applied to normal subjects who were studied over a wide range of physiological variation. This design also afforded an opportunity to examine in more detail the well-known effects of sodium depletion on aldosterone excretion. In addition, it enabled a
much needed analysis of daily changes in aldosterone excretion as they might occur in response to higher sodium intakes.

The results obtained validated the radioimmunoassay as a reliable, simpler alternative method, and they further characterized aldosterone excretory responses to changes in sodium balance. In particular, the data suggested a dynamic role for the hormone in governing renal sodium excretion, even at very high levels of sodium intake.

Methods

SUBJECTS
Fifty-two normal volunteers, ages 20-40 years, were the subjects of this study. Twelve were studied on the metabolic ward, 22 were not hospitalized but adhered to a constant dietary regimen, and 18 were on unrestricted diets. Most patients in the first two groups maintained a constant sodium intake for 5 days, and aldosterone excretion was measured in urine collected on the final day. Several of the volunteers were subjected to more than one dietary regimen. Three different diets were used: (1) a low-sodium diet of less than 20 mEq/day, (2) a normal-sodium diet of 100 mEq/day, and (3) a high-sodium diet of 200 mEq/day. A few subjects ate 200 mEq/day of sodium chloride in addition to the salt intake of an unrestricted random diet.

In five normal subjects who maintained a constant dietary regimen for 5 consecutive days of 3 consecutive weeks, aldosterone excretion was measured in urine collected on the final day. Several of the volunteers were subjected to more than one dietary regimen. Three different diets were used: (1) a low-sodium diet of less than 20 mEq/day, (2) a normal-sodium diet of 100 mEq/day, and (3) a high-sodium diet of 200 mEq/day. A few subjects ate 200 mEq/day of sodium chloride in addition to the salt intake of an unrestricted random diet.

In five normal subjects who maintained a constant dietary regimen for 5 consecutive days of 3 consecutive weeks, aldosterone excretion was measured daily on the last 3 days of the 5-day period. On the weekends, these subjects were on unrestricted diets.

Four additional normal subjects received 270 or 300 mEq/day of sodium for 11 consecutive days after 9 days of sodium depletion without diuretic treatment. Aldosterone excretion was measured each day.

HYDROLYSIS AND EXTRACTION
Because of the possibility of contamination, only disposable glassware was used throughout the analysis. All glassware was rinsed with methanol before use.

During collection of a complete 24-hour urine sample, the urine was stored at 4°C without addition of preservatives. The urine volume was measured, and then sodium and potassium concentrations were analyzed by flame photometry. Ten ml of urine was stored at -20°C for measurement of aldosterone.

For determination of aldosterone, the urine was thawed and, after thorough mixing, a 3-ml sample was extracted in a 17 x 150-mm glass test tube containing 15 ml of spectroanalyzed methylene chloride. The extracted urine was acidified to pH 1.0 with a minimum volume of a combination of 6N, 3N, and 1N HCl. After 24 hours, 1 ml of hydrolyzed urine was transferred (Clay Adams Selectapette) to a 17 x 150-mm glass test tube containing 2000 counts/min of [3H] aldosterone (40 mc/m mole). After 30 minutes, the urine was extracted with 15 ml of methylene chloride. The extract was washed consecutively with two 1.5-ml portions of 0.1N NaOH, 1.5 ml of 0.1N acetic acid, and 1.5 ml of distilled water.

After evaporation under nitrogen at 37°C, the dried extract was dissolved in 2 ml of ethanol, and 1.0 ml was transferred to a counting vial, dried, dissolved in 10 ml of toluene phosphor, and counted in a scintillation counter. The remaining solution was divided into two 0.4-ml samples, which were stored at 4°C until radioimmunoassay.

The radioactivity in the 1 ml of extract was compared with that in the standard [3H] aldosterone, which was added to the hydrolyzed urine, and losses due to the extraction procedure were calculated.

Radioimmunoassay

Antibodies.—Aldosterone antibodies, induced in sheep by the injection of lyophilized aldosterone-21-hemisuccinate bovine serum albumin,1 together with Freund adjuvant, have been characterized by Katz and co-workers (10). We found the cross-reactivity of other steroids to be 0.7% for corticosterone, 0.8% for cortisone, and 0.4% for hydrocortisone.

Prior to radioimmunoassay, the antibody was diluted in 0.1M Tris buffer, pH 8.0 (14). The antibody has been stored at this dilution at -20°C without loss of activity for up to 6 months. The Tris buffer, which was the same as that used throughout the radioimmunoassay procedure, was made from Trizma base (Sigma Chemical Company) containing 0.3% bovine serum albumin (Fraction V, Pentex), 0.3% lysozyme (three-times crystallized, General Biochemicals), 0.2% neomycin sulfate, and 0.125% phenylmercuric acetate. The pH was adjusted to 8.0, and the buffer was filtered before use.

Dilution of Urine Extract.—One of the 0.4-ml samples of urine was evaporated to dryness and then redissolved in 0.2 ml, 1 ml, or 2 ml of ethanol, depending on the anticipated aldosterone excretion rate and also on the 24-hour urine

1Aldosterone antibodies were donated by Dr. William Kelly. They are also commercially available from Research Plus Laboratories, Denville, N. J.
volume. The second sample was saved for a repeat of the determination, if necessary.

Incubation.—Ten and 20 μl of aldosterone standards (0.5, 2, and 8 ng/ml in 95% ethanol) were pipetted in duplicate, and both 10 and 20 μl of each unknown sample were also pipetted (Eppendorf pipette) into 12 x 75-mm glass test tubes. These samples were evaporated to dryness in a vacuum oven at 37°C. Tris buffer, 0.5 ml, at pH 8.0 containing 2000 counts/min of [3H] aldosterone and a 1:1,000,000 dilution of antibody was added to each tube. A similar mixture, 0.5 ml, was added to two pairs of duplicates, but the antibody and sample were omitted. These latter tubes were used to determine the nonspecific binding of [3H] aldosterone to protein in the buffer solution and also to measure the total radioactivity added to each tube. A third pair of tubes was set up with both antibody and [3H] aldosterone but with no added sample or standard aldosterone. This last pair of tubes determined the zero point on the standard curve. The samples were mixed thoroughly, warmed at 37°C for 10 minutes, and then incubated at 4°C for 18 hours.

Addition of Charcoal.—Using a Selectapette, 0.5 ml of 0.5% charcoal in water containing 0.05% dextran was added quickly to each tube, except the pair set up for determination of total radioactivity. The charcoal was stirred vigorously on a magnetic stirrer throughout the addition. Charcoal was added to half of the duplicates used for determining the standard curve at the beginning and to the other half at the end of the series. Water, 0.5 ml, was added to the tubes set up for measurement of total radioactivity. All of the tubes were mixed on a Vortex mixer for about 3 seconds and then centrifuged together at 4°C and 1000 g. The supernatant fluid was decanted into counting vials containing 10 ml of 20% BBS-3 Bio-solv (Beckman) in toluene phosphor, and then two drops of 15% ascorbic acid were added to each vial. After an hour for equilibration, the samples were counted for 10 minutes each.

Calculations.—The relationship of bound aldosterone to free aldosterone was calculated using a logit plot. This plot was convenient because it formed a straight line (15) (Fig. 1).

\[
Y = \frac{B}{T} = \frac{B_0}{T}
\]

where \(Y\) = bound over total ratio relative to the initial bound over total ratio, \(B = \) bound counts for each standard or sample, \(B_0 = \) bound counts for the zero point on the curve, and \(T = \) total counts added to each tube.

\[
\text{logit } Y = 2.303 \log_{10} \frac{Y}{1-Y}
\]

In practice, only \(Y/(1-Y)\) was calculated for all samples, and the values of the standard samples were plotted on log-log paper against the amount of added aldosterone. Because a log scale was used, the standards were chosen so that the amounts were approximately doubled at each consecutive point on the curve.

After the amount of aldosterone in the unknown samples was read from the curve, the amount of aldosterone in 1 ml of urine was calculated for both of the duplicates, and then the mean value was determined. Finally, the result was expressed as micrograms of aldosterone excretion per 24 hours by multiplying by the urine volume.

Quality Control.—To continually check the accuracy and the reproducibility of the radioimmunoassay, two urine samples of known aldosterone concentration were routinely hydrolyzed, extracted, and assayed with each set. The dilutions of these samples were such that between the two of them they spanned the whole range of the standard curve. If the results from either of these samples were more than ± 15% away from a previously determined mean, the whole series was discarded.

If the nonspecific binding rose above 8% of the total radioactivity, the set was discarded. Sometimes, the duplicates of the nonspecific binding were variable. This variability appeared to be caused by inconsistencies in the addition of the charcoal; when this occurred the entire set was discarded.
In the assay of individual samples, the determination was repeated if the duplicates varied by more than 15% of the mean of the two values and also if one of the duplicates fell outside the standard curve. Every result was the mean of at least two values.

Results

Radioimmunoassay

The standard curve (Fig. 1) was sensitive from 4 to 120 pg and was quite consistent from day to day. Thus, on 14 consecutive series set up on different days, the addition of 4 pg of aldosterone reduced the [3H] aldosterone binding by $6.9 \pm 3.7\%$ (SD), and 8 pg lowered it by $13.1 \pm 3.7\%$.

Twenty-five aqueous samples which contained 1.0-50 ng of aldosterone were carried through the complete hydrolysis and extraction procedure. Only the extraction before hydrolysis was omitted. The mean recovery was $110 \pm 11\%$ (SD). When a water blank was carried through the whole procedure, the values ranged from 0 to 6 pg.

Comparison of Radioimmunoassay and Double-Isotope Dilution

Table 1 presents data from multiple assays of five different urine samples which were passed through the complete hydrolysis and extraction procedure. Mean values of the results ranged from normal (9.8 μg/day) to high (52 μg/day). The mean standard deviation was 7.7%, indicating a high degree of reproducibility. The lower limit of sensitivity of the method was 0.4 ng aldosterone/ml urine or 0.4 μg/day when the urine volume was 1000 ml/day.

The results derived from radioimmunoassay were compared with 72 determinations on the same samples measured by a technique of double-isotope dilution developed in this laboratory (3, 16). The striking correlation obtained can be seen in Figure 2. The ratio of the two assays was 1.06, and the correlation was highly significant ($P < 0.001$).

The correlation of the results of radioimmunoassay and double-isotope dilution extended over the whole range of physiological variation which occurred in response to induced changes in sodium balance. When the aldosterone results determined by radioimmunoassay were plotted against sodium excretion, the values all fell on the curve which was derived from aldosterone determinations by double-isotope dilution (Fig. 3). There was no apparent difference in the relationship between aldosterone and urinary sodium excretion when aldosterone was measured in samples collected under balance conditions or in random samples.

Further Evaluation of Radioimmunoassay

In an attempt at simplification of the method, extraction before hydrolysis was omitted in 12 assays (Fig. 4). In each determination, the result obtained from unextracted urine was higher than that in urine which had been extracted; the mean value was 32% higher.

Another attempt at simplification was the omission of extraction after hydrolysis. The urine was adjusted to pH 7.0 after hydrolysis, and samples of unextracted urine were added to the radioimmunoassay incubation medium. In this study, the mean aldosterone determination was 109% higher than that derived from urine extracted with methylene chloride (Fig. 4).

Time Course of Changes in Aldosterone Excretion with Changes in Dietary Sodium

Table 2 presents data for aldosterone excretion rates in five normal subjects who were on identical low-, normal-, or high-sodium diets for 5 consecutive days of 3 consecutive weeks. Only data for the last 3 days of each dietary period are presented. Several features should be noted. First, on the low-sodium diet, the expected directional

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**Table 1**

<table>
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<th>Mean (μg/day)</th>
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<th>N</th>
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N = number of determinations.
Relationship between changes in sodium excretion and aldosterone excretion were found in every study. However, during sodium deprivation, every subject exhibited a striking increase in aldosterone excretion on either day 4 or day 5 of the diet. In the three subjects in

**TABLE 2**

Aldosterone Secretion on Low-, Normal-, and High-Sodium Diets

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Day of diet</th>
<th>Low sodium (mEq/day)</th>
<th>Urinary aldosterone (µg/day)</th>
<th>Normal sodium (mEq/day)</th>
<th>Urinary aldosterone (µg/day)</th>
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**Aldo** = aldosterone.

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Relationship of urinary aldosterone excretion to daily urine sodium excretion; 198 aldosterone determinations were made by radioimmunoassay and 75 by double-isotope dilution. There was complete overlap of the two methods of measurement over the entire range. No difference was apparent in aldosterone measurements derived from random samples or those collected under balance conditions.

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All subjects had previously been on a sodium intake of 10 mEq/day. Italic numbers indicate that sodium excretion was greater than sodium intake. UNαV = urinary sodium excretion in mEq/day, Aldo = aldosterone excretion in μg/day, and Bal = cumulative sodium balance in mEq (mean of previous day and day of study).
Methylene chloride extraction was omitted either before hydrolysis or after hydrolysis. The results of both sets of analyses by radioimmunoassay were falsely high when these simplifications of the assay procedure were used.

whom the increase occurred on day 4, there was no further increase on day 5; urinary sodium excretion remained almost identical on day 5, suggesting that no further increase in the rate of aldosterone excretion was demanded for these subjects to maintain sodium balance. In four of the five subjects, this large increase in aldosterone excretion was associated with a sharp fall in the rate of sodium excretion. It thus appeared that aldosterone secretion responded adequately only after considerable sodium loss and that the rise in aldosterone was associated with a marked and fully appropriate fall in renal sodium excretion.

A second feature of the aldosterone secretory response was observed during sodium loading. In subjects 1 and 3, aldosterone excretion fell progressively with sodium loading. However, in subjects 4 and 5, there appeared to be a sharp secondary rise in aldosterone excretion, and, in subject 2, aldosterone excretion remained at a somewhat higher level than that observed in 1 and 3. Subjects 2, 4, and 5 also exhibited a much higher rate of urinary sodium excretion than did subjects 1 and 3.

To investigate these fluctuations in aldosterone excretion further, four additional normal subjects were depleted of sodium by consuming only 10 mEq/day of sodium for 9-11 days, and they were then placed on a fixed high-sodium diet of either 270 or 300 mEq/day. The results of these studies are presented in Table 3 and Figure 5. Since the subjects had been depleted of sodium during the previous 9-11 days, it was felt that meaningful balance studies could be carried out because all subjects were beginning at a relatively low level of body sodium. Previous studies described above demonstrated that the body defends a certain critical mass of sodium by responding with high levels of aldosterone excretion, and it was felt that each of these subjects was beginning at this low basal level of total body sodium. Cumulative balance was calculated at the end of each daily period from the difference between intake and daily urinary sodium excretion, assuming a 10-mEq/day imperceptible loss. Since aldosterone excretion is a reflection of the summated daily rate of adrenal secretion and the balance is calculated at the end of each day, it was felt that a more appropriate balance period to relate aldosterone to would be the mean of the two sodium balances determined at the end of the day before the collection and at the end of the day of the aldosterone measurement. Balance data in Table 3 and Figure 5 were so derived.

Each subject underwent an initial period of sodium retention which lasted either 3 or 4 days. This was followed by natriuresis, in which each subject excreted sodium in excess of intake for 3–5 days. A recovery period consistently followed in all four subjects, and in the period sodium excretion was again lower than sodium intake.

In general, urinary aldosterone excretion
Fluctuations in sodium excretion, sodium balance, and aldosterone excretion during a main-
tained high-sodium diet. Mean values ± 1 sd from the three normal subjects who ate 270 mEq sodium/day are presented. Each subject exhibited similar and corresponding fluctuations in sodium and aldosterone. The highest levels of sodium excretion were associated with the lowest rates of aldosterone excretion.

was inversely related to the rate of sodium excretion so that, during the initial period of sodium retention, aldosterone excretion was highest and, during the second period of excessive sodium excretion, the rate of aldosterone excretion was lowest. Mean aldosterone excretion for all four subjects was 23.4 μg/day during sodium retention, 3.6 μg/day during natriuresis, and 4.5 μg/day during the second more moderate period of sodium retention.

In all subjects, positive sodium balance reached a peak during the initial phase of sodium retention. This ranged from 293 mEq in subject 6 to 581 mEq in subject 8. Urinary sodium loss during the next natriuretic period
ranged from 108 mEq (subject 6) to 314 mEq (subject 8). Thus, there appeared to be a relationship between the amount of sodium retention and the level of the ensuing natriuresis.

In general, there was an inverse relationship between the rate of sodium excretion and the daily rate of aldosterone excretion, even at the high levels of sodium excretion (Fig. 5). However, no such relationship was apparent when sodium balance was related to urinary aldosterone excretion.

**Discussion**

Aldosterone, secreted solely by the adrenal cortex, is metabolized by the liver and the kidneys (17, 18), and approximately fifteen different metabolites are excreted into the urine (19). When [3H] aldosterone is given intravenously, about 40% of the injected radioactivity is excreted as 3, 5-tetrahydroaldosterone (20, 21). The rate of excretion of this compound after injection of a known amount of tracer aldosterone was originally used to measure the adrenal cortical secretory rate of aldosterone in man (1). About 10% of the injected radioactive aldosterone is excreted as the 18-C-glucuronide, the so-called "acid-labile" or "3-oxo-conjugate" (22). This latter compound has been widely used for the measurement of both excretion and secretion rates of aldosterone (2, 3). In man, complete formation and excretion of this metabolite can occur within 6 hours (23). In patients with impaired renal function, the time necessary for excretion of the acid-labile conjugate may be increased (24). Studies measuring both secretion and excretion rates indicate that the excretion rate of the conjugate is a reliable index of the daily adrenal secretory rate in normal subjects (3). Under normal conditions, half of the acid-labile conjugate excreted into the urine is made by the kidney, and the other half is formed by the liver. In the presence of liver disease, as much as 50% of the secreted aldosterone may be metabolized and excreted in the urine as this conjugate (18). However, such special situations are usually readily apparent clinically.

Antibodies to aldosterone have been prepared by several laboratories. However, all of these exhibit significant cross-reactions with other corticosteroids which are present in both blood and urine in concentrations far greater than that of aldosterone. In this regard, there are two unique properties of the urinary 3-oxo-conjugate which can be used to advantage. (1) The original steroid can be reconstituted by acid hydrolysis, and (2) the conjugate is insoluble in organic solutions. Accordingly, in the present method, as in that of Wahlen and co-workers (13), all of the immunologically cross-reacting corticoids were extracted into methylene chloride and discarded prior to acid hydrolysis. Then the methylene chloride-insoluble acid-labile conjugate of aldosterone was treated with acid to liberate the free hormone, which was extracted into methylene chloride. Thus, separation of aldosterone from immunologically cross-reacting compounds was accomplished by extraction, acid hydrolysis, and then reextraction.

Radioimmunoassay can be as accurate as double-isotope dilution only if rigid quality control is maintained. For many reasons such as steroid contamination, changes in the binding characteristics of the antibody due to storage, breakdown of the [3H] aldosterone, and poor storage of the aldosterone standard, all of the values can be too high or too low in any particular assay. In addition, falsely high results can occur when the urine extracts are not washed thoroughly after extraction. Because of these problems, we included two standard urines in every batch of urine samples and only if they both were within ±20% of a previously determined mean was the series accepted. In addition, the results of a sample were accepted only if the duplicates of each sample agreed within ±20%.

It is our view that the measurement of aldosterone in urine is useful when compared with the alternate measurement of plasma aldosterone, because the 24-hour urinary measurement is an integrated reflection of the daily adrenal secretion of aldosterone (3, 25).
In this context, it may often provide more useful information than a plasma value which reflects only a single point in time. Furthermore, the collection of a 24-hour urine sample is necessary to decide whether a plasma or a urinary aldosterone measurement is normal or abnormal in relationship to the concurrent level of sodium balance. No practical alternative to the use of the daily rate of urinary sodium excretion as an indicator of sodium balance is readily available. Thus, in the absence of an alternative index of normalcy, 24-hour urine collections may be a necessity even for plasma methods. Since the measurement of aldosterone in urine is relatively simple, it is a convenient tool for analysis of aldosterone secretory behavior in man.

The values for urinary aldosterone measured by radioimmunoassay or by double-isotope dilution were interchangeable and superimposable over the physiological range which occurred in relationship to induced changes in sodium balance (25-27) (Figs. 2, 3). This approach of developing a nomogram for comparing the aldosterone excretion of different subjects was inherently more accurate than the more conventional method of grouping various ranges of sodium intake and giving a range of aldosterone values for the sodium range. The greater accuracy resulted because any chosen range could span a portion of the curve in which a small increment in sodium excretion was associated with a large change in aldosterone excretion.

For example, between sodium intakes of 25 and 75 mEq/day the normal value for aldosterone excretion can range from 8 to 48 μg/day, but at 25 mEq/day the range is only from 23 to 48 μg/day, and at 75 mEq/day the range is only from 8 to 27 μg/day. There is, therefore, a much greater chance of detecting an abnormality when the continuous-curve approach is used.

In the present study, serial observations were made in normal subjects maintained on different, but constant, levels of dietary sodium. When normal subjects were placed on sodium-restricted diets (20 mEq/day), they excreted sodium at amounts well in excess of intakes for a few days. This excretion was accomplished by increases in aldosterone which, however, were inadequate to prevent appreciable sodium loss, on the order of 100–200 mEq of sodium. Early in sodium depletion, the moderate increases in aldosterone secretion were insufficient to effect adequate sodium conservation. However, after considerable sodium or volume depletion had been induced, aldosterone secretion then reacted sharply and appropriately. The system, therefore, seemed to be designed to defend a critical mass rather than to respond in a continuous or linear fashion to sodium loss.

A second characteristic of the aldosterone secretory response was defined in studies of normal subjects placed on a fixed, but high, sodium intake (Table 3, Fig. 5). In all four subjects, there was an initial period of sodium retention lasting for 3 or 4 days in which intake exceeded output. In each of the four subjects, this excess intake was followed by a second period in which the rate of sodium excretion actually exceeded intake. This period lasted for 5–3 days and was then followed by another period of mild positive sodium balance lasting for at least the next 3 days, when the study terminated.

Aldosterone excretion, measured daily, fell serially as urinary sodium excretion increased so that the highest rates of urinary sodium excretion were associated with the lowest levels of aldosterone excretion. Note that in the third period when urinary sodium excretion began to fall from its maximum, aldosterone exhibited a slight, but consistent rise. The data strongly suggested that the phasic, albeit relatively slight, changes in sodium excretion which occurred during circumstances of prolonged sodium loading were in fact under the direction of changes in aldosterone secretion.

In addition to providing evidence for a role for changes in aldosterone secretion in modulating salt balance during sodium loading, these data indicated that the rate of aldosterone secretion was more closely related to the rate of sodium excretion by the kidney than it
was to the state of overall sodium balance. Thus, the highest level of sodium balance was associated with an aldosterone excretion rate double that of the lowest value observed. Moreover, during the natriuretic period, cumulative sodium balance declined while aldosterone excretion remained constant, but low. When one also considers the errors inherent in the calculation of balances, the results indicated that the urinary sodium excretion was a simpler, more reliable guide for evaluation of normalcy of hormone activity. This correlation of urinary sodium excretion and aldosterone secretory activity is, perhaps, not unexpected since the urinary sodium excretion reflects the activity of the hormone on its target organ, the kidney. These studies do not invalidate the classical concept that, in a steady state, sodium intake and sodium balance are regulators of the rate of aldosterone secretion. Some function of a change in sodium balance obviously was the stimulus which induced a change in hormonal activity. However, the hormonal response to the stimulus appeared sluggish so that large overshoots in balance occurred both positively and negatively before equilibrium was achieved. This delay might be caused either by a lag in the renin response to sodium balance or by a delay in the adrenal response to changes in plasma renin levels (14, 25). In analyzing data, this time lag and overshoot was less of a problem when aldosterone secretory activity was related to the concurrent urinary sodium excretion.

Further confirmation of the consistent relationship between aldosterone excretion and urinary sodium excretion was derived from studies on all of the normal subjects. In some of them, aldosterone was measured in samples collected during random sodium intake. In others, aldosterone was measured in samples collected on a fixed high-, normal-, or low-sodium diet before balance was achieved and again after 5 days or more on the same fixed dietary regimen. Complete overlap of the data was observed (Fig. 3). Accordingly, the normalcy of aldosterone excretion can be determined with as much accuracy in outpatients resorting to random diets as in those who are studied under metabolic ward conditions, as long as collection of the 24-hour urine samples is complete and kidney function is normal.

The aldosterone secretory response to sodium deprivation is described in this paper as a continuous process expressing the direct involvement of the hormone in the function of salt conservation. On the other hand, the aldosterone secretory response to sodium loading appears to be an oscillating one, responding to fluctuations in sodium balance, but more consistently related in time to induced changes in the rate of salt excretion. Fluctuations in hormone secretory activity in relation to sodium balance are still apparent at high levels of sodium intake when adrenal cortical participation often has been thought to be minimal. These serial observations of aldosterone changes during sodium deprivation and sodium loading provide an additional dimension—that of time—to our understanding of the secretory behavior of aldosterone.

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Aldosterone Excretion: PHYSIOLOGICAL VARIATIONS IN MAN MEASURED BY RADIOIMMUNOASSAY OR DOUBLE-ISOTOPE DILUTION
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