STUDIES ON THE MECHANISM OF EXPERIMENTAL PROTEINURIA INDUCED BY RENIN*

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In recent years, especially since the advent of the electron microscope, there has been a renewed interest in the morphologic and functional aspects of a variety of experimental and human renal diseases associated with proteinuria. An excellent experimental model for such studies has been the renin-induced proteinuria in the rabbit and the rat. In 1940, Pickering and Prinzmetal (1) reported that parenteral administration of renin, the renal enzyme implicated in experimental renal hypertension, induced a marked diuresis in the rabbit, accompanied by increased rate of sodium and chloride excretion and a significant degree of proteinuria. Brandt and Gruhn (2) confirmed this observation, and by studying the simultaneous excretion of injected hemoglobin, concluded that the proteinuria was caused by diminished tubular reabsorption of protein from the glomerular filtrate and not by altered glomerular permeability. Shortly afterwards, Addis *et al.* (3) discovered that renin produced an intense proteinuria in the rat also and, from a combination of morphological and functional data obtained by these workers and by others, it was concluded that renin produced this effect mainly by increasing glomerular permeability. Thus, conflicting views have been expressed concerning the mechanism by which renin produces an increased excretion of protein in the urine.

Previous studies on the relationship between the pressor and proteinuric activities of renin have also led to similar conflicting results. Thus, Addis *et al.* (3) suggested that the proteinuria was dependent on the pressor activity of renin, since inhibition of this activity by heat also abolished the proteinuric effect. On the other hand, Sellers *et al.* (4) concluded that "the proteinuric property of renin is not related to the ability of this compound to elevate

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arterial blood pressure," since renin, administered intramuscularly to the rat, produced massive proteinuria without causing elevation of arterial blood pressure. They noted also that, in adrenalectomized rats, renin, administered in amounts causing a significant elevation of blood pressure, failed to produce proteinuria. Since the renin used in all these studies was of heterologous (hog) origin and of relatively low purity, it was possible that the proteinuric effect was due to non-specific contaminants in the renin preparation. Administration of heterologous proteins is known to cause proteinuria in the rat (5).

In view of these considerations, it was decided to explore the problem of renin-induced proteinuria with special emphasis on the relationship between the enzymatic activity and proteinuric effect of renin. Furthermore, the morphologic nature of the renal lesion associated with this proteinuria was investigated also in this study with the help of the electron microscope.

Materials and Methods

Renins.—Hog, rat, beef, sheep, rabbit, and dog renins were prepared according to the procedures previously described (6–8).

Angiotensin.—The valine[5], aspartyl-β-amide, synthetic derivative of angiotensin II was obtained through the courtesy of Dr. R. Schwyzer, Ciba Laboratory, Basle, Switzerland.

Antirenin.—This was prepared by immunization of rats with hog renin as described previously (9).

Aldosterone.—This was obtained through the courtesy of Dr. R. Gaunt, Ciba Laboratory Pharmaceutical Products Inc., Summit, New Jersey.

Desoxycorticosterone Acetate.—This was a commercial preparation obtained from Schering Corporation, Bloomfield, New Jersey.

Bioassay.—Angiotensin, renin, and antirenin were assayed according to the procedures previously described (10, 9). One unit of renin and of angiotensin is defined as that amount, which when injected intravenously in a normal, trained, unanesthetized dog, raises the mean arterial blood pressure by 30 mm Hg. The terms biological or enzymatic activity of renin, used throughout this manuscript, refer to its pressor activity in the dog. For antirenin, the amount of antiserum, which neutralized one unit of renin, is said to contain one unit of antirenin.

Animals.—Female, Wistar rats weighing between 175 and 225 gm were used in this study. This choice of the rat strain and sex was based on the observation that the daily urinary protein excretion in this animal is fairly low (less than 10 mg) and fairly constant.

Collection of Urine.—Renin was administered intraperitoneally. Beginning on the day prior to the injection of renin and through the test period each animal was given 1 per cent saline for drinking. Immediately after the injection, all food was withdrawn and the urine produced during the test period (4 hours in renin-treated animals or 24 hours in untreated animals) was collected by placing the rat in a metabolism cage. At the end of the test period complete evacuation of the bladder was effected by placing an ether cone over the nose of the rat, for an instant.

Determination of Protein.—This was carried out according to the procedure described by Addis *et al* (3). Urinary protein was precipitated by adding Tsuchiya's reagent (phosphotungstic acid in acid alcohol). The precipitate was dissolved in 3.2 per cent NaOH and the amount of protein was determined by the biuret reaction.

Adrenalectomy.—The left adrenal gland was removed first, by the paravertebral approach,

and 3 to 4 days later the right gland was removed in the same manner. Following adrenalectomy, all rats were given 1 per cent saline for drinking as routine.

Preparations of Renin Derivatives.—Hog renin was modified through a variety of chemical reactions. In some cases (first four reactions described below) there was 95 per cent or greater inhibition of the original biological activity of renin, whereas, in others (last three), the degree of inactivation varied from 0 to 50 per cent. In each case appropriate controls were run to demonstrate that the change in biological activity was caused by the specific reagent and not by the conditions (pH, temperature, time, etc.) of the reaction.

1. Reaction with p-chloromercuribenzoate: This was carried out according to the procedure recommended by Dr. Erwin Haas,[1] Mount Sinai Hospital, Cleveland. This reagent is known to combine with free sulfhydryl groups. To 6.0 ml of hog renin (330 units, specific activity 2.8 units/mg) were added 3.0 ml of 0.5 M phosphate buffer (pH 6.9), 21.0 ml water, and 45.0 mg of *p*-chloromercuribenzoate (0.004 M). The solution was incubated at 37°C for 15 hours and later dialyzed against cold (2°C) water for 16 hours. The final product showed approximately 4 per cent of the original renin activity.

2. Reaction with iodoacetic acid: This reagent, in addition to its reaction with free sulfhydryl groups, also combines with certain other groups containing free hydrogen atoms. This procedure also was recommended by Dr. Haas. To 2.0 ml of hog renin (110 units, specific activity 2.8 units/mg) were added 3.0 ml of 0.5 M phosphate buffer (pH 8.0), 25.0 ml of water, and 235 mg (0.04 M) of iodoacetic acid. The solution was kept at 2°C for 4 hours and later dialyzed against cold (2°C) water for 16 hours. The final product had approximately 5 per cent of the original renin acitivity.

3. Reaction with iodine: To 6.0 ml of hog renin (52 units, specific activity 2.6 units/mg) was added 2.5 ml of 0.5 M phosphate buffer (pH 8.0) followed by 0.5 ml of 0.1 N iodine and 0.1 ml of 0.1 N NaOH added dropwise with stirring. The solution was allowed to stand at 2°C for 30 minutes and then the excess iodine was destroyed by the addition of appropriate amounts of 0.1 N sodium sulfite. The solution was then dialyzed for 16 hours against cold (2°C) water. The final product showed less than 1 per cent of the original renin activity.

4. Reaction with 10 per cent formaldehyde: To 26.0 ml. of hog renin (260 units, specific activity 2.6 units/mg) were added 4.0 ml of 0.5 M phosphate buffer (pH 8.0) and 10.0 ml of 40 per cent formaldehyde to give a final formaldehyde concentration of 10 per cent. The solution was incubated at 30°C for 1 hour and later dialyzed as before. The final product had approximately 4 per cent of the original renin activity.

5. Reaction with 2 per cent formaldehyde: This was carried out in the same manner as above with the exception that the final formaldehyde concentration was adjusted to 2 per cent. There was no detectable loss of renin activity by this procedure.

6. Acetylation of renin: The procedure for acetylating renin with acetic anhydride has been described previously (8). The biological activity of acetylated renin was 64.0 per cent of that of the untreated renin.

7. Coupling of renin with the diazonium salt of p-aminohippuric acid: The diazonium salt of *p*-aminohippuric acid was prepared by adding solid sodium nitrite to a solution of 120 mg of *p*-aminohippuric acid in 6 ml of 0.3 N HCl at 2°C. The excess nitrous acid was destroyed by adding solid ammonium sulfamate. The diazonium salt was then added to a solution of hog renin (150 units, specific activity 2.6 units/mg) in 30 ml of 0.2 M Na_2HPO_4 also kept at 2°C. The solution was allowed to stand for 1 hour and then dialyzed as usual. The final product retained 75 per cent of the original renin activity.

Morphologic Studies.—All rats were anesthetized 20 minutes prior to sacrifice with intraperitoneal injections of 2 per cent sodium pentobarbital given in a dose of 0.2 ml per 100 gm

[1] Personal communication.

total body weight. Ten minutes before sacrifice many of the rats received single intravenous injections (tail vein) of 1 ml of saccharated ferric oxide (SFO), proferrin® Merck, Sharp and Dohme, West Point, Pennsylvania, used as a tracer substance to study glomerular capillary permeability. At the time of sacrifice, the kidneys were removed quickly and the right kidney was sectioned and allowed to fix in formalin. Paraffin sections were stained as routine with hematoxylin and eosin and were treated also by the periodic acid-Schiff (PAS) reaction and the Berlin blue reaction for ferric ion. The sections were examined with a light microscope. Small pieces of the left kidney (less than 1 mm^3) were immediately placed in cold (4°C) 1 per cent buffered osmium tetroxide with sucrose (pH 7.4) and allowed to fix for 1 hour. The tissue blocks were dehydrated and embedded in methacrylate, epon 812, or maraglas. Thin sections were cut on a Porter-Blum microtome using glass knives and photographed either unstained or stained with lead hydroxide (11) in an RCA EMU-2D electron microscope.

RESULTS

In a large series of determinations (total 112), the average daily (24 hour) urinary protein excretion in normal, untreated, female, Wistar rats was found to be 4.8 mg with a range of 1.45 to 9.8 mg. Following a single, intraperitoneal injection of 4 units of hog renin (specific activity 2.8 units/mg) in 2 ml of normal saline, there was a marked increase in the rate of protein excretion and within 4 hours after the injection a total of 98 mg (average of 43 determinations with a range of 25 to 162 mg) protein was excreted in the urine. The rate of excretion was maximum within the 1st hour after renin injection and accounted for 70 to 80 per cent of the total protein lost during the 4 hours. It decreased gradually during the next 3 hours and returned to the normal level by the end of the 4th hour. Similar results have been reported by previous workers who also used hog renin in their studies (2–5). Because of the heterologous nature and the low purity of the renin preparations used in these studies, it was possible that the proteinuric effect might be related to a foreign protein contaminant in the renin preparation. To investigate this possibility, it was decided to use hog renin preparations of widely varying purity in the study of proteinuria. As shown in Table I, the degree of proteinuria was approximately the same in spite of a 40-fold difference in the specific activity of renin when the same number of pressor units (4) of renin was administered. These results clearly indicate that the proteinuria is related to the biological activity of renin and not to the total amount of non-reactive protein administered.

Table II summarizes the results on the effects of renins of various animal species on proteinuria in the rat. In these studies 4 units of each type of renin was administered in the usual manner. Renin from each of the animal species tested was effective in inducing proteinuria. It is important to note that rat renin was also active in the rat, since this further argues against the possibility that a heterologous, "foreign" protein might be responsible for the proteinuric effect of renin.

The relationship between the enzymatic activity and proteinuric activity of renin was further explored by subjecting hog renin to a variety of chemical

modifications which inhibited the enzymatic activity (*a*) almost completely, (*b*) partially, or (*c*) not at all, and then comparing the proteinuric effect of these renin derivatives. The preparations of these derivatives have been described previously. The reaction between renin and antirenin (Table III) was carried out by incubating at room temperature for 2 minutes, 10 units of hog

TABLE I

Proteinuria Induced by Hog Renin Preparations of Different Purity

Specific activity of hog renin	Protein excreted in 4 hrs.*
units/mg	*mg*
1.2	86
2.8	102
12.8	94
48.0	98

From each preparation of hog renin a total amount of protein, equivalent to 4 pressor units, was injected intraperitoneally and the urinary protein was determined as previously described.

* Average in two animals.

TABLE II

Proteinuria Induced by Renins of Various Animal Species

Source of renin	Specific activity	Protein excreted in 4 hrs.*
	units/mg	*mg*
Dog	0.2	76
Beef	0.6	62
Rabbit	2.0	90
Rat	0.19	104
Sheep	0.07	58

From the renin preparation of each animal species indicated in the above table, an amount of protein, equivalent to 4 pressor units, was injected as described previously.

* Average in two animals.

renin with 0.5 ml of rat serum containing 20 units of antirenin (hog) in a total volume of 1 ml. The solution was then injected in the usual manner. The results of these experiments are summarized in Table III. It is readily apparent from these results that in every case the proteinuric effect of renin is correlated with its enzymatic activity. Thus, all the evidence accumulated so far strongly suggests that the proteinuria induced by renin is related specifically in some manner to its enzymatic activity. Since renin is known to manifest its enzymatic (pressor) action through the formation of the octapeptide, angiotensin II, one would expect the latter to have a proteinuric effect as well. Indeed,

when synthetic angiotensin II was administered in the same manner, a significant degree of proteinuria was observed. However, even with large doses of angiotensin II (50 to 500 units given in a single or multiple doses), the proteinuria induced was highly variable and of a much lower order (average of 25 determinations was 18 mg/4 hours with a range of 1.5 to 37 mg) as compared to that induced by renin. Similar results have been reported by Addis *et al.* (3) who used a much cruder preparation of angiotensin in their studies. The most probable explanation of this apparent discrepancy lies in the known observa-

TABLE III

Modification of Renin Activity and Its Effect on Proteinuria

Reaction of renin with	Inhibition of pressor activity	Total units of renin administered*	Protein excreted in 4 hrs.‡
	Per cent		*mg*
p-Chloromercuribenzoate	96	10	2.5
Iodoacetic acid	95	10	3.4
Iodine	99	10	2.6
10 per cent formaldehyde	96	10	4.2
Antirenin (20 units)	100	10	1.8
2 per cent formaldehyde	0	4	88
Acetic anhydride	36	4	28
Acetic anhydride	36	8	106
Diazonium salt of *p*-aminohippuric acid	25	4	56

The preparations of the various hog renin derivatives used in these experiments have been described previously (see Materials and Methods). Renin was administered in the usual manner, in varying doses of pressor units as indicated above.

* Total units present prior to reaction with respective reagent.

‡ Average in two to four animals.

tion that angiotensin, administered intraperitoneally, is destroyed rapidly by a group of enzymes called angiotensinase and, therefore, may never reach an adequately high concentration at the site where it produces its proteinuric effect. On the other hand, renin, by causing a slow, steady release of angiotensin at the appropriate site may attain a higher, effective concentration of the latter and thereby produce its marked proteinuric effect. The other, less likely, possibility is that active renin, in addition to and independent of its role in producing angiotensin, may produce yet another effect causing proteinuria. However, no evidence has yet come forth to suggest that renin has any biological effect, apart from those attributed to angiotensin.

Relationship of the Adrenal Gland to the Renin-Induced Proteinuria.—Previous studies (4, 12) had shown that in adrenalectomized rats, renin no longer induced proteinuria and that the treatment of these animals with desoxycorti-

costerone acetate (DOCA) or cortisone restored their ability to respond to renin with a marked proteinuria. We were able to confirm these observations and demonstrate further that treatment with aldosterone also had the same effect as that produced by DOCA or cortisone (Table IV). In adrenalectomized animals, which were not treated, even high doses (4 to 30 units) of renin failed to induce proteinuria during a period of about 4 weeks after the removal of the adrenal glands. However, in some animals, after 7 to 8 weeks following surgery, a pronounced proteinuria was again observed with the usual (4 units) dose of renin. This suggested that by 7 to 8 weeks after adrenalectomy, adequate accessory adrenal tissue had regenerated to permit the usual degree of

TABLE IV

Effect of Adrenalectomy and Adrenal Steroid Therapy on Renin-Induced Proteinuria

Exp. No.	No. of observations	Treatment	Renin administered	Average protein excreted in 4 hrs.
			units	*mg*
1	22	Adrenalectomy	4 to 30	0.8
2	10	Same as Exp. 1 + DOCA*	4	110
3	6	Same as Exp. 1 + aldosterone*	4	92
4	8	8 wks. after adrenalectomy	4	84

All adrenalectonized animals were given normal saline for drinking. Animals in Exp. 2 and 3 were given, in addition, DOCA or aldosterone as indicated above. Administration of hog renin and determination of urinary protein were carried out as previously described.

* DOCA and aldosterone were administered subcutaneously in doses of 0.5 mg and 0.14 μg respectively, given daily for 6 days prior to renin treatment.

proteinuria with renin. A careful search at the postmortem examinations of these animals invariably revealed a significant amount of adrenal tissue usually located somewhere in the perirenal region.

Relationship between Systemic Pressor Response and Proteinuric Activity of Renin.—Sellers *et al.* (4) had shown previously that renin induced marked proteinuria when administered in a dose which was insufficient to cause a rise in the systemic arterial blood pressure. They also observed that in adrenalectomized rats, renin administered intravenously elevated mean arterial blood pressure without causing proteinuria. We have confirmed these observations and have found that in normal rats, 4 units of renin, administered intraperitoneally, did not produce a detectable rise in the mean arterial blood pressure as measured by the indirect, plethysmographic (tail cuff) method. A marked proteinuria, however, was induced in these animals (Tables I and II). On the other hand, in four of the adrenalectomized rats studied, doses of renin as high as 25 to 30 units failed to produce proteinuria, although, they did cause a significant (40 to 60 mm Hg) rise in the mean arterial blood pressure. Thus, it

appears that the systemic pressor effect and the proteinuric effect of renin are independent of each other but that renin has to be biologically (enzymatically) active in order to produce either of these effects.

Morphologic Studies.—In all of the previous studies, as well as in our studies, light microscopy failed to reveal any significant glomerular or tubular alterations to account for the renin-induced proteinuria. Therefore, ultrastructural studies with the electron microscope were undertaken. This study was carried out in the following manner. Twenty-eight female, Wistar rats were used.

TABLE V

Treatment and Grouping of Rats for Electron Microscopy

Treatment	Saccharated ferric oxide injections	No. of rats
None (controls)	None given	2
None (controls)	Given	2
Renin, 20 min. before sacrifice	Given	2
Renin, 40 min. before sacrifice	Given	2
Renin, 1 hr. before sacrifice	Given	6
Renin, 2 hrs. before sacrifice	None given	2
Renin, 24 hrs. before sacrifice	Given	4
DOCA-renin, 1 hr. before sacrifice	Given	4
Adrenalectomy-renin, 1 hr. before sacrifice	Given	4
Total		28

Four pressor units of hog renin was administered in the usual manner. For electron microscopy, the kidney tissue was processed as previously described (see Materials and Methods).

Renin was administered to twenty-four of these rats, some of which had received priming doses of DOCA and some of which had been adrenalectomized, as noted in Table V. Four similar rats, not injected with renin, served as controls.

Light Microscopy.—

Controls: No renal pathologic alterations were observed in the control rats. In those injected with SFO a positive reaction for the ferric ion was noted in the glomerular and the peritubular capillaries. No SFO was identified outside of the capillaries or within the tubules.

Renin injections alone: No definite glomerular or tubular alterations were identified. Stainable SFO was identified within glomerular and peritubular capillaries. In addition more stainable iron was located in the glomerular capillary walls than in the controls. The localization of this staining reaction was identical with the location of the glomerular basement membrane and appeared

to outline this structure within the glomeruli. This increase in stainable iron within the glomeruli was noted in those rats receiving renin 20, 40, or 60 minutes prior to sacrifice but was not as apparent in those rats receiving renin 24 hours prior to sacrifice. No definite iron was identified within tubular cells, tubular lumina, or glomerular visceral epithelial cells.

DOCA primed followed by renin injections: Slight focal swelling of the glomerular epithelial cells was suggested. A slight increase in PAS-positive inclusion droplets was identified within the proximal tubular cells. The glomeruli and tubules were otherwise unaltered. The stainable iron was similar in location and extent to that in the rats receiving renin alone, again tending to outline the glomerular basement membrane.

Adrenalectomy followed by renin injections: No glomerular or tubular alterations were identified. Stainable iron was located in glomerular and peritubular capillaries. Less SFO was identified within the glomerular capillary walls than in the rats injected with renin with or without DOCA.

Electron Microscopy.—

Controls: (Figs. 1 and 2). Good preservation was noted in the glomerular and tubular cells. The glomerular epithelial, endothelial, and mesangial cells contained the expected amount of cytoplasm and organelles. A few single membrane–limited inclusions were identified within the visceral epithelial cells. The foot processes were thin and discrete. The basement membrane of the capillary loops was uniform and thin. The three layers of the basement membrane could be defined; the inner less dense lamina rara interna, the central dense lamina densa, and the outer less dense lamina rara externa. Scattered single membrane–limited vacuoles and inclusions were present in the proximal tubular epithelial cells.

No alterations in the ultrastructure of the glomeruli or tubules were noted in the rats injected with SFO. The SFO appeared as discrete, small electron-opaque particles within the glomerular capillary lumina and in the lamina rara interna of the basement membrane. The particles appeared to aggregate against the lamina densa. Very few were observed in the lamina rara externa, slit pores and foot processes of the overlying visceral epithelial cells. None was observed in Bowman's space. Discrete particles of SFO were present within the fenestrations of the endothelial cells and between endothelial and mesangial cells. Very few SFO particles were present within these cells. No SFO was identified within the tubules. Some was observed within the lumina of the interstitial capillaries; however, none appeared to be moving through the walls of the capillaries.

Renin injections alone: (Figs. 3 and 4). The visceral epithelial, endothelial, and mesangial cells of the glomeruli of the renin-treated rats were swollen. The Golgi network and endoplasmic reticulum, especially of the epithelial cells, were

very prominent. Many single membrane–limited inclusions containing material of variable density were located within the cytoplasm of the visceral epithelial cells. The foot processes were focally thickened, blunted, and fused, narrowing or obliterating the slit pores. The lamina densa of the basement membrane was focally thickened; however, no defects or fibrillary structures were noted. The endothelial cells were also swollen, but less prominently so. The peripheral endothelial cytoplasm and fenestrations remained intact. The mesangial cells were prominent with an increase in size and number of their subendothelial pseudopods.

The SFO particles were abundant within the capillary lumina and walls. A rather striking increase in the number of particles within the lamina densa and lamina rara externa was noted. Large aggregates of particles were located between the endothelial cells and basement membrane and also between adjacent endothelial and mesangial cells. A few aggregates (sometimes, but not always, membrane-limited) were located within endothelial, mesangial, and rarely visceral epithelial cells. Scattered SFO particles were found free within Bowman's space. These changes appeared in the rats injected with renin 20 minutes prior to sacrifice but were much more prominent in those rats injected 60 minutes before sacrifice. Relatively fewer alterations were noted in the rats injected 24 hours before sacrifice.

The proximal tubular cells contained an increase of apical vacuoles in those animals injected 60 minutes prior to sacrifice and an increase in dense, heterogeneous, membrane-limited inclusions toward the mid- and basilar portions of the cells in those injected 2 and 24 hours prior to sacrifice. No SFO was identified within the tubular cells. Particles of SFO were identified within the fenestrations of the endothelial cells and within the underlying basement membranes of the interstitial capillaries. No other interstitial alterations were identified.

DOCA primed followed by renin injections: No significant difference could be established between the alterations produced in this group and those rats given renin alone 60 minutes before sacrifice. Again the glomerular cells and basement membranes were focally swollen, and an increased amount of SFO particles appeared to be filtered by the glomeruli. The tubules were also similar to those described in the rats injected with renin alone.

Adrenalectomy followed by renin injections: Fewer inclusions were observed within glomerular and proximal tubular cells. The glomerular cells were less swollen, and fewer SFO particles were identified in the lamina rara externa and the epithelial cells, indicating less filtration or permeability of the glomerular capillary wall. The tubules, blood vessels, and interstitial areas were within normal limits.

DISCUSSION

All the evidence that we have accumulated so far is consistent with the idea that the proteinuric effect of renin is dependent on its enzymatic activity and

that, most likely, renin acts through angiotensin in producing this effect. Two possible sites of action, namely, glomerular and tubular, must be considered in explaining this proteinuric effect. In other words, the proteinuria could result from increased glomerular filtration and/or decreased tubular reabsorption of protein. It appears from our results and those of others (4, 13) that the proteinuria caused by the renin-angiotensin system results mainly from glomerular alterations. Using intravenously injected hemoglobin (13) and Evan's blue dye (4) as tracers, these workers found significant collections of these materials in Bowman's space, and lumina of proximal convoluted tubules after treatment with renin and suggested increased glomerular capillary permeability as the probable mechanism. In our studies such increased permeability was shown clearly by using intravenously injected SFO particles as tracer. Following treatment with renin, large aggregates of these particles were seen streaming through the lamina densa of the glomerular capillary basement membrane and accumulating in significant numbers in Bowman's space. Certain other morphological changes, although of a somewhat subtle and focal nature, were appreciated also with the help of the electron microscope. These changes included swelling and vesicle formations in epithelial and endothelial cells of glomerular capillaries and focal flattening, blunting, and fusion of epithelial foot processes. The exact significance of these changes in relation to proteinuria is not yet known. Similar changes have been reported in a variety of experimental and human renal diseases associated with proteinuria (14, 15), and it is not clear at the present time as to whether these changes reflect the cause or result of protein leakage through glomerular capillaries. However, the latter is thought to be more likely. Frequently, in cases of experimental and human proteinuria, these are the only changes seen in glomeruli which by light microscopy appear entirely normal. It is interesting to note that in our studies, these changes appeared to be readily reversible, since, 24 hours after renin injection they were not detectable. The possibility that the renin-induced proteinuria is caused, in part, by decreased tubular reabsorption of protein cannot be ruled out completely on the basis of our studies. We made no attempts to study quantitative, functional tubular alterations, although, certain other investigators (1, 2) have reported evidence to suggest inhibition of tubular reabsorptive mechanisms caused by renin. On purely morphological grounds, however, we found no tubular alterations, with light and electron microscopes, to suggest decreased reabsorptive mechanisms. In fact, the increased vacuolization of proximal convoluted tubular cells noted, suggests a compensatory increased reabsorption of filtered substances.

The mechanism, by which the renin-angiotensin system produces glomerular capillary injury with resulting increased basement membrane permeability to protein, presents an interesting problem. Angiotensin may, in some unknown manner, act directly on a glomerular capillary structure and increase capillary permeability or it may produce this effect through intense vasoconstriction

and transient ischemia. We have recently studied the effect of angiotensin on vascular permeability in guinea pig skin by using a technique described by Miles and Wilhelm (16) and Ratnoff and Lepow (17). This compound markedly enhanced vascular permeability when administered in concentrations similar to those required for histamine.[2] Previous reports (18, 19) on the production of vascular lesions in the rat by renin and angiotensin also illustrate the injurious effect of these compounds on blood vessels.

The mechanism of the permissive role played by the adrenal gland and adrenal cortical steroids in renin-induced proteinuria is not yet clear. It is possible that part of the explanation lies in the altered glomerular filtration rate (GFR) that may be produced by adrenalectomy. It is well known that, in man, adrenal insufficiency is often accompanied by reduced renal blood flow and GFR. With a decreased GFR, one might conceive of a situation in the rat where (even after renin treatment) the amount of protein in the glomerular filtrate at all times would be well below the tubular maximum (Tm) for protein reabsorption. In such a case proteinuria would not occur. While GFR measurements were not carried out in our study, others (20, 21) have reported that, in adrenalectomized rats maintained on saline, renal blood flow and GFR are essentially unchanged. Perhaps, the observation that the adrenal gland appears to be involved in the production of the morphologic glomerular lesions by renin deserves greater consideration. In adrenalectomized rats, treatment with renin did not produce the morphologic glomerular alterations similar to those seen in normal animals treated with renin. In this connection, it is interesting to note that in the production of vascular lesions in the rat with renin and angiotensin, as reported by previous workers, adrenal cortical hormones were found to have a definite enhancing or aggravating effect (18, 22). The mechanism of this effect remains entirely unclear.

SUMMARY

Renin-induced proteinuria in the rat was investigated, with special emphasis on the relationship between the enzymatic activity and the proteinuric effect of renin. The dependence of the proteinuric effect on the enzymatic activity was shown by using (*a*) renin preparations of widely varying purity and (*b*) chemically modified "active" and "inactive" renin derivatives.

Angiotensin II, the pressor product of the enzymatic action of renin, also produced significant proteinuria.

Adrenalectomy abolished the proteinuria induced by renin. Proteinuria, however, occurred as a result of pretreatment with DOCA, or aldosterone, or without treatment, 7 to 8 weeks after adrenalectomy.

Electron microscopic studies of the kidney at the time of maximal proteinuria

[2] Unpublished observations.

showed focal flattening and fusion of epithelial foot processes, as well as swelling and vesicle formation in endothelial and epithelial cells of the glomeruli. Studies with intravenously injected saccharated iron oxide showed increased permeability of the glomerular capillary basement membrane to these particles. These changes were transient and were not seen 24 hours after renin injection. Adrenalectomy prevented these changes.

It is concluded that renin, acting through angiotensin, causes glomerular capillary damage with increased permeability of these structures to protein and resultant proteinuria. The adrenal glands participate in a permissive role in this phenomenon.

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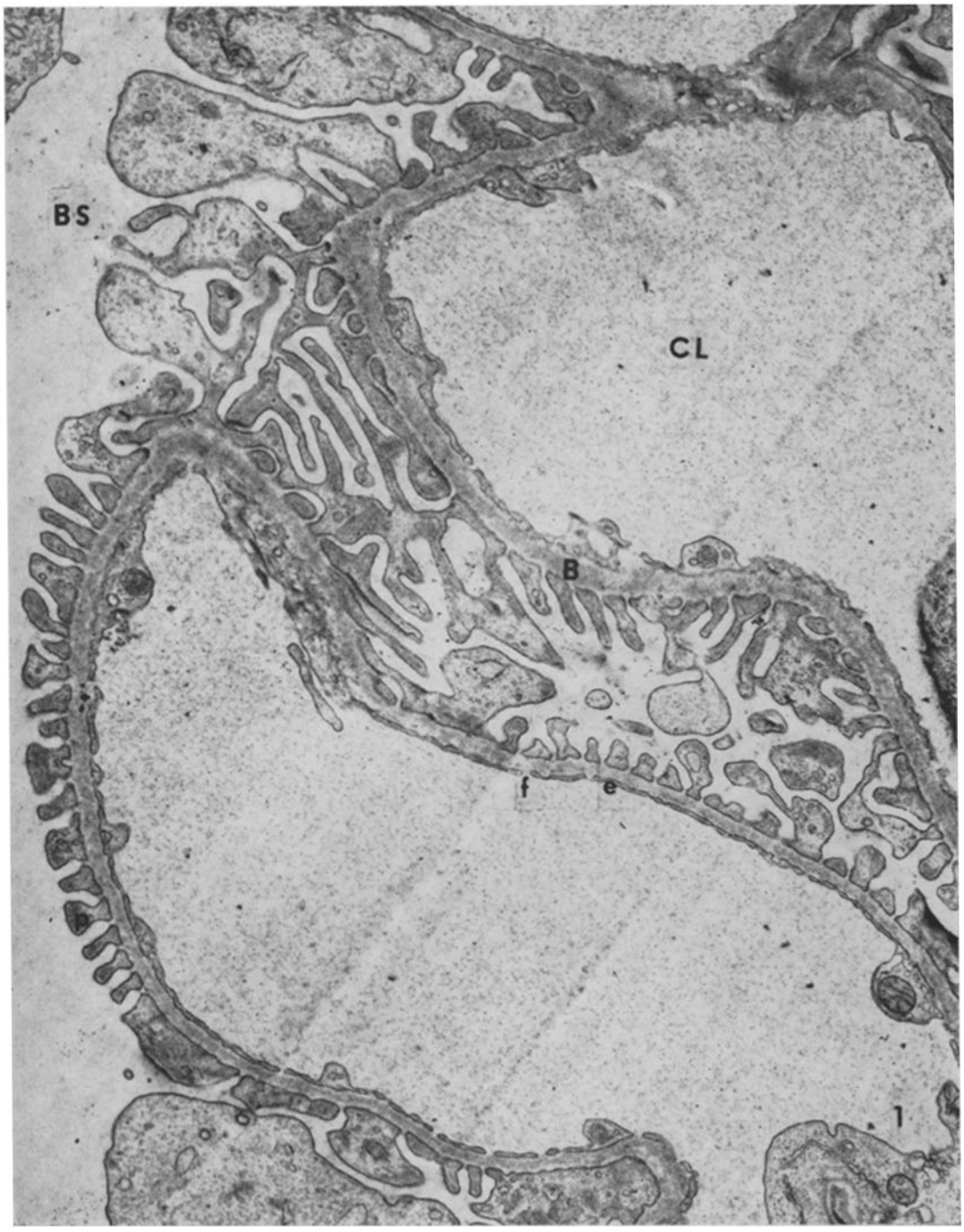
EXPLANATION OF PLATES

PLATE 68

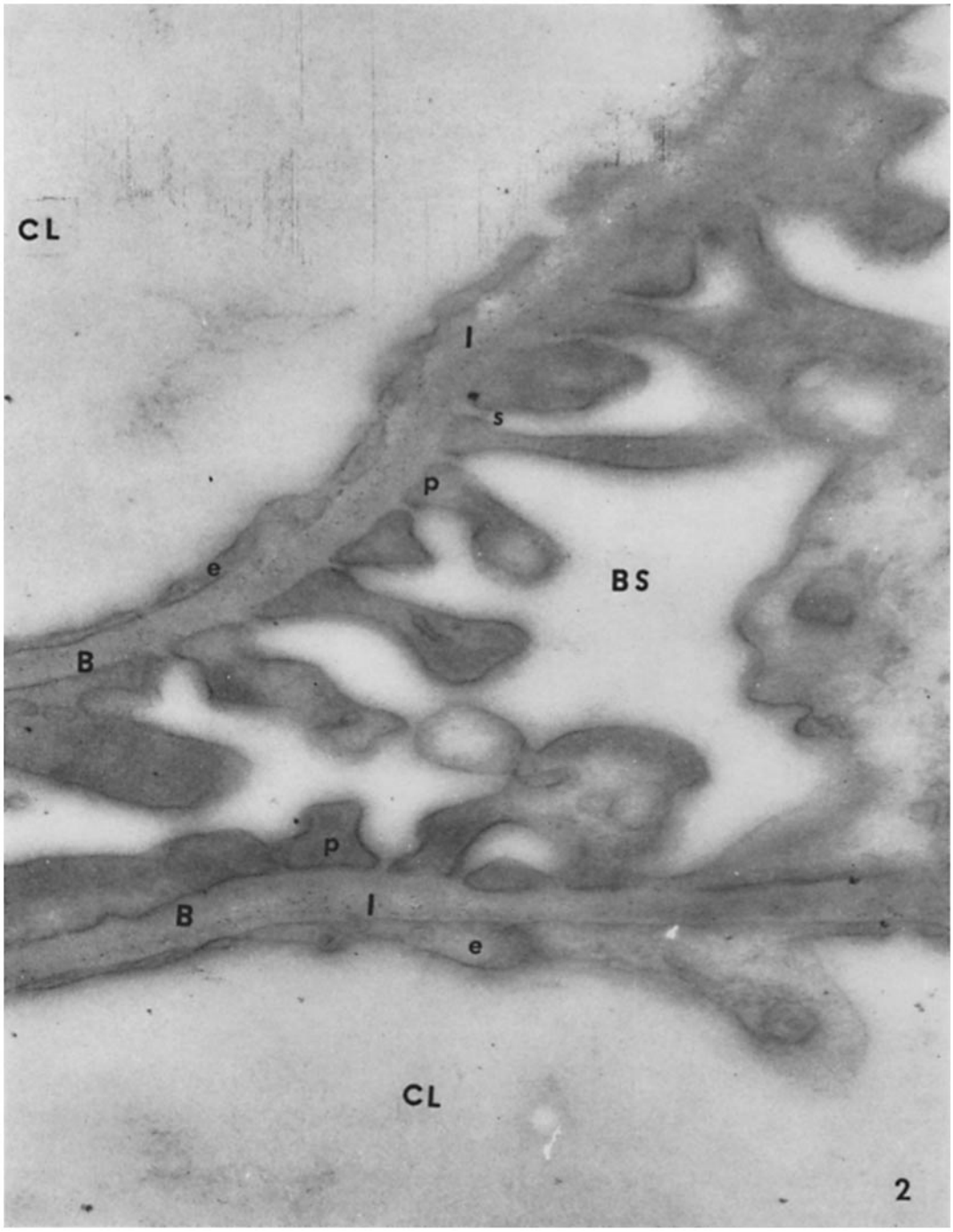
FIG. 1. Portion of glomerulus from uninjected control rat. The basement membrane (*B*) is thin and uniform. The epithelial foot processes (*p*) are discrete. The peripheral endothelial cytoplasm (*e*) is thin with small uniform fenestrations (*f*). *CL*, capillary lumen; *BS*, Bowman's space. Epon; lead hydroxide. × 15,800.



(Deodhar *et al.*: Proteinuria induced by renin)

PLATE 69

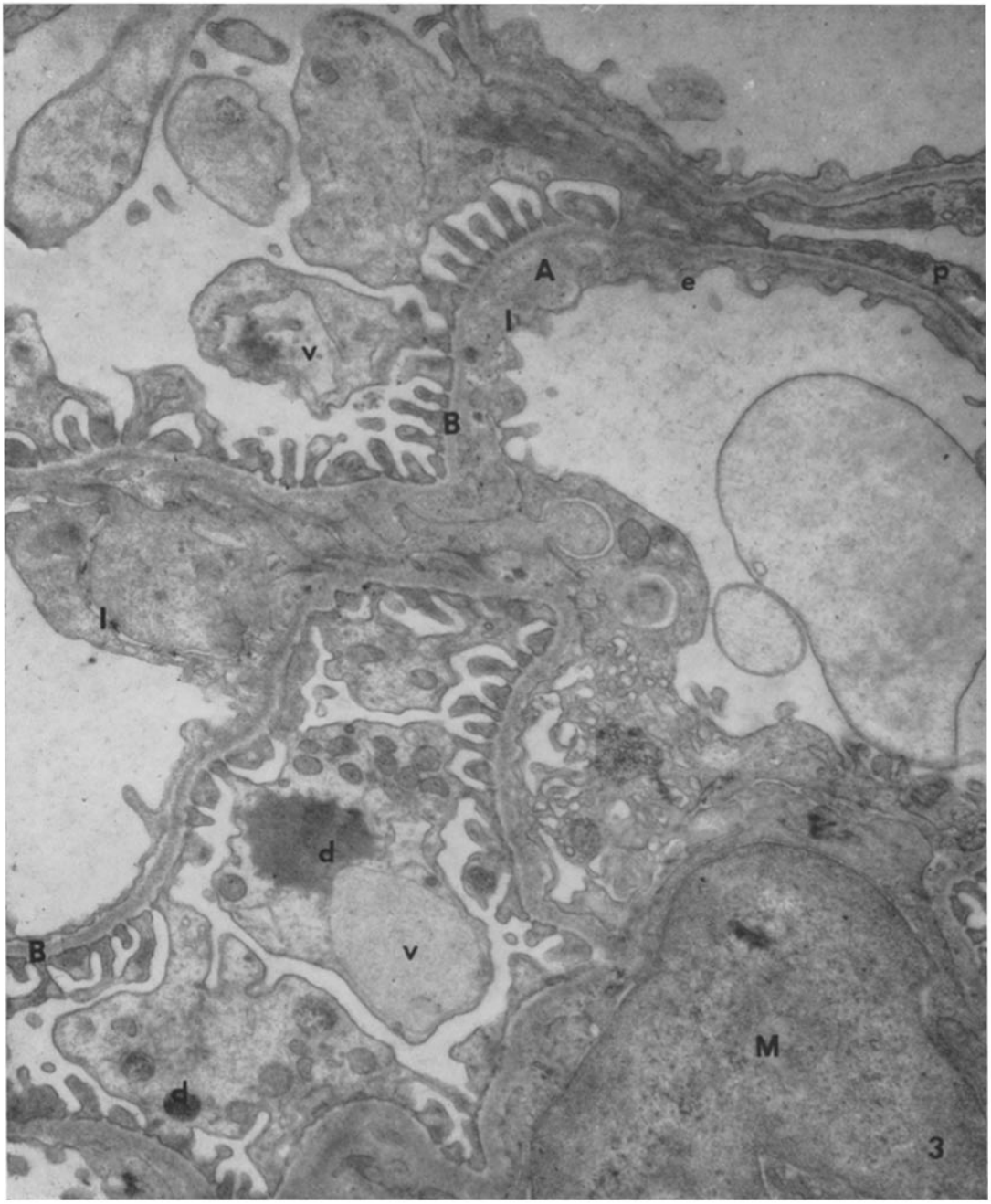
FIG. 2. High magnification of a portion of glomerulus of control rat injected with saccharated iron oxide. Iron particles (*I*) are concentrated on the inner side of the basement membrane (*B*). Only a few are present in the outer aspect on the slit pores (*s*) between adjacent epithelial foot processes (*p*). *e*, endothelium; *CL*, capillary lumen; *BS*, Bowman's space. Methacrylate; unstained. × 56,000.



(Deodhar *et al.*: Proteinuria induced by renin)

PLATE 70

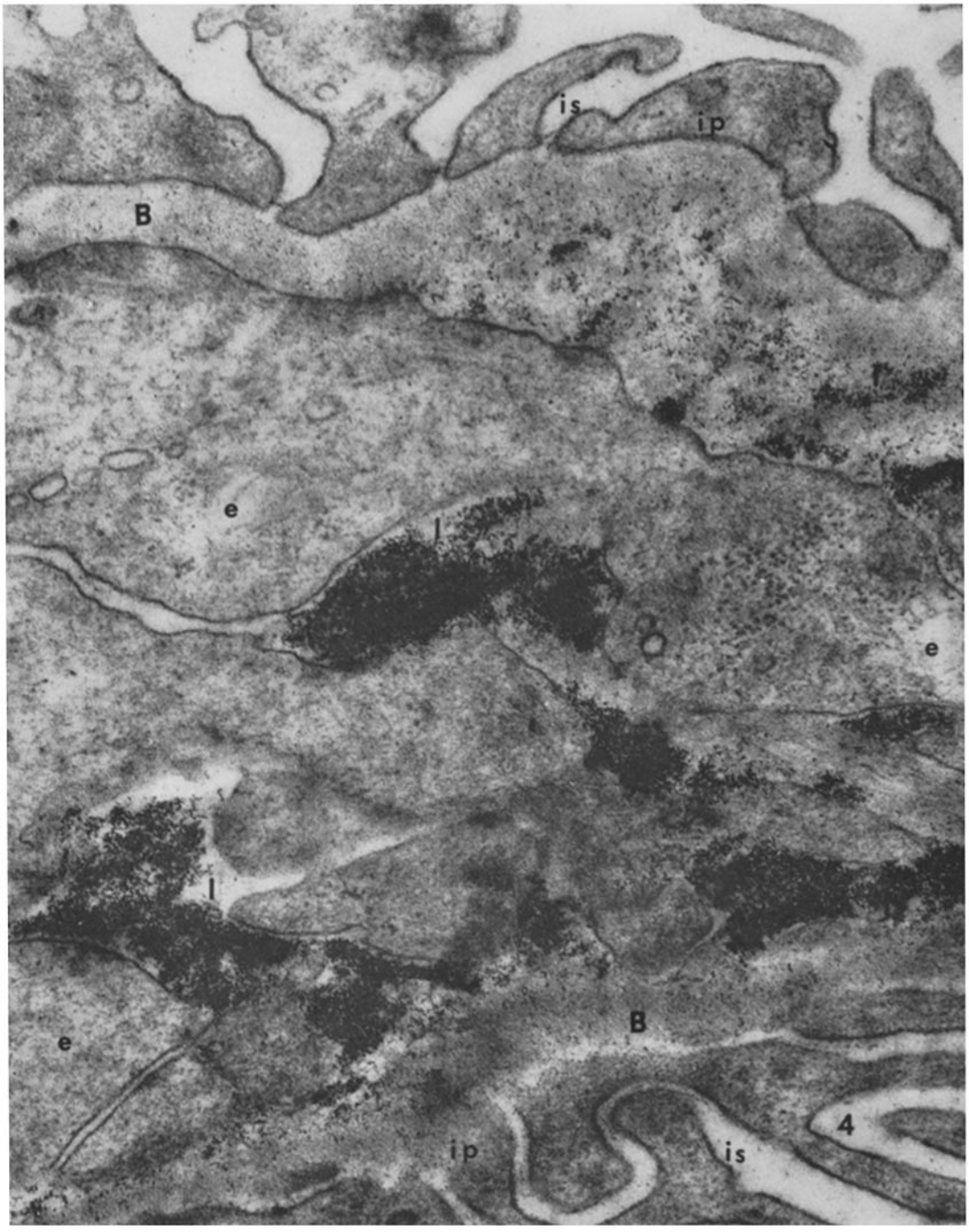
FIG. 3. Portion of glomerulus of rat injected with renin 1 hour and saccharated iron oxide 10 minutes prior to sacrifice. Isolated particles and aggregates of dark staining iron (*I*) are located below and within the basement membrane (*B*). Amorphous material (*A*) has accumulated focally between the endothelial cell cytoplasm (*e*) and the basement membrane. Foot processes (*p*) are widened focally. Vesicles (*v*) and reabsorption droplets (*d*) are present in the visceral epithelial cytoplasm. *M*, mesangial cell. Maraglas; lead hydroxide. × 15,800.



(Deodhar *et al.*: Proteinuria induced by renin)

PLATE 71

FIG. 4. High magnification of a portion of a glomerulus of a rat receiving both renin and iron oxide. Large aggregates of iron (*I*) are located among portions of endothelial cells (*e*), in the amorphous accumulated material between the endothelial cells and basement membrane (*B*), and through the thickness of the basement membrane. A few isolated particles are located within the epithelial foot processes (*ip*) and in the slit pores (*is*). Maraglas; lead hydroxide. × 57,000.



(Deodhar *et al.*: Proteinuria induced by renin)