Acta Medica et Biologica
Vol. 33, No. 4, 183-191, 1986

REDUCTION OF URINARY CHLORIDE EXCRETION BY PRORIFERATED MESANGIAL CELLS IN RESPONSE TO WALKING IN CHILDREN

TADASHI ASAMI, M. D., KIN-YA HASHIMOTO,
AND
KAORU SAKAI, M. D.

Department of Pediatrics, School of Medicine, Niigata University, Asahimachi-dori 757, Niigata, Japan.

(Received September 17, 1985)

INTRODUCTION

In a normal kindney, urinary chloride (Cl) excretion is regulated by glomerular filtration and renal tubular reabsorption, which is called glomerulotubular feedback mechanism[1)2)]. In a diseased kidney, proliferated mesangial cells have been studied and revealed to have contractile activities which reduce the surface area of the glomerular capillary wall for filtration and lead to a reduction in the glomerular filtration coefficient[3),4),5)].

However, the mechanism of renal handling of chloride is not so well understood as urinary sodium excretion. Decreases in fractional urinary sodium excretion upon orthostatic loading and walking in patients with mesangial cell proliferations have been reported, and considered to be caused by the reduction of glomerular filtration[6),7)].

This study was designed to examine the roles of mesangial cells in the regulation of urinary Cl excretion in children with chance proteinuria and/or hematuria and with biopsy-proved chronic glomerulonephritis (GN).

METHOD

SUBJECTS

1. Chance Proteinuria and/or Hematuria

219 patients aged 6 to 18 years (90 males and 129 females) found to have chance

proteinuria and/or hamaturia on annual urinary mass-screening tests for renal diseases were classified into four large groups (I, II, III, and IV) according to their urinary findings as follows.

Group I (n=89, 30 males and 59 females): negative urinary protein with urinary red blood cell (RBC) counts less than 20/high-pouer (×400) field (HPF) in the centrifuged sediment on microscope.

Group II (n=63, 25 males and 38 femles): negative urinary protein with urinary RBC counts more than 20/HPF.

Group III (n=36, 21 males and 15 females): positive urimary protein with urinary RBC counts less than 20/HPF.

Group IV (n=31, 14 males and 17 females): positive urinary protein with urinary RBC counts more than 20/HPF.

The patients in each of the four groups were further subdivided according to whether the urinary RBC counts were more or less than 5/HPF, which is generally considered as the normal limit as shown in Fig. 1.

In group, I, the patients with urinary RBC counts less than 5/HPF were further subdivied into subgroup I'. In group II, the patients with urinary RBC too high to be counted were subdivied into subgroup II'. In group III, the patients with urniary RBC counts counts less than 5/HPF were subdivied into subgroup III'. In group IV, the

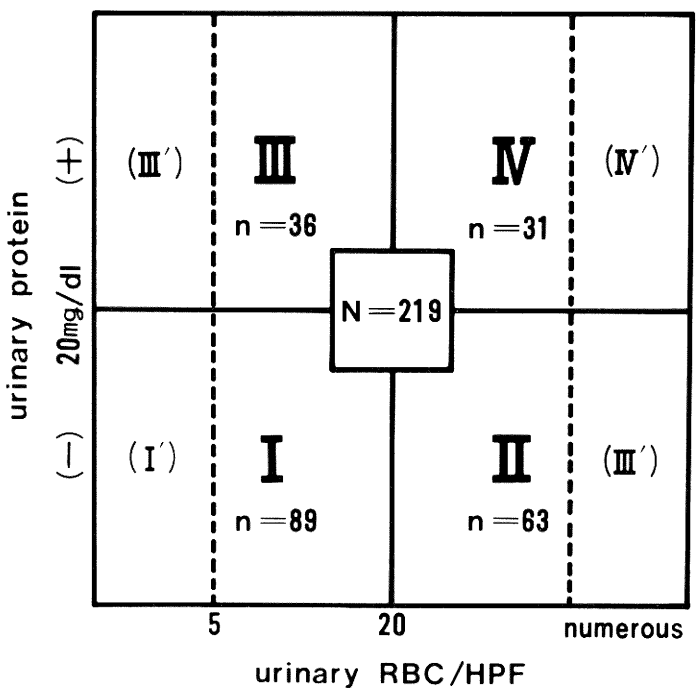

Fig. 1. Classification of renal glomerular lesions.

patients with numerous urinary RBC counts in the sediment were subdivided into subgroup IV'. Thus subgroup I' was considered an almost normal urine group, and subgroup IV' the most severe urine group.

2. Biopsy-Proved Chronic Glomerulonephritis (GN)

27 patients aged 6 to 18 years (14 males and 13 females) with biopsy-proved renal glomerular lesions other than above mentioned subjects were classified according to the severity of mesangial cell proliferations based on a Table by one of our co-workers who had not been informed of the patient's data on urinary findings or urinary electolytes.

1 minimal change GN (n=5, 4 males and 1 female)

2-A-a mild proliferative GN (n=14, 8 males and 6 females)

2-A-b moderate proliferative GN (n=3, 1 male and 2 females)

4 membranoproliferative GN (MPGN,n=5, 1 male and 4 females)

STUDY PROTOCOL (Fig. 2)

The protocol of the study is shown in Fig. 2. Hereafter, following abbreviations are used.

FVM-urine; the first-voided morning urine, indicating the urine taken after sustained recumbency (sleep).

OPC-urine; the urine voided at the outpatient clinic (OPC) of our hospital, indicating the urine taken after orthostatic loading (standing, walking, or sitting in upright possition which are all done when visiting the hospital).

All 246 subjects were instructed by the physicians not to restrict any salts, meats, or vegetables. Therefore the amount of sodium, potassium, and chloride intake were

Table Classification of Renal Glomerular Lesions

Classification of Renal Glomerular Lesions

1	normal or minimal change
2-A-a	mild proliferative GN
2-A-b	moderate proliferative GN
2-A-c	severe proliferative GN
2B	proliferative GN with focal crescents
2C	proliferative GN with generalized crescents
3	membranous nephropathy
4	membranoproliferative GN
5	focal GN
6	too advanced to be classified
7	unclassified

GN:glomerulonephritis

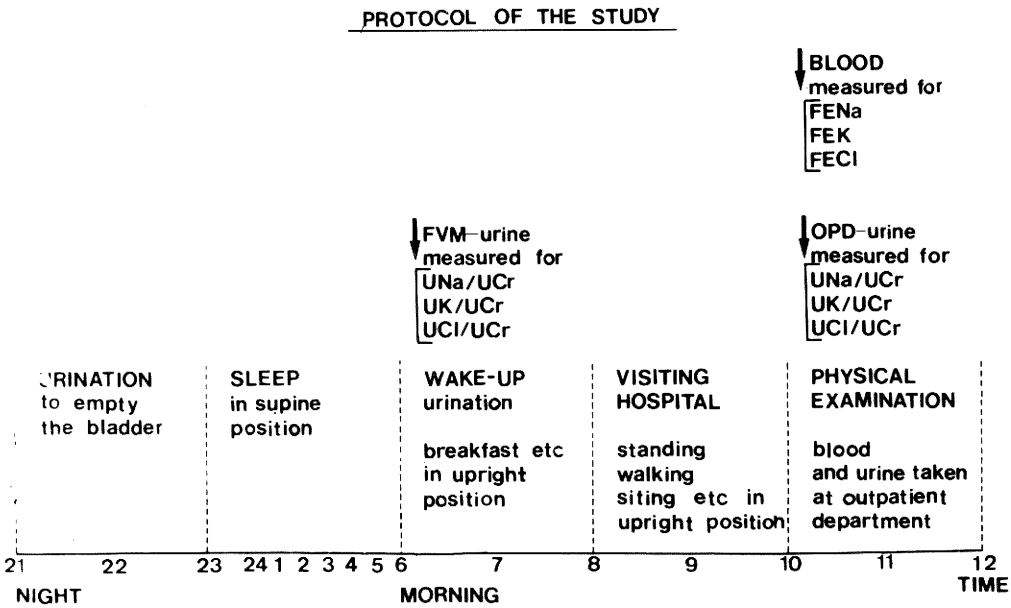

Fig. 2. Protocol of the study.

considered to be comparable for statistical analysis by using a large number of subjects in a localized area in Japan.

Patient were instructed to empty the in bladders before going to bed at night on the day prior to visiting the hospital. FVM-urine was obtained between 6:00 am and 8:00 am at home, then the patient visited the out-patient clinic of our hospital, and venous blood and urine samples were taken simultaneavsly betwen 10:00 am and 12:00 am by informed consent to measure chloride (SGI), creatinine (SCr), urinary chloride (UCr), and creatinine (UCr).

ASSESMENT OF URINARY CHLORIDE EXCRETION

Using values obtained above, the following indices were calculated: Fractional Chloride Excretion Rate (FECl)=UCl/SCl/UCr×SCr×100%, Urinary Cl/Creatinine Ratio (UCl/UCr)=UCl (mEq/L)/UCr (mg/L)

The ratio of UCl/UCr in OPC-urine to UCl/UCr in FVM-urine was also calculated using the following formula to evaluate the effects of walking on urinary chloride excretion.

(UCl/UCr in OPC-urine)÷(UCl/UCr in FVM-urine)

RESULTS

In the following data, ± values represent the mean ± SD.

RELATION BETWEEN FECL MEASURED AT OUTPATIENT CLINIC AND URINARY FINDING (Fig. 3).

FECl in subgroup I' with normal urinary findings was 1.80±0.65%, in which statistically significant decreases were noted as the urinary findings became more severe in groups II, II', III', III, and IV'.

RELATION BETWEEN THE RATIO OF UCL/UCr IN OPC-URINE TO THAT IN FVM-URINE AND URINARY FINDINGS (Fig. 4)

The ratio in subgroup I' was 2.35±1.74, in which a significant decrease was noted as the urinary findings became more severe in groups II' II', and IV.

RELATION BETWEEN THE RATIO OF (UCL/UCR IN OPC-URINE) TO (UCL/UCR IN FVM-URINE) AND THE SEVERITY OF MESANGIAL CELL PROLIFERATION IN CHRONIC GN (Fig. 5)

In patients with minimal glomerular lesions, UCl/UCr in OPC-urine was 3. 28-fold higher than it was in FVM-urine. These ratios decreased as the glomerualr mesangial cell proliferations became more severe: 1. 45-fold higher in mild proliferative GN; 1. 30 -fold higher in moderate proliferative GN; and 0.80-fold lower in MPGN. In contrast to

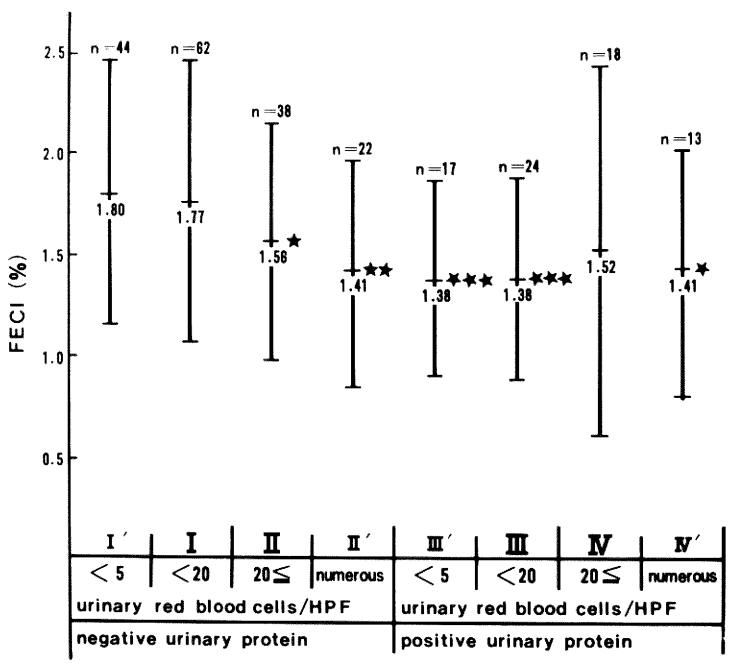

Fig. 3. Relation between FECl measured after walking at the outpatient clinic.
(★★represents p values; ★0.05<p<0.10, ★★0.01<p<0.05, ★★★0.001<p<0.01)

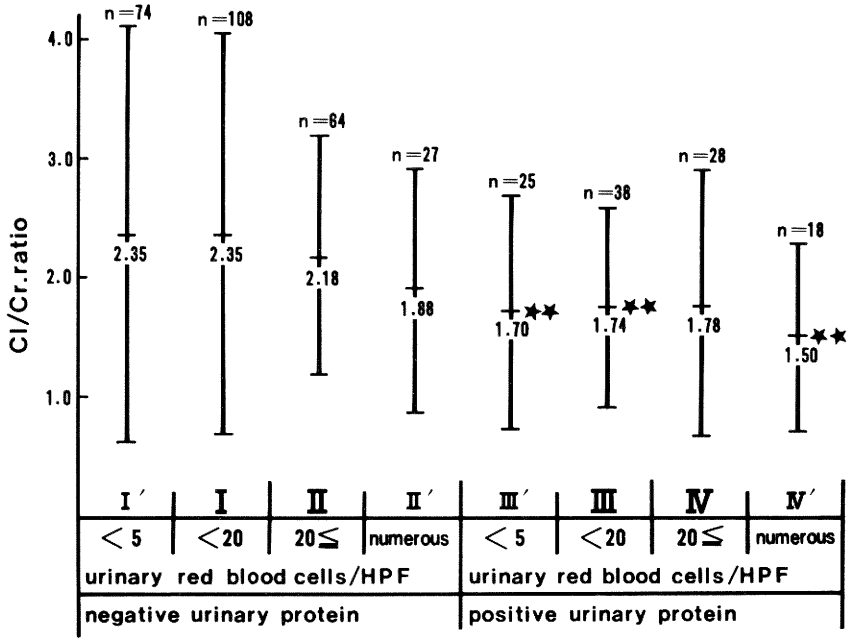

Fig. 4. Relation between the ratio of UCl/UCr in OPC-urine to UCl/UCr in FVM-urine and urinary findings.
(★★represents $0.01<p<0.05$)

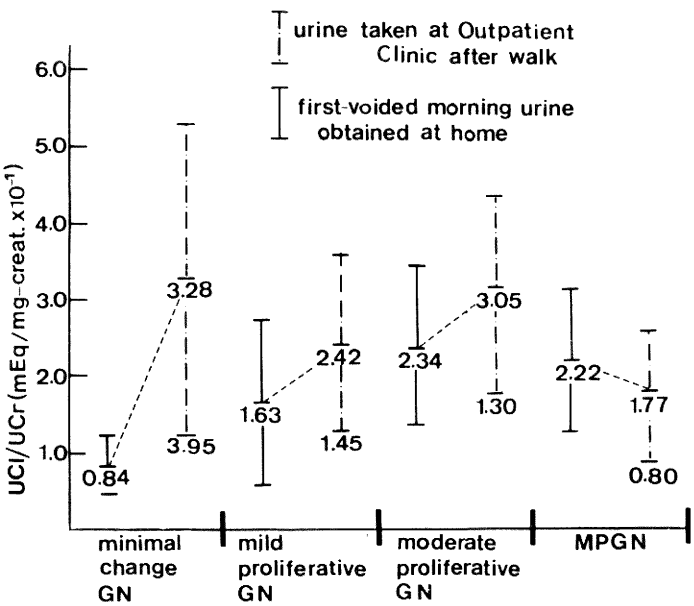

Fig. 5. Relation between the ratio of UCl/UCr in OPC-urine to UCl/UCr in FVM-urine and mesangial cell proliferations. These ratios were more decreased as the mesangial cell proliferations become more severe, and inverted in MPGN (1.77/2.22=0.80).

OPC-urine, UCl/UCr in FVM-urine was significantly higher in patients with mild proliferative GN ($0.01<p\ 0.05$), moderate proliferative GN ($0.001<p<0.01$), and MPGN ($0.001<$ p) than it was in patients with minimal change GN, which implied that a compensatory increase of urinary Cl excretion occured in the supine position at night for the transient Cl retention in the upright position. The sum total of UCl/UCr in FVM- and OPC-urine was 4.12 (0.84+3.28) in minimal change GN and 3.29 (2.22+1.77) in MPGN, which suggested that the total daily intake and elimination of Cl of the patients in each GN group might have been very close despite the differences in the seerity of mesangial cell proliferations.

RELATION BETWEEN FECL AND UCL/UCR

In addition to the results presented above, we also examined the relation of FECl to UCl/UCr in 25 randomly selected subjects. A significant correlation was noted ($r=0.87$, $p<0.01$) between them. Although a blood and urine smaple is required to measure, FECl, it's rather difficult to obtain, simultaneously, a morning blood and urine sample from an outpatient at home. So the highly significant correlation between FECl and UCl/UCr may allow us to use "UCl/UCr" as a practical altenative to FECl.

Discussion

In the first part of this study, we showed that significant decreases of FECl and UCl/UCr in OPC-urine were induced upon standing and walking in the groups with severe urinary findings, which suggested some relations of urinary Cl excretion to urinary findings and renal pathological lesions, because a nation-wide study conducted in Japan[8] revelaed that diffuse mesangial cell proliferation and MPGN are more frequent in children with severe urinary findings than in those with microhematuria.

In the second part of this study, a significant correlation between the severity of mesangial cell proliferations and the simultaneous reductions in urinary Cl excretion induced by the positional change from supine to walking was shown.

Urinary chloride excretion is regulated by the glomerular filtration rate, renal tubular reabsorption, and excretion. However, the precise mechanism for renal handling of chloride is not so well understood as urinary sodium. Orita et al[6] reported that in patients with mesangial proliferative GN the change of position from supine to upright for two hours induced sigificantly greater decreases in creatinine clearance, FENa, and urine Na excretion than in healthy subjects, followed by increases in tubular Na reabsorption, serum and plasma aldosterone concentration.

Morphological studies have demonstrated fine structures of smooth muscle fibers in the cytoplasma of mesangial[9),10)] cells. Cultures of adult human glomeruli were observed by Bernik [11] using microcinematography, and contractile activities of cuman glomeruli were shown. Becker[12] demonstrated actomyosin in mesangial cells. In rat excperiments, Blantz et al[13] showed the effects of angiotensin II upon the glomerular microcirculation and ultrafiltration coefficient. In Blantz's study[13], contraction of mesangial cells

by stimulation of arginine, vasopressin, and angiotensin II were visualized for the first time by Ausiello[4].

Ultrastructural studies have revealed that glomerular epithelial and visceral epithelial cells also posses contractile elements in the cytoplasma[14),15)]. However, both Ausiello[4] and Mahieu[3] found that whereas cultured mesangial cells exhibited a contratile response when exposed to physiologic concentration of angiotensin II, ADH, or norepinephrine, no response was elicited in cultured epithelial cells exposed to these same hormones. Threfore, at present, the available evidence is most consistent with the view that the mesangial cells are the predominant regulators of glomerual capillary surface area and, thereby, of the filtration coefficient[5].

The influences of posture on kideny function were reported in healthy [16),17),18)] subjects and ascribed to changes in renal hemodynamics[19]. In the upright position, hydrostatic pressure of the lower vessels below the heart increases and extravavastion of plasma component other than protein occurs[18], which leads to increases in hematocrit and plama protein concentrations, and decreases in cirulation volume[20]. In these processes, rises in plasma renin-angiotensin-aldosterone concentrations have been reported[21),22),23),24),25)].

An injection of low-dose angiotensin II has been reported to decrease the glomerular filtration coefficient[26]. Our study showed that in patients with moderate to severe mesangial proliferative GN including MPGN, urinary Cl excretion was significantly more decreased upon standing and walking than in minimal change GN. The observed significant decrease in the ratio of UCl/UCr in OPC-urine to those in FVM-urine in severe urine groups also supports the possible role of proliferated renangial cells in the regulation of urinary Cl excretion, because glomerular measangial cell proliferations in biopsied kindey specimens were reported to be more frequent and more severe in patients with severe urinary finding than in those with only slight hematuria[8].

In the patients with severe mesangial proliferative GN and MPGN, the transient retention of Cl induced by an orhtostatic positional change was found to be compensated for by the increased urinary excretion in the supine position during sleep, as reflected by the increased urinary Cl excretion in FVM-urine. Thus clinically neither weight gain nor fluid retention occurs in these patients.

Glomerular filtration is now considered to be regulated by the contraction of mesangial cells stimulated by angiotensin II, as described above, which makes it possible to consider the fractional urinary Cl excretion rate and the decrease induced by walking as one of the indices of glomerular function in association with meangial cell proliferations.

References

1) Schnermann, J., Ploth, D. W. and Hermie, M.: Activation of tubulglomerular feedback by chloride transport. *Pfluegers Arch.*, **362**: 229)240, 1975.
2) Weight, F. S. and Briggs, J. P.: Feedback regulation of glomerular filtration rate. *Am. J. Physiol.*, **233**: F1-F7, 1979.

3) Mahieu, P. R., Foidart, J. B., DuBois, C. H., Dechenne, C, A., and Deheneffe, J: Tissue culture of normal rat glomeruli: contractile activity of the cultured mesangial cells. *Invest. Cell Pathol.,* **3**: 121-128, 1980.
4) Ausie, D. A., Kerisberg, J. I., Roy, C., and Karnovsky, M.J.: Contraction of cultured glomerular cells of apparant mesangial origin after stimulation. *J. Clin. Invest.,* **65**: 754-760, 1980.
5) Lance, D., Ichikawa, I., and Brenner, B. M.: Hormonal modulation of glomerular funtion. *Am. J. Physiol.,* **244**: F95-F104, 1983.
6) Orita, Y., Fujiwara, Y., Sasaki, E., Ochi, S., Takamitsu, Y., and Abe, H.: Posturally induced antinatriuresis in patients with mesangial proliferative glomerulonephritis. In: Seki, Casley -Smith, J. R., and Andreoli, T. E. eds. Edema, Recent Advances. Miura Fund for Medical Research, Tokyo, 1982, pp. 253.
7) Asami, T., Hashimoto, k., and Sakai, k.: A decrease in urinary sodium excretion induced by walking in children with mesangial cell proliferations. Acta Medica et Biologica, in press.
8) Kitagawa, T.: Chronic glomerulonephritis of childhood. *Acta Pedtiatrica Japonica,* **83**: 625-630, 1979.
9) Yamada, E.: The fine structure of the renal glomerulus of the mouse. *J. Biophys. Biochem. Hytol.,* **1**: 551-586, 1955.
10) Latta, H.: Ultrastructure of the glomerulus and juxtaglomerular apparatus. *Handb. Physiol. (Sect. 8, Renal Rhysiology)*: 1-31-1973.
11) Bernik, M. C.: Contractile activity of human glomeruli in culture. *Nephron,* **6**: 1-10, 1969.
12) Becker, C. G.: Demonstration of actyomysin in mesangial cells of the renal glomerulus. *Am. J. Pathol,* **66**: 97-110, 1972.
13) Blantz, R. C., Ronnen, K. S., and Tucker, B. J.: Angiotensin II effects upon the glomerular microcirulation and ultrafiltration on coefficient of the rat. *J. Clin. Invest.,* **56**: 419-434, 1976.
14) Pease, D., C.: Myoid features of renal corpuscles and tubuless. *J. Ultrasturuct. Res.,* **23**: 304-320, 1968.
15) Andrews, P. M.: Investigations of cytoplasmic contractile and cytoskletal elements in the kidney glomerulus. *Kidney Int.,* **20**: 549-562, 1981.
16) White, H. L., Rosen, I. T., Fisher, S.S., and Wood, G. H: The influence of posture on renal activity. *Am. J. Physiol.,* **78**: 185-200, 1926.
17) Brun, C., Knudsen,E. O. E., and Raaschou, F.: The influence of posture on kidney function. *Acta Med. Scand.,* **122**: 332-341, 1945.
18) Pearce, M. L., and Newman, E. V.: Some postural adjustments of salt and water excretion. *J. Clin. Invest.,* **33**: 1089-1094, 1954.
19) Rosenbaum, J. D., Ferguson, B. C., Davis, R. K., and Rossameisl, E. C.: The influence of cortisone upon the diurnal rthythm of renal excretory function. *J. Clin. Invest.,* **31**: 507-520, 1952.
20) Hagan, R. D. Difz, F. J., and Horvath, S. M.: Plasma volume changes with movement to supine and standing positions. *J. Appl. Physiol,* **45**: 414-418, 1978.
21) Gowenlock, A. H., Mills, J. N., and Thomas, S.: Acute postural changes in aldosterone and electrolyte excretion in man. *J. Phtsiol.,* **146**: 133-141, 1959.
22) Cohen, E.L, Conn, J. W., and Rovener, D. R.: Postural augmentation plasma renin activity and aldosterone excretion in normal people. *J. Clin. Invest.,* **46**: 418-428, 1967.
23) Nielsen, I., and Moller, I.: On the mechanism of renin stimulation: the effect of postural change, salt depletion, and exercise. *Acta Med. Scand.,* **186**: 493-497, 1969.
24) Oparil, S., Vassaux., Sanders, C. A., and Harber,E.: Role of renin in acute postural hemostasis. *Circulation.,* **41**: 89-95, 1970.
25) Sassard, J., Vincent, M., Annat, G., and Bizollon, C. A.: A kinetic study of plasma renin and aldosterone during changes of posture in man. *J. Clin. Dndocrinol. Metab.,* **42**: 20-27, 1976.
26) Ichikawa, I., Miele, J. F., and Brenner., B.: Reversal of renal cortical actions of angiotensin II by verapamil and manganese. *Kidney Int.,* **16**: 137-147, 1979.