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# DYNAMIC ALDOSTERONE AND 18-HYDROXYDEOXYCORTICOSTERONE STUDIES IN LABILE AND STABLE BENIGN ESSENTIAL HYPERTENSION

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## SUMMARY

Under baseline conditions of recumbency and normal sodium intake there was a small significant hyperaldosteronism evident from elevated plasma concentration and aldosterone excretion in presence of suppressed metabolic clearance rate (MCR) in benign essential hypertension (BEH).

BEH patients, particularly those with hyperkinetic circulation often excreted more oxo-conjugate and present greater than normal response in plasma aldosterone to upright posture. Upright posture decreased aldosterone MCR in controls, but effect was negligible in BEH.

In BEH, decreased MCR and excretion of hepatic metabolite (tetrahydroaldosterone) may be due to impaired hepatic extraction related to increased aldosterone binding to a transcortin-like plasma fraction (TLPF). Concomitant fluctuations of TLPF and plasma concentration during circadian rhythm, the menstrual cycle and pregnancy suggest a possible role of TLPF in aldosterone distribution and dynamics. Recumbent BEH patients had increased plasma aldosterone around midnight.

BEH patients showed hyperresponsiveness to ACTH infusion in plasma aldosterone with simultaneous sharp decrease of TLPF binding, while aldosterone MCR increased significantly and the ratio of the urinary and hepatic metabolites of aldosterone changed.

Changes in binding degree probably contribute to perturbated aldosterone metabolism in BEH.

In the majority of BEH patients with normal or low plasma renin activity 18-hydroxydeoxycorticosterone secretion is increased. ACTH stimulation or dexamethasone suppression of 18-hydroxydeoxycorticosterone is comparable in controls and patients. This hormone circulates in unbound form.

## INTRODUCTION

Modifications in the metabolism of aldosterone in benign essential hypertension (BEH) already described [1–7] present a new aspect of this disease. The plasma concentration of aldosterone depends on the balance between the rate of secretion by the adrenals and the metabolic clearance rate (MCR) determined mainly by the liver [8] but also by the kidney [9]. In addition, the plasma concentration is markedly influenced by posture because the hepatic blood flow (and the MCR, which in normal subjects approximates the hepatic blood flow) is lower in the upright posture, than during recumbency [8]. The hepatic extraction and therefore the MCR are also affected by degree of binding of the steroid to circulating proteins [10–12]. It can probably be assumed that the concentration of non-protein-bound, and possibly of albumin-bound aldosterone will govern the rate of hepatic extraction and of plasma clearance of aldosterone [12]. Our earlier studies indicated a higher mean plasma concentration of aldosterone in patients with BEH and a lower MCR of aldosterone in most patients, even though many of them had low-normal or even subnormal aldosterone secretion rates [1, 2]. The low MCR of aldosterone has been confirmed in larger numbers of patients with both stable and labile BEH [6, 7, 13, 14].

At the same time, a lower urinary excretion of tetrahydroaldosterone-glucuronide (a hepatic metabolite of aldosterone) and a higher excretion of the 18-oxo-conjugate (renal metabolite of aldosterone) has been found, especially in the labile form of BEH [1–7] (Fig. 1).

For these reasons, it is not always possible to assess the degree of hyperaldosteronism by single measurements of the usual parameters (urinary excretion, plasma concentration or secretion rate); a dynamic approach (such as sodium depletion or loading, blood

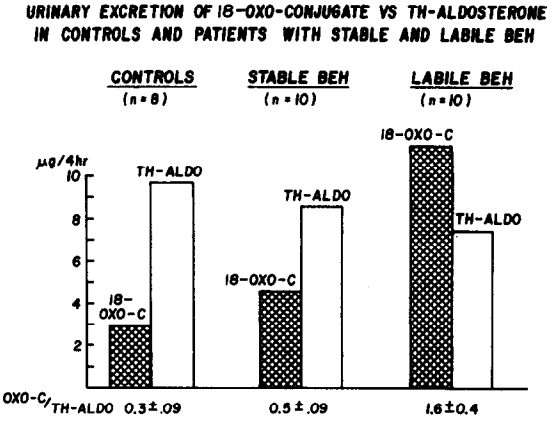


Fig. 1. Mean urinary excretion (µg/4 h) of aldosterone 18-oxoconjugate (renal metabolite) and tetrahydroaldosterone glucuronide (hepatic metabolite) in recumbent (0800–1200 h) control subjects and patients with stable and labile BEH, with their ratios.

volume manipulation or posture) is often necessary to reveal the defect.

Recent observations [6] suggest that many of the alterations in the metabolism of aldosterone may be due to impaired hepatic extraction, related—at least in part—to an increase in the binding of aldosterone to a transcortin-like plasma fraction (TLPF) in BEH.

This report describes an assay for the degree of TLPF binding. It also reports new data on the MCR and binding of aldosterone to plasma proteins, the effect of upright posture and ACTH on the MCR, the urinary excretion pattern of the oxo-conjugate, plasma aldosterone concentrations, circadian rhythms of aldosterone in BEH, and changes in plasma aldosterone concentration and binding during the menstrual cycle. Some new data on the metabolism of 18-hydroxydeoxycorticosterone (18-OH-DOC) in BEH are also reported.

## METHODS AND PATIENTS

The MCR of aldosterone was determined by continuous infusion of the labelled hormone [2] while the urinary excretion of the 18-oxo-conjugate and tetrahydro-glucuronide of aldosterone were determined by the double-isotope dilution procedure, with [$^3$H]-labelled oxo-conjugate and tetrahydroaldosterone-glucuronide as markers [15]. Plasma total aldosterone was determined by radioimmunoassay [16] and the secretion rate of 18-OH-DOC by double-isotope dilution assay.

### 18-*Hydroxydeoxycorticosterone* (18-OH-DOC)

Briefly, the procedure for 18-OH-DOC involves the use of [$^3$H]-labelled 18-OH-DOC as the marker and $^{14}$C-labelled acetic anhydride as the acetylating agent. The secretion rate was calculated from the specific activity of an exclusive urinary metabolite, 18-hydroxytetrahydro-11-desoxycorticosterone (18-OH-TH-DOC) which was measured following the formation of a $\gamma$-lactone by periodic acid oxidation. About 2 $\mu$Ci of [$^3$H]-18-OH-DOC were injected and 48-h urine was collected. Following the enzymatic hydrolysis and extraction, the dry sample was first chromatographed on a paper partition system consisting of benzene–hexane–methanol–water (33:17:40:10 by vol). The isolated 18-OH-TH-DOC was oxidized by a mixture of periodic acid in aqueous pyridine and chromatographed on a paper partition system consisting of benzene–hexane–methanol–water (17:22:40:10 by vol.). Following the acetylation with $^{14}$C-acetic anhydride in pyridine, the 3-monoacetate of the lactone of 18-OH-TH-DOC was purified to constant S.A. by the following paper partition systems consisting of: (a) methanol–water–decaline (10:1:10 by vol.) (reverse-phase); (b) methanol–water–mesitylene (10:1:10 by vol.) (reverse phase).

The next purification was achieved by silica gel thin layer chromatography using 4% methanol in benzene as mobile phase. The last purification was performed on the same reverse phase system as described under (b).

### *Binding of aldosterone to TLPF*

This estimation was based on the demonstration by Daughaday [17] that the high-affinity binding by specific steroid-binding globulins can be destroyed by heating the plasma at 60°C for 20 min. This allows the determination of the TLPF binding by comparison of the binding in heated and intact plasma. We separated free from bound aldosterone by a method similar in principle to equilibrium dialysis, but offering milder and more rapid conditions. Unbound aldosterone was quantitatively and almost instantly absorbed to Dextran-coated charcoal while protein-bound hormone remained in the supernatant. Dextran-coated charcoal is superior to other adsorbents in that it remains well suspended until centrifuged. Binding was estimated in 1:5 diluted plasma at the physiological temperature of 37°C. The following conditions gave maximum binding with the highest efficiency in the removal of free aldosterone.

#### *Materials*

(a) Aldosterone: crystalline d-aldosterone (Ciba) and [1,2,6,7,(n) $^3$H]-aldosterone (100 Ci/mol, Amersham) were used without further purification. The purity of [$^3$H]-aldosterone was verified as described before [16].

(b) A Dextran–coated charcoal suspension was prepared every month [16] by adding 500 mg of Dextran T 70 (Pharmacia, Uppsala, Sweden) and 250 mg of Darco G 60 charcoal (Matheson, Coleman and Bell) to 100 ml of 0·05 M phosphate buffer at pH 8·0. It was stored at 4°C.

(c) All the water used throughout this procedure was double-distilled in all-quartz apparatus without any added preservative [16].

(d) [$^3$H]-aldosterone solution was made up in 0·05 M phosphate buffer, pH 7·0, at a concentration of about 6,000 c.p.m. in 0·1 ml.

#### *Method*

The procedure consisted in determinations of total aldosterone [16], of total bound aldosterone in untreated plasma (or serum), and of albumin-bound aldosterone in plasma (or serum) heated to 60°C.

For the determinations were needed:

(A) A series of tubes labelled P (plasma). To each tube (disposable 12 × 75 mm) in duplicate was added: 0·1 ml of 0·05 M phosphate buffer, pH 8·0 (kept at room temperature); 0·2 ml of saline (0·9 g/100 ml water); 0·1 ml plasma (chilled in ice bath); 0·1 ml [$^3$H]-aldosterone solution.

(B) A series of tubes labelled P′ with half the amount of [$^3$H]-aldosterone to provide evidence of the dose-response relationship. To each tube, the same reagents were added under the same conditions, in duplicate: 0·1 ml buffer; 0·25 ml saline; 0·1 ml plasma; 0·05 ml [$^3$H]-aldosterone solution.

(C) A series of tubes labelled H (heated). To each tube in duplicate, the same reagents under the same conditions were added: 0·1 ml buffer; 0·2 ml saline;

0·1 ml plasma; 0·1 ml [$^3$H]-aldosterone solution. The H tubes were then incubated for 25 min in a water bath at 60°C.

(D) Two tubes containing 0·1 ml of buffer, 0·3 ml of saline and 0·1 ml of the same [$^3$H]-aldosterone solution were also prepared (d.p.m. std, Standard).

All tubes were covered with parafilm (American Can Co.) and incubated at 37°C for 3 h. Ten tubes at a time were then placed in an ice bath and the free aldosterone was removed by adding 0·5 ml (Oxford Automatic Pipette) of Dextran-coated charcoal suspension to each tube. During the addition, the particles were kept suspended by the slow rotation of a magnetic stirrer. After 10 min in the ice bath, the tubes were centrifuged for 10 min at 3500 rev/min in a refrigerated centrifuge. The resulting supernatants (bound fraction) were decanted directly into the counting vials containing 15 ml of Bray's solution and immediately counted as described before [16].

*Calculations*

The percentages of bound aldosterone were calculated as follows: bound aldosterone as % of total

$$= \frac{\text{d.p.m.P} \times 100}{\text{d.p.m. std.}}$$

likewise, albumin-bound aldosterone as % of total

$$= \frac{\text{d.p.m.H} \times 100}{\text{d.p.m. std.}}$$

and,

% TLPF-bound = % total bound − % albumin bound.

The concentration of TLPF-bound aldosterone was calculated by the equation:

$$\text{ng TLPF-bound/100 ml plasma} = T_A \times \% \text{ TLPF-bound}$$

and the fraction of non-protein bound aldosterone may be calculated as follows:

$$\text{non-protein bound} = T_A - \text{TLPF-bound}$$

where $T_A$ is totally measured endogenous aldosterone (in nanograms) [16], d.p.m.P is the fraction of counts in the supernatant of tubes labelled P, d.p.m. std. are the counts measured in tubes containing [$^3$H]-aldosterone standard and d.p.m.H are the counts in the supernatant of tubes labelled H.

*Assessment of the method*

(a) The percentage of aldosterone bound to TLPF measured in freshly drawn serum did not change after storage for 1 day, 2 months or 6 months, in the frozen state or after freeze and thawing several times.

(b) The percentage binding to TLPF decreased when the plasma was diluted (Fig. 2a).

(c) When the assay was performed at 37°C, equilibrium with the labelled marker was reached after 2 h and remained unchanged for at least 4 h (Fig. 2b).

(d) If the 3 h incubation was performed at 4°C the percentage bound to TLPF was slightly lower.

(e) Heating plasma diluted 1:5 reduced TLPF binding at temperatures between 45 and 60°C (Fig. 2c).

(f) The plasma was diluted with 0·1 M phosphate buffers at various pH values; the percentage binding increased gradually up to pH 6 and remained constant until pH 9 (Fig. 2d).

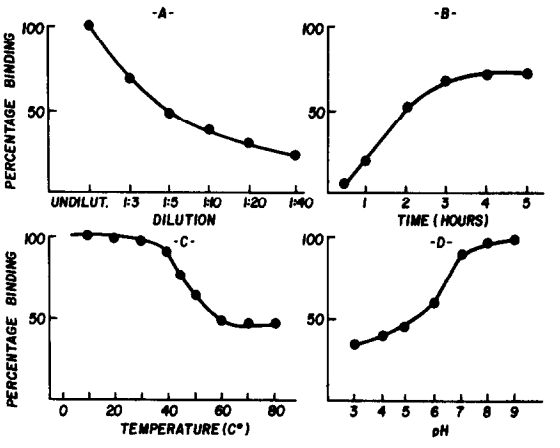


Fig. 2. (A) Effect of plasma dilution on percentage of aldosterone bound to TLPF at 37°C in 3-h incubation. (B) Effect of duration of incubation at 37°C on percentage bound (plasma diluted 1:5). (C) Effect of heating plasma diluted 1:5 on the percentage bound after incubation at 37°C for 3 h. (D) Effect of pH on percentage bound after incubation at 37°C for 3 h.

The reproducibility of the procedure is illustrated by the following values for the amount of aldosterone bound to the TLPF in 0·1 ml of plasma: 1·71 ± 0·15 pg (S.E., $n = 9$), or as a percentage of the total aldosterone 7·72 ± 0·20% (S.E., $n = 9$).

## CONTROL SUBJECTS AND PATIENTS

The control subjects were healthy volunteers with blood pressure below 140/90 mm Hg on numerous occasions, whose medical history and physical examination revealed no abnormality. In the patients, the diagnosis of benign essential hypertension was made on the basis of a thorough physical examination and numerous tests as described [7, 13, 14], which exclude any known causes of hypertension. The patients were divided into groups of labile or stable BEH, as defined earlier [7, 14]. In patients with labile BEH blood pressure was over 140/90 mm Hg but declined to below this limit with rest; in the group with stable BEH, the blood pressure remained constantly above this limit. Patients with low-renin BEH were selected as follows. Every patient had at least two renin determinations, one recumbent with a normal sodium and potassium intake (135 m-equiv of sodium and 90 m-equiv of potassium daily) and another after stimulation either by upright posture or low salt diet and/or furosemide. Patients showing plasma renin activities [7] of less than 0·5 ng/ml/h during stimulation by upright posture and/or less

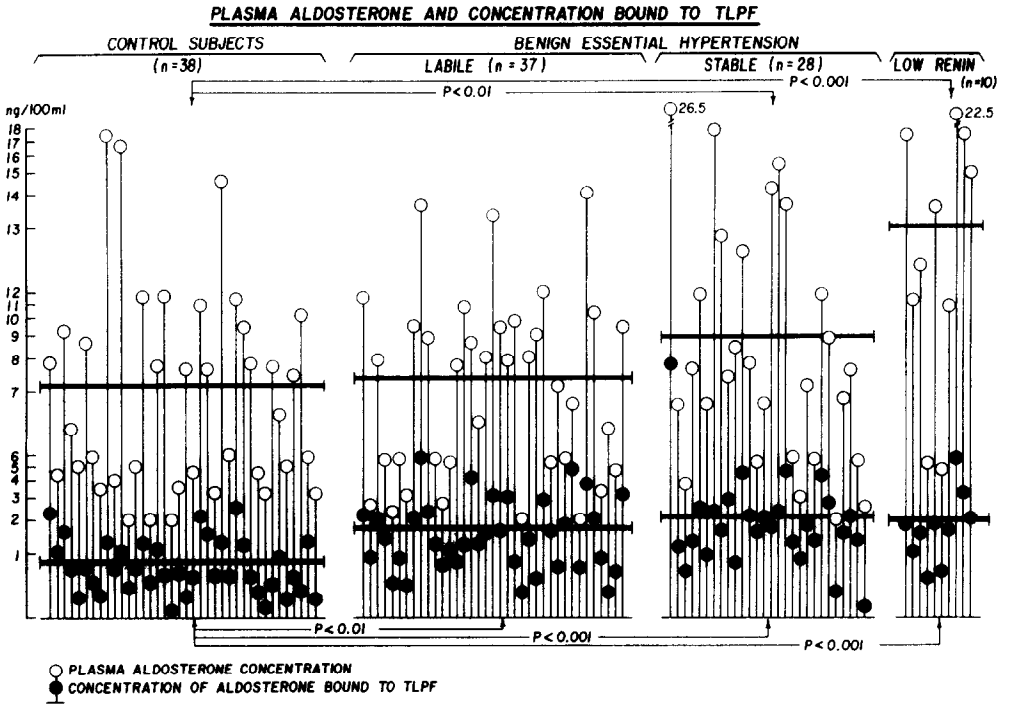


Fig. 3. Plasma concentrations of total and TLPF-bound aldosterone in control subjects and patients with labile and stable BEH. Samples taken in recumbent posture at 0830 h.

than 1·5 ng/ml/h after stimulation by low-salt diet or furosemide and upright posture were considered as having low-renin BEH.

All the control subjects and patients underwent baseline steroid determinations (unless otherwise noted) in the recumbent posture between 0830 and 0900 h on the 4th day of a diet containing 135 m-equiv of sodium and 90 m-equiv of potassium per day. All the women studied were in the early follicular phase of the menstrual cycle [7, 16]. The majority of patients with labile BEH had never been treated. The treatment of the other patients was discontinued during the study whenever possible.

Urinary samples for the experiments involving postural adaptation in the excretion of the oxo-conjugate were collected at the same time of day (0800–1200 h) on 2 consecutive days—on 1 day in the recumbent, and on the other in the upright posture—the subjects having been upright or leisurely walking, but never recumbent. The postural sequence was taken at random. Blood samples for aldosterone determinations were taken at the end of each 4-h period.

## RESULTS

The metabolic clearance rate (MCR) of aldosterone and its plasma concentration, determined in six control subjects and 29 patients with BEH, were negatively correlated ($r = -0{\cdot}44$, $P < 0{\cdot}01$, $y = -24 \times + 1059$) [6, 7].

Since previous evidence has suggested [6] that TLPF binding may affect aldosterone distribution and dynamics, we investigated this binding to see whether it could explain the lower metabolic clearance rate of aldosterone in BEH.

The mean concentration of aldosterone bound to TLPF was higher in labile, stable and low-renin BEH than in controls (Fig. 3) and to about the same extent (1·7 ± 0·21 S.E., 2·1 ± 0·29 and 2·0 ± 0·46 ng/100 ml vs 0·8 ± 0·09 ng/100 ml in controls). There was no

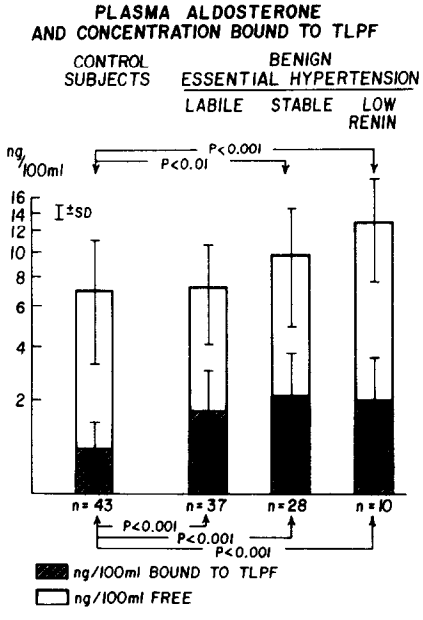


Fig. 4. Concentration of total, free and TLPF-bound plasma aldosterone in control subjects and patients with labile and stable BEH with normal and low plasma renin activity. Vertical bars, ±S.D.

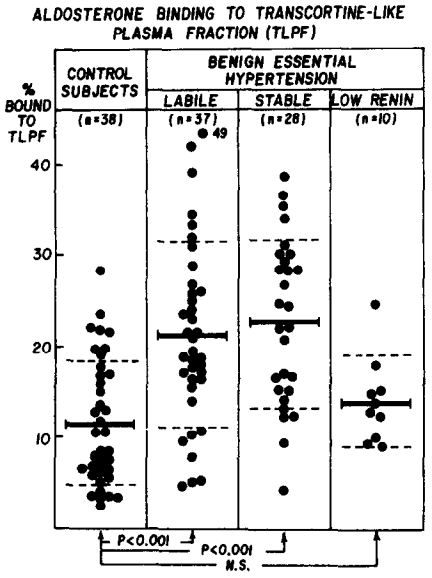


Fig. 5. Percentage of aldosterone bound to TLPF relative to total amount of aldosterone present.

difference in the albumin binding between the patients and the controls.

The mean total plasma concentration of aldosterone (Fig. 4) was significantly higher in stable BEH with or without low-renin than in labile BEH or controls.

When the results from Fig. 4 are expressed as the percentage of aldosterone bound to TLPF (Fig. 5), the mean is significantly higher ($P < 0.001$) for the patients with labile, with stable hypertension than in control subjects (21·3 ± 1·7 S.E., 22·8 ± 1·7 and 14·0 ± 1·5% bound to TLPF vs 11·6 ± 1·1% bound to TLPF in controls). All assays for TLPF binding were conducted with reference to a plasma pool collected from 29 of the above control subjects. The value obtained for TLPF percentage binding in this normal pool was 12·8% vs 13·3 obtained as a mean of all individual determinations in the same 29 control subjects in our initial study [6].

*Percentage binding of aldosterone to TLPF in various physiological and clinical conditions*

(*a*) *Primary hyperaldosteronism.* In one case of surgically proven primary hyperaldosteronism the following values relating to aldosterone metabolism were found: urinary excretion of the 18-oxo-conjugate of aldosterone, 58 μg/24 h (high) before and 0·22 μg/24 h after surgery; plasma aldosterone before surgery, 36·9 ng/100 ml (high) and TLPF binding 11% (normal); in a different experiment, the urinary 18-oxo-conjugate was 8·5 μg/4 h (high) (0800 h–noon) and concomitantly determined tetrahydroaldosterone-glucuronide 26·0 μg/4 h (high), giving a normal ratio between these values of 0·32. The measured MCR was normal at 1303 l/24 h/m$^2$.

(*b*) *Secondary aldosteronism with renin-producing ectopic tumor.* In a single case of an ectopic renin-producing tumor (pulmonary), plasma renin activity on two occasions was very high at 61 and 76 ng/ml/h,

Table 1. Aldosterone plasma concentration and degree of binding to TLPF during pregnancy

| Aldosterone | Trimester 1st | 2nd | 3rd | Post partum |
|---|---|---|---|---|
| Total in plasma | 46·3 | 46·1 | 70·0 | 6·5 |
| (ng/100 ml) | — | 21·3 | 23·8 | 12·9 |
| | 77·0 | 25·7 | — | 12·3 |
| | — | 28·0 | — | 5·0 |
| | — | 32·0 | 22·5 | 2·0 |
| | 24·5 | 46·3 | 64·6 | 8·0 |
| | 22·7 | 27·9 | 42·6 | 5·3 |
| Mean | 42·6 | 32·4 | 44·7 | 7·4 |
| ±S.E. | 12·6 | 3·7 | 9·9 | 1·5 |
| Per cent bound | 36·0 | 28·0 | 18·0 | 41·0 |
| to TLPF | — | 35·0 | 35·9 | 29·3 |
| | 16·9 | 11·7 | — | 16·8 |
| | — | 29·2 | — | 20·6 |
| | — | 7·2 | 17·0 | 25·4 |
| | 5·6 | 8·7 | 9·5 | 22·0 |
| | 31·0 | 28·1 | 24·9 | 42·5 |
| Mean | 22·3 | 21·1 | 21·0 | 26·1 |
| ±S.E. | 6·9 | 4·3 | 4·4 | 3·7 |
| Concentration of | 16·8 | 13·1 | 12·9 | 2·7 |
| TLPF-bound form | — | 7·5 | 8·5 | 3·8 |
| (ng/100 ml) | 13·1 | 3·0 | — | 2·1 |
| | — | 8·2 | — | 1·0 |
| | — | 2·3 | 3·8 | 0·5 |
| | 1·4 | 4·0 | 6·1 | 1·8 |
| | 7·2 | 7·8 | 10·6 | 2·2 |
| Mean | 9·6 | 6·5 | 8·3 | 2·0 |
| ±S.E. | 3·3 | 1·4 | 1·6 | 0·4 |

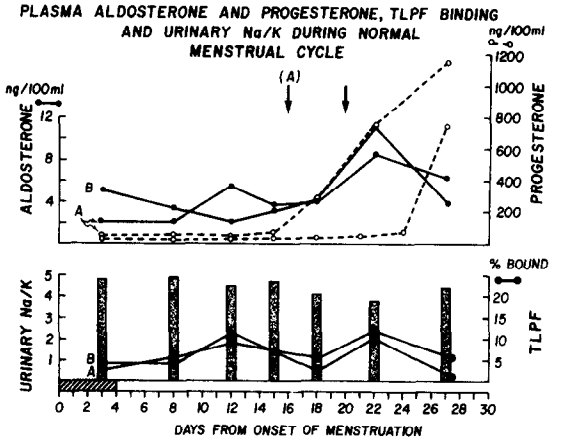


Fig. 6. Plasma aldosterone, progesterone, concentration of TLPF-bound aldosterone and urinary Na/K during ovulatory menstrual cycles in two normotensive subjects (A and B). Vertical arrows indicate the day of ovulation.

as was plasma aldosterone at 238 and 302 ng/100 ml. The percentage of aldosterone bound to TLPF was low at 0·0 and 1·1%. The histology of the adrenal glands of this patient revealed an exceptionally marked hyperplasia of the zona glomerulosa.

(*c*) *Pregnancy.* In seven cases of normal pregnancy, total plasma aldosterone concentration and concentration of aldosterone bound to TLPF were much higher (Table 1) than in control plasma, as shown in Fig. 3. Plasma aldosterone and the concentration of bound aldosterone decreased sharply during the 1–3 days after delivery while the percentage of bound aldosterone remained elevated.

(*d*) *Menstrual cycle.* In four subjects, plasma aldosterone (0800 h in recumbent posture since the night before) and TLPF binding were found to fluctuate with a characteristic pattern through the menstrual cycle. The plasma concentration increased slightly in the midfollicular phase and rose to a major peak again in the midluteal phase.

The urinary sodium/potassium ratio varied inversely with aldosterone levels during the menstrual cycle. Two of the cycles were ovulatory (Fig. 6) and two were anovulatory (Fig. 7). Aldosterone fluctuated in all four cycles but its peak was accompanied by an increase in progesterone in one ovulatory cycle only (patient A, Fig. 6). The percentage of aldosterone bound to TLPF seemed to vary directly with aldosterone levels in all four subjects.

(*e*) *Circadian rhythms.* Circadian rhythms of plasma aldosterone and TLPF binding in supine posture around the clock were also studied in controls and patients with BEH (Fig. 8). The mean plasma aldosterone concentrations obtained by sampling at 4-h intervals indicate that in our six control subjects the same time variation occurred as previously reported [6, 18, 19]. The levels were highest at 0400 h and higher again between 1000 h and noon than at 0800 h and 1600 h, with a lower value at 2000 h and a minimum value at midnight. In patients with BEH, the variations usually occurred at the same time but were of lesser amplitude. In five out of six patients with BEH, in contrast to normal controls, however, the aldosterone levels started to increase at 2000 h to give, by midnight, values similar to or even higher than the peak values.

In seven control subjects, the mean recumbent plasma aldosterone at midnight was 3·3 ng/100 ml ±0·6 S.E. vs a mean of 11·2 ± 1·0 in seven patients with BEH (Table 2). The difference was significant at $P < 0{\cdot}001$.

In addition, the binding of aldosterone to TLPF seemed to have a similar circadian periodicity to that of aldosterone (Fig. 8) in both control subjects and patients with BEH.

(*f*) *Postural response in MCR, urinary excretion of the 18-oxo-conjugate, and plasma concentration of total and TLPF-bound aldosterone.* The MCR of aldosterone decreased in control subjects by a mean of 24% in response to upright posture (sitting, standing and quiet ambulation, before and during the continuous infusion), as compared to values obtained on the previous day in recumbent posture following a full night recumbent in bed. In contrast, in 9 out of 13 patients with either stable or labile BEH, the same postural change did not induce any significant decrease in the MCR of aldosterone (Fig. 9). The TLPF binding did not change in response to posture in either the control subjects or patients with BEH.

In a further series of experiments, the effect of upright posture on the urinary excretion of the 18-oxo-conjugate, the most rapidly eliminated metabolite of aldosterone, was studied. In response to upright posture, the 4-h excretion of the oxo-conjugate increased consistently in control subjects (Fig. 10), increased slightly and with two exceptions in stable BEH, and decreased in labile BEH. Expression of the results per gram of creatinine [6] did not change the pattern, so that the observed differences are probably not due to variations in the glomerular filtration rate during postural adaptation.

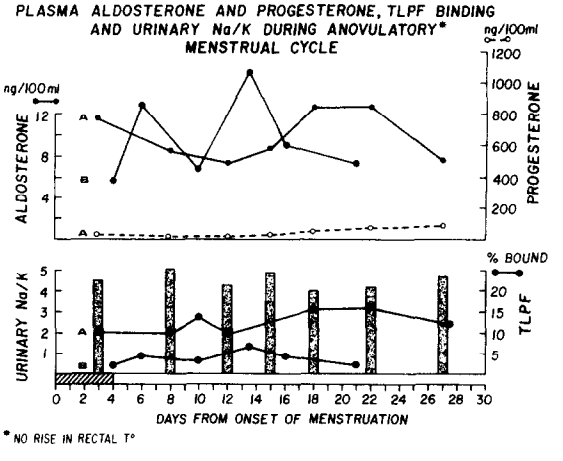


Fig. 7. Same as in Fig. 6, but during two anovulatory menstrual cycles (no rise in rectal temperature).

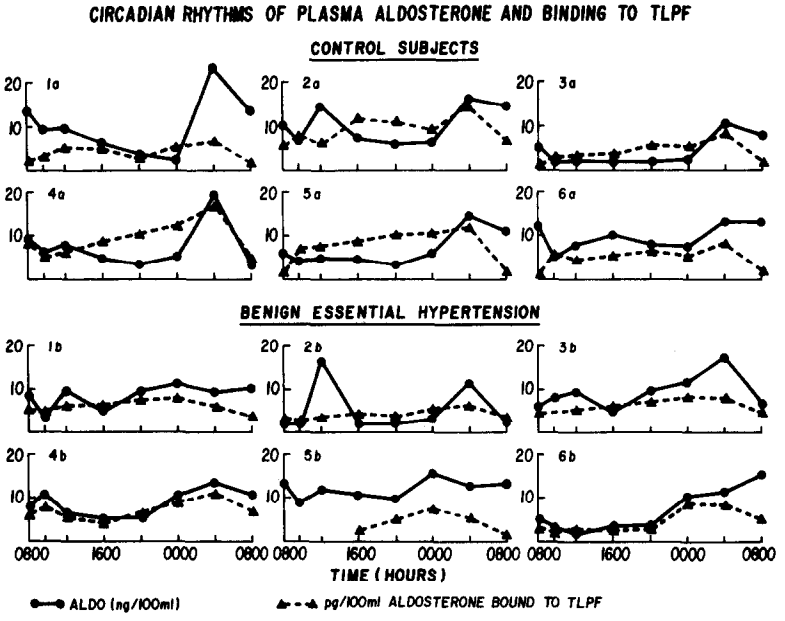


Fig. 8. The 24-h changes in recumbent posture, of plasma concentration and TLPF binding of aldosterone in control subjects and patients with BEH. The first 0800 h sample was obtained after the subjects had remained recumbent since the previous evening.

The upright posture resulted in a rise in plasma aldosterone concentration (at noon) in all control subjects (Fig. 10).

The same stimulus caused a greater mean increase in 17 patients with labile and 15 patients with stable BEH.

The increase of plasma aldosterone during upright posture, relative to the respective supine values, gave a mean ratio of 2·1 in control subjects and showed a greater increase in hypertensives, with a mean ratio of 3·4 ($P < 0{\cdot}002$) in labile and 2·6 in stable BEH. There was no change in the percentage of TLPF-bound aldosterone in response to upright posture.

(*g*) *Effects of ACTH administration on plasma free and TLPF-bound aldosterone.* Eight-hour intravenous infusions of ACTH (25 i.u.) resulted in a moderate increase in the plasma concentration of aldosterone in five control subjects and in a greater increase in five patients with low-renin BEH, as well as in three patients with BEH and normal renin activity (Fig. 11).

Table 2. Recumbent plasma aldosterone at midnight (ng/100 ml)

| Control subjects ($n$ = 7) | Benign essential hypertension ($n$ = 7) |
|---|---|
| 2·1 | 11·5 |
| 6·0 | 3·4 |
| 2·2 | 11·7 |
| 3·2 | 10·3 |
| 4·1 | 15·0 |
| 3·5 | 10·0 |
| 2·0 | 16·8 |
| Mean: 3·3 ± 0·54 S.E. | 11·2 ± 1·61 S.E. |

The difference between the three groups was more evident after the first 4 h than at the end of the infusion. The TLPF binding measurements indicated a sharp decrease in both the percentage binding and the concentration of the TLPF-bound fraction already after 4 h of the infusion in all three groups of subjects. The binding to this protein fraction was virtually absent at the end of the infusion. The inverse relationship between the unbound and bound fraction means that ACTH has increased the concentration of the free, physiologically active plasma aldosterone.

There seems to be a less pronounced decrease in binding to TLPF in patients with low-renin BEH than in control subjects.

After 4 h of ACTH infusion, the MCR of aldosterone increased by 60, 44, 50, 64 and 36% in five BEH patients respectively and by 50% in one control sub-

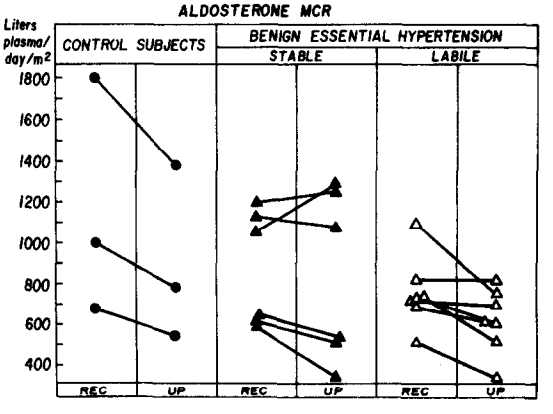


Fig. 9. The MCR of aldosterone in control subjects and patients with stable and labile BEH in recumbent and upright postures.

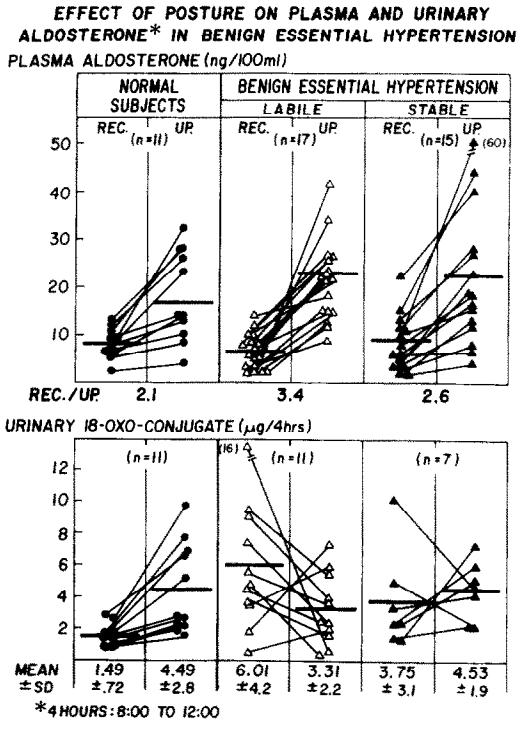


Fig. 10. Effect of posture on urinary excretion, from 0800 h to noon, of the 18-oxo-conjugate of aldosterone in control subjects and patients with stable or labile BEH and on plasma aldosterone concentration (samples taken at noon in either recumbent or upright posture) in normal controls and patients with labile BEH. The samples in upright posture were taken after 4 h in this position.

ject while the hepatic blood flow, measured by constant infusion of indocyanine green, remained unchanged in all subjects. The ACTH infusion induced a sodium retention in five control subjects and two BEH patients reflected by a 43% mean decrease in the urinary sodium excretion. In contrast, in seven other patients with BEH there was no change in the urinary sodium content. Simultaneously determined urinary 24-h excretion of the oxo-conjugate increased more and of the tetrahydroaldosterone increased less in response to ACTH in patients with BEH (18-oxo-conj./TH-aldo. = 0·06) than in control subjects (18-oxo-conj./TH-aldo. = 0·007).

(*h*) *Dexamethasone administration.* Dexamethasone administration resulted in a fall of plasma aldosterone, in both controls and patients with BEH, to very low levels (about 2 ng/100 ml from a mean of 9 ng/100 ml) with a very marked increase in percentage of aldosterone bound (40–50%) to TLPF and almost no change in the concentration of bound aldosterone which indicates that the decrease occurred mainly in the unbound aldosterone fraction.

*Dynamic studies on 18-Hydroxydeoxycorticosterone*

The secretion rate of 18-OH-DOC was measured after 8-h infusion of 25 i.u. of ACTH in control subjects and patients with BEH (Fig. 12). The 18-OH-DOC secretion rate rose in most patients with stable

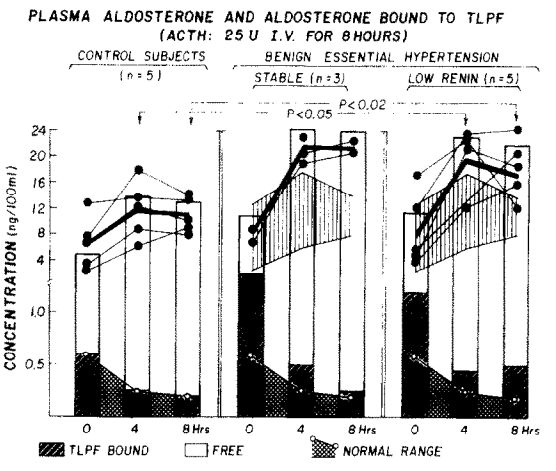


Fig. 11. Total, free and TLPF-bound plasma aldosterone in five control subjects (shaded areas), three patients with stable BEH and five patients with low-renin BEH at time 0 (0800 h) and after 4 h and 8 h of an intravenous infusion of 25 i.u. of ACTH. The means are shown by the thick lines.

BEH, whether with low or normal plasma renin activity, and there was thus an essentially normal response to ACTH infusion which resulted in some patients in very high values (Fig. 12).

Dexamethasone administration decreased the 18-OH-DOC secretion rate in all patients studied.

In addition, the protein binding of 18-OH-DOC (determined by means of Dextran-coated charcoal in variable proportions) was found to be negligible.

Dietary sodium restriction caused a significant increase in the 18-OH-DOC secretion rate in three control subjects while two patients with stable BEH had a similar response, a third patient showed a decrease and a fourth no change.

In two control subjects, potassium loading (250 m-equiv/day for 4 days) did not change the 18-OH-DOC

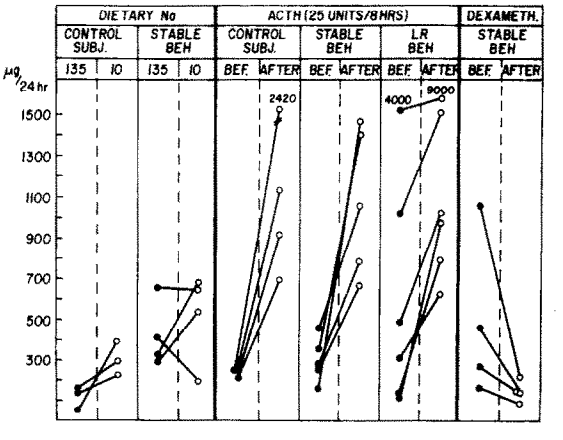


Fig. 12. 18-OH-DOC secretion rate (μg/day) in control subjects and patients with stable BEH on normal (135 m-equiv/day) and low (10 mEq/day) sodium intake, as well as the response to ACTH infusion (25 i.u. infused intravenously during 8 h) or dexamethasone in control subjects and patients with stable BEH or low-renin BEH.

secretion rate while plasma aldosterone increased from a mean of 13 to 54 ng/100 ml.

## DISCUSSION

Although the heat lability of the compound to which aldosterone binds allows a simple and specific assay of this binding, we do not yet know what (presumably protein) molecule is involved. However, its heat lability and persistence in diluted plasma are suggestive of specific high affinity protein binding. It is not known, for example, whether there is any relationship between the TLPF binding of aldosterone and, the higher than normal concentrations of immunoglogulins recently found in about 30% of hypertensive patients [20].

The above results suggest that the method used and the TLPF binding of aldosterone are indeed specific, and it is probably significant that the degree of binding to a heat-stable substance, probably albumin, does not change in any of the metabolic situations we have investigated.

Our studies suggest that the hepatic metabolism of aldosterone in BEH is modified [6, 7] because of an increased binding of aldosterone to TLPF and also because of a small but significant decrease in the hepatic blood flow [21, 22], both probably contributing to a significant decrease in the MCR of aldosterone [1–4, 6, 7]. The significant negative correlation between the MCR and the plasma concentration of aldosterone [6, 7] is compatible with this reasoning and emphasizes the importance of evaluating the MCR of aldosterone as a factor influencing plasma concentration in BEH.

The above results indicate a significant increase in the mean percentage binding of aldosterone to TLPF in a group of patients with low-renin BEH studied for the first time.

The inverse relationship between the MCR of aldosterone and the percentage of aldosterone bound to TLPF [6] on the one hand, and the plasma concentration of aldosterone on the other, suggest a possible regulatory role of the TLPF binding in maintaining the plasma aldosterone level. Low TLPF binding in cases of secondary aldosteronism, and normal TLPF binding in a case of primary aldosteronism, suggest that the increased TLPF binding in BEH is not merely a consequence of increased levels of circulating aldosterone and its increase in patients with labile BEH, the majority of which never had any medication, indicates that it is not a consequence of the treatment.

Aldosterone bound to TLPF is probably not metabolized by the liver and its metabolism is probably impaired in the tubules [9, 12]. The unbound portion appears to be significantly reduced by higher TLPF binding in patients with labile BEH (Fig. 4) as compared to control subjects even though the total concentration of aldosterone is apparently normal. The amount of bound aldosterone is as high or even higher in stable or low-renin BEH as in labile BEH, but because the total aldosterone concentration is higher the free aldosterone concentration is as high as in control subjects. In labile or stable BEH, transient excessive levels of plasma aldosterone occurred in response to dynamic challenges such as change of posture, diurnal rhythm or stimulation by ACTH.

Extra-renal control of sodium retention and potassium excretion by aldosterone has been demonstrated in a variety of tissues such as sweat glands, intestinal mucosa, muscle, colon, salivary glands, spleen, liver and brain (Ref. [23]).

It is not certain how aldosterone is reaching these target tissues without being metabolized. It has been shown that the distribution patterns of steroids bound to a globulin-like fraction with high binding affinity were qualitatively similar in blood and target tissues [24, 25]. Aldosterone, therefore, could be transported to some of the tissues as a TLPF complex and would then be transferred to the tissue proteins, having only a very short existence as free hormone. Since TLPF-bound aldosterone could be expected to have modified dynamics and probably a reduced circulation in the outer pool of distribution (out of the extracellular fluid), this could result in a different, and probably enhanced efficiency of conversion at the target sites. By this mechanism, the aldosterone complex could be affecting the sodium transport and determining the site of action of aldosterone in some area of the outer pool tissues where the receptors are located.

The tendency to lower values for the secretion rates (SR) of aldosterone, and the apparently higher peripheral plasma concentration [2–7], especially of the unbound fraction, in BEH [6] suggest that a negative feedback is operating in patients with BEH. Since only the unbound moiety is believed to be effective as a negative feedback signal, and since the increased peripheral level does not constitute this negative feedback *per se* [26, 27], an increased activity of aldosterone at some receptor site, resulting in a positive sodium balance, may represent the feedback loop. Observations indicating that the fall in mean arterial pressure seen in some hypertensive patients when on a low sodium diet is closely related to their loss of exchangeable sodium from non-plasma tissues [28] are therefore in favor of the above conclusion.

Studies of the percentage of aldosterone bound under various physiological and clinical conditions suggest that TLPF binding has some regulatory function.

The data obtained in a single case of primary hyperaldosteronism indicated that despite higher than normal production and plasma concentration of aldosterone, its TLPF binding remained normal, as did its hepatic metabolism (normal MCR and normal ratio of the two urinary metabolites). An additional conclusion is that the increased binding observed in BEH is probably not secondary and therefore does not constitute a defense mechanism against the increased plasma concentration of aldosterone. A similar conclusion can be drawn from the case of ectopic renin-producing tumor.

During pregnancy, increased total aldosterone concentration was accompanied by increased TLPF binding. This is in agreement with the previous observation [29] of binding of aldosterone during pregnancy to a globulin-like plasma fraction. The abrupt post-partum fall in plasma aldosterone concentration was not reflected by the same fall in the percentage binding. Increased TLPF binding may partially account for the absence of the effects of increased aldosterone during pregnancy.

In four female subjects, the plasma aldosterone concentrations showed sometimes, but not always, a moderate increase in the mid follicular phase and, invariably, a more marked increase in the mid or late luteal phase of the cycle. Both peaks were reflected by variations in the urinary sodium and potassium concentration in direct relationship to aldosterone, indicating that both peaks are of physiological importance. The TLPF binding seemed to follow the changes in aldosterone concentration. Both variations occurred in ovulatory as well as anovulatory cycles and were not related to changes in plasma progesterone.

The circadian rhythm study is compatible with a slight excess of circulating aldosterone in patients with BEH during the first part of the night, especially around midnight, in contrast to control subjects showing the lowest level in late evening.

The early morning peak of plasma aldosterone which normally occurs at 0400 h, comes at about midnight in patients with BEH. This could be a consequence of the apparent inability of patients with BEH to increase their MCR of aldosterone in response to recumbent posture [6, 7, confirmed in this study]. This unresponsiveness might be related to the increased TLPF binding of aldosterone. This increased binding may also, by reducing renal and hepatic aldosterone extraction, affect the plasma concentration and urinary excretion of aldosterone and its metabolites during stimulation or suppression of its production by the adrenals. Perhaps this explains, by delaying its elimination from the blood, the small excess of circulating aldosterone [6] in patients with BEH in the morning after rising.

Increased binding to TLPF may also explain the decreased degradation of aldosterone by the liver to the tetrahydrometabolite by decreasing its hepatic extraction [3–6], and resulting in a greater secretion of the 18-oxo-conjugate of aldosterone in BEH.

We have demonstrated that plasma aldosterone in three patients with normal-renin BEH and in five with low-renin BEH increased in response to ACTH infusions to a greater extent than in five control subjects. The difference was more evident between controls and low-renin BEH after 4 h of infusion ($P < 0{\cdot}02$) than after 8 h ($P < 0{\cdot}05$). The infusion of ACTH resulted in a sharp decrease in the percentage binding and the concentration of bound aldosterone to TLPF in all subjects and in a simultaneous marked increase in the MCR of aldosterone. This binding was even lower after 8 h. The mean decrease was less marked in patients with low-renin BEH. This decreased binding and a higher 18-oxo-conj./TH-aldosterone ratio in BEH than in control subjects are compatible with the observed increased MCR of aldosterone during ACTH infusion. This is in agreement with a very recent report by Pratt and Melby[30] who showed in healthy adult subjects on 200 m-equiv/day of sodium a five- and four-fold increase respectively in the urinary oxo-conjugate and tetrahydroaldosterone with almost no change in plasma concentration in response to chronic ACTH administration, while the MCR was increased from 46 to 91%.

In our control subjects, the acute ACTH stimulation resulted in only a slight increase in plasma aldosterone concentration. It is likely that the greater response in plasma aldosterone in patients was due in part to a greater increase in the secretion of aldosterone but at the same time to a less marked decrease in the TLPF binding and a smaller increase in the MCR. Besides, a nearly identical increase in plasma cortisol in the same control subjects and patients [7] with low-renin BEH indicates that the ACTH response of the adrenal gland was no different in the two groups of subjects. It would therefore be surprising that the increase in the secretion of aldosterone, a steroid dependent predominantly on the renin-angiotensin system, should be greater in patients than the response in plasma cortisol. Evidence for the dual control of aldosterone secretion has been given in recumbent control subjects. Aldosterone secretion was influenced primarily by ACTH during high sodium intake, in the presence of a suppressed renin-angiotensin system, and by renin during low sodium intake and by both ACTH and renin during intermediate sodium intake [31].

The latter experiments suggest that some patients with low-renin BEH may have an excess of mineralocorticoid activity because of a significant increase in the unbound aldosterone fraction and transient excesses of circulating aldosterone when exposed to stress.

In addition, the plasma of four dexamethasone treated subjects showed a significantly greater binding affinity for aldosterone than in normal plasma in the presence of a greatly reduced unbound fraction.

The metabolism of 18-OH-DOC, another mineralocorticoid with a frequently increased secretion rate in BEH [32], has been examined with a similar dynamic approach. It can be concluded that 18-OH-DOC secretion is significantly increased in about 60% of patients with normal plasma renin activity and even more so with the same frequency in patients with low renin activity [32]. The response to stimulation by ACTH or suppression by dexamethasone seems to be the same for control subjects and patients with BEH. This is in agreement with apparent virtual absence of binding of 18-OH-DOC to plasma protein fractions in both control subjects and patients and with the previously found normal MCR of 18-OH-DOC in patients with BEH [33]. Control subjects

responded to dietary sodium restriction with an increase in 18-OH-DOC secretion rate while in patients, this response was inconsistent.

## CONCLUSION

Aldosterone is present in the blood in both a free physiologically active form and in a protein-bound form. The blood level of aldosterone fluctuates markedly during stress, pregnancy, the menstrual cycle and in response to posture, dietary sodium intake, or ACTH infusion and dexamethasone administration. It has been shown that under most of these conditions, the response of patients with benign essential hypertension (BEH) differs from that in control subjects. This may be related at least in part to a reduced metabolic clearance rate of aldosterone in BEH. In addition, the response is often different between groups of patients with labile or stable BEH, or in patients with normal or low plasma renin activity. For further elucidation of these mechanisms, it becomes necessary to determine simultaneously several parameters of aldosterone under dynamic conditions and to fractionally determine the free and protein-bound aldosterone in plasma.

The data strongly suggest that the binding of aldosterone to a heat-labile component of plasma has some regulatory effect on the concentration of the physiologically active form of aldosterone in plasma and that changes in the degree of binding contribute to the perturbed metabolism of aldosterone in BEH.

We postulate that the increase in the protein-bound aldosterone may result, because of modified dynamics, in an enhanced tissue concentration and activity in an unidentified metabolic pool. The significance of this in the pathogenesis of essential hypertension is yet to be defined.

Blunted responses of plasma concentrations of this hormone to adrenal stimulation may occur in BEH without necessarily being due to abnormal responses of secretion or excretion.

In addition, patients with BEH have a hyperresponsiveness to ACTH in plasma aldosterone concentration with a simultaneous sharp decrease of the binding to the heat-labile component of plasma and a significant increase in the MCR of aldosterone.

The secretion rate of 18-hydroxydeoxycorticosterone is higher in about 60% of BEH patients with normal and especially with low plasma renin activity. ACTH stimulation or dexamethasone suppression of this hormone is the same in control subjects and patients. This hormone circulates in unbound form in control subjects and BEH patients.

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