

THE EFFECTS OF INTRAVENOUS INFUSIONS OF VALINE-5
ANGIOTENSIN II AND OTHER PRESSOR AGENTS ON
URINARY ELECTROLYTES AND CORTICO-
STEROIDS, INCLUDING ALDOSTERONE *

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Previous studies from this laboratory (1-4) have shown that: 1) there is a statistically significant increase in the mean urinary aldosterone excretion from groups of patients with essential, renal or malignant hypertension, as compared with that of normal subjects similarly studied; 2) 43 per cent of patients with essential hypertension have urinary aldosterone values above normal limits; 3) there is an excessive fluctuation of daily urinary aldosterone in hypertensive patients, from normal to above normal levels, which is most prominent in the early phase of the disease; and 4) mean urinary pregnanetriol is significantly decreased ($p < 0.001$) in the same groups of hypertensive patients, and the pregnanetriol/aldosterone ratio is below the lower limits of normal range in 92 per cent of all patients studied with arterial hypertension.

The findings of a mean increase in urinary aldosterone and of its excessive daily fluctuation in hypertensive patients have recently been confirmed by Romanelli, Biancalona and Matterazzi (5), Venning, Dyrenfurth, Dossetor and Beck (6) and Schwartz (7). However, Laragh and co-workers (8), using a method devised by Ulick, Laragh and Lieberman (9) for the measurement of aldosterone secretion rate, found normal values in 8 patients with benign essential hypertension and significantly increased secretion rates in 5 of 8 pa-

tients with advanced hypertension and in 14 of 15 patients with malignant hypertension. On the other hand, in a preliminary study, Muller found normal aldosterone secretion rates in patients with malignant hypertension (10).

It appears however, that this disturbance in adrenocortical regulation may not be the basic or the sole factor in the pathogenesis of hypertension. Both clinical and experimental evidence strongly suggest that the kidney plays a definite role. We therefore studied the possibility of a relationship between the renal pressor system and aldosterone.

Our preliminary results (11), showing a marked stimulatory effect of angiotensin II on urinary aldosterone, have been confirmed in the present study.

Seven normal subjects were studied. Continuous intravenous infusions of glucose, angiotensin, norepinephrine and phenylephrine were administered for periods of 7 to 14 hours, and urines were analyzed for sodium, potassium, aldosterone, cortisol, cortisone and their tetrahydro derivatives. The results obtained show a marked and specific tropic effect of angiotensin on aldosterone excretion.

SUBJECTS AND METHODS

The subjects were 7 healthy male volunteer medical students, maintained on a fixed sodium and potassium intake (102 and 90 mEq per day, respectively) for 5 days prior to and during the experimental period. They were admitted to hospital for metabolic balance for the duration of the experiments. Urine was collected every 24 hours, except on infusion days, when it was collected during the period of infusion and again separately for the rest of the 24 hour period (post-infusion period). Six infusions were carried out with synthetic valine-5 angiotensin II, two with norepinephrine¹ and two with phenylephrine.² The pressor substances were diluted in 5 per cent

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¹ Winthrop, Levophed.

² Winthrop, Neosynephrine.

TABLE I
Effects of *i. v.* valine-5 angiotensin II infusions in normal male volunteers *

Patients	Dose of angiotensin	Aldosterone† (μg/day)			
		Day 1	Day 2		Day 3
			Angiotensin infusion	Post-angiotensin infusion	
J.M.	2 mg/14 hrs	14	52	69	68
R.A.	3.1 mg/7 hrs	10	14	47	
	3.4 mg/13 hrs	11	105	98	28
F.M.	1.2 mg/8 hrs	19	32	42	23
J.-P. D.	0.9 mg/7 hrs	5	36	27	16
H.N.	0.9 mg/12 hrs	23	84	29	25

* On fixed Na and K intake (102 and 90 mEq/day, respectively).

† Nowaczynski, Koiw and Genest's procedure (21).

glucose in water and infused at a rate sufficient to maintain a constant elevation of diastolic pressure of at least 30 to 40 mm Hg above control levels, for periods ranging from 7 to 14 hours. Control infusions of 5 per cent glucose in water were given to 5 subjects.

Urinary sodium and potassium were determined with a model 52 C Perkin-Elmer flame photometer. The procedures used for hydrolysis, extraction, purification and determination of the steroids, as well as the technical details, have been previously described (3, 12, 13). These

TABLE II
Effects of *i. v.* infusions of angiotensin and norepinephrine in normal male subjects *

		Urinary excretion								
		K	Na	Aldosterone	Tetrahydroaldosterone	Cortisol	Cortisone	Tetrahydrocortisol	Tetrahydrocortisone zone†	Sum of "F" + "E" THF + THE
		mEq/day			μg/day		μg/day		μg/day	mg/day
R.A. (22 yrs)										
Day 1	Control	84	85	11	76	70	133	3,017	1,407	4.6
Day 2	Angiotensin inf. (3.4 mg/13 hrs)	78	31	105	505	218	227	2,382	6,792	9.6
	Post-inf. period	46	23	98	383	444	133	907	2,129	3.6
Day 3	Control	90	108	28	138	125	153	3,744	1,815	5.8
Day 4	Control	75	78	19	65	180	74	2,099	803	3.2
Day 5	Norepinephrine inf. (6.3 mg/7 hrs)	60	230	41	493	508	511	2,348	3,259	6.6
	Post-inf. period	65	26	26	73	106	246	990	1,445	2.8
J.M. (26 yrs)										
Day 1	Control	39	39	14	195	210	89	1,229	790	2.3
Day 2	Angiotensin inf. (2 mg/13.6 hrs)	81	14	52	336	248	97	1,916	1,357	3.6
	Post-inf. period	73	12	69	595	102	137	3,752	2,358	6.3
Day 3	Control	48	35	68	226	193	197	3,989	3,404	7.8
J.P.D. (21 yrs)										
Day 1	Control	67	58	5	71	107	140	1,024	1,768	3.0
Day 2	Angiotensin inf. (0.9 mg/7.3 hrs)	50	6	36	389	218	249	931	1,852	3.2
	Post-inf. period	69	31	27	249	93	85	699	966	1.8
Day 3	Control	95	39	16	56	229	197	1,954	1,162	3.5
H.N. (23 yrs)										
Day 1	Control	75	53	23	66	85	183	2,199	1,806	4.3
Day 2	Angiotensin inf. (0.9 mg/12.4 hrs)	70	16	84	113	137	311	876	3,955	5.3
	Post-inf. period	44	6	29	458	117	291	585	1,211	2.2
Day 3	Control	74	88	25	138	141	239	3,317	912	4.6

* On a fixed Na and K intake (102 and 90 mEq/day, respectively).

† The measurement of the "tetrahydrocortisone zone" by the blue tetrazolium reaction includes also that of tetrahydroaldosterone and of *allo*-tetrahydrocortisol.

procedures are essentially based on isolation of the various steroids in a high degree of purity. Identification of the individual steroids is based on chromatographic mobilities in two or three paper systems in comparison to mobilities of standard reference substances, on ultraviolet light absorption, reaction with blue tetrazolium (14) and isonicotinic acid hydrazine (15), absorption spectra in concentrated sulfuric acid (16-18) and in 100 per cent phosphoric acid (19-21).

Since the blue tetrazolium reaction was used for determination of tetrahydrocortisone, the indicated values include also those of the ring-A reduced metabolite of aldosterone, tetrahydroaldosterone (pregnane 3α -18,21-triol-11,20-dione) and of *allo*-tetrahydrocortisol (3α ,11 β ,17 α ,21-tetrahydroxy-*allo*-pregnane-20-one).

Mean recovery of added aldosterone to urine aliquots from normal subjects was 83 per cent. As we have emphasized in the past (21) the recovery is a direct function not only of the procedure itself, but mainly of the meticulousness in the technique of completely solubilizing residues, of their total transfer from beaker (or other container) to paper chromatograms and of complete elution of "steroids spots" from the chromatograms. Reproducibility in the hands of a well trained, experienced

and meticulous technician is within 10 to 20 per cent, depending on the amount of steroid present in urine.

Peterson, Karrer and Guerra's modification of Silber and Porter's procedure was used for determination of urinary 17-hydroxycorticosteroids (22). Pregnanetriol was measured according to Bongiovanni and Eberlein's procedure (23). For the measurement of the ring-A reduced metabolite of aldosterone (24), the material eluted from the zone containing this compound in the ethylene glycol: toluene system was acetylated and chromatographed in the iso-octane, 1: cyclohexane, 2: ethylene glycol system (24). The reduced metabolite of aldosterone was measured as a triacetate using the blue tetrazolium reaction. Our assumption that the material measured is the ring-A reduced metabolite of aldosterone is based on the following facts: 1) its similar chromatographic mobility to the metabolite A2 of aldosterone described by Sandor, Nowaczynski and Genest (24) in the following paper chromatographic systems—(a) for the free compound in toluene: ethylene glycol, and (b) for the acetate derivatives in toluene, 1: iso-octane, 1:70 per cent methanol (Bush B 1 system) and iso-octane, 1: cyclohexane, 2: ethylene glycol; 2) the same chromatographic mobility as the compound described by Ulick and Lieberman (25)

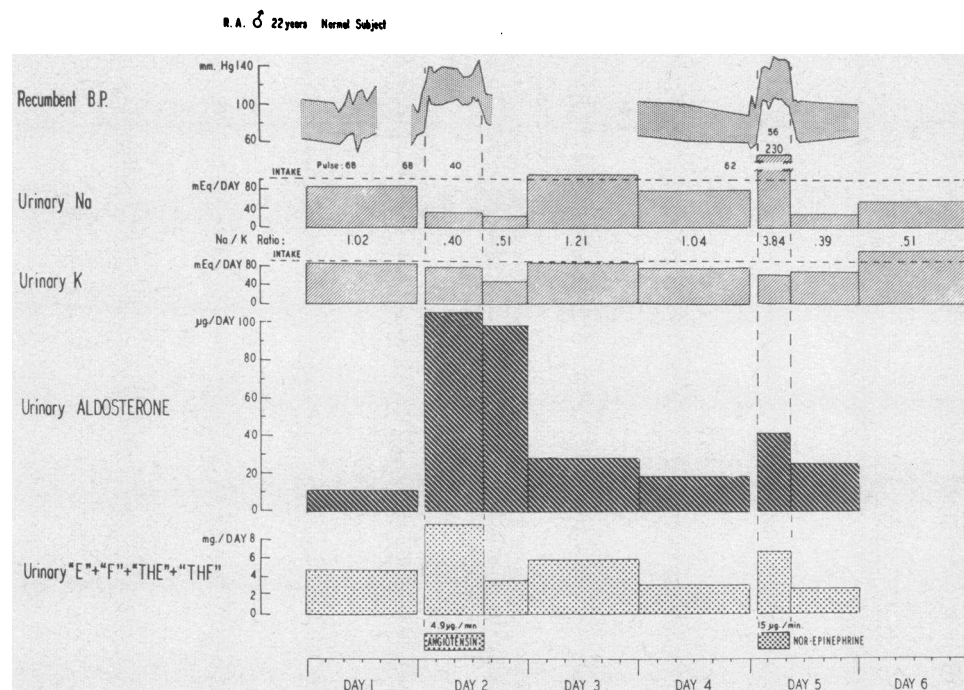


FIG. 1. EFFECT OF INFUSION OF ANGIOTENSIN ($4.9 \mu\text{g}$ PER MINUTE FOR 13 HOURS) AND OF NOREPINEPHRINE ($15 \mu\text{g}$ PER MINUTE FOR 7 HOURS) ON BLOOD PRESSURE, URINARY SODIUM, NA: K RATIO, POTASSIUM, ALDOSTERONE AND THE SUM OF SEPARATELY DETERMINED CORTISONE, CORTISOL AND THEIR TETRAHYDRO DERIVATIVES. Note the extreme stimulation of aldosterone which persisted for 10 hours after the infusion of angiotensin and which was accompanied by marked fall in urinary sodium and Na: K ratio and a slight rise in the 17-hydroxycorticosteroids. In contrast, the norepinephrine infusion produced a slight rise in urinary aldosterone coincident with a marked increase in urinary sodium and Na: K ratio.

in the benzene:50 per cent methanol system; 3) the identical spectrum in concentrated sulfuric acid to that given by metabolite A2 of aldosterone as described by Sandor and associates (24) and; 4) the decreased polarity of the compound after acetylation, which indicates formation of a triacetate.

RESULTS

The over-all effect of the angiotensin infusions on urinary aldosterone of normal males is shown in Table I. The rate of angiotensin infused varied from 1.2 to 7.4 μg per minute. There is a two- to tenfold increase in urinary aldosterone during and/or immediately following the angiotensin infusions. This increase can persist for 1 day after the infusion. The mean urinary aldosterone excretion of the group was 12 μg per day during the control period, 54 during the period of infusion, and 52 during the post-infusion period.

The results of the daily excretion of sodium, potassium, and of cortisol, cortisone and their tetrahydro derivatives in four subjects in whom they were analyzed are shown in Table II. During the infusion of angiotensin, there is a very slight but consistent rise in the sum of these steroids above control levels. In two subjects, the sum of these steroids increased two- to threefold, but only once was the value definitely above the range of normal variations. The increase is mostly evident in the tetrahydrocortisone zone, which includes the tetrahydroaldosterone. The latter, when separated, accounted for part of the rise and showed changes parallel to those of aldosterone. The twofold increase in the sum of 17-hydroxycorticosteroids in Subject R.A. is still within the range of normal variations.

The infusion of angiotensin (3.4 mg per 13 hours) in Subject R.A. was accompanied and followed by a fall in urine volume, a sodium retention, and a tenfold increase in urinary aldosterone (Figure 1). Smaller increases in cortisol, cortisone and their tetrahydro derivatives were noted (Table II). The sum of the 17-hydroxycorticosteroids went up from 4.6 to 9.6 mg per day. The norepinephrine infusion (15 μg per minute for 7 hours) provoked a very marked natriuresis accompanied by an increase in aldosterone (twofold), tetrahydroaldosterone (eightfold), cortisol (threefold), cortisone (sevenfold) and "tetrahydrocortisone zone" (threefold). This occurred only during the period of the infusion.

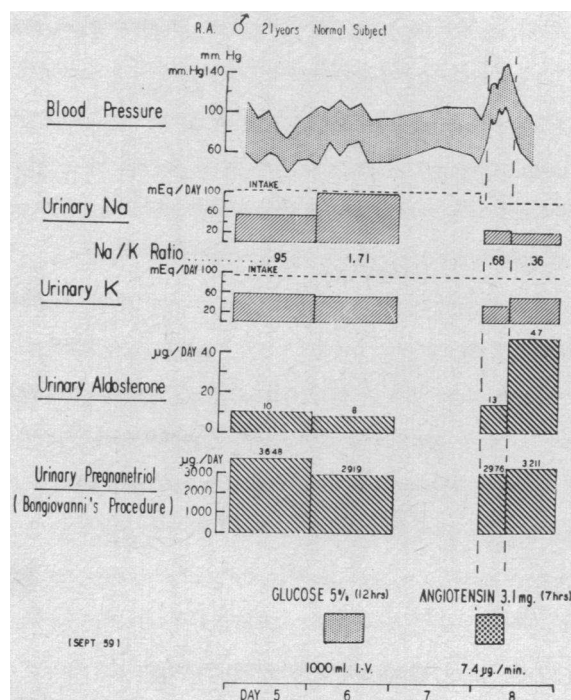


FIG. 2. EFFECTS OF INFUSIONS OF 5 PER CENT GLUCOSE IN WATER (1 L FOR 12 HOURS) AND OF ANGIOTENSIN (7.4 μg PER MINUTE FOR 7 HOURS) ON BLOOD PRESSURE, URINARY SODIUM, NA:K RATIO, POTASSIUM, ALDOSTERONE AND PREGNANETRIOL. Glucose infusion was accompanied by an increase in sodium output without any change in urinary aldosterone. In contrast, the angiotensin infusion was immediately followed by a fourfold rise in urinary aldosterone accompanied by a marked fall in sodium output and in the Na:K ratio.

Another experiment was performed on the same subject who received a control infusion of 5 per cent glucose for 12 hours and a second infusion of angiotensin (3.1 mg per 7 hours). No change in urinary aldosterone is observed during the glucose infusion, but the angiotensin administration is accompanied by a marked sodium retention and followed by a large increase in aldosterone (Figure 2).

In Subject J.M. (Figure 3), the infusion of angiotensin (2 mg per 14 hours) is accompanied and followed by sodium retention and a fivefold increase in urinary aldosterone excretion which persists through the day after infusion. The sum of the 17-hydroxycorticosteroids rose progressively from 2.3 to 7.8 mg per day.

In Table III are shown the results of two phenylephrine infusions on aldosterone excretion, which fell markedly during and immediately fol-

J.M. ♂ 26 years Normal Subject

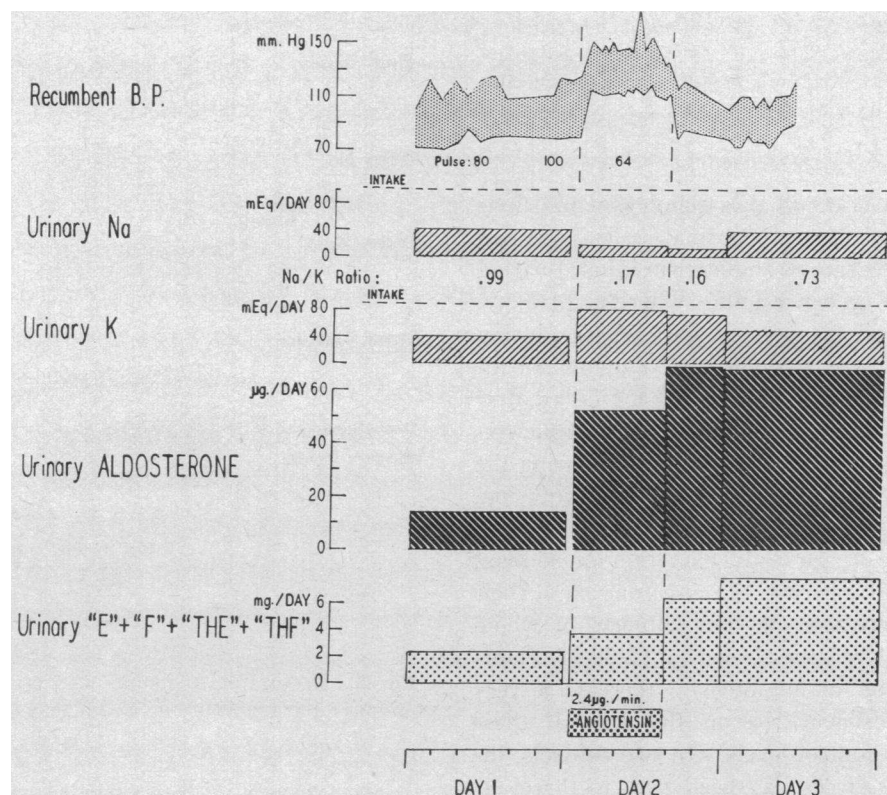


FIG. 3. EFFECTS OF ANGIOTENSIN INFUSION ($2.4 \mu\text{g}$ PER MINUTE FOR 14 HOURS) ON BLOOD PRESSURE, URINARY SODIUM, NA:K RATIO, POTASSIUM, ALDOSTERONE AND THE SUM OF SEPARATELY DETERMINED CORTISONE, CORTISOL AND THEIR TETRAHYDRO DERIVATIVES. The very marked rise in urinary aldosterone during the infusion persisted immediately after the infusion and the following day and was accompanied by a marked fall in urinary sodium and Na:K ratio, and a significant increase in potassium. Urinary 17-hydroxycorticosteroids increased slightly and progressively.

lowing the infusions, while a pronounced natriuresis was produced.

Subject P.M., a hyper-reactor with blood pressure readings occasionally above 150/90 mm Hg, received an intravenous phenylephrine infusion at the rate of $10 \mu\text{g}$ per minute for 8 hours. Aldosterone fell significantly, while sodium excretion increased from a control level of 60 to 274 mEq per day (Figure 4). This subject did not show significant change in urinary aldosterone during a control 5 per cent glucose infusion.

Intravenous infusion of phenylephrine at the rate of $8.3 \mu\text{g}$ per minute for 8 hours in Subject G.T. (Figure 5) was accompanied by a fall in urinary aldosterone coinciding with a very marked natriuresis. This subject also received, 2 days

before, a norepinephrine infusion, but unfortunately the urine aliquot collected during the infusion was accidentally lost before steroid determinations. There was no change in sodium

TABLE III
Effects of phenylephrine infusions in normal male volunteers *

Subjects	Urinary aldosterone†			
	Day 2			Day 3
	Day 1	Phenyleph. infusion	Post-phenyleph. infusion	
	$\mu\text{g/day}$	$\mu\text{g/day}$	$\mu\text{g/day}$	$\mu\text{g/day}$
G.T.	32	11	16	34
P.M.	26	12	17	12

* On fixed Na and K intake (102 and 90 mEq/day).

† Nowaczynski, Koiv and Genest's procedure (21).

P. M. ♂ 22 years Hyper-reactor

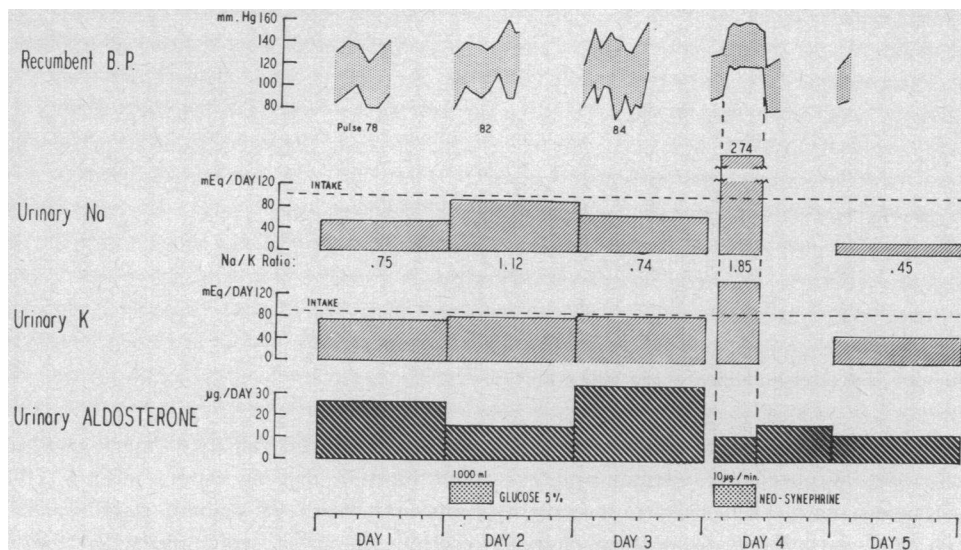


FIG. 4. EFFECTS OF INFUSIONS OF 5 PER CENT GLUCOSE IN WATER (1 L FOR 8 HOURS) AND OF PHENYLEPHRINE (10 μ G PER MINUTE FOR 8 HOURS) ON BLOOD PRESSURE, URINARY SODIUM, NA:K RATIO, POTASSIUM AND ALDOSTERONE. In contrast to the effects of angiotensin, phenylephrine, infused at a rate sufficient to produce the same increase in diastolic pressure, provoked an important natriuresis accompanied by a marked rise in Na:K ratio and potassium, and by a significant fall in aldosterone.

excretion, and no increase in aldosterone was noted during the post-infusion period.

In another series of experiments previously done in our laboratory (26), we have studied the

effects of 8-hour intravenous infusions of norepinephrine and of epinephrine in 5 per cent glucose on the urinary excretion of aldosterone and 17-hydroxycorticosteroids. Three normotensive sub-

G.T. ♂ 22 years Normal Subject

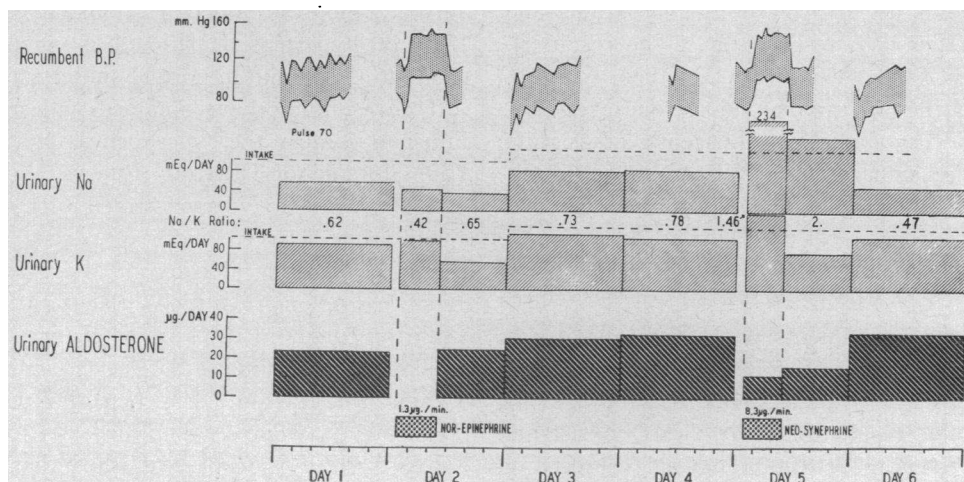


FIG. 5. EFFECTS OF INFUSION OF NOREPINEPHRINE (1.3 μ G PER MINUTE FOR 8 HOURS) AND OF PHENYLEPHRINE (8.3 μ G PER MINUTE FOR 8 HOURS) ON BLOOD PRESSURE, URINARY SODIUM, NA:K RATIO, POTASSIUM AND ALDOSTERONE. The patient's response to phenylephrine is exactly superimposable upon the one illustrated in the previous figure.

TABLE IV
Effects of 8-hour i. v. infusions of norepinephrine and epinephrine (in 5 per cent glucose)

Patients*	Age	Diagnosis	Aldosterone†			17-Hydroxycorticosteroids‡		
			Control glucose 5%	Norepin.	Epin.	Control glucose 5%	Norepin.	Epin.
			µg/day	µg/day	µg/day	mg/day	mg/day	mg/day
R.B.	25	Benign ess. hypert.	9	3	3	4.3	5.5	6.6
A.C.	18	Benign ess. hypert.	3	3	7			
W.C.	41	Benign ess. hypert.	8	8	9	6.3	8.0	7.9
C. de B.	18	Normal subject	7	8	11	4.2	5.6	5.2
T.P.	35	Schizophrenia	9	12	4			
A.G.	28	Obesity	5	5		1.6	5.5	

* Unrestricted diet.

† Nowaczynski, Koiw and Genest's procedure (21).

‡ Peterson and associates' modification of Silber-Porter (22).

jects and three patients with early benign essential hypertension were admitted to hospital and received unrestricted diets. On Day 1, a control infusion of 5 per cent glucose was administered for 8 hours. On Day 2, an 8-hour norepinephrine infusion in 5 per cent glucose was given at a rate sufficient to produce a slight increase in systolic (mean, 25; range, 12 to 41) and diastolic (mean, 15; range, 7 to 24 mm Hg) blood pressure, and an average decrease of 11 pulsations per minute (range, 5 to 16). On Day 3, an 8-hour epinephrine infusion in 5 per cent glucose was given at a rate sufficient to produce an average increase of 29 pulsations per minute (range, 19 to 40). Urines were collected only during the time of infusion and all results of urinary aldosterone and 17-hydroxycorticosteroids are expressed in micrograms and milligrams per day, respectively. The results illustrated in Table IV indicate that there is essentially no change in urinary aldosterone excretion. There is a very slight, but apparently not significant, rise in urinary total 17-hydroxycorticosteroids with equal distribution in both free and conjugated fractions.

DISCUSSION

In the six experiments performed, angiotensin markedly reduced urine volume, sodium excretion and the urinary sodium:potassium ratio. This confirms the results of Peart who consistently observed the same effect in normal subjects receiving angiotensin infusions (27-29). The effects of angiotensin administration in normal subjects appear to be twofold: 1) a hemodynamic one

with pronounced fall in glomerular filtration rate and filtered load of water, sodium, chloride and potassium with subsequent reduction in their excretion, an effect seen only when there is a significant rise in blood pressure; and 2) a direct effect on the tubular reabsorption of water and sodium either per se or through an increase in aldosterone secretion, as the present experiments strongly suggest. Peart has demonstrated that the same effect on water and sodium excretion could be obtained without any change in glomerular filtration rate when angiotensin is infused at a very slow rate—insufficient to raise the blood pressure. It is also of great interest that, according to Peart's experiments, hypertensive patients show an increase in urine volume and natriuresis during angiotensin infusion. This effect, noted in 17 of 20 hypertensive patients, is completely opposite to that found in normal subjects (29).

The results we have obtained with angiotensin infusions confirm the experimental data of Tobian (30, 31) and of Hartroft, Pitcock and Hartroft (32, 33), who have demonstrated in rats a parallel relationship between width of adrenal zona glomerulosa secreting aldosterone and granularity of renal juxtaglomerular cells elaborating renin or renin precursor in response to changes in sodium intake. Our findings also agree with those of Deane and Masson (34), recently confirmed by Hartroft, Newmark and Pitcock (35) and by Gross (36, 37), who established that renin administration produces a rapid and marked increase in width of the adrenal zona glomerulosa in rats.

Another aspect of these studies is given empha-

sis by the recent experiments of Hilton (38). Using *in vivo* perfusion of adrenal glands in dogs, Hilton has demonstrated that vasopressin specifically stimulates cortisol secretion and does not influence that of aldosterone. Since vasopressin is closely related chemically to angiotensin, it is of interest to note that these two related polypeptides can specifically and differentially stimulate the secretion of the two main corticosteroid hormones.

If the studies of Laragh and co-workers (8) on the increased aldosterone secretion rate in human malignant hypertension are confirmed, despite the negative preliminary results of Muller (10), it is difficult to reconcile the urinary values found, for example, in patients with malignant hypertension, where 21 of 38 determinations in 26 patients were within normal range by our method (3, 21), and those of Laragh who found aldosterone secretion rates above 700 μg per day (normal range, 150 to 330 μg per day) in 11 out of 15 patients with malignant hypertension. This is somewhat surprising, since in the cases of primary aldosteronism in which high urinary aldosterone levels are almost always found, secretion rates below 650 μg per day have been found in 3 of 5 patients studied by the same authors. This would indicate quite important differences in metabolism of aldosterone in these two diseased states. The solution of these discrepancies must await further work, especially the serial measurement of secretion rate in the same patients as well as the simultaneous plasma level and urinary excretion of aldosterone. More study is also necessary to elucidate the factors responsible for the excessive fluctuation in urinary aldosterone of patients with benign hypertension.

The stimulatory effect of angiotensin on aldosterone appears quite marked and specific. Such effect was noted only once (a nearly twofold rise) in seven experiments with norepinephrine and not with phenylephrine or epinephrine. The increase in urinary aldosterone is probably not the result of the induced hypertensive state, since it is not encountered with phenylephrine and norepinephrine (with the one exception), although significant increases in blood pressure were achieved in all instances. Since some experimental data suggest that hyperfunction of the adrenal zona glomerulosa is accompanied by increased granularity of juxta-

glomerular cells and renin content of kidneys, it would be of the utmost interest to determine plasma angiotensin levels in patients with proven primary hyperaldosteronism. Studies are actually under way in our laboratory to do simultaneous determinations of blood angiotensin (39), urinary aldosterone, pregnanetriol and pregnanetriol: aldosterone ratio in hypertensive patients.

SUMMARY AND CONCLUSION

Seven healthy young men were studied, under conditions of metabolic balance for sodium and potassium, for the effect of intravenous infusions of glucose, angiotensin, norepinephrine and phenylephrine on urinary electrolytes, aldosterone, cortisol, cortisone and their tetrahydro derivatives. Six angiotensin infusions done in five subjects were accompanied or followed by sodium retention, antidiuresis, a two- to tenfold increase in aldosterone excretion and a small and probably not significant increase in cortisol, cortisone and their tetrahydro derivatives. Two phenylephrine infusions stimulated diuresis and sodium excretion, and decreased aldosterone excretion. Urinary aldosterone did not increase during similar infusions of 5 per cent glucose in water, and in six out of seven infusions with norepinephrine.

These experiments establish that angiotensin has a marked and quite selective effect on aldosterone excretion in man. Its effect on urinary 17-hydroxycorticosteroids is very slight and probably not significant from the physiological point of view. This strongly suggests a direct evidence, in humans, of a definite correlation between the adrenal zona glomerulosa secreting aldosterone and the renal juxtaglomerular apparatus elaborating renin or renin precursor. It also opens new areas of research in the field of relationships between hormones of polypeptidic and steroidal nature.

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