

## PLASMA RENIN ACTIVITY IN HYPERTENSIVE DISEASES

YASUNOBU ITASAKA

*2nd Department of Surgery, School of Medicine Nagoya University  
(Director: Prof. Shin Hoshikawa)*

### ABSTRACT

Plasma renin activity was determined in normotensive and hypertensive human subjects by a modification of the method of Boucher and co-workers. Simultaneous determination of plasma volume, serum electrolyte concentration and plasma renin activity was performed. The plasma renin activity was normal in essential hypertension and in pheochromocytoma, normal or subnormal in Cushing's syndrome with adrenal hyperplasia, increased in Cushing's syndrome with adrenal carcinoma and in renal or renovascular hypertension and decreased in primary aldosteronism. Three hours after taking upright posture, patients with essential hypertension showed two to fivefold increase of plasma renin activity, while those with primary aldosteronism did not. In renovascular hypertension and primary aldosteronism, plasma renin activity returned to normal range after operation. Diurnal variation of plasma renin activity was investigated under a standard condition. In normotensive subjects, highest value was observed in the forenoon and lowest value in the afternoon. As respects the diurnal variation of plasma renin activity, an inverse relationship between plasma renin activity and plasma volume was exhibited.

### INTRODUCTION

Since the existence of renin, as vasopressor substance, was suggested by Tigerstedt and Bergman (1898)<sup>1)</sup>, many works about renin have been done. Goldblatt *et al.* (1934)<sup>2)</sup> demonstrated that hypertension was experimentally able to be produced by renal artery constriction. Brown-Menendez *et al.* (1940)<sup>3)</sup> postulated that when renal artery was narrowed renin was released from the kidney into circulation and acted upon plasma substrate to produce some pressor peptide, which raised the arterial pressure by direct pressor effect. Since renin and angiotensin were identified by Kohlstaedt<sup>4)</sup> and Page<sup>5)</sup>, many investigators have reported the results of assay for the pressor substance in hypertensive diseases.

Fasciolo *et al.*<sup>6)</sup> reported normal renin level in the blood of patients with benign hypertension and increased level in patients with severe hypertension. Helmer<sup>7)</sup> reported that the highest value of renin was observed in accelerated hypertension.

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Received for publication December 17, 1969.

In subsequent reports<sup>8)9)</sup>, it was demonstrated that an aldosterone producing factor was present in the renin fraction of kidney extracts, and that infusion of renin or angiotensin-II<sup>10)</sup> resulted in increased aldosterone excretion.

Following the observation of Conn *et al.*<sup>11)</sup>, determination of plasma renin activity was found to be important and useful procedure in distinguishing either the adrenal or the kidney as the instigator, since plasma renin activity in primary aldosteronism was extremely suppressed.

In the course of studies attempting to correlate plasma renin activity with aldosterone excretion, Gordon *et al.*<sup>12)</sup> found that the plasma renin activity and the urinary aldosterone excretion rose in the forenoon and diminished in the afternoon. Although they demonstrated a diurnal rhythm in plasma renin activity in normotensive subjects, no work has been done on the study in hypertensive subjects.

Since feed-back mechanism is not yet certainly recognized in the renin-angiotensin-aldosterone system, it seems to be important to study the diurnal variation of plasma renin activity in hypertensive diseases, especially in primary aldosteronism and in renovascular hypertension.

In this report, a study was made on the values of plasma renin activity in hypertensive diseases, on their diurnal variations and on the relationship among plasma renin activity, serum electrolyte concentration and plasma volume.

#### MATERIALS AND METHODS

*Materials:* Twelve normotensive and twenty-three hypertensive patients were investigated. The normotensive group consisted of 3 patients with nodular goiter, 1 patient with thyroid cancer, 2 patients with breast tumor and 6 patients with gastroduodenal diseases. The hypertensive group consisted of 4 patients with essential hypertension, 8 patients with renal or renovascular hypertension, 5 patients with Cushing's syndrome, 5 patients with primary aldosteronism and 1 patient with pheochromocytoma.

These subjects were all hospitalized and given standard diet containing 15 g of salt per day. Blood was drawn in the supine position usually between 8.00 a.m. and 9.00 a.m.

On the experiment of diurnal variation of plasma renin activity, samples were obtained at constant intervals, 4 times a day in normotensive subjects and twice a day in hypertensive subjects.

From at least 6 h before the first sampling to the final, the subjects were laid in bed in controlled recumbent position.

For determination of plasma volume, the subjects received 10 drops of Lugol's solution on the previous day to block the thyroid gland<sup>13)</sup>. All medications including antihypertensive drugs were discontinued at least 1 week prior to study.

*Measurement of plasma renin activity:* Plasma renin activity was determined by a modification of the method of Boucher and co-workers<sup>14</sup>. Approximately 20 ml of peripheral venous blood was obtained by the disposable syringe, immediately transferred to the tube already containing  $10^{-2}$  M EDTA ( $\text{NH}_4$ ) and chilled in an ice bath. After centrifugation for 20 min. at 3,000 r.p.m. at  $0-5^\circ\text{C}$ , the plasma was collected, filtered on glass wool and volume was measured. Four of moist Dowex 50 W  $\times$  2 ( $\text{NH}_4$ ) resin was added to plasma and the mixture was adjusted to pH 5.5 by addition of 1 N HCl under the control of pH-meter. The sample was incubated for 3 h at  $37^\circ\text{C}$  using mechanical shaker. Following incubation, the mixture was transferred to a chromatographic column (1 cm  $\times$  10 cm) already containing 1 ml of Dowex 50 W  $\times$  2 ( $\text{NH}_4$ ) resin and maintained at  $0-5^\circ\text{C}$  in specially designed apparatus. The column was washed first with 15 ml of 0.2 N ammonium acetate, secondarily with 20 ml of 10% acetic acid and thirdly with 15 ml of distilled water. These eluates were discarded. At this point, the column was transferred to room temperature. Angiotensin was eluted with 15 ml of 0.1 N diethylamine and then with 0.2 N ammonium hydroxide. The eluate was evaporated to dryness in a conical flask connected to a rotary evaporator. The eluate was not acidified to avoid the formation of ammonium acetate<sup>15</sup> which usually showed depressor effect in the bioassay, but immediately evaporated under vacuum. The temperature during evaporation was kept below  $48^\circ\text{C}$ . The dry residue was dissolved into about 2 ml of 80% ethanol and evaporated to dryness. This was repeated 6 times to remove all traces of ammonium acetate. The final dry residue was dissolved in 1 ml of 0.9% saline. Angiotensin generated and eluted from each sample was assayed by its pressor activity using the male Donryu rat (weighing about 150 g) anesthetized with pentobarbitrate (50 mg/kg) intraperitoneally. After tracheotomy, blood pressure changes were measured by a catheter in the carotid artery connected with a transducer and a recorder. Infusions of standards and samples were done through the cannulated jugular veins. The bracket method<sup>16</sup> was used throughout the assay.

Reproducibility in same samples yielded a standard deviation of 10%. The mean recovery using 50 ng of synthetic Val-5-angiotensin-II was 80%. Detectable renin activity was approximately over 20 ng/dl, which was mainly dependent upon response of artery.

*Determination of plasma volume:* Plasma volume was measured using Volëmetron. The venous blood was withdrawn from antecubital vein before and after administration of  $5 \mu\text{C}$   $\text{R}^{131}\text{ISA}$ . Each 8 ml of blood was put into Volëmetron and counted. The standard deviation of this system was within 0.1 liter.

Hematocrit was measured at the same time to calculate plasma volume.

*Measurement of serum electrolyte concentration:* Serum Na and K concentra-

tion were measured using flame-photometer. The standard deviation was within 0.3%.

## RESULTS

The results of normotensive control subjects were listed in Table 1. The range of plasma renin activity was 144 to 400 ng/dl with a mean of 228 ng/dl

TABLE 1. Plasma renin activity in normotensive subjects

Case No.	Age	Sex	Sampling time	Renin Activity (ng/dl)	Remarks
1	41	M	8.00 a.m.	200	Duodenal ulcer
2	30	F	8.00 a.m.	175	Struma nodosa
3	41	F	8.00 a.m.	194	Struma nodosa
4	30	F	8.00 a.m.	180	Gastric leiomyoma
5	28	M	8.00 a.m.	400	Duodenal ulcer
6	36	M	8.00 a.m.	150	Duodenal ulcer
7	41	M	8.00 a.m.	315	Gastric ulcer
8	39	F	8.00 a.m.	208	Thyroid cancer
9	41	M	8.00 a.m.	275	Gastric ulcer
10	45	F	8.00 a.m.	144	Breast cancer
11	52	F	8.00 a.m.	165	Breast cancer
12	19	M	8.00 a.m.	333	Struma nodosa

TABLE 2. Plasma renin activity in hypertensive subjects

Case No.	Age	Sex	Sampling time	Renin Activity (ng/dl)	Remarks
13	37	M	8.00 a.m.	307	Essential hypertension
14	21	M	8.00 a.m.	170	Essential hypertension
15	20	M	8.00 a.m.	220	Essential hypertension
16	30	F	6.00 a.m.	187	Essential hypertension
			4.00 p.m.	156	
17	41	F	8.00 a.m.	undetectable	Primary aldosteronism
18	36	F	8.00 a.m.	147	Primary aldosteronism
19	37	M	8.00 a.m.	undetectable	Primary aldosteronism
20	22	F	8.00 a.m.	undetectable	Primary aldosteronism
21	39	M	8.00 a.m.	50	Primary aldosteronism
22	10	M	8.00 a.m.	3,180	Renal hypertension
23	19	M	8.00 a.m.	1,500	Renovascular h.
24	23	F	8.00 a.m.	438	Renovascular h.
25	18	M	8.00 a.m.	4,650	Renovascular h.
26	12	F	8.00 a.m.	1,640	Renal hypertension
27	28	M	6.00 a.m.	420	Renal hypertension
			4.00 p.m.	480	
28	20	M	6.00 a.m.	714	Renal hypertension
			4.00 p.m.	430	
29	19	F	6.00 a.m.	420	Renal hypertension
			4.00 p.m.	500	
30	16	F	6.00 a.m.	62	Cushing's syndrome
			4.00 p.m.	30	(Hyperplasia)
31	22	F	6.00 a.m.	185	Cushing's syndrome
			4.00 p.m.	250	(Hyperplasia)
32	24	F	6.00 a.m.	125	Cushing's syndrome
			4.00 p.m.	90	(Hyperplasia)
33	22	M	6.00 a.m.	2,250	Cushing's syndrome
			4.00 p.m.	1,000	(Carcinoma)
34	34	M	6.00 a.m.	4,270	Cushing's syndrome
			4.00 p.m.	5,000	(Carcinoma)
35	52	M	6.00 a.m.	288	Pheochromocytoma
			4.00 p.m.	21	

(SD 80 ng/dl). The results of hypertensive subjects were epitomized in Table 2. All of the values of renin activity in essential hypertension were within the range of normotensive subjects. Average value (232 ng/dl) is not significantly different from that of normotensive subjects ( $p=0.01$ ). In 4 of 5 patients with primary aldosteronism, plasma renin activity was undetectable or extremely low compared with normotensive subjects.

In another case (No. 18), plasma renin activity was at lowest limit of the normal range. In all patients with renal or renovascular hypertension, the values of plasma renin activity were above the normal range. In 4 patients

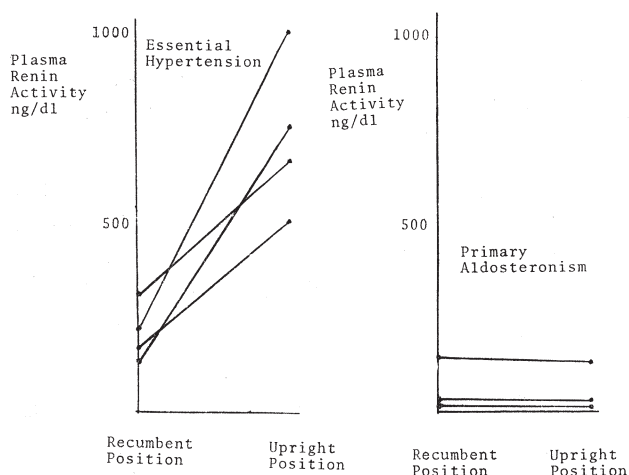


FIG. 1. Effect of alteration of posture in Essential hypertension and in primary aldosteronism.

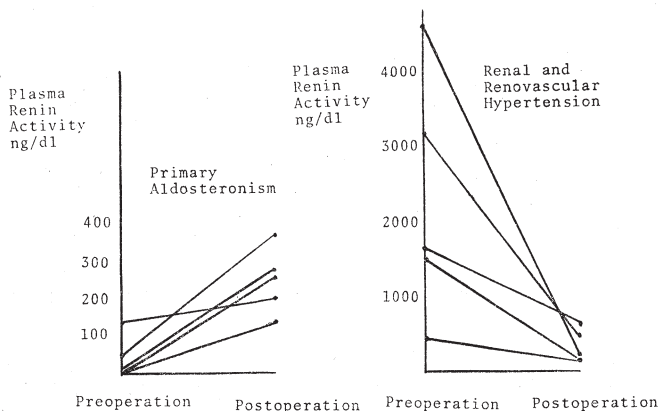


FIG. 2. Plasma renin activity in primary aldosteronism and in renal or renovascular hypertension.

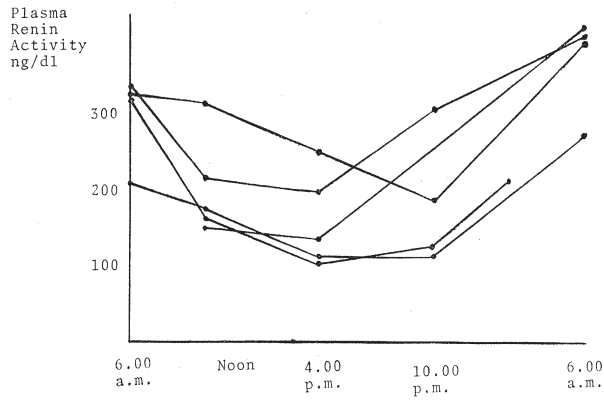


FIG. 3. Diurnal variation of plasma renin activity in normotensive subjects.

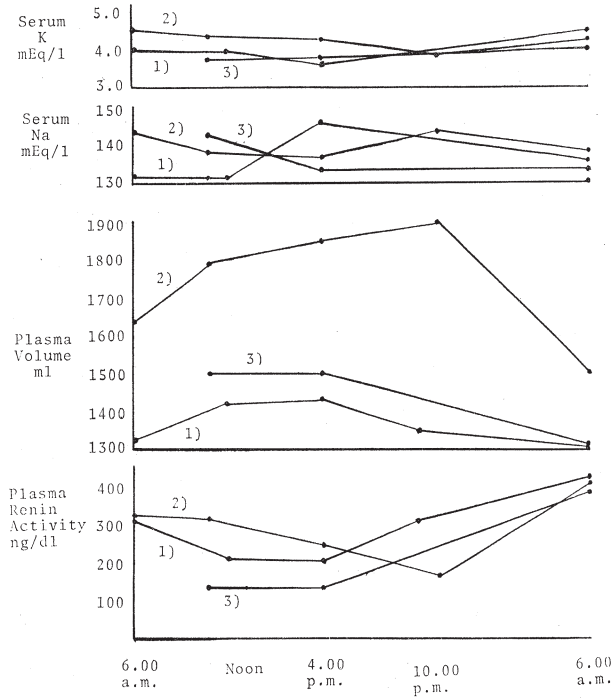


FIG. 4. Diurnal variation of plasma renin activity, plasma volume and serum electrolyte concentration in normotensive subjects.

1): No. 8, 2): No. 12, 3): No. 10

(Nos. 22, 23, 25 and 26), especially, extremely high values were manifested. The range of plasma renin activity in this disease was considerably wide (420-4,650 ng/dl).

The results with Cushing's syndrome and pheochromocytoma was described later.

Effect of upright posture on plasma renin activity was depicted in Fig. 1. Patients took upright posture for 3 h starting at 8.00 a.m. in this test. Two to fivefold increase of plasma renin activity was seen in patients with essential hypertension, but no increase in those with primary aldosteronism.

Effect of surgery was illustrated in Fig. 2. In 4 of 5 patients with renal or renovascular hypertension, nephrectomy was performed. In a patient (No. 23) with renovascular hypertension, the repair of stenotic renal artery was performed. In those with primary aldosteronism, adrenal gland including

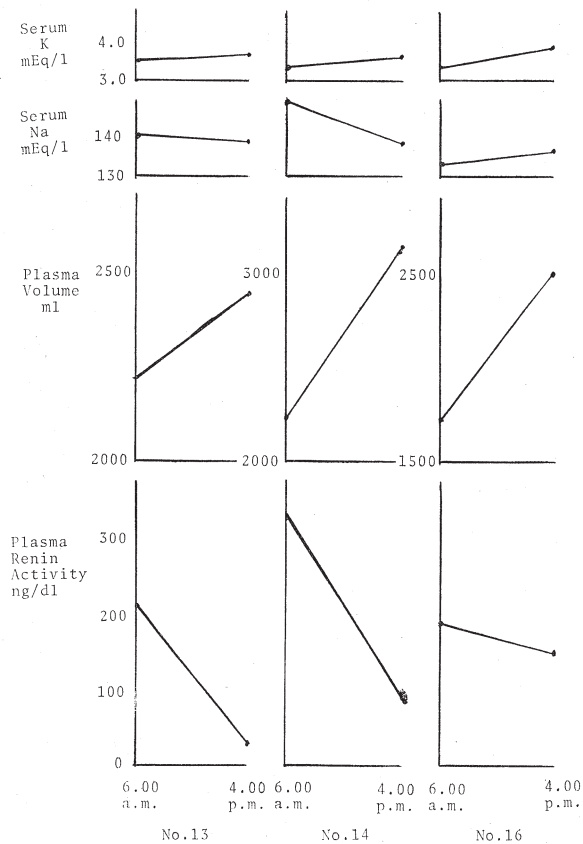


FIG. 5. Diurnal variation of plasma renin activity, plasma volume and serum electrolyte concentration in essential hypertension,

adenoma was removed. Three patients with renal or renovascular hypertension and all patients with primary aldosteronism exhibited normal values at the assay in postoperation. Although two other nephrectomized patients showed slightly high values at the assay in postoperation, their blood pressure improved greatly.

Diurnal variation of plasma renin activity in 5 normotensive subjects was illustrated in Fig. 3. Plasma renin activity showed higher values in the forenoon than in the afternoon. It reached maximum at 6.00 a.m., gradually decreased and dropped to minimum at 4.00 p.m.. In 3 normotensive subjects, plasma renin activity, plasma volume and serum electrolyte concentration were determined simultaneously (Fig. 4). Plasma volume was inversely proportional to plasma renin activity (Correlation coefficient of No. 8 and No. 12 was  $-0.75$  and  $-0.70$  respectively). Whereas serum electrolyte concentration did not

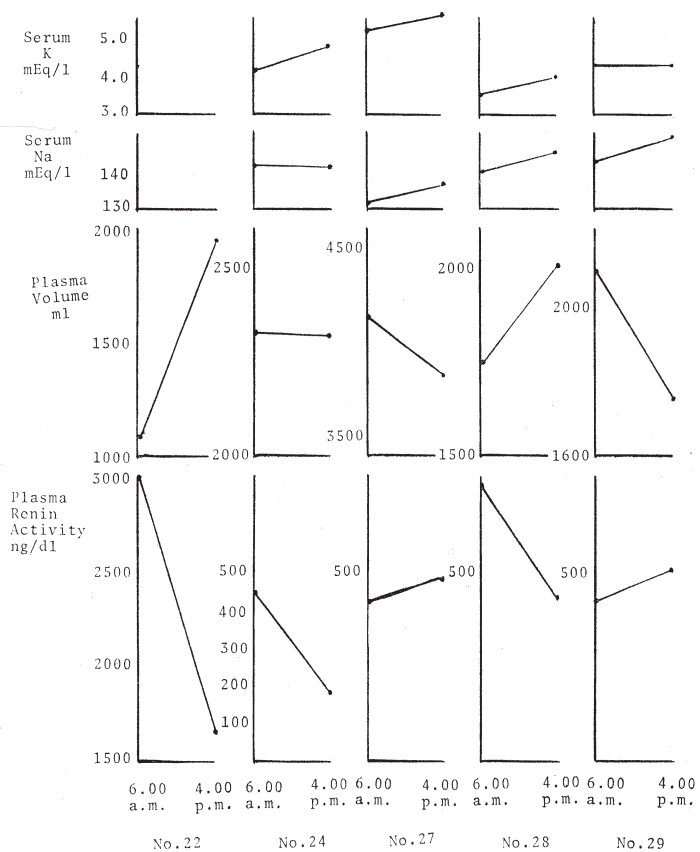


FIG. 6. Diurnal variation of plasma renin activity, plasma volume and serum electrolyte concentration in renal hypertension and in renovascular hypertension.



correlate with plasma renin activity.

The average value for plasma renin activity of 5 normotensive subjects at 6.00 a.m. and 4.00 p.m. was  $305 \pm 26$  ng/dl and  $185 \pm 49$  ng/dl respectively. The difference was statistically significant at 1% level. Therefore we decided to take samples at 6.00 a.m. and 4.00 p.m. to evaluate diurnal variation of plasma renin activity in hypertensive patients. If the value in the forenoon was higher than that in the afternoon, we designated it as normal pattern, and if not, as abnormal pattern. In 3 patients with essential hypertension (Fig. 5), plasma renin activity exhibited normal pattern. Plasma renin activity was inversely correlated with plasma volume; however, a constant relation was not seen between plasma renin activity and serum electrolyte concentration. Fig. 6 illustrates the data of 3 patients with renal hypertension and 2 patients with renovascular hypertension. In these patients, 2 showed abnormal pattern of plasma renin activity, 3 did normal. Inverse relationship between plasma renin activity and plasma volume was seen in 4 patients, but not in one patient

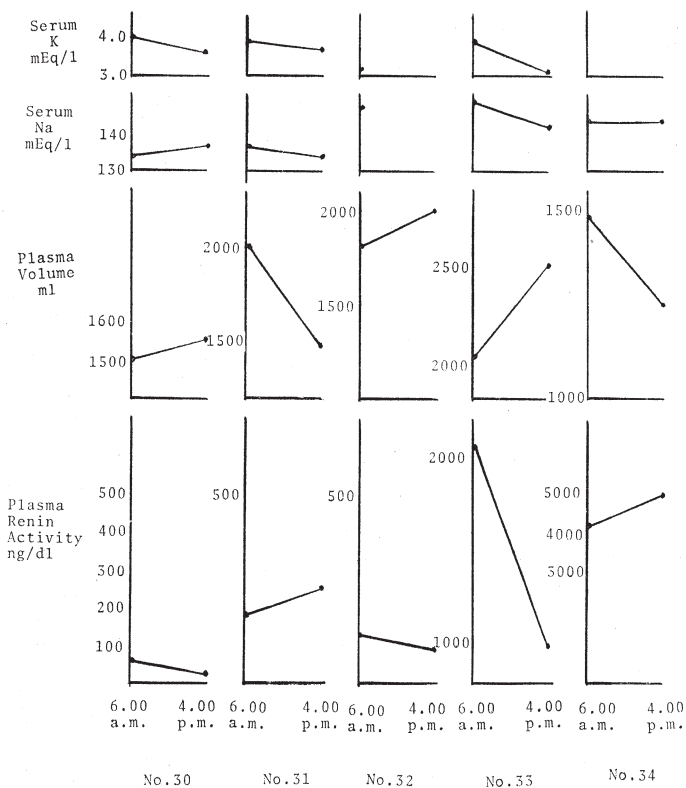


FIG. 7. Diurnal variation of plasma renin activity, plasma volume and serum electrolyte concentration in Cushing's syndrome.

(No. 24). Fig. 7 presents the results of Cushing's syndrome, which consists of 3 cases (Nos. 30, 31 and 32) of adrenal hyperplasia and 2 cases (Nos. 33 and 34) of adrenal cancer. Plasma renin activity of the patients with adrenal hyperplasia was normal or subnormal, while that of the patients with adrenal cancer was extremely high. Diurnal variation showed abnormal pattern in 2 cases and normal in the other 3 cases. An inverse relationship was seen between plasma renin activity and plasma volume.

Fig. 8 summarizes the data of 3 patients with primary aldosteronism. No renin activity was detectable in 2 patients. One patient (No. 18) showed normal value of plasma renin activity, however, diurnal variation of renin activity was abnormal.

The plasma volume increased significantly in all cases. These patients showed no increase of plasma renin activity not only after ingesting the low

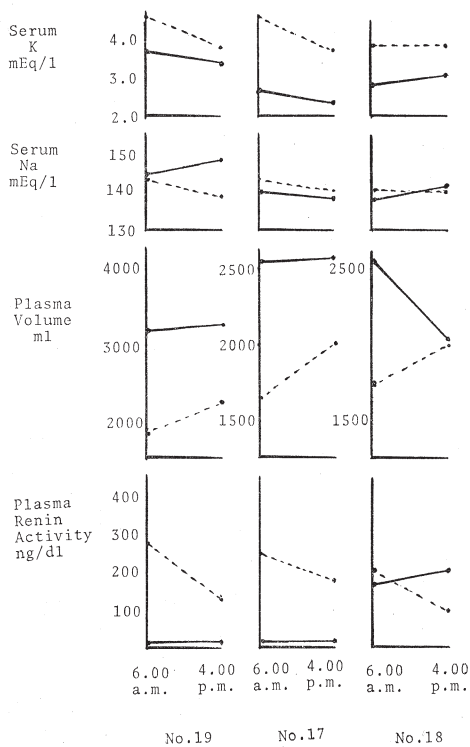


FIG. 8

FIG. 8. Diurnal variation of plasma renin activity, plasma volume and serum electrolyte concentration in primary aldosteronism.

solidline: preoperation  
dottedline: postoperation

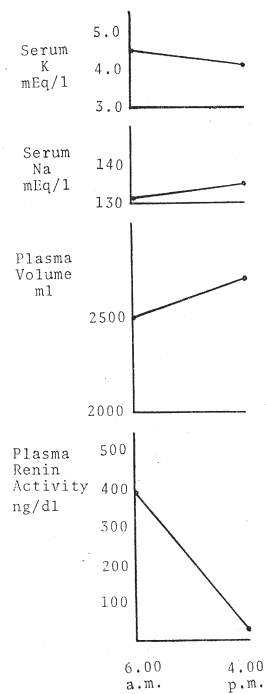


FIG. 9

FIG. 9. Diurnal variation of plasma renin activity, plasma volume and serum electrolyte concentration in pheochromocytoma.

salt diet (10 mEq/day) for 3 days but even after 3 h upright position. Adrenal exploration was performed in all cases, and adrenocortical tumor, typical of primary aldosteronism, was found. Prognoses after operation were all satisfactory and the diurnal variation returned to normal range as it was depicted with dotted line in Fig. 8. The results of the patient with pheochromocytoma were illustrated in Fig. 9. The plasma renin activity exhibited normal value, normal pattern and inverse relationship with plasma volume.

#### DISCUSSION

Although Goldblatt<sup>17)</sup> proposed that multiple intrarenal arterial constrictions should be present in essential hypertension and that this was responsible for the genesis of this disease, subsequent workers<sup>18)19)</sup> failed to prove increased renin in essential hypertension.

Four patients in this report exhibited normal renin activity, and it seems, therefore, reliable that renal pressor system plays no pathologic role in these patients. Helmer<sup>7)</sup> reported that correctable renal hypertension was frequently associated with significant increase in peripheral renin and that these determinations would be advocated for the screening of hypertensive patients. Recently, however, other investigators<sup>20)21)</sup> reported that some patients with correctable renal hypertension had normal renin levels. On our patients with renal or renovascular hypertension, plasma renin activity was high in preoperative period and lowered by surgery. Renal pressor system is probably substantial for the genesis of this disease.

Brown<sup>22)</sup> reported that hypernatremia and abnormally low plasma renin concentration might occur in primary aldosteronism and Cushing's disease. In his statement, the depression of plasma renin concentration was showed to be closely connected to the increase in serum sodium concentration. Three patients with Cushing's syndrome associated with hyperplasia exhibited normal or subnormal renin activity, normal sodium concentration and increased plasma volume.

Two patients with adrenal cancer exhibited extremely high plasma renin activity, normal level of serum sodium concentration and decreased plasma volume. This discrepancy in plasma renin activity is difficult to explain by the difference in sodium concentration, but it might be due to the difference of plasma volume.

Four of 5 patients with primary aldosteronism exhibited extremely low plasma renin activity, although their serum sodium concentration had been already normalized by "renal escape"<sup>23)24)</sup>.

Conn<sup>25)</sup> demonstrated that the combination of greatly elevated secretion of aldosterone with zero-value for plasma renin activity was unique and that it was characteristic only of primary aldosteronism. Further studies were carried

out by many workers and it was established that this criteria was quite adequate to distinguish primary aldosteronism from other diseases.

One of the 5 patients with primary aldosteronism, however, exhibited normal value of plasma renin activity, although it was at lowest limit of normal range. Suppressed renin activity in this disease is probably due to the increase of intravascular volume<sup>26)</sup>.

Provided the expansion of intravascular volume remains in relative low level, it may be reasonable that plasma renin activity is not suppressed. Slaton and co-workers<sup>27)</sup> demonstrated in their 10 cases of primary aldosteronism that the plasma volume ranged from +5% to +75% above predicted normal values calculated on the basis of height and weight. In the patient who showed normal level of plasma renin activity in spite of primary aldosteronism, the plasma volume was +25%, however, in another 2 patients whose plasma renin activity was undetectable, the plasma volume was +50% and +80% respectively. This level of expansion in plasma volume (+25%) is apparently not sufficient to suppress plasma renin activity.

On the other hand, there is a possibility of coexistence of hemodynamic abnormality in the renal artery in this case.

Kaplan<sup>28)</sup> reported a case of primary aldosteronism with malignant hypertension, and Greco<sup>29)</sup> also demonstrated a similar case.

However, the determination of plasma renin activity was not described in their reports. Brown<sup>30)</sup> reported a case of primary aldosteronism with arteriolar fibrinoid lesion in the kidney. In his case, plasma renin concentration was depressed, and raised by administration of spironolactone to normal range. Although such a possibility may not be denied, arteriogram taken in my case revealed no abnormality in the main branch of renal artery.

A number of studies have been done on the response of the kidney to catecholamine under various conditions<sup>31) 32)</sup>, and it is suggested that infusion of catecholamine raised plasma renin activity through renal hemodynamic changes. The fact that a patient with pheochromocytoma exhibited normal value in the morning and low in the evening, might explain only that reduction of renal blood flow followed by excessive catecholamine secretion did not occur in the sampling time. On the experiment by Brown<sup>33)</sup>, which pointed out the diurnal variation of renin concentration in normal subjects, no attempt was made to limit physical activity and dietary intake (salt content), both of which might possibly influence on plasma renin concentration. As Gordon<sup>12)</sup> postulated, normotensive recumbent subjects exhibited a constant diurnal rhythm. The factors which regulate the diurnal variation of plasma renin activity were not investigated in previous reports. It is well-known that sodium-deprivation is great stimulus to secretion of renin. An inverse relationship between serum sodium concentration and renin concentration was documented by Brown *et al*<sup>22)</sup>. Recently, it was pointed out that the conditions, in which renin concentration

apparently related to serum sodium concentration, tended to show characteristic aberrations in extracellular fluid volume.

Fraser *et al.*<sup>34)</sup> demonstrated a rise in plasma renin concentration without change in serum sodium concentration. Newsome *et al.*<sup>35)</sup> suggested that changes in body fluid volume might supersede serum sodium concentration as a determinant of plasma renin activity.

Since there was found an inverse relationship between plasma volume and plasma renin activity in majority of my cases, it is suggested that plasma volume plays significant role in diurnal control of plasma renin activity. No work, however, has been done on the study of diurnal variation of plasma volume.

Aldosterone is considered to be one of the factors which participate homeostatically in the regulation of body fluid by means of sodium retaining effect.

Biglieli<sup>26)</sup> reported a case of primary aldosteronism, in which abnormal diurnal variation of aldosterone was speculated, that is, urinary aldosterone determination revealed a nocturnal excretion three times above the day time output.

Abnormal diurnal variation of plasma renin activity in primary aldosteronism is probably due to abnormal aldosterone secretion from adrenocortical tumor.

#### CONCLUSION

1. For the evaluation of plasma renin activity, it is necessary to prescribe the sampling time, patient's posture and salt content in diet, because these factors will influence on the plasma renin activity.

2. Some case of primary aldosteronism may exhibit normal value of plasma renin activity when the expansion of plasma volume is not sufficient to suppress plasma renin activity.

3. Determination of diurnal variation of plasma renin activity and evaluation of the effect of upright posture will be a useful aid for the diagnosis of primary aldosteronism.

4. Patients with essential hypertension exhibited normal values of plasma renin activity, those with renal or renovascular hypertension increased values, those with Cushing's syndrome normal or subnormal values, those with adrenal carcinoma increased values, those with primary aldosteronism suppressed values and a patient with pheochromocytoma normal value.

5. Plasma volume, probably acting intermediately for renal blood flow, controls diurnal variation of plasma renin activity, however, serum electrolyte concentration has no effect on this.

## ACKNOWLEDGMENT

The author wishes to express his deep gratitude to Prof. Shin Hoshikawa and Dr. Hiroshi Yogo for suggestions and criticisms. And also to all the members of the research group on endocrinology in 2nd department of surgery for their co-operation.

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