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Further Studies on Urinary Aldosterone in Human Arterial Hypertension.* (23843)

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Two years ago, using a bioassay determination of a purified urinary aldosterone fraction, we reported a significant 2-fold increase in mean urinary aldosterone excretion of groups of severe essential and malignant hypertensive patients as compared to a group of normal subjects(1). Subsequent chromatographic study of this fraction revealed the possible occurrence of 6 other ultraviolet absorbing substances(2). This finding permitted elaboration of a new specific physico-chemical method for determination of aldosterone isolated from urinary extracts(3).

Because of extensive clinical and experimental evidence showing that adreno-cortical hormones with a predominantly mineralocorticoid activity may be directly concerned with the mechanism of arterial hypertension (4), we have applied this new chemical method to the study of 39 patients with essential, renal and malignant hypertension and another group of 11 patients with pheochromocytoma, Cushing's syndrome, primary aldosteronism, and coarctation of the aorta.

Method and subjects. The method used was previously reported(3). It is based essentially on isolation of aldosterone in a very high degree of purity and on its determination and identification by ultra-violet absorption at 239 mμ, reduction of blue tetrazolium and absorption spectra in 100% phosphoric acid and concentrated sulfuric acid. In the last 2 years we have had repeated difficulties with eluates from Whatman paper 1 and 2, whether it was specially selected for chromatography or not. Residues of blank eluates from these papers contain a number of interfering substances which react readily with a yellowish brown color after addition of tetramethylammonium hydroxide and blue tetrazolium(5). It has therefore been necessary to add a final purification procedure for separation of aldosterone from these interfering substances eluted from the Whatman paper.† A chromatographic glass column (internal diameter: 7 mm) is filled with Kieselgur (Hyflo-Super Cell, Johns Manville) 500 mg/500 mg water. The eluate residue of the aldosterone zone obtained in the last system (Bush B 5) is dissolved in 5 ml of benzene saturated with water. 15 ml of petroleum ether (D 0.67-0.69) are added and the mixture transferred to the column. The first eluate is discarded. Aldosterone is eluted by the second solvent fraction containing benzene (30 ml) and petroleum ether (10 ml). The Kieselgur and the solvents used are purified according to Hegedüs *et al.*(6) and Schindler *et al.*(7). Each patient was studied as follows: complete history with special emphasis on psychosomatic and emotional aspect of the patient's personality; detailed physical examination with special attention to the state of arteries, fundi and heart, electrocardiogram and teleoroentgenogram of heart, urine analysis with special attention to sediment, blood urea, phenolsulfonphtalein ex-

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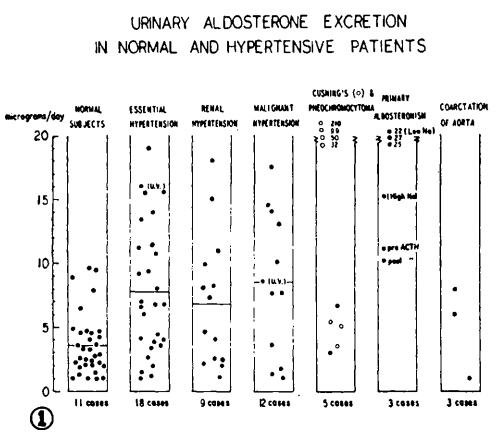
† We found recently that these contaminants interfere little with the isonicotinic acid hydrazide reaction described recently by Weichselbaum and Margraf and which depends on the presence of a Δ^4-3 ketone group (8).



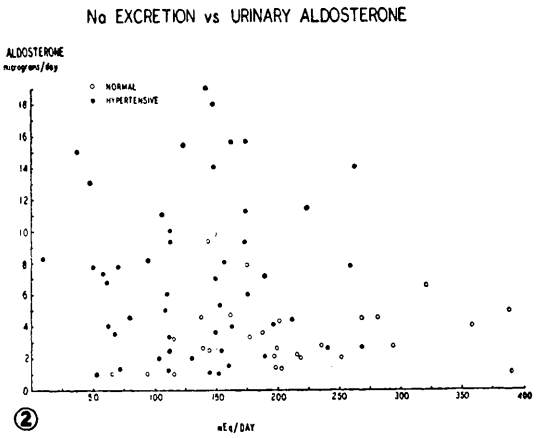


FIG. 1. Results of individual "spot" determinations done on normal subjects and patients with the various types of hypertensive cardiovascular disease.

FIG. 2. Urinary aldosterone excretion shows no correlation with urinary sodium in normal subjects or hypertensive patients on a self-selected diet containing 50 and 400 meq of sodium per day.

cretion, urea and endogenous creatinine clearance, serum potassium and intravenous pyelography. Retrograde pyelography, Rogitine and benzodioxane tests, and aortography were done when indicated. Patients were on ward diet without any restriction and all urine collections were done after a minimum of 48 and, in most cases, 72 hours of hospital admission. Exception was made for most patients with malignant hypertension who were on a low protein diet (30 g/day) and were not permitted to use the salt-shaker. Only one patient with essential hypertension was in congestive heart failure with generalized edema, and his aldosterone excretion was 3.3 μg/day. No other presented clinically detectable edema or venous distension. Venous pressure was taken in several patients and was within normal limits. In the clinical histories, 22 of the 39 patients complained of some degree of dyspnea on exertion. The presence of papilledema and of a diastolic pressure constantly above 130 mm of Hg were main criteria for differential diagnosis of the 12 patients with malignant hypertension. Nine of these were of renal origin and had very poor renal function with nitrogen retention and a high serum creatinine level. Hypertension of renal origin was diagnosed only when it was clear from the case history that the renal disease preceded the appearance of hypertension.

Results. Results are presented in 2 ways: (1) "spot" determinations done on normal subjects and patients with various types of hypertension (Fig. 1); (2) serial determinations done on one normal subject and 2 patients with early asymptomatic hypertension, one of benign essential variety and the other of renal origin secondary to repeated toxemia of pregnancy.

Thirty-three urinary aldosterone determinations in 11 normal subjects give a mean daily excretion of 3.6 μg/day, range 1 to 9.5 μg/day. Fifty-four aldosterone determinations were done in 39 patients with essential, renal

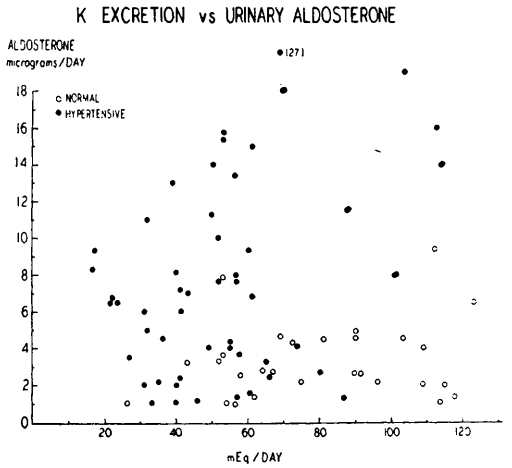


FIG. 3. Many patients with arterial hypertension excrete more aldosterone on a lower potassium intake than do normal subjects.

TABLE I. Aldosterone Excretion in Arterial Hypertension.

	Normal subjects	Hypertension		
		Essential	Renal	Malignant
No. of patients	11	18	9	12
No. of determinations	33	28	14	12
Mean (μg/24 hr)	3.6 $\pm$.42	7.8 $\pm$.96	6.9 $\pm$ 1.4	8.4 $\pm$ 1.4
"t"		4.1	2.99	4.22
"p"		<.001	<.001	<.001

and malignant hypertension. The mean excretion of each of these 3 groups was about twice the average normal excretion and about 55% of the patients showed an excretion higher than normal or in the higher range of normal. The significance of the differences between the means by the "t" test is such that the "p" value for the essential, renal and malignant groups is below 0.001 (Table I). These findings fully confirm our previous report(1a.b). Although these results show that in more than half of the patients with arterial hypertension, urinary aldosterone is in the high range or higher than normal, their significance is limited since they represent results of "spot" determinations. Therefore, one normal subject carrying his usual activities and on a self-selected diet was studied for 2 consecutive days in June 1956 and for 9 consecutive days in November 1956. His aldosterone excretion was quite constant between 1.3 to 6.5 μg/day despite marked fluctuations of urinary sodium from 66 to 386 meq/day. On the other hand, one patient with asymptomatic benign essential hypertension was studied for 5 consecutive days and her aldosterone excretion was 7, 15.7, 3.6, 9.3 and 14 μg/day with a fairly constant urinary sodium output between 150 and 170 meq/day.

A second patient with asymptomatic renal hypertension secondary to repeated toxemia of pregnancy showed similar variations of 12.1 (average of 2 days) 2.4, 4.5 and 7.2 μg/day. No reasons of acute stress, anxiety state, changes in sodium or potassium intake could be found to account for these variations in the 2 patients.‡

Two patients with proven pheochromocytoma had values within the normal range. Three patients with Cushing's syndrome showed variable results. One of these, who presented severe hypertension, hypernatremia (153 meq/liter), hypokalemia (2.45 meq/liter) due to bilateral adrenal hyperplasia, had a urinary aldosterone of 99, 50, 210, 32 μg/day(9). Because the critical state of patient's condition did not permit early bilateral adrenalectomy, he was given daily X-ray therapy over the pituitary region and testosterone propionate (25 mg 3 times a week). After 4 weeks of this treatment, the aldosterone excretion was 4.7 μg/day. A second patient with bilateral adrenal hyperplasia and with a normal blood pressure and normal sodium and potassium serum levels, had an aldosterone excretion of 5.3 μg/day which rose to 15.6 during intravenous ACTH infusion (25 units during 8 hours). The third patient with Cushing's syndrome due to a chromophobe adenoma of the pituitary had values of 3.5 and 5 μg/day. This patient was moderately hypertensive.

Three patients with primary aldosteronism were studied. One patient was studied by Dr. Arnold Relman at Mass. Memorial Hospital, Boston, under conditions of low and high sodium intake and before and during ACTH treatment. Results are indicated in Fig. 1. Three patients with coarctation of the aorta had values within the normal range. The pre-operative value on one such patient (followed by Dr. Nathan Sims, University of Vermont) was 7.8 and following surgical correction the values were 7.3 μg eight days post-operatively and 2.6 μg/day 26 days post-operatively.

Among the factors which could be related to aldosterone excretion in normal and hypertensive patients, the following were studied:

‡ Since submission of manuscript, a third patient with benign essential hypertension was studied for serial urinary aldosterone determinations and presented similar variations of 10.2, 1.2, 1.6, 15.5, 14.5, 4, 8.8 μg/day. This patient's only complaints were periods of dizziness, scotomas and nycturia of two months duration. No symptoms or signs of cardiac failure. Venous pressure: 70 mm H_2O. Endogenous creatinine clearance 100 ml/min. PSP excretion: 61% in 60 minutes.

urine volume, urinary sodium and potassium, urinary Na/K and K/Na ratios and cardio-thoracic ratio. No correlation could be observed between aldosterone excretion and sodium intake as reflected by urinary sodium which varied between 50 and 400 meq/day (Fig. 2). No correlation could be made with the Na/K or the K/Na ratios. Similar findings were also reported in normal subjects on unrestricted diets by Tait and his group(10).

No correlation could be made between aldosterone excretion and the urine volume, cardio-thoracic ratio or presence or absence of dyspnea on exertion. One finding of interest shows that many hypertensive patients have a higher aldosterone output on a lower potassium intake than the normal subjects (Fig. 3). This is in contrast with the findings of the stimulating effect of high potassium intake on the aldosterone excretion(3,11,12,13). These observations serve to emphasize that aldosterone excretion in hypertensive patients or in normal subjects on self-selected diets is the result of the interaction of many factors, several as yet unknown.

Conclusion. Our results indicate 1) increased aldosterone excretion in about 55% of patients, 2) a very significant difference between mean aldosterone excretion of patients with essential, renal or malignant hypertension as compared with normal subjects, 3) a much greater degree of fluctuation in serial daily aldosterone determinations in 2 early asymptomatic hypertensive patients as compared to a normal subject. These observations bring additional and direct evidence for an adrenal cortical disturbance in hypertensive cardio-vascular disease. Although the evidence is suggestive, it cannot be established at the present time if these findings play an etiological role in pathogenesis of arterial hypertension. They may provide the explanation for the successful use of salt depletion by chlorothiazide and mercurials as essential adjunct to the "effectiveness" of the present anti-hypertensive therapy.

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