PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 178, 297–303 (1985)

Multiple Defects in the Renin–Angiotensin System in Alloxan-Diabetic Kidney (42013)

SIU-CHEONG HO AND ANDREW M. MICHELAKIS

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan 48824

Abstract. A defect in the renin–angiotensin system has been shown in diabetic patients and experimental animals, in particular with nephropathy or autonomic neuropathy. The mechanism for this low plasma renin activity (PRA) is poorly understood. In order to clarify this defect, the renin–angiotensin system was studied in alloxan-induced diabetic and age-match control mice. In diabetic animals, kidney renin activity (KRA) was significantly lower than that of the controls, while plasma renin substrate (PRS) concentration was slightly higher and PRA was normal. The amount of injected radiolabeled renin extracted by the kidney was normal, but the amount extracted by the liver was significantly decreased in diabetic animals. On the other hand, the degradation of the extracted renin by both the kidney and the liver was elevated as compared to the controls. This high degradation rate was accompanied by a slight increase in lysosomal protease activity in the kidneys. In *in vivo* studies, isoproterenol-induced PRA was 20-fold in control animals. In diabetics, isoproterenol-induced PRA was attenuated and rose only four- to fivefold over basal level. The angiotensin converting enzyme (ACE) activity in the kidney was significantly decreased in the diabetic state. It is concluded that there were multiple defects in the renin–angiotensin system in this diabetic model, namely, a depletion of renin storage with subsequent loss of maximal responsiveness to the adrenergic agonist in renin release, an elevation of intrarenal renin degradation together with a deficiency in ACE which would possibly lead to a decrease in intrarenal formation of angiotensin II.

Renal failure is one of the major causes of death in patients with diabetes mellitus. This condition is often associated with high blood pressure (1). The renin–angiotensin system has been extensively studied by Christlieb's group and others in diabetic patients and experimental-induced diabetic animals (2–7). In diabetic patients, whether hypertensive or normotensive, the plasma renin activity (PRA) has been variously reported as high, normal, or low in uncomplicated, nonketotic conditions (4, 5, 7). When the disease is complicated by nephropathy or autonomic neuropathy, most studies show a suppression of PRA (7–9). The mechanisms responsible for the decreased PRA in diabetics have not been clearly defined.

In regard to studies on hemodynamics in diabetes, Mogensen has reported an increase in renal size, glomerular filtration rate, and filtration fraction (10). Micropuncture studies also indicate an increase in glomerular capillary pressures (11). An increase in urinary albumin excretion in patients and experimental animals with diabetes is suggested as a result of hemodynamic alterations or changes in the functional characteristics of the filtration barrier (12).

The renin–angiotensin system has recently been emphasized as an intrarenal hormonal system in regulating renal functions, such as autoregulation and electrolyte balance (13) and we have reported that the kidney is endowed with a specific mechanism for renin degradation (14). In this report, we present results for the catabolic activity for renin in the diabetic kidney and liver. We further compared the degrees of renin release induced by isoproterenol, and the angiotensin converting enzyme (ACE) activity in the plasma and the kidney of diabetic animals against controls. These data suggest that in the diabetic state, the renin–angiotensin system was greatly inhibited in this model.

Methods. *Induction of diabetes by alloxan.* This procedure for alloxan-induced diabetes was followed as reported by Duncan and Bond (15). Male ICR albino mice (7 weeks old) from Harlan, Indiana, fasted for 18 hr were intraperitoneally injected with a single dose (170 mg/kg body wt) of freshly prepared alloxan (Eastman Organic Chemicals, Roch-

0037-9727/85 $1.50

ester, N.Y.) in saline. Control mice received an equivalent amount of saline. All mice were then given free access to water and food. Diabetes was confirmed by glycosuria determined by glucose enzymatic test strip (Lilly, Indianapolis, Ind.). Experiments were performed between 3 to 5 weeks after alloxan injection.

Renin–angiotensin system. PRA was determined in the presence of exogenous renin substrate (plasma from rats nephrectomized 48 hr previously) by 10-min incubation at 37°C to assure linear generation of angiotensin I (2). The angiotensin I generated was determined by radioimmunoassay (16). Kidney renin activity (KRA) was determined as described previously (2). Plasma renin substrate (PRS) concentration was determined from 0.1 ml plasma in the presence of 4 μg purified renin A (16) from mouse submaxillary glands incubated at 37°C for 30 min. Angiotensin I formation was assayed as above. Angiotensin converting enzyme (ACE) activity was determined from the plasma samples at pH 8.0 and from the kidney samples at pH 6.0 (17).

Determination of lysosomal enzyme activities. Acid phosphatase activity was determined according to the procedure supplied by Sigma (St. Louis, Mo.). One unit represents 1 μmole of product formed per 15 min at 37°C. Cathepsin D activity was determined by incubating the samples with bovine hemoglobin as substrate at pH 3.0 (18). One unit is defined as the quantity that would produce an increase in absorbance of 1.0 at 280 nm in 60 min. Cathepsin B activity was assayed as previously described (19). One unit was defined as the quantity releasing 1 nmole of 2-naphthylamine per minute under the assay conditions. *N*-Acetyl-β-glucosaminidase (NAG) and β-glucuronidase activities were assayed with substrates, phenolphthalein-β-D-glucuronide and *p*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside, respectively, as previously described (20).

Radioiodination of renin. Purified mouse kidney renin was prepared by pepstatin-affinity, immunoaffinity, and CM-cellulose chromatographic techniques (16). The purity of this protein was judged by a single protein band in polyacrylamide gel electrophoresis. This renin was labeled with Na ^{125}I by chloramine-T technique (14). Free ^{125}I was removed by BioRad AG1X8 resin and dialysis. Preparations with more than 99% of the radioactivity precipitable by 10% trichloroacetic acid (TCA) would be used for further experiments. The specific activity of the radioactive renin was 100–120 mCi/mg protein.

Accumulation and degradation of labeled renin. Control or diabetic mice were under pentobarbital anesthesia (60 mg/kg, ip) throughout the experiment. This procedure was adopted from the method of Mego *et al.* (21). Control and diabetic mice were injected intravenously with labeled renin (10^6 cpm) under anesthesia as stated above. *In vivo* uptake of labeled renin by the kidneys and livers was allowed for 15 min. After that, these organs were washed by perfusion, removed, and homogenized separately with 20 ml of ice-cold unbuffered 0.25 *M* sucrose. The amounts of renin extracted by the kidneys and the livers were determined by the radioactivity in the homogenates. Homogenates were centrifuged at 500*g* for 5 min. The supernatant fractions were centrifuged again at 12,000*g* for 30 min. The lysosomal fractions obtained from the pellets were suspended in 0.25 *M* sucrose, 40 m*M* mercaptoethanol, 10 m*M* monoiodotyrosine, and 25 m*M* Tris–acetate, pH 5.0. Samples of 1-ml aliquots were incubated at 37°C for 0, 10, 20, 40, and 60 min. At the end of the incubation, 1 ml of 20% TCA was added to each sample. After 10 min on ice, the soluble and insoluble fractions were separated by centrifugation at 3000 rpm for 30 min. Radioactivity was then determined in the pellet and the TCA-soluble supernatant fractions. The acid-soluble radioactivity has been demonstrated to consist of monoiodotyrosine as a protein digestive product in this laboratory and in others (21, 22). Therefore, the rate of labeled renin degradation in the lysosomal preparation was determined by the increase in TCA-soluble radioactivity over incubation time.

Isoproterenol-induced renin release. Under pentobarbital anesthesia, 0.1 ml of saline or DL-isoproterenol HCl (Sigma) at a dose of 1 μg/g body wt was injected through the jugular vein through a catheter in the control and diabetic mice. After 30 min, 0.1 ml blood was drawn from the inferior vena cava below

the renal veins by a syringe with a hypodermic needle G30 and then put into the heparinized tubes. The plasma was separated by centrifugation at 4°C. Plasma renin activity was determined as described above.

Protein assay and others. Protein content was determined by Coomassie brilliant blue G-250 binding with bovine serum albumin being used as the standard (23). Blood urea nitrogen (BUN) concentration was determined by the method of Kaplan (24). Blood glucose was determined enzymatically by Statzyme glucose assay kits (Worthington Diagnostics, Elk Grove Village, Ill.).

Statistics. The significance of differences between groups was determined using Student's t test. The 0.05 level of probability was used as the criterion of significance.

Results. Alloxan-induced diabetes in these mice was confirmed by the exhibition of glucosuria and polyuria. The variation in the susceptibility of these mice in the alloxan treatment was indicated by the large standard errors in the BUN concentration and the blood glucose levels in the diabetic mice (Table I). The wet weight of the kidney in the diabetics was significantly increased by 32%. This renal hypertrophy has been well documented in patients and experimental animals with diabetes mellitus.

A comparison of the renin–angiotensin system between the control and diabetic mice is shown in Table II. PRA in the diabetic mice fluctuated widely and was not significantly different from control. Mean PRS in the diabetic mice was 607 μg AI/ml, which was slightly, but not significantly, higher than that of the control mice. On the other hand, kidney renin activity (KRA) in the diabetics was significantly lower than that of the controls both in total activity and in specific activity.

TABLE I. ORGAN WEIGHTS, PROTEIN CONTENTS, BLOOD UREA NITROGEN CONCENTRATIONS (BUN), AND PLASMA GLUCOSE LEVELS

	Control	Diabetes
Organ wet weights (g)		
Kidney	0.52 ± 0.01	0.69 ± 0.11
Liver	1.98 ± 0.32	1.89 ± 0.27
Protein content (mg)		
Kidney	62.8 ± 4.6	81.9 ± 31.6
Liver	196.1 ± 32.1	214.9 ± 38.4
Plasma glucose (mg/dl)	157.1 ± 4.6	841.0 ± 337.6
BUN (mg %)	28.3 ± 1.7	35.1 ± 7.4

Note. n = 12 for both control and diabetes. Data are expressed as means ± SE.

TABLE II. RENIN–ANGIOTENSIN SYSTEM IN CONTROL AND DIABETIC MICE

	Control	Diabetes	P
PRA (ng AI/hr/ml)	44.6 ± 3.5	41.2 ± 22.5	NS
PRS (ng AI/ml)	378 ± 28	607 ± 145	NS
KRA			
Total activity (μg AI/hr)	205 ± 12	176 ± 16	<0.05
Specific activity (μg AI/hr/mg protein)	3.28 ± 0.24	1.95 ± 0.58	<0.05

Note. n = 12 for both controls and diabetes.

In order to quantitate the rate of renin degradation out of renal stored renin interferences, a method using pure radiolabeled renin was adopted from another system (21). This method required an intravenous injection of labeled renin into the animals. The percentage of labeled renin extracted from the blood circulation by the kidney or liver could then be determined. Furthermore, the renin degradation could be easily followed by the formation of TCA-soluble radioactivity in the lysosome that was incubated in *in vitro* conditions optimized for lysosomal proteolytic activity. Table III shows the effect of alloxan-induced diabetes on the renin extraction in the kidney and the liver. The liver played a major role in labeled renin extraction. In the nondiabetic control animals, after 15 min of *in vivo* uptake, 37.6% of the injected radioactivity was found in the liver, while only 9.4% was found in the kidneys. In the diabetic animal, the amount of labeled renin extracted by the liver was significantly suppressed. However, the percentage of labeled renin extracted by the kidneys was relatively, but not significantly, higher in the diabetic mice. On the other hand, when the rates of renin degradation in the lysosomal fractions were examined (Figs. 1 and 2), both the liver and kidney samples from the diabetic animals were significantly increased, by 45.3% ($P < 0.05$) for the kidney and 46.6% ($P < 0.05$) for the liver after 1-hr incubation.

TABLE III. ACCUMULATION OF LABELED RENIN BY THE LIVERS AND THE KIDNEYS

	% Accumulated	% Accumulated/g
Kidney		
Control	9.4 ± 1.2	15.7 ± 1.8
Diabetes	12.2 ± 3.1	16.8 ± 2.8
P	N.S.	N.S.
Liver		
Control	37.6 ± 2.7	17.8 ± 2.5
Diabetes	29.3 ± 4.7	11.4 ± 2.6
P	<0.05	<0.01

Note. Labeled renin was intravenously administered into the anesthetized animals. After 15 min of *in vivo* uptake the organs were washed by perfusion. Values are expressed as percentages of total injected radioactivity. $n = 8$ for both controls and diabetes.

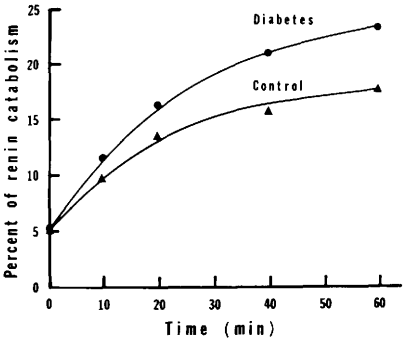

FIG. 2. *In vitro* degradation of radiolabeled renin by the liver lysosomal fraction after *in vivo* uptake for 15 min. Each point represents the mean of triplicate.

Since changes in degradation properties of the tissue may be accompanied by the changes in the lysosomal enzyme activities, the activities of different lysosomal enzymes were measured. The results are shown in Table IV. There was no significant effect of the diabetic state on the specific activities of most proteases tested, except for β-glucuronidase which was significantly decreased in the diabetic kidney. As a whole, the total activities of these lysosomal enzymes were either normal or slightly increased. Cathepsin D was demonstrated previously to be responsible for the degradation of proteins that had been taken up by endocytosis from the plasma (21). In this study, the specific activity of this enzyme was only slightly increased in animals with diabetes. However, the total activity of this enzyme was significantly increased. This result may be due to the diabetic state itself or to the renal hypertrophy.

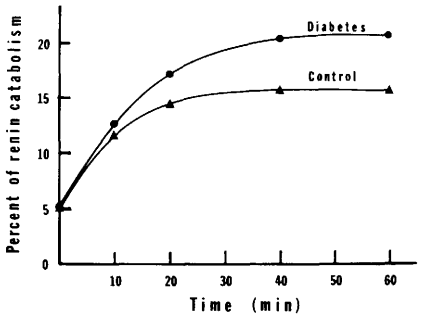

FIG. 1. *In vitro* degradation of radiolabeled renin by the kidney lysosomal fraction after *in vivo* uptake for 15 min. Each point represents the mean of triplicate.

Figure 3 shows the effect of isoproterenol on renin release in control and diabetic mice. The basal PRA levels were comparable between the control and diabetic group. In control mice, PRA was increased by 20-fold after isoproterenol stimulation. However, in the diabetic mice, this stimulation was significantly attenuated. Only a four- to fivefold increase in PRA was observed.

Intrarenal formation of angiotensin II is of crucial importance in regulating renal function. Angiotensin converting enzyme (ACE) activity was determined in the kidney and the plasma. In the diabetic animals, ACE activity was significantly decreased, while in the plasma, this activity was not significantly different from the control (Fig. 4).

Discussion. Alloxan, similar to the other diabetogenic drug, streptozotocin, resulted in lowering the kidney renin content (2). However, PRA remained normal while PRS was slightly elevated. Christlieb and Long (3) have demonstrated that decrease of KRA by alloxan was not due to glucosuria, osmotic diuresis, or the direct toxic effect of alloxan (2, 3). Phlorizin, which induces glucosuria and osmotic diuresis, failed to affect the renal renin content. Furthermore, a lowering of KRA was also observed even when the kidney was protected by renal artery clamping during alloxan treatment (2). This decrease in KRA may reflect a metabolic defect in renin synthesis which is secondary to the diabetic state. Theoretically, a defect in renin synthesis

TABLE IV. LYSOSOMAL ENZYMATIC ACTIVITIES IN KIDNEYS AND LIVERS

	Total activity		Specific activity	
	Control	Diabetes	Control	Diabetes
Kidney				
Cathepsin D	20.1 ± 3.4	37.6 ± 15.2*	0.31 ± 0.02	0.48 ± 0.29
Acid phosphatase	2.85 ± 0.39	3.79 ± 0.44*	41.5 ± 1.2	46.6 ± 9.9
NAG	614 ± 20	660 ± 103	9.7 ± 0.4	9.2 ± 2.4
β-Glucuronidase	56.7 ± 10.5	40.5 ± 18.0	9.12 ± 2.36	5.61 ± 1.73*
Cathepsin B	1489 ± 166	1577 ± 197	23.6 ± 0.8	20.3 ± 7.2
Liver				
Cathepsin D	42.8 ± 2.5	50.2 ± 16.1	0.21 ± 0.01	0.21 ± 0.03
Acid phosphatase	14.1 ± 0.9	16.0 ± 3.1	72.4 ± 5.5	68.2 ± 10.8
NAG	652 ± 56	904 ± 68**	3.3 ± 0.2	4.2 ± 0.5
β-Glucuronidase	491 ± 11	563 ± 111	2.37 ± 0.14	2.36 ± 0.57
Cathepsin B	4444 ± 342	4403 ± 475	22.6 ± 1.5	21.1 ± 3.5

Note. n = 12 for both controls and diabetes.
* $P < 0.05$ vs control.
** $P < 0.01$ vs control.

or a suppression of renal renin synthesis should lead to a decrease in basal PRA. However, PRA in this and some other studies was normal despite a decrease in KRA. These discrepancies may suggest that other mechanisms besides the basal renin release were responsible for controlling PRA.

Radiolabeled renin extraction data in this study (Table III) showed that the total amount of renin uptake by the diabetic kidneys insignificantly surpassed that of the controls. Furthermore, renin degradation rates were enhanced by 45% (Fig. 1). The renin extraction by the liver of diabetic animals decreased in spite of a similar enhancement of renin degradation as compared to that of the controls. Increase in catabolic rates of proteins has been well established in diabetes. In this study, this increase in renin catabolism was associated with a slight increase in lysosomal enzymatic activities of cathepsin D and acid phosphatase. In diabetic animals, the decrease in renin extraction in the liver would suggest a decrease in receptor sites at the cellular membrane. Since the liver is the major organ

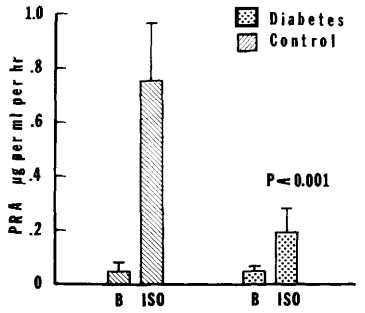

FIG. 3. Induction of PRA by isoproterenol in control and diabetic mice. Saline or isoproterenol (1 μg/g body wt) was injected through the jugular vein in control and diabetic mice. After 30 min, PRA was determined from the plasma obtained from the inferior vena cava. B, for basal PRA after saline injection. ISO, for isoproterenol-induced PRA. n = 12 for each group. A significance with $P < 0.001$ was obtained when comparing isoproterenol-induced PRA in diabetic and control mice.

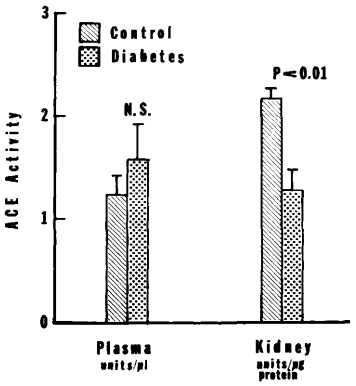

FIG. 4. Quantitation of ACE activity in plasma and kidney homogenates of control and diabetic mice (n = 12 for each group).

responsible for the plasma renin removal, the significant decrease in plasma renin extraction may in part explain the normal levels of PRA in spite of a significant decrease in kidney renin content.

In a previous report, we have shown that the kidney is endowed with a specific machinery for renin degradation (14). We speculated that some renin molecules after secretion from the afferent arterioles would somehow be extracted by the glomerulus or by the proximal tubules after being filtered through the glomerulus and then would be degraded. The slight increase in renin extraction by the diabetic kidneys could simply be explained either by the increase in glomerular uptake due to renal hypertrophy, or by an increase in glomerular filtration which would result in an elevation of proximal tubular uptake and degradation.

Figure 3 shows that the isoproterenol-induced renin release was significantly attenuated. This result could be interpreted in a number of ways. A defect in the catecholamine responsiveness, such as a decrease in the number of β-adrenergic receptors, as found in the diabetic hearts (26), or a defect in the renin synthesis in the diabetic state leading to a depletion of the readily releasable renin pool may contribute to this result. Therefore, under the stimulation of the adrenergic agonist, the maximum amount of renin available for release would be significantly decreased as compared to controls. In other studies with diabetic patients with orthostatic hypotension, PRA does not respond normally to the stimulus of upright posture (27). In diabetic patients with stable nonazotemic conditions, the furosemide-induced renin release was significantly lower as compared to that of controls (28). However, no significant difference was observed between the two groups in the responsiveness of PRA to isoproterenol infusion (28). Whether the blunted renin responses to standing and furosemide is due to severe nephropathy is unknown.

Quantitation of ACE activity in the plasma and in the kidney homogenates showed that ACE activity was significantly decreased in the diabetic kidneys (Fig. 4). In the plasma samples of the diabetic animals, this activity was slightly elevated. There are strong evidences in the literature supporting the idea that angiotensin II plays a significant role in regulating renal hemodynamics, such as glomerular filtration rate, tubuloglomerular feedback, and vascular resistance (29). The formation of angiotensin II depends on the rate-limiting conversion of renin substrate into angiotensin I, which is further converted into angiotensin II by the ACE. Using the immunohistochemical technique, ACE was found to be located mainly at the brush border of the proximal convoluted tubules (30). Receptor sites for angiotensin II have been identified at the glomerular mesangial cells and also at the brush border membrane (31, 32). The actual sites of intrarenal angiotensin II formation as well as the sites of its action remain speculative. The decrease in ACE activity in the kidneys of the diabetic animals in this study suggests that there is a defect in the angiotensin II formation within the kidney. This together with the other defects in the renin–angiotensin system, such as decrease in renin storage, elevation of renin degradation, and attenuation of the isoproterenol-induced PRA suggest that there are multiple derangements in the renin–angiotensin system in diabetes.

This work was supported by NIH grant HL-25995.

1. Drury PL. Diabetes and arterial hypertension. Diabetologia **24**:1–9, 1983.
2. Christlieb AR. Renin, angiotensin, and norepinephrine in alloxan diabetes. Diabetes **23**:962–970, 1974.
3. Christlieb AR, Long R. Renin–angiotensin system in phlorhizin compared with alloxan diabetes in the rat. Diabetes **28**:106–109, 1979.
4. Burden AC, Thurston H. Plasma renin activity in diabetes mellitus. Clin Sci **56**:255–259, 1979.
5. Chatel RD, Weidmann P, Flammer J, Ziegler WH, Beretta-Piccoli C, Vetter W, Reubi F. Sodium, renin, aldosterone, catecholamines, and blood pressure in diabetes mellitus. Kidney Int **12**:412–421, 1977.
6. Weidmann P, Beretta-Piccoli C, Keusch C, Cluck Z, Mujagic M, Grimm M, Meier A, Ziegler W. Sodium-volume factor, cardiovascular reactivity and hypotensive mechanism of diuretic therapy in mild hypertension associated with diabetes mellitus. Amer J Med **67**:779–784, 1979.
7. Christlieb AR, Kaldany A, D'Elia JA. Plasma renin activity and hypertension in diabetes mellitus. Diabetes **25**:969–974, 1976.
8. Christlieb AR. Nephropathy, the renin system and hypertensive vascular disease in diabetes mellitus. Cardiovasc Med **2**:417–431, 1978.
9. Campell IW, Ewing DJ, Anderton JL, Thompson

JH, Horin DB, Clarke BF. Plasma renin activity in diabetic autonomic neuropathy. Eur J Clin Invest **6**:381–385, 1976.
10. Mogensen CE, Andersen MJF. Increased kidney size and glomerular filtration rate in early juvenile diabetes. Diabetes **22**:706–712, 1973.
11. Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int **19**:410–415, 1981.
12. Michels LD, Davidman M, Keane WF. Glomerular permeability to neutral and anionic dextrans in experimental diabetes. Kidney Int **21**:699–705, 1982.
13. Hall JE, Guyton AC, Jackson TE, Coleman TG, Lohmeier TE, Tripodo NC. Control of glomerular filtration rate by renin–angiotensin system. Amer J Physiol **233**:F366–F372, 1977.
14. Ho SC, Izumi H, Cojocel C, Michelakis AM. Role of kidney and liver in degradation of circulating submaxillary renin in mice. J Lab Clin Med **103**:125–136, 1984.
15. Duncan WE, Bond JS. Decreased turnover of soluble liver proteins in mice with alloxan induced diabetes. Amer J Physiol **241**:E151–E159, 1981.
16. Ho SC, Izumi H, Michelakis AM. The purification and characterization of multiple forms of mouse submaxillary gland renin. Biochim Biophys Acta **717**:405–413, 1982.
17. Burghardt W, Schweisfurth H, Dahlheim H. Juxtaglomerular angiotensin II formation. Kidney Int **22**(Suppl 12):S49–S54, 1982.
18. Barrett AJ. Cathepsin D. Purification of isoenzymes from human and chicken liver. Biochem J **117**:601–607, 1970.
19. Barrett AJ. A new assay for cathepsin B1 and other thiol proteinase. Anal Biochem **47**:280–293, 1972.
20. Hoff FV, Hers HG. The abnormalities of lysosomal enzymes in mucopolysaccharidases. Eur J Biochem **7**:34–44, 1968.
21. Mego JL, Barnes J. Inhibition of heterolysosome formation and function in mouse kidneys by injection of mercuric chloride. Biochem Pharmacol **22**:373–381, 1973.
22. Christensen EI, Maunsbach AB. Intralysosomal digestion of lysoszyme in renal proximal tubule cells. Kidney Int **6**:396–407, 1974.
23. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**:248–254, 1976.
24. Kaplan A. Urea nitrogen and urinary ammonia. In: Reiner M, ed. Standard Methods of Clinical Chemistry. New York, Academic Press, pp245–256, 1965.
25. Kreisberg JI. Insulin requirement for contraction of cultured rat glomerular mesangial cells in response to angiotensin II: Possible role for insulin in modulating glomerular hemodynamics. Proc Nat Acad Sci **79**:4190–4192, 1982.
26. Savarese JJ, Berkowitz BA. β-Adrenergic receptor decrease in diabetic rat hearts. Life Sci **25**:2075–2078, 1979.
27. Beretta-Piccoli C, Weidmann P, Ziegler WH, Glueck Z, Keusch G. Plasma catecholamines and renin in diabetes mellitus: Relationship with posture, age, sodium and blood pressure. Klin Wochenschr **57**:681–691, 1979.
28. Beretta-Piccoli C, Weidmann P, Keusch G. Responsiveness of plasma renin and aldosterone in diabetes mellitus. Kidney Int **20**:259–266, 1981.
29. Thurau K, Mason J. The intrarenal function of the juxtaglomerular apparatus. In: Guyton AC, Thurau K. eds. MTP International Review of Science, Physiology Series 1, Kidney and Urinary Tract Physiology. London/Baltimore, Univ Park Press, Vol 6:pp357–389, 1974.
30. Taugner R, Hackenthal E, Rix ER, Nobiling R, Paulsen K. Immunocytochemistry of the renin–angiotensin system: Renin, angiotensinogen, angiotensin I, angiotensin II, and converting enzyme in the kidneys of mice, rats and tree shrews. Kidney Int **22**(Suppl 12):S33–S43, 1983.
31. Osborne MJ, Droz B, Meyer P, Morel F. Angiotensin II: Renal localization in glomerular mesangial cells by autoradiography. Kidney Int **8**:245–254, 1975.
32. Cox HM, Munday KA, Poat JA. The binding of [^{125}I]-angiotensin to rat renal epithelial cell membranes. Brit J Pharmacol **79**:63–70, 1983.

Received April 2, 1984. P.S.E.B.M. 1985, Vol. 178.
Accepted October 29, 1984.