Studies of the Control of Plasma Aldosterone Concentration in Normal Man

I. RESPONSE TO POSTURE, ACUTE AND CHRONIC VOLUME DEPLETION, AND SODIUM LOADING

GORDON H. WILLIAMS, JOHN P. CAIN, ROBERT G. DLUHY, and RICHARD H. UNDERWOOD

From the Endocrine-Metabolic Unit, Peter Bent Brigham Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT The peripheral plasma levels of aldosterone, renin activity (PRA), potassium, corticosterone, cortisol, and in some cases angiotensin II, were measured in normal subjects undergoing postural changes, acute diuretic-induced volume depletion, and alterations in dietary sodium. On a 10 mEq sodium/100 mEq potassium intake, subjects supine for 3 consecutive days had identical diurnal patterns of PRA, angiotensin II, aldosterone, cortisol, and corticosterone, with peaks at 8 a.m. and nadirs at 11 p.m. With an increase in sodium intake to 200 mEq, plasma levels of aldosterone and PRA fell to one-third their previous levels but the diurnal pattern in supine subjects was unchanged and again parallel to that of cortisol and corticosterone. There was no diurnal variation of plasma potassium on either sodium intake in the supine subjects. On a 10 mEq sodium/100 mEq potassium intake, supine 8 a.m. plasma aldosterone (55±7 ng/100 ml) and PRA (886±121 ng/ 100 ml per 3 hr) increased by 150–200% after subjects were upright for 3 hr. However, even though the patients maintained an upright activity pattern, there was a significant fall in plasma aldosterone to 33±5 ng/100 ml at 11 p.m. Potassium levels varied in a fashion parallel to aldosterone and PRA. Plasma cortisol and corticosterone had a diurnal pattern similar to that found in supine subjects. In response to acute diuretic-induced volume depletion, the nocturnal fall in aldosterone levels did not occur. The 11 p.m. value (102±20 ng/100 ml) and the 8 a.m. value postdiuresis (86±15 ng/100 ml) were both significantly greater than the prediuresis levels. PRA showed a similar altered pattern while potassium levels fell throughout the day. In some but not all studies, changes in plasma aldosterone coincided with changes in plasma cortisol, corticosterone, and/or potassium. However, in all studies, changes in plasma aldosterone were invariably associated with parallel changes in plasma renin activity and/or angiotensin II levels. These findings support the concept that PRA is the dominant factor in the control of aldosterone when volume and/or dietary sodium is altered in normal man.

INTRODUCTION

Alterations of aldosterone secretion in response to sodium restriction, posture, acute diuresis, or hemorrhage have been widely considered to be mediated by the renin-angiotensin system (1–4). Other factors, such as potassium (5–7), adrenocorticotropin (ACTH) (7–9), and serotonin (10) have been shown to alter aldosterone secretion in vivo and/or in vitro, but presumably, they play only a minor role in comparison to the renin-angiotensin system in controlling the secretion of aldosterone in response to volume changes. However, some investigators (11–13) have suggested that the renin-angiotensin system is not necessarily the primary mechanism leading to increased secretion of aldosterone during sodium or volume changes. Disagreement on this point could be related to the usual method of studying these two parameters. Most previous studies in man have related either

urinary excretion or secretion rates of aldosterone determined over hours to single or multiple but instantaneous determinations of renin activity or angiotensin II levels (3, 4, 6, 8, 14). The exact relationship of renin activity to plasma aldosterone when changes in volume are produced have not been entirely clear from the few previous studies when both have been measured simultaneously (13, 15, 16) and none have measured both potassium and ACTH levels, directly or indirectly, at the same time. In order to clarify this relationship the present paper reports the simultaneous measurement of plasma corticosterone, cortisol, aldosterone, potassium, angiotensin II, and peripheral renin activity (PRA)[1] in a group of normal subjects undergoing postural changes, acute diuretic-induced volume depletion and alterations in dietary sodium.

METHODS

Protocols

13 normal subjects (11 males, 2 females) between the ages of 21 and 30 yr were studied on The Clinical Research Center of the Peter Bent Brigham Hospital. All subjects were normotensive, had normal physical examinations, and no evidence by history or laboratory testing for clinical disease. All denied the use of drugs. All were informed of the nature of the studies and signed consent forms were obtained. Each subject underwent one or more of the following protocols.

CONSTANT ACTIVITY—CONSTANT DIET

Chronic (low dietary sodium) volume-depletion study (10 mEq Na, 100 mEq K diet). Five subjects (four males, one female) were maintained on a constant activity pattern simulating normal daily activity, i.e., supine from 11:30 p.m. until 8 a.m.; up from 8 a.m. to 11:30 p.m. with one 300 yard walk at least hourly. All subjects on a constant 10 mEq sodium/100 mEq potassium, 2500 ml, isocaloric diet divided into three meals a day and a small snack at bedtime. On the day of admission, 400–500 ml of blood was drawn from each subject in a sterile acid citrate dextrose blood bank container and maintained in the blood bank for reinfusion later in the study.

After they had achieved metabolic balance (usually on the 5th or 6th day), blood was obtained for sodium, potassium, hematocrit, total protein, plasma cortisol, corticosterone, aldosterone, and PRA at 8 a.m., 11 a.m., 5 p.m., 11 p.m., and at 8 a.m. the following morning. PRA was not determined at 11 p.m. After each sample of blood was drawn, an equivalent volume (approximately 60–80 ml) of the patient's blood was reinfused. All blood samples were drawn premeal or presnack. Urines were collected every 6 hr and analyzed for creatinine, sodium, and potassium.

Acute diuretic-induced volume-depletion study (10 mEq Na, 100 mEq K diet). This study began the day after the chronic volume depletion-constant activity protocol with the same five subjects maintained on the same dietary intake and activity program. Blood was drawn and replaced and urine collected for the same determinations in an identical

---

[1] *Abbreviation used in this paper:* PRA, plasma renin activity.

fashion as in the previous study. At 9 a.m., the subjects were given oral furosemide every 6 hr (mean total dose = 190 mg/24 hr) to maintain a sufficient urine output to produce a 1.5–2 kg weight loss over the 24 hr period (17).

SUPINE STUDY—CONSTANT DIET

Chronic (low dietary sodium) volume-depletion study (10 mEq Na, 100 mEq K diet). On the day of admission 700–800 ml of blood was drawn from five male subjects and stored in the blood bank for reinfusion later in the study. When the subjects had achieved metabolic balance on the 10 mEq sodium, 100 mEq potassium diet, they were placed in the supine position for 82 consecutive hr, beginning at 10 p.m. the day before sampling. Daily blood samples for plasma potassium, corticosterone, cortisol, aldosterone, and PRA and in some cases angiotensin II levels were drawn at 8 a.m., 11 a.m., 5 p.m., and 11 p.m. on 3 consecutive days. After each sampling an equivalent amount of blood was replaced.

Volume expansion study (200 mEq Na/200 mEq K diet). Five subjects (four males, one female) were phlebotomized upon admission. When they achieved metabolic balance on the 200 mEq sodium/200 mEq potassium diet, they were placed in the supine position for 82 consecutive hr. Blood samples were drawn at 8 a.m., 11 a.m., 5 p.m., and 11 p.m. for plasma cortisol, corticosterone, aldosterone, potassium, and PRA. Equivalent amounts of blood were reinfused as described in the previous protocol.

Laboratory procedures

All blood samples were immediately spun and the plasma separated and frozen until time for assay. The samples for PRA and angiotensin II levels were drawn using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant and the samples for potassium, corticosterone, cortisol, and aldosterone used sodium heparin as the anticoagulant. Sodium and potassium in urine and blood were measured by flame photometry using lithium as an internal standard. Plasma cortisol and corticosterone levels in the two constant activity studies were measured by a modification of the double-isotope derivative technique of Kliman and Peterson (18). In this method the nonspecific blank was 200 pg/2 ml, the coefficient of variation at the 20 and 2 ng/ml level, respectively was 15–20%, and the recovery of added steroid ranged from 75 to 105%. The plasma aldosterone levels in the two constant activity studies were measured by a double-isotope derivative technique as described by Brodie, Shimizu, Tait, and Tait (19). The nonspecific blank for this method in our laboratory is 105±85 (SD) pg/20 ml with a coefficient of variation at the 5 ng/100 ml level of ±15%.

The plasma aldosterone, corticosterone, and cortisol in the two supine studies were measured by displacement analysis techniques as previously described from this laboratory (20). In brief, the plasma is extracted with methylene chloride, chromatographed in a Bush 5 system and the three steroids eluted. The corticosterone and aldosterone are determined by radioimmunoassay using antibodies specifically directed against them. The cortisol is measured by a competitive protein-binding method similar to that described by Nugent and Mayes (21). The levels found in the plasma of adrenalectomized subjects are below the sensitivity of the method for cortisol and corticosterone, and at the 2 pg/4 ml level for aldosterone. The sensitivity of the cortisol assay system is 0.2 ng/binding tube; for the corticosterone assay, 0.02 ng/binding tube; and for the aldosterone, 0.002 ng/binding tube. The coefficient of variation is between 7 and

11% at a level 10 times the sensitivity for the three assays. Recovery of added steroid from each assay is the same ranging from 85 to 110%. The cortisol, corticosterone, and aldosterone values in 12 samples were compared with the above mentioned double-isotope derivative method which produced a correlation coefficient of 0.99 with a P value of correlation < 0.001 for all three steroids.

Angiotensin II values were measured by a double-antibody radioimmunoassay system (22). This assay is sensitive to a level of 10 pg/ml with a coefficient of variation at the 20 pg/ml level of 6.8%. Recoveries of added angiotensin II at three different levels range from 88 to 108%.

PRA was measured by a modification of the Boucher method as previously described (17). Statistical analyses were performed as described by Snedecor and Cochran (23), utilizing a General Electric 635 computer. The results are expressed as mean ±standard error of the mean and significance as $P < 0.02$ unless otherwise indicated. Nonsignificant differences were those with $P > 0.05$.

RESULTS

Constant activity

Chronic (low dietary sodium) volume depletion. Table I summarizes the results from this study. The mean 24 hr urinary sodium excretion was 6±1.4 mEq/24 hr and potassium excretion, 94±4 mEq/24 hr. There was no significant variation in the 6 hr sodium excretion (range 0.6–2.4 mEq). However, the 7 a.m. to 1 p.m. and 1 to 7 p.m. potassium excretion (28±2 mEq each) was significantly greater than the 1 to 7 a.m. excretion (14±4 mEq).

The mean 8 a.m. supine plasma aldosterone level (55±7 ng/100 ml) rose significantly at 11 a.m. (100±17 ng/100 ml) after the subjects had been upright for 3 hr. However, even though the patients maintained an upright activity pattern from 11 a.m. to 11 p.m., there was a significant fall to a 11 p.m. value of 33±5 ng/100 ml. The 11 p.m. value was also significantly less than both the 5 p.m. upright level (68±4 ng/100 ml) and the 8 a.m. supine value.

The mean plasma cortisol value showed the expected diurnal fall during the day. The 8 a.m. value (12.3±1.1 μg/100 ml) was significantly greater than the 11 p.m. value (4.1±1.3 μg/100 ml). The plasma corticosterone values showed a similar fall with the 8 a.m. level (0.66± 0.09 μg/100 ml) being significantly greater than the 11 p.m. value (0.9±0.04 μg/100 ml).

The mean 8 a.m. supine PRA (886 ± 121 ng/100 ml per 3 hr) was significantly less ($P < 0.05$) than the 11 a.m. value (1200±185 ng/100 ml per 3 hr) when the subjects had been upright for 3 hr. However, there was no significant difference between the 5 p.m. value (1348± 226 ng/100 ml per 3 hr) and either the 8 a.m. or the 11 a.m. levels even though the mean level was greater. The greater standard error caused by some subjects showing a fall and others a rise from 11 a.m. to 5 p.m. is the reason for this. PRA was not determined at 11 p.m.

Potassium changed during the day in a pattern similar to the plasma aldosterone with a rise at 11 a.m. and then a gradual fall throughout the rest of the day. The 11 a.m. value (4.8±0.1 mEq/liter) was significantly greater ($P < 0.05$) than the 11 p.m. value (4.4±0.2 mEq/liter). No significant changes in serum sodium, protein, or hematocrit occurred during the study.

Acute diuretic-induced volume depletion. The mean weight loss produced by the diuretic was 1.8±0.1 kg over the 24 hr period. There was an increase in the mean sodium excretion to 202±11 mEq/24 hr and the mean potassium excretion to 152±6 mEq/24 hr. In all 6-hr time periods there was a significantly greater sodium excretion on the diuretic day than on the control day. Also, there was a significantly greater sodium and potassium excretion during the first 12 hr compared with the second 12 hr on the diuretic day. This is probably explained by the fact that most of the total furosemide was given during the first 12 hr period.

As in the control study, there was a significant in-

TABLE I
Diurnal Plasma Cortisol, Corticosterone, Aldosterone and Renin Activity, and Serum Potassium in Normal Subjects on a 10 mEq Na/100 mEq K Intake with Normal Activity

Sub-jects	Cortisol				Corticosterone				Aldosterone				Potassium				Renin activity		
	8a*	11a	5p	11p	8a*	11a	5p	11p	8a*	11a	5p	11p	8a*	11a	5p	11p	8a*	11a	5p
	μg/100 ml				μg/100 ml				ng/100 ml				mEq/liter				ng/100 ml per 3 hr		
1	11.7	7.1	4.0	1.4	0.38	0.31	0.22	0.09	70	165	87	39	4.5	4.8	4.4	4.2	784	1350	1150
2	8.2	4.9	2.9	1.1	0.64	0.49	0.46	0.19	33	52	16	14	4.2	4.6	4.6	4.1	1140	1200	1000
3	14.1	—	6.6	3.5	0.85	—	0.45	0.32	42	94	52	36	5.1	5.0	4.6	4.2	1210	1730	770
4	14.9	7.7	—	6.0	0.92	0.22	—	—	80	109	84	34	4.7	4.8	4.7	5.0	975	1100	1920
5	12.6	10.2	8.8	8.4	0.49	0.22	0.15	0.17	46	78	100	44	4.6	4.7	4.8	4.4	325	620	1900
Mean	12.3	7.5	5.6	4.1	0.66	0.29	0.32	0.19	55	100	68	33	4.6	4.8	4.6	4.4	886	1200	1348
±SEM	±1.1	±0.9	±1.1	±1.3	±0.09	±0.06	±0.07	±0.04	±7	±17	±14	±5	±0.1	±0.1	±0.1	±0.2	±121	±185	±226

* Mean of two determinations.
Subjects were supine 11 p.m. to 8 a.m., upright 8 a.m. to 11 p.m.

TABLE II

Diurnal Plasma Cortisol, Corticosterone, Aldosterone, Renin Activity and Serum Potassium in Normal Subjects on a

Sub-ject	Cortisol					Corticosterone				
	8a*	11a	5p	11p	8a	8a*	11a	5p	11p	8a
	μg/100 ml					*μg/100 ml*				
1	11.7	8.7	10.6	12.8	15.6	0.38	0.31	0.24	1.36	0.94
2	8.2	—	7.3	5.2	8.5	0.64	—	0.81	0.61	1.08
3	14.1	9.0	12.2	4.8	11.2	0.85	0.40	0.98	0.50	1.10
4	14.9	7.2	16.9	23.4	16.1	0.92	0.48	1.28	—	1.65
5	12.6	11.3	11.8	10.8	19.6	0.49	0.26	0.14	0.45	0.72
Mean	12.3	9.1	11.8	11.5	14.2	0.66	0.36	0.69	0.73	1.10
±SEM	±1.1	±0.7	±1.4	±3.1	±1.8	±0.09	±0.02	±0.19	±0.18	±0.14

* Mean of two determinations.

crease ($P < 0.05$) in the plasma aldosterone when the subject had been up for 3 hr (84±6 ng/100 ml) (Table II). However, in contrast to the control day, there was no significant fall in the plasma aldosterone levels during the evening. The 11 p.m. value (102±20 ng/100 ml) was significantly greater than both the 8 a.m. prediure-sis supine value (55±7 ng/100 ml) and the 11 p.m. value on the control day (33±5 ng/100 ml). The 8 a.m. postdiuresis supine value (86±15 ng/100 ml) was also significantly greater ($P < 0.05$) than the 8 a.m. prediuresis supine value.

In contrast to the control day, both plasma cortisol and

TABLE III

Diurnal Plasma Cortisol, Corticosterone, Aldosterone, Renin Activity, Angiotensin II,

Sub-ject	Day of study	Cortisol				Corticosterone				Aldosterone			
		8a	11a	5p	11p	8a	11a	5p	11p	8a	11a	5p	11p
		μg/100 ml				*μg/100 ml*				*ng/100 ml*			
6	1	11	8	8	3	0.67	0.91	0.53	0.12	71	38	33	39
	2	10	8	8	1	0.68	0.30	0.48	0.04	48	32	34	41
	3	10	5	4	—	0.51	0.32	0.27	0.16	64	37	49	—
	4	11				0.89				51			
7	1	12	10	6	1	1.38	0.24	0.29	0.15	86	61	—	36
	2	10	—	8	1	1.04	—	0.69	0.11	164	160	82	65
	3	12	—	3	1	0.79	—	0.09	0.05	89	62	56	68
	4	15				—				115			
8	1	13	8	7	3	0.73	0.23	0.18	0.11	80	43	24	15
	2	14	—	10	—	1.11	—	0.34	—	132	—	25	—
	3	14	7	6	—	1.1	0.24	0.28	—	—	35	34	20
	4	11				0.63				51			
9	1	10	6	3	1	—	0.27	0.18	0.06	101	43	21	21
	2	10	6	7	3	0.39	0.04	0.04	0.02	61	47	25	24
	3	14	8	—	8	0.32	—	0.13	0.10	65	38	54	19
	4	11				0.22				65			
10	1	8	6	6	1	0.88	0.59	0.80	0.14	66	35	27	7
	2	10	8	4	1	1.24	0.57	0.80	0.54	103	63	67	18
	3	14	7	9	1	—	0.34	0.21	0.11	134	90	40	—
	4	10				0.52				118			
Mean		11	7	6	2	0.77	0.37	0.35	0.14	88	56	41	31
±SEM		±1	±1	±1	±1	±0.08	±0.07	±0.06	±0.03	±7	±5	±5	±5

* 10 mEq sodium/100 mEq potassium diet.

1734   G. H. Williams, J. P. Cain, R. G. Dluhy, and R. H. Underwood

Aldosterone					Potassium					Renin activity			
8a*	11a	5p	11p	8a	8a*	11a	5p	11p	8a	8a*	11a	5p	8a
ng/100 ml					*mEq/liter*					*ng/100 ml per 3 hr*			
70	69	91	133	131	4.5	4.5	3.9	3.9	3.9	784	1340	1780	1371
33	—	45	37	32	4.5	4.4	3.9	3.9	3.7	1140	1280	1960	3800
42	—	112	70	74	5.1	4.9	4.7	4.4	4.3	1210	1330	3260	2471
80	93	63	162	110	4.7	4.6	5.0	4.6	3.8	975	1570	2410	2000
46	190	66	109	83	4.6	4.0	3.8	3.1	4.7	325	730	1600	2000
55	84	73	102	86	4.6	4.5	4.3	4.0	4.1	886	1250	2202	2328
±7	±6	±10	±20	±15	±0.1	±0.1	±0.3	±0.3	±0.2	±121	±102	±268	±408

corticosterone failed to show the same magnitude of fall during the day. The 11 p.m. cortisol level (11.5±3.1 μg/100 ml) on the diuresis day was significantly greater than on the control day (4.1±1.3 μg/100 ml). Although the 8 a.m. value postdiuresis (14.2±1.8 μg/100 ml) was greater than the 8 a.m. value prediuresis (12.3±1.1 μg/100 ml), the difference was not significant. Plasma corticosterone showed a similar altered pattern. However, the 8 a.m. level postdiuresis (1.1±0.14 μg/100 ml) was significantly greater ($P < 0.05$) than prediuresis (0.66±0.09 μg/100 ml).

The PRA pattern was also altered. There was a sig-

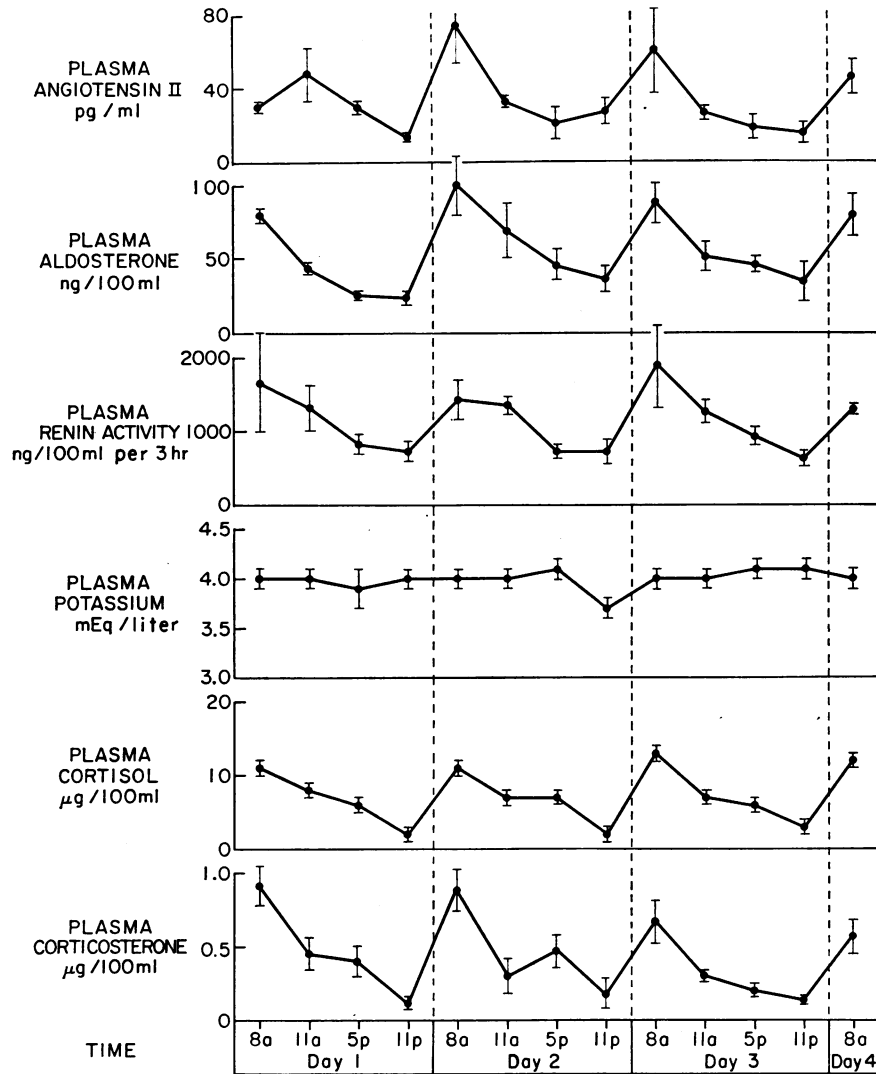| Potassium				Renin activity				Angiotensin II			
8a	11a	5p	11p	8a	11a	5p	11p	8a	11a	5p	11p
mEq/liter				*ng/100 ml per 3 hr*				*pg/ml*			
3.8	3.9	3.4	—	600	910	730	630	40	65	37	—
3.8	3.7	—	3.6	970	1040	650	840	—	—	—	—
4.0	—	3.9	3.8	1120	1035	690	692	120	42	—	—
—				1200				—			
—	4.0	4.2	4.2	1200	1390	880	630	28	—	29	—
4.0	—	4.2	3.7	1125	1300	890	670	49	31	—	37
—	—	4.3	4.4	1140	1760	1136	945	43	—	34	45
4.1				1500				70			
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—				—				—			
4.1	4.0	4.0	4.1	3992	2315	1120	1139	30	69	26	12
4.3	4.0	4.1	3.8	2254	1833	964	1100	45	42	32	35
4.2	3.9	4.0	4.2	—	1400	1270	640	—	28	14	31
4.0				1228				25			
4.2	4.2	4.1	3.8	914	627	536	533	22	<10	—	16
3.8	4.2	4.0	3.8	1353	1271	467	294	125	25	<10	<10
3.9	4.0	4.0	4.1	3462	867	583	367	24	<10	<10	<10
3.9				1338				47			
4.0	4.0	4.0	4.0	1560	1312	826	707	51	36	24	24
±0.1	±0.1	±0.1	±0.1	±240	±132	±72	±72	±9	±7	±4	±5

FIGURE 1 Diurnal variation in plasma angiotensin II, aldosterone, renin activity, potassium, cortisol, and corticosterone in supine normal subjects on a 10 mEq sodium/100 mEq potassium diet.

nificant rise ($P < 0.05$) when the subjects went from supine to 3 hr upright (1250±102 ng/100 ml per 3 hr), similar to the control day. However, the 5 p.m. level (2202±268 ng/100 ml per 3 hr) was significantly greater than both the 11 a.m. level and the 8 a.m. supine level. Furthermore, the 8 a.m. PRA postdiuresis (2328±408 ng/100 ml per 3 hr) was significantly greater than the 8 a.m. value prediuresis (886±121 ng/100 ml per 3 hr).

In contrast to the control day, there was a steady significant fall in the potassium levels from an 8 a.m. value of 4.6±0.1 mEq/liter to an 11 p.m level of 4.0±0.3 mEq/liter and a postdiuresis 8 a.m. level of 4.1±0.2

mEq/liter. The hematocrit and serum protein rose slightly but not significantly.

Supine study—constant diet

Chronic (low dietary sodium) volume depletion. The mean daily sodium excretion was 11±4, 8±3, and 5±1 mEq/day on the 3 consecutive days of the study. The mean potassium excretion was 86±6, 87±5, and 84±4 mEq/day.

The mean plasma aldosterone fell significantly during the course of the day (Table III, Fig. 1). The 8 a.m. level (88±7 ng/100 ml) was significantly greater than the 11 a.m. value (56±5 ng/100 ml) which was signifi-
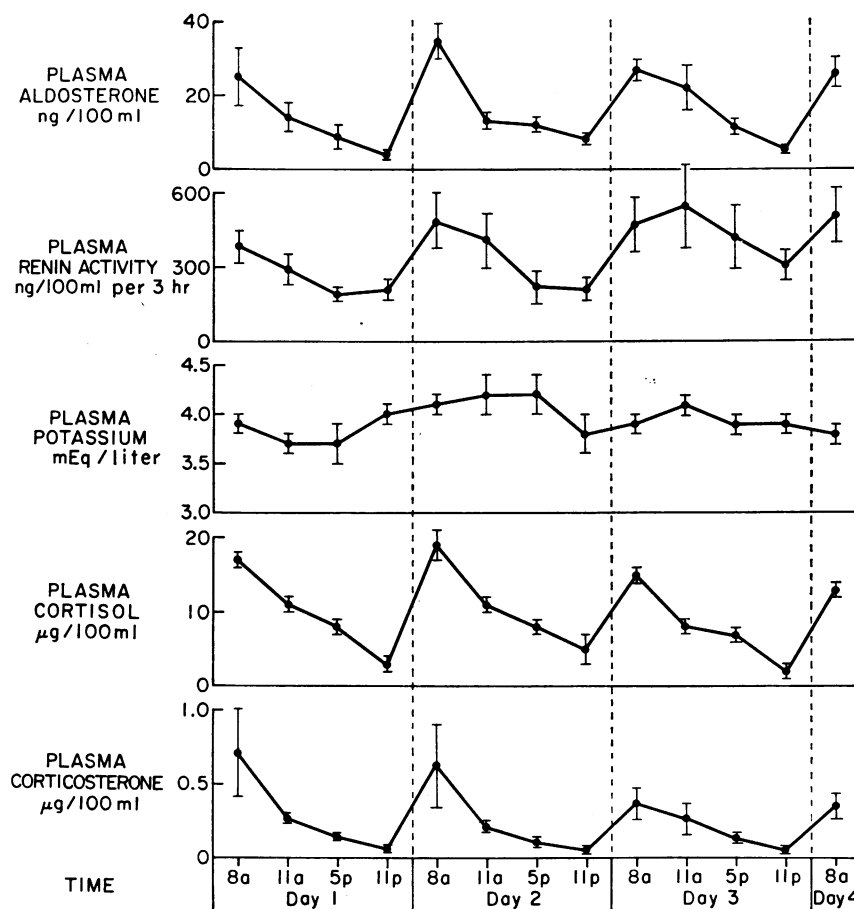
FIGURE 2 Diurnal variation in plasma aldosterone, renin activity, potassium, cortisol, and corticosterone in supine normal subjects on a 200 mEq sodium/200 mEq potassium diet.

cantly greater than the 11 p.m. value (31±5 ng/100 ml). The 11 a.m. supine value was also significantly less than the 11 a.m. upright value (100±17 ng/100 ml) from the previous protocol. Likewise, the 5 p.m. supine value (41±5 ng/100 ml) was significantly less than the 5 p.m. upright value (68±4 ng/100 ml). However, there was no significant difference between the 11 p.m. supine and upright values. In some subjects, this circadian rhythm was more evident on the 2nd and 3rd days of this 3 day study. The 8 a.m. supine aldosterone was greater in this study than in the constant activity study (55±7 ng/100 ml), probably because the hemorrhage induced 5–7 days before each study was 50% greater in the supine study.

Plasma cortisol showed the expected decline throughout the day with the 8 a.m. value (11±1 μg/100 ml) being significantly greater than the 11 p.m. value (2±1 μg/100 ml). The mean plasma corticosterone at 8 a.m. (0.77±0.08 μg/100 ml) was also significantly greater

than the 11 p.m. value (0.14±0.03 μg/100 ml). All three steroids, cortisol, corticosterone, and aldosterone, showed almost identical relative changes throughout the day and the correlation coefficient between them was highly significant ($P < 0.001$).

PRA showed a similar gradual fall throughout the day with the 8 a.m. value (1560±240 ng/100 ml per 3 hr) significantly greater than the 11 p.m. value (707±72 ng/100 ml per 3 hr). The 8 a.m. value was also significantly greater than the 5 p.m. value (826±82 ng/100 ml per 3 hr), and the 11 a.m. value (1312±132 ng/100 ml per 3 hr) was significantly greater than both the 5 p.m. and the 11 p.m. levels (Table III, Fig. 1). The 8 a.m. plasma angiotensin II levels (51±9 pg/ml) were significantly greater ($P < 0.05$) than the 11 p.m. level (25 ± 5 pg/ml). Thus, changes in PRA and angiotensin levels significantly correlated with changes in cortisol, corticosterone, and aldosterone. In contrast, the potas-

TABLE IV
Diurnal Plasma Aldosterone, Cortisol, Corticosterone, Potassium, and Renin Activity

Sub-ject	Day of study	Cortisol				Corticosterone			
		8a	11a	5p	11p	8a	11a	5p	11p
		μg/100 ml				μg/100 ml			
6	1	18	7	9	4	1.75	—	0.21	0.07
	2	—	8	—	—	1.84	0.31	—	—
	3	18	8	8	2	0.82	0.75	0.23	0.04
	4	—				0.60			
7	1	16	15	9	4	0.38	0.13	0.06	0.05
	2	21	12	6	2	0.20	0.16	0.12	0.05
	3	15	8	5	1	0.20	0.16	0.04	0.06
	4	15				0.26			
11	1	18	10	7	4	0.46	0.33	0.20	0.08
	2	13	10	6	3	0.38	0.25	0.15	0.06
	3	14	7	8	4	0.26	0.15	0.07	0.08
	4	15				0.46			
12	1	17	16	8	2	0.24	0.28	0.16	0.07
	2	17	14	11	8	0.22	0.13	0.07	0.05
	3	—	9	5	2	0.13	0.12	0.13	0.04
	4	—				0.10			
13	1	—	9	5	2	—	0.36	0.11	0.04
	2	23	12	7	6	0.53	0.23	0.08	0.04
	3	14	7	7	2	0.47	0.17	0.22	0.06
	4	10				0.36			
Mean		16	10	7	3	0.51	0.25	0.13	0.05
±SEM		±1	±1	±1	±1	±0.11	±0.04	±0.02	±0.01

sium levels showed no diurnal changes throughout the day.

Volume expansion study. The daily mean sodium excretion on the 3 days of this study was 245±31, 209±11, and 185±23 mEq/day. Although there was a tendency for the sodium excretion to be greater on the 1st supine day, this was not significant. Mean daily potassium excretion was 171±7, 157±10, and 154±13 mEq/day on each one of the days.

Mean plasma aldosterone concentration declined throughout the day. The mean 8 a.m. level (28±3 ng/100 ml) was significantly greater than the 11 a.m. level (16±3 ng/100 ml), the 5 p.m. level (11±2 ng/100 ml), and the 11 p.m. level (6±1 ng/100 ml). The plasma corticosterone and cortisol mean levels showed a similar fall during the day; the mean levels at 8 a.m. were significantly greater than at 11 p.m. (Table IV, Fig. 2).

As in the low sodium-supine study, PRA showed a constant decline parallel to that of cortisol, corticosterone, and aldosterone. The mean 8 a.m. PRA value (471± 80 ng/100 ml per 3 hr) was significantly greater than the 11 p.m. value (240±28 ng/100 ml per 3 hr) (Table IV, Fig. 2). Potassium levels similarly showed no significant changes during the course of the study and were not significantly correlated with either PRA, cortisol, corticosterone, or aldosterone.

In comparing the plasma steroid levels on the high and low sodium diets, cortisol showed no significant differences and aldosterone was always significantly higher on the low sodium intake at all time periods. In contrast, while corticosterone levels were not significantly different at 8 and 11 a.m., they were significantly greater on the low sodium diet at 5 and 11 p.m.

DISCUSSION

In the present study, upright posture, diuresis, or alterations in sodium balance were associated with parallel changes in PRA and aldosterone. Under some circumstances, changes in plasma aldosterone also coincided with changes in plasma cortisol, corticosterone, or potassium. However, under all circumstances, changes in plasma aldosterone were associated with parallel changes in PRA and/or angiotensin II levels. These findings support the concept that angiotensin II is the dominant factor in the control of aldosterone with volume manipulation in normal man. Some investigators have not always found such a close association (11–13, 24). In some instances, the experimental design or methodologic differences might explain this. The present study was designed to carefully control other factors which could alter the levels of renin activity or aldosterone and

Aldosterone				Potassium				Renin activity			
8a	11a	5p	11p	8a	11a	5p	11p	8a	11a	5p	11p
ng/100 ml				*mEq/liter*				*ng/100 ml per 3 hr*			
11	8	4	4	3.8	3.9	3.2	—	260	220	110	210
—	11	—	—	—	3.5	—	—	770	450	470	270
16	14	14	5	3.4	3.9	3.6	3.8	556	883	640	420
19				3.6				760			
10	3	4	1	3.9	3.9	3.8	3.9	360	170	150	63
21	12	7	7	4.0	3.8	3.9	3.8	250	150	100	160
24	13	4	1	4.0	4.1	3.9	3.7	240	260	170	94
26				3.6				270			
48	15	7	6	3.5	3.6	3.7	3.8	650	488	192	223
46	10	7	7	3.7	4.5	3.6	3.2	486	385	142	194
39	16	16	7	3.8	3.8	3.8	—	357	80	194	253
40				3.9				760			
29	29	22	5	4.0	3.9	4.3	4.2	450	460	310	350
39	21	15	13	4.5	4.6	4.5	4.5	790	850	330	280
30	44	16	5	4.3	4.1	4.3	4.1	920	1140	860	480
15				4.2				500			
—	17	7	6	4.3	3.1	3.7	4.1	260	140	200	180
32	9	18	6	4.3	4.4	4.7	3.7	180	220	83	150
24	24	7	9	4.1	4.6	3.9	4.1	371	400	267	277
28				3.8				220			
28	16	11	6	3.9	4.0	3.9	3.9	471	420	281	240
±3	±3	±2	±1	±0.1	±0.1	±0.1	±0.1	±50	±78	±56	±28

thereby artifactually produce dissociation between them. Dietary intake of sodium and potassium as well as the timing of sampling to eating were rigidly regulated since it has been shown that changes in either one can produce significant changes in the plasma levels of aldosterone and/or PRA (4, 6, 25). Because hemorrhage has been shown to induce changes in renin activity and aldosterone secretion (26), an equal volume of the subject's blood was reinfused during the course of the study as samples were obtained. Activity of the patients before and during the time of sampling was also standardized. In studies of over 50 normal subjects on supine protocols, we have observed that as many as 25% excrete a significantly greater amount of sodium on the 1st day supine than on previous days with normal activity or subsequent supine days. Because this increased natriuresis might alter the normal pattern of aldosterone in some subjects, two additional days of sampling were performed.

The absolute plasma aldosterone values on both high and low sodium intake are in reasonable agreement with other investigators (Table V). The higher plasma level on a high sodium diet in the present study is probably the result of the greater daily potassium intake (200 mEq) and to a lesser extent the hemorrhage induced 5–8 days before sampling. The slightly higher

mean value in the present study on a 10 mEq sodium/100 mEq potassium intake could reflect the greater potassium intake, the lower sodium intake and/or the previous hemorrhage. The mean PRA on the low sodium study of Michelakis and Horton (13) was only 25% of the value obtained in the present study and in the study of Gordon, Wolfe, Island, and Liddle (29). There is no apparent explanation for this difference, particularly since PRA in Michelakis' and Horton's study, like the angiotensin II levels in the study of Best, Coghlan, Bett, Cran, and Scoggins (24), did not significantly change when sodium intake was restricted.

It has been well established that there is a diurnal pattern to the excretion of aldosterone with a greater excretion in the daytime than at night (14, 30, 31). Whether this pattern was anything more than a reflection of the diurnal posture of the subjects was unclear since most investigators had found no consistent change in excretion of aldosterone when posture was eliminated (32, 33). Analysis of plasma values of aldosterone have produced conflicting results. An early study (34) showed no significant variation in the level of aldosterone (obtained every 6 hr) in recumbent subjects. More recently, Michelakis and Horton (13) demonstrated that there is a diurnal variation of plasma aldosterone independent of posture in normal subjects.

TABLE V
Comparison of Plasma Aldosterone and Renin Activity Measured in Several Studies

	Urine excretion		Plasma aldosterone	*Method	Renin activity
Investigator	Sodium	Potassium			
	mEq/day		ng/100 ml		ng/100 ml per 3 hr
Michelakis and Horton (13)	150	82	9.8	DID	197
	10–20	82	24.1	DID	274
Balikian et al. (27)	128	50	2.15	DID	—
Ford et al. (28)	146	65–73	9.1	Calc.	—
	16	65–73	45.5	Calc.	—
Gordon et al. (29)	10	Unspecified	—		800
Present study	161	213	28	RIA	471
	6	94	55	RIA	866

* DID, double-isotope derivative; Calc., calculated from secretion rate and metabolic clearance rate; RIA, radioimmunoassay.

However, the timing of the circadian rhythm was dependent upon dietary intake of sodium. With intake of normal amounts of sodium, the peak plasma aldosterone value occurred at midnight, while on a low sodium intake, it occurred between 6 a.m. and noon (similar with that of cortisol). PRA also showed a circadian rhythm, but peak values occurred at 12 midnight on both diets. Thus, there was an unexplained dissociation between PRA and plasma aldosterone on a low sodium intake. The present study confirms that there is a diurnal variation of plasma aldosterone independent of the upright posture. However, in contrast to what had previously been reported (13), there was no alteration in the diurnal pattern of plasma aldosterone when dietary sodium intake was changed from 10 mEq to 200 mEq/day. Thus, no dissociation between PRA and aldosterone was seen in the present study. The PRA pattern is similar to what Gordon et al. reported in sodium restricted supine subjects with a peak value between 4 and 8 a.m. (29). The difference between the present study and the previous ones may reflect methodologic differences. In Michelakis' and Horton's study (13), a single day's sampling was obtained to determine the pattern of plasma aldosterone and PRA. As has been indicated earlier in some of our subjects, the plasma aldosterone cycle obtained during the 1st day supine was sometimes different than during the subsequent days. Secondly, they did not replace the blood lost during the sampling period. Since the last sample drawn in their study was at midnight, the increased levels found at that time for both PRA and aldosterone may in part be a reflection of the hemorrhage induced.

Gordon et al. (29) have also measured PRA throughout the day in subjects maintaining normal upright posture. In spite of this constant stimulus for renin release, the circadian rhythm of PRA in their study and the plasma aldosterone in the present one were maintained with a low value occurring in the late evening. The mechanism by which the circadian pattern in plasma aldosterone is produced and how it is maintained even in the face of upright posture is unclear from the present or previous studies. It has been amply demonstrated that ACTH can stimulate both excretion, secretion, and plasma levels of aldosterone (1, 17, 35). Since in the supine studies, the plasma levels of aldosterone, cortisol, and corticosterone all changed in parallel fashion, they may all be related to the same stimulus, i.e., the circadian variation in ACTH release. Alternatively, because PRA varied in a fashion parallel to aldosterone, it may be the direct stimulus. It has been shown in the rat (36), but not in man (37), that ACTH can alter renin release. Since PRA varied in a manner parallel to plasma aldosterone, ACTH may be affecting both renin release and adrenal glucocorticoid secretion or another, presumably central nervous system factor, may be producing a synchronous release of both ACTH and renin.

There are circadian changes in the levels of a variety of substances in the blood or urine of normal man (38). Therefore, the parallel changes in PRA and aldosterone in supine subjects do not necessarily mean that aldosterone follows renin activity, but may simply reflect part of a general diurnal cycling. Acute alterations in the cycle were therefore produced to determine if plasma aldosterone would still follow alterations in PRA. Assuming the upright posture produced a significant 150–200% parallel increase in both PRA and plasma aldosterone without any alteration in plasma corticosterone and cortisol. These changes are in agreement with those of Michelakis and Horton (13) for subjects on a low sodium intake and with Balikian, Brodie, Dale,

Melby, and Tait (27) for subjects on a high sodium intake.

The second alteration of the normal circadian pattern was produced by acute volume depletion. Previous studies have shown that when a 2–3% decrease in body weight is produced by acute diuresis, there is a two to fivefold increase in PRA and aldosterone secretion in normal subjects (17, 39). Rosenthal, Boucher, Nowaczynski, and Genest (16) have shown that acute administration of furosemide produced a three to fourfold increase in PRA after 60 min and a doubling of plasma aldosterone after 2 hr. Results of the present study show a similar parallel increase in renin activity and aldosterone in response to acute diuresis. Furthermore, the acute diuresis completely abolished the usual evening fall in PRA and aldosterone. Although cortisol and corticosterone diurnal patterns were also altered by the diuresis, the changes were quantitatively less significant than those observed for PRA and aldosterone.

At times in the present study, changes in plasma aldosterone paralleled presumed changes in ACTH secretion as reflected in changes in plasma levels of corticosterone and cortisol. In the supine study, there was a strong correlation between plasma levels of corticosterone, cortisol, and aldosterone. However, in the constant activity study where the normal circadian release of PRA was altered, plasma aldosterone but not cortisol and corticosterone followed the changes in PRA. Changes in aldosterone could also be dissociated from changes in potassium, another aldosterone stimulus. Serum potassium levels during the supine study did not change when there was a significant fall in both plasma aldosterone and PRA. Likewise, in the volume depletion study, there was a negative correlation with the serum potassium falling during the day, while the plasma aldosterone rose.

It is clear from the present study that changes in volume, dietary sodium, or posture produce parallel changes in plasma aldosterone, plasma angiotensin II, and PRA. While other factors may modify the response and presumably the synchrony between PRA and aldosterone secretion under the assigned criteria of the present study, there was a very close correlation between renin activity, angiotensin II, and aldosterone in young, healthy individuals. The data suggest that plasma aldosterone is under the dominant control of renin activity and angiotensin II in these circumstances. This is critically important since in some diseases (15, 17, 40–43) a dissociation between PRA and aldosterone secretion has been reported with sodium manipulation and/or volume depletion. The results of the present study would imply that in these diseases other factors are influencing the levels of PRA and aldosterone.

REFERENCES

1. Liddle, G. W., L. E. Duncan, Jr., and F. C. Bartter. 1956. Dual mechanism regulating adrenocortical function in man. Amer. J. Med. 21: 380.
2. Laragh, J. H., and H. C. Stoerk. 1957. A study of the mechanism of secretion of the sodium retaining hormone (aldosterone). J. Clin. Invest. 36: 383.
3. Binnion, P. F., J. O. Davis, T. C. Brown, and M. J. Olichney. 1965. Mechanisms regulating aldosterone secretion during sodium depletion. Amer. J. Physiol. 208: 655.
4. Gross, F. 1968. The regulation of aldosterone secretion by the renin-angiotensin system under various conditions. Acta Endocrinol. Suppl. 124: 41.
5. Laragh, J. H., and H. C. Stoerk. 1955. On the mechanism of secretion of the sodium-retaining hormone (aldosterone). J. Clin. Invest. 34: 913.
6. Dluhy, R. G., R. H. Underwood, and G. H. Williams. 1970. Influence of dietary potassium on plasma renin activity in normal man. J. Appl. Physiol. 28: 299.
7. Ganong, W. F., E. G. Bigleri, and P. J. Mulrow. 1966. Mechanisms regulating adrenocortical secretion of aldosterone and glucocorticoids. Recent Prog. Horm. Res. 22: 381.
8. Williams, G. H., R. G. Dluhy, and R. H. Underwood. 1970. The relationship of dietary potassium intake to the aldosterone stimulating properties of ACTH. Clin. Sci. (London). 39: 489.
9. Venning, E. H., I. Dyrenfurth, J. B. Dossetor, and J. C. Beck. 1962. Influence of alterations in sodium intake on urinary aldosterone response to corticotropin in normal individuals and patients with essential hypertension. Metab. (Clin. Exp.). 11: 254.
10. Müller, J., and W. H. Ziegler. 1968. Stimulation of aldosterone biosynthesis in vitro by serotonin. Acta Endocrinol. 59: 23.
11. Müller, J. 1971. Regulation of Aldosterone Biosynthesis. Springer-Verlag New York Inc., New York. 108.
12. Blair-West, J. R., M. Cain, K. Catt, J. P. Coghlan, D. A. Denton, J. W. Funder, B. A. Scoggins, and R. D. Wright. 1969. Aldosterone: regulation and biosynthesis. In Progress in Endocrinology. C. Gual, editor. Excerpta Medica Foundation Publishers, Amsterdam. 276.
13. Michelakis, A. M., and R. Horton. 1970. The relationship between plasma renin and aldosterone in normal man. Circ. Res. Suppl. 26-27: I-185.
14. Luetscher, J. A., Jr., and A. H. Lieberman. 1958. Aldosterone. Arch. Intern. Med. 102: 314.
15. Luetscher, J. A., M. H. Weinberger, A. J. Dowdy, G. W. Nokes, H. Balikian, A. Brodie, and S. Willoughby. 1969. Effect of sodium loading, sodium deprivation and posture on plasma aldosterone concentration and renin activity in hypertensive patients. J. Clin. Endocrinol. Metab. 29: 1310.
16. Rosenthal, J., R. Boucher, W. Nowaczynski, and J. Genest. 1968. Acute changes in plasma volume, renin

activity, and free aldosterone levels in healthy subjects following furosemide administration. *Can. J. Physiol. Pharmacol.* **46**: 85.

17. Williams, G. H., L. I. Rose, R. G. Dluhy, D. McCaughn, P. I. Jagger, R. B. Hickler, and D. P. Lauler. 1970. Abnormal responsiveness of the renin aldosterone system to acute stimulation in patients with essential hypertension. *Ann. Intern. Med.* **72**: 317.

18. Kliman, B., and R. E. Peterson. 1960. Double isotope derivative assay of aldosterone in biologic extracts. *J. Biol. Chem.* **235**: 1639.

19. Brodie, A. H., N. Shimizu, S. A. S. Tait, and J. F. Tait. 1967. A method for the measurement of aldosterone in peripheral plasma using ³H-acetic anhydride. *J. Clin. Endocrinol. Metab.* **27**: 997.

20. Underwood, R. H., and G. H. Williams. 1972. Simultaneous determination of aldosterone, corticosterone and cortisol in peripheral plasma by displacement analysis techniques. *J. Lab. Clin. Med.* **79**: 848.

21. Nugent, C. A., and D. M. Mayes. 1966. Plasma corticosteroids determined by use of corticosteroids binding globulin and dextran-coated charcoal. *J. Clin. Endocrinol. Metab.* **26**: 1116.

22. Emanuel, R., J. P. Cain, and G. H. Williams. 1972. Simultaneous determination of angiotensin I and angiotensin II in unextracted plasma by double antibody radioimmunoassay. *J. Lab. Clin. Med.* In press.

23. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition. 91, 172.

24. Best, J. B., J. P. Coghlan, J. H. N. Bett, E. J. Cran, and B. A. Scoggins. 1971. Circulating angiotensin II and aldosterone levels during dietary sodium restriction. *Lancet.* **2**: 1353.

25. Dluhy, R. G., L. Axelrod, R. H. Underwood, and G. H. Williams. 1972. Studies of the control of plasma aldosterone concentration in normal man. II. Effect of dietary potassium and acute potassium infusion. *J. Clin. Invest.* In press.

26. Skillman, J. J., D. P. Lauler, R. B. Hickler, J. H. Lyons, J. E. Olsen, M. R. Ball, and F. D. Moore. 1967. Hemorrhage in normal man. *Ann. Surg.* **166**: 865.

27. Balikian, H. M., A. H. Brodie, S. L. Dale, J. C. Melby, and J. F. Tait. 1968. Effect of posture on the metabolic clearance rate, plasma concentration and blood production rate of aldosterone in man. *J. Clin. Endocrinol. Metab.* **28**: 1630.

28. Ford, H. C., H. P. Pieters, and R. E. Bailey. 1968. Aldosterone and sodium conservation, metabolic clearance and secretion-excretion rates of aldosterone in normal subjects. *J. Clin. Endocrinol. Metab.* **28**: 451.

29. Gordon, R. D., L. K. Wolfe, D. P. Island, and G. W. Liddle. 1966. A diurnal rhythm in plasma renin activity in man. *J. Clin. Invest.* **45**: 1587.

30. Bartter, F. C., C. S. Delea, and F. Halberg. 1962. A map of blood and urinary changes related to circadian variations in adrenal cortical function in normal man. *Ann. N. Y. Acad. Sci.* **98**: 969.

31. Venning, E. H., I. Dyrenfurth, and C. J. P. Giroud. 1956. Aldosterone excretion in healthy persons. *J. Clin. Endocrinol. Metab.* **16**: 1326.

32. Muller, A. F., E. L. Manning, and A. M. Riondel. 1958. Influence of position and activity on secretion of aldosterone. *Lancet.* **1**: 711.

33. Wolfe, L. K., R. D. Gordon, D. P. Island, and G. W. Liddle. 1966. An analysis of factors determining the circadian pattern of aldosterone excretion. *J. Clin. Endocrinol. Metab.* **26**: 1261.

34. Wolff, H. P., and M. Torbrica. 1963. Determination of plasma aldosterone. *Lancet.* **1**: 1346.

35. Horton, R. 1969. Stimulation and suppression of aldosterone in plasma in normal man and primary aldosteronism. *J. Clin. Invest.* **48**: 1230.

36. Hanger-Klevene, J. H., H. Brown, and N. Fleischer. 1969. ACTH stimulation and glucocorticoid inhibition of renin release in the rat. *Proc. Soc. Exp. Biol. Med.* **131**: 539.

37. Newton, M. A., and J. H. Laragh. 1968. Effect of corticotropin on aldosterone excretion and plasma renin in normal subjects, in essential hypertension, and in primary aldosteronism. *J. Clin. Endocrinol. Metab.* **28**: 1006.

38. Mills, J. N. 1966. Human circadian rhythms. *Physiol. Rev.* **46**: 128.

39. Espiner, E. A., J. R. Tucci, P. I. Jagger, and D. P. Lauler. 1967. The effect of acute diuretic induced volume depletion on aldosterone secretion in normal man. *Clin. Sci. (London).* **33**: 125.

40. Collins, R. D., M. R. Weinberger, A. J. Dowdy, D. W. Nokes, C. M. Gonzales, and J. A. Luetscher 1970. Abnormally sustained aldosterone secretion during salt loading of patients with various forms of benign hypertension; relationship to plasma renin activity. *J. Clin. Invest.* **49**: 1415.

41. Christlieb, A. R., R. B. Hickler, D. P. Lauler, and G. H. Williams. 1969. Hypertension with inappropriate aldosterone stimulation: a syndrome. *N. Engl. J. Med.* **281**: 128.

42. Chin, R. H., J. A. Brown, R. Fraser, S. M. Heron, A. F. Lever, L. Murchison, and J. I. S. Robertson. 1970. The natriuresis of fasting: relationship to changes in plasma renin and plasma aldosterone concentration. *Clin. Sci. (London).* **39**: 437.

43. Mitra, S., S. M. Genuth, L. B. Berman, and V. Vertes. 1972. Aldosterone Secretion in anephric patients. *N. Engl. J. Med.* **286**: 61.