Sodium Depletion Proteinuria: Experimental and Electronmicroscopic Studies[1,2] (35036)

HILARY ZARNOW AND CONRAD L. PIRANI

The Otto Saphir Department of Pathology, Michael Reese Hospital & Medical Center, Chicago, Illinois 60616; and the University of Illinois, College of Medicine, Chicago, Illinois 60612

Pickering and Prinzmetal (15) in 1940 observed that there was an increased urinary excretion of protein following the injection of exogenous renin into experimental animals. This observation was confirmed by many other investigators (1, 8, 9, 11, 13,).

Fisher and Masson (9) in 1961 were the first to examine by electron microscopy the kidneys of animals with renin-induced proteinuria. They found changes primarily in the visceral epithelial cells of the glomerulus. These changes consisted of cytoplasmic swelling, increased prominence of the Golgi zone, and focal fusion of epithelial cell foot processes. Deodhar *et al.* (8) in 1964 confirmed these results and in addition observed, by electron microscopy, an increased passage across the glomerular capillary wall of the electron dense tracer saccharated ferric oxide following the injection of renin. They also demonstrated that the proteinuric effect of renin disappeared if the enzymatic activity of renin, which results in the release of angiotensin, was inhibited.

During the past 20 years many studies have related increased urinary protein excretion to an increase in circulating renin in such varied clinical states as congestive heart failure, cirrhosis of the liver, prolonged orthostasis, and after heavy exercise (2, 3, 6, 17, 20). The knowledge that the injection of exogenous renin into an experimental animal produces marked proteinuria adds significance to this clinical relationship, but does not provide proof of a direct causal relationship.

It remained for Tobian (19) in 1967 to demonstrate an increased rate of urinary protein excretion in the rat following acute sodium depletion which is a well-established stimulus for an increased endogenous renin production (4, 5, 18).

It was the purpose of this study to confirm Tobian's experimental findings of an increased urinary protein excretion produced by acute sodium depletion, as well as to observe by light and electron microscopy any renal changes which might be related to the increased urinary protein excretion produced by sodium depletion. It was beyond the scope of this study to determine whether an increased endogenous renin production was the basis for an increase in urinary protein excretion. However, it was important to compare any morphologic changes in the glomeruli with those obtained by others after injection of heterologous renin.

Materials and Methods. Female Wistar rats ranging in weight from 120 to 275 g were used. The rats were maintained on a standard laboratory diet with 1% sodium chloride in the drinking water for 2 weeks prior to any experimentation. The animals were placed in metabolic cages and urine was collected for 24-hr periods into a jar containing thymol as a preservative.

Animals were randomly selected to under-

[1] Supported by HEW U.S. Public Health Service Grant #10314. Computation was accomplished with the assistance of the Biomedical Computation Facilities, University of Chicago, under U.S. Public Health Grant FR00013 from the Division of Research Facilities and Resources of the National Institutes of Health.

[2] Submitted by Dr. H. Zarnow in partial fulfillment of the requirements for a Master of Science Degree in Pathology, Graduate College, University of Illinois, Chicago, Illinois 60612.

TABLE I. Composition of Dialysis Fluids.[a]

	Fluid used for Na depleting dialyses (mmoles/liter)	Fluid used for nondepleting dialyses (mmoles/liter)
NaCl	None	130
$NaHCO_3$	15	15
$KHCO_3$	5	5
$CaCl_2$	2.5	2.5
$MgSO_4$	0.8	0.8
Glucose	277.5	277.5

[a] pH was adjusted to 7.3 by adding 0.1 *N* HCl.

go either the sodium depleting or the nondepleting dialyses. On the day before dialysis the regular diet was changed to one low in sodium with distilled water for drinking. The composition of the dialyzing fluids is given in Table I. Peritoneal dialysis was performed by placing animals under light ether anesthesis and then injecting slowly a volume of dialyzing fluid, heated to a temperature of 37°, equal to 20% of the rat's weight. After 1 hr the rats were again placed under light ether anesthesia and the fluid was removed.

The 24-hr urinary protein excretion was measured on the day before dialysis (day −1) as well as on the day of dialysis (day 0) and the day after dialysis (day +1). Animals were weighed on day −1 and day +1. For purposes of comparison, the urine protein excretion on the day before dialysis (day −1) was compared to that on the day after dialysis (day +1). Urine protein concentrations were determined by a modification of the biuret method (14) using tungstic acid to precipitate out the protein.

The urine Na content and pH as well as the Na content of the removed dialyzing fluid were also monitored.

Kidney specimens from animals submitted to depleting and nondepleting dialyses were obtained under light ether anesthesia at the end of the 24-hr urine collection on day +1. In some animals colloidal carbon was injected intravenously at this time. The left kidney was exposed and buffered 2.5% glutaraldehyde was dripped onto the suface. A thin midline horizontal wedge was resected and immersed in 2.5% glutaraldehyde. A second wedge was removed and placed in 60 ml of Zenker's formol solution. The tissue fixed in glutaraldehyde was postfixed in OsO_4 and embedded in Epon 812. Ultrathin sections were cut with a Porter-Blum Mt-2 ultramicrotome using a diamond knife, stained with uranyl acetate and lead citrate and examined with an RCA EMU 3H electron microscope. The tissue fixed in Zenker's formol was embedded in paraffin. Two-μ thick sections were stained with hematoxylin and eosin, alcian blue–PAS and silver methenamine. The juxtaglomerular apparatus (JGA) was evaluated by determining the number of JGA's visualized/100 glomeruli (a function of JGA size) and the average number of cells per JG body (19). These counts were repeated by an independent observer who was unaware of the experimental results.

Several animals were sacrificed a few hours after the end of dialysis to look for early changes. In addition, several kidney specimens from normal rats were taken as an additional morphological control.

Results. Physiologic studies. Normal rats. This group consisted of 37 normal female Wistar rats ranging in weight from 120 to 270 g. Three random nonconsecutive 24-hr urine protein excretions were determined for each rat. The average protein excretion varied from 0.1 to 4.4 mg/100 g of body weight/24 hr (Fig. 1). The protein excretion of a single animal was fairly constant and showed a maximum range of daily variation of ±25% of its mean 24-hr protein excretion. These values are in close agreement with data reported by Addis *et al.* and Deodhar *et al.* (8) for normal rats of similar sex, strain, and weight. The urine pH was measured on each sample and was found to be generally acid with a mean of 6.9, and a range from 6.2 to 7.4. Urine sodium concentration was measured randomly in about 25% of the samples and was found to be always greater than 100 meq/liter with a mean of 120 meq/liter and a range of 100 to 145 meq/liter.

Dialysis group. A total of 31 rats underwent 70 peritoneal dialyses with some rats having only one dialysis and others as many

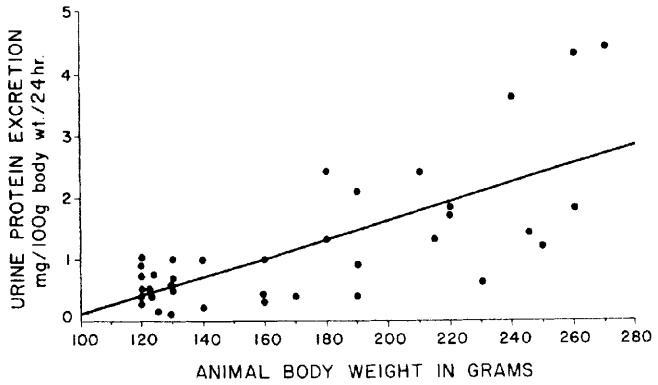

FIG. 1. Average urine protein excretion (mg/100 g of body wt/25 hr) versus body weight in 37 normal rats. The calculated regression line has been plotted. The formula for the line is urine protein excretion = 1.45 body weight −1.36 (SE = ±0.243); calculated correlation, $r = 0.7097$. A similar relationship was observed when the log urine protein excretion was plotted against body weight. The data indicates a relative increase in the rate of urine protein excretion with increasing body weight since the protein excretion was already expressed in terms of 100 g of body weight.

as four dialyses. The selection of whether an animal underwent a control dialysis was always random. Animals who underwent multiple dialyses were given at least 2 weeks on a regular diet with 1% NaCl drinking water to recover between dialyses. There was no difference in the results of animals undergoing only one dialysis and those who underwent multiple dialyses. There was no difference in the animal body weight between day −1 and day +1 following either type of dialysis.

The results in the two groups are given in Table II. In the control group, 0 out of 33 dialyses were followed by a 3-fold increase in the protein excretion. In the experimental group 19 out of 37 dialyses were followed by a 3-fold or greater increase in urinary protein excretion.

To test whether there was significant quantitative difference in urinary protein excretion between the sodium depleted and the nondepleted groups, the change in urinary protein excretion for each single dialysis, control or experimental, was determined by dividing the urinary protein excretion on the day after dialysis (A) by the urinary protein excretion on the day before dialysis (B). Because of the wide range of urinary protein excretion of the animals used in this study, all values were converted to logarithms (base 10) in order to minimize sample variability. The mean log before (log B), the mean log after (log A), and the mean log of the ratio (log A/B) were determined for both the control and the experimental groups, and then a t test was performed comparing the two groups (Table IIIa). There was no significant difference in the protein excretion of the two groups before dialysis ($p > 0.5$), but there was a highly significant difference in the two groups following dialysis ($p < 0.001$). This difference was greatest when log A/B which is the expression of the change in protein excretion, was compared between the two groups.

TABLE II. Urine Protein Excretion Following Dialysis with Na Depleting and Nondepleting Solutions.

Changes in 24-hr protein excretion	No. of dialyses with	
	Control solution	Na depleting solution
No change	28	7
1.5–3 × inc.	5	11
3–8 × inc.		13
8 × inc.		6

The closeness in absolute numbers of the log A/B in the experimental group (0.5402) to the log A/B experimental minus log A/B control (0.5378) indicates that the degree of change in the control group was so low that

TABLE III. 24-hr Urinary Protein Excretion Before and After Dialysis.

Log	Control	Expt.	Diff. expt.—cont.	SE of diff.	t	p
		a. All animals (31 rats, 70 dialyses)				
Before	−0.069 ± 0.416[a]	−0.171 ± 0.359[a]	−0.102	0.092	1.107	>0.5
After	−0.067 ± 0.357	0.369 ± 0.420	0.436	0.093	4.669	<0.001
After/before	0.002 ± 0.176	0.540 ± 0.322	0.538	0.063	8.549	<0.001
		b. Paired dialyses (19 rats, 38 dialyses)[b]				
Before	−0.088 ± 0.427	−0.119 ± 0.378	−0.031	0.041	0.557	>0.5
After	−0.065 ± 0.381	0.401 ± 0.302	0.466	0.072	7.250	<0.001
After/before	0.023 ± 0.174	0.520 ± 0.313	0.497	0.074	6.739	<0.001

[a] Standard deviation.

[b] Control and experimental dialysis in the same animal analyzed by the method of paired comparison.

the ratio A/B control approached unity and log A/B control was near zero.

Nineteen animals underwent both a sodium depleting and a control dialysis. It was decided to make a subgroup of these animals and to analyze these results by the method of paired comparisons utilizing a single t test. Ten of these underwent a control dialysis prior to the sodium depleting one, while nine underwent a control dialysis following the sodium depleting dialysis, so that the sequence of the type of dialysis was not significant in determining the results. The results (Table IIIb) showed a highly significant increase in protein excretion following the sodium depleting dialysis compared to the control dialyses ($p < 0.001$). The closeness of the absolute value of log A/B experimental minus log A/B control of the paired group to that of the entire group (0.487 vs 0.540) is a good indicator that the two groups were indeed similar in behavior.

In an attempt to estalbish the maximal period of proteinuria, the 24-hr urinary protein excretion on day 0 was also determined. In all cases except one after a control dialysis, the level of urinary protein excretion on day 0 was less than on day +1.

The total Na (meq) removed by peritoneal dialysis was compared to the change in urinary protein excretion, but no direct relationship was demonstrated (Fig. 2). The ur-

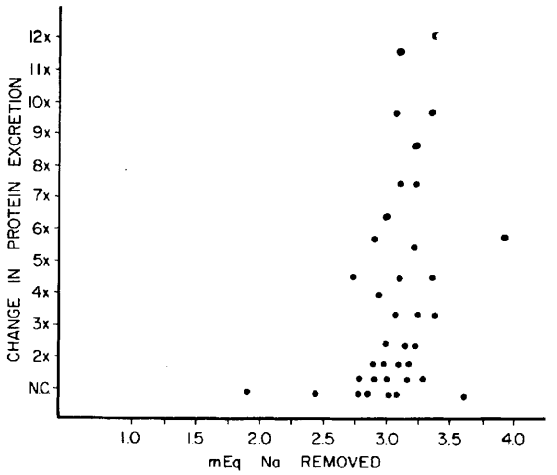

FIG. 2. Change in protein excretion following 37 sodium depleting dialyses versus sodium (meq) removed per dialysis.

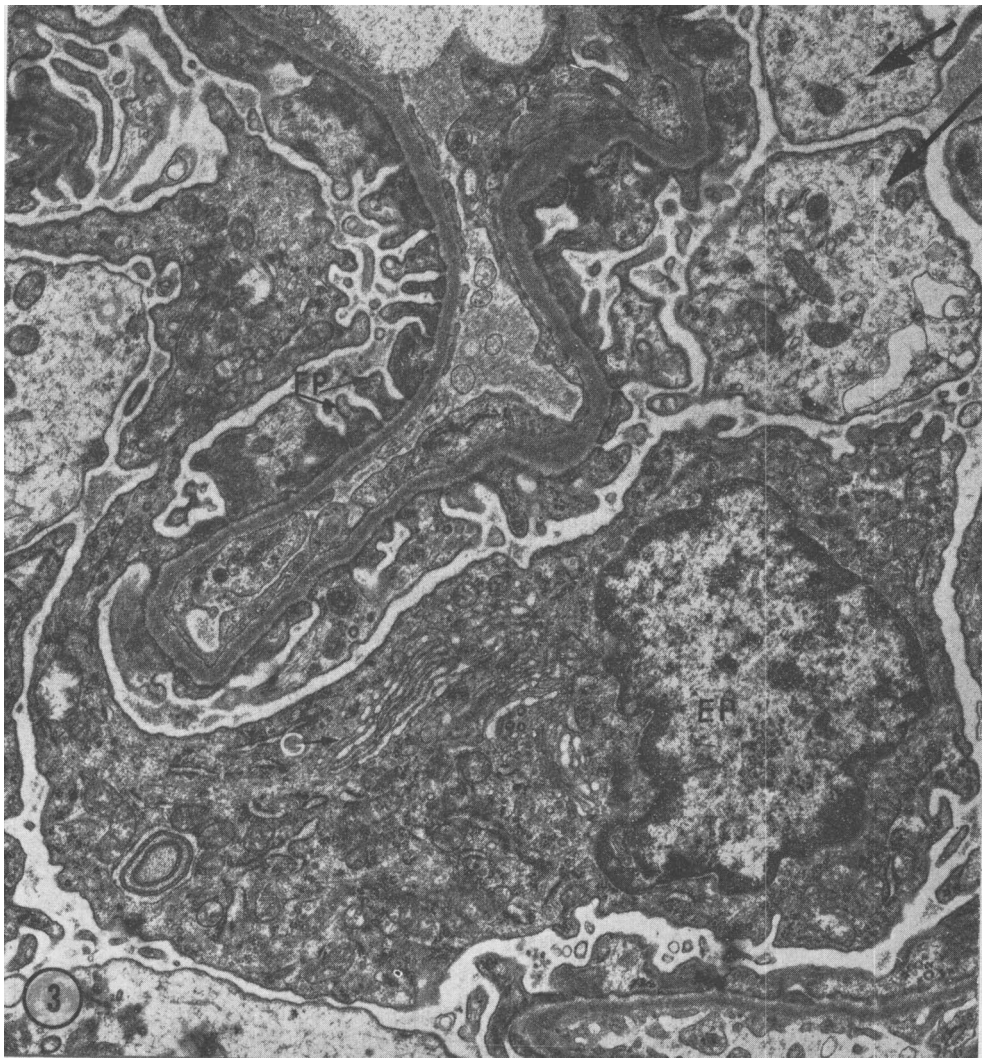

FIG. 3. Active appearing visceral epithelial cell (EP) from an experimental control animal. The Golgi zones (G) are clearly seen. The number of cytoplasmic organelles is slightly increased. There is a focal area of cell swelling (arrows) which was rarely seen in the control group; ×11,720.

inary pH following dialysis was more acid than before dialysis with a mean 6.2 vs 6.9. There was no difference in this between the sodium depleted group and the controls. There was also no significant difference in the 24-hr urine volumes of both groups. The mean urine Na predialysis was 120 meq/liter. On day + 1 in the sodium depleted group the mean urine Na was 20 meq/liter, whereas in the nondepleted group, there was no change in the urine Na concentration.

Morphologic studies. Light microscopy. Controls. The control group consisted of three subgroups: (i) normal nonmanipulated animals; (ii) animals who underwent the nondepleting dialyses; (iii) animals who were sodium depleted but did not show an increased urinary protein excretion. No renal morphologic alterations were observed. In the animals injected with colloidal carbon, the carbon particles were visible within the capillary lumens. In none of the three groups were changes seen in the glomeruli, vessels, renal tubules, or JGA.

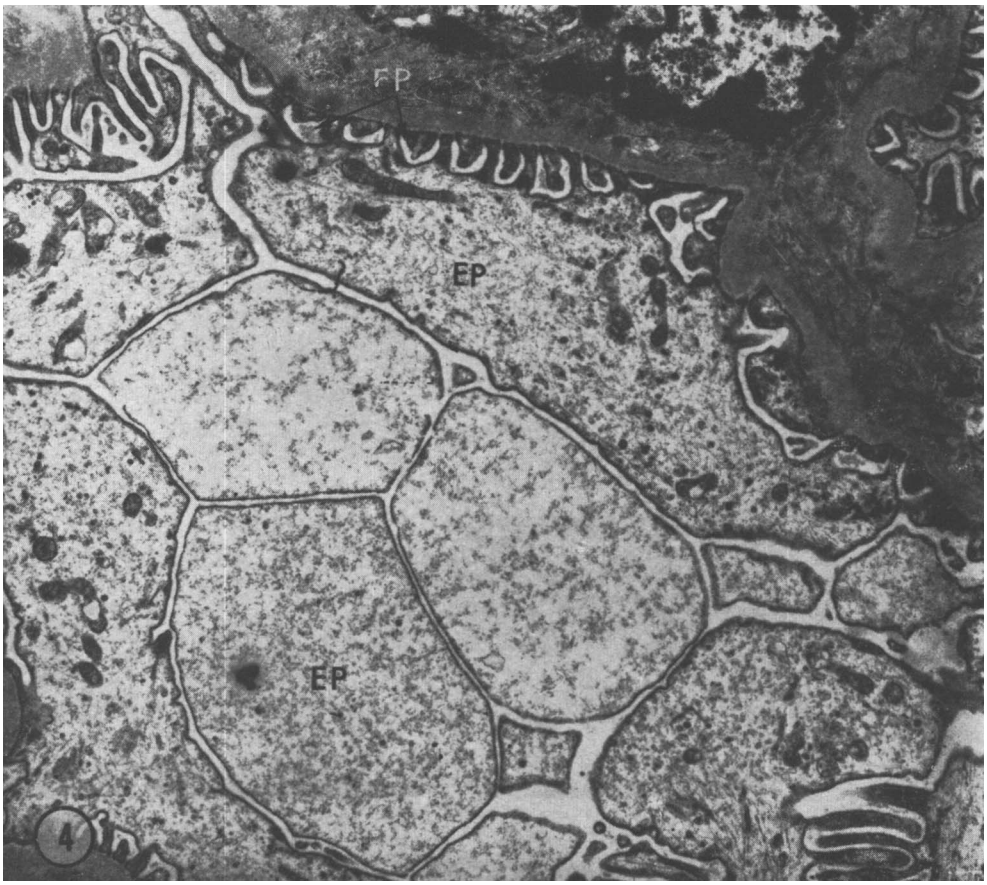

FIG. 4. Marked swelling of visceral epithelial cells (EP) in a sodium-depleted rat. In the swollen cytoplasm there is almost a complete absence of cytoplasmic organelles. The foot processes (FP) are normal; ×5960.

Sodium depleted rats. No renal morphologic alterations were observed. There were no differences by light microscopy between the experimental and control groups in either glomeruli, tubules, vessels, or in the number and cellularity of the JGA's.

Electron microscopy. Controls. In all 3 control groups the glomerular epithelial, endothelial, and mesangial cells were of normal size and contained the expected number of organelles (Fig. 3). The foot processes were discrete. The basement membrane was thin and uniform. The tubular cells were normal in size, and no changes were detected in the number and appearance of the cytoplasmic organelles. The vessels were unremarkable. The juxtaglomerular regions showed small to moderate numbers of granules in the cells of the juxtaglomerular body and the smooth muscle cells of the arteriolar wall. In these structures neither the number of cells nor that of intracytoplasmic organelles was increased. In the animals injected with colloidal carbon before sacrifice carbon particles were seen within the capillary lumens and in the mesangial zones. No carbon particles were seen within the visceral epithelial cells or in Bowman's spaces.

Sodium depleted rats with increased urinary protein excretion. The visceral epithelial cells of the sodium depleted rats were enlarged and swollen. Except in a few of the most severely swollen cells (Fig. 4), the Golgi zone and the endoplasmic reticulum were

especially prominent (Fig. 5). In general there was an increased number of intracytoplasmic organelles. The foot processes were focally thickened, blunted, and occasionally fused (Fig. 6). There was an increased number of dense osmiophilic intracytoplasmic deposits. The endothelial and mesangial cells showed no significant changes from the controls. In some animals there was an increased number and swelling of the mitochondria within the cells of the proximal tubules. The majority of animals showed no changes in the tubules. The juxtaglomerular regions in only a few animals presented a greater number of granules in the cells of the juxtaglomerular body and arteriolar wall, than seen in the controls. The number of cells and of intracytoplasmic organelles was not increased. Colloidal carbon particles were seen in those animals which were injected, within the capillary lumens and the mesangial zones, and also on occasion within the cytoplasm of the visceral epithelial cells. No free carbon was ever seen in the urinary space.

Discussion. The results clearly indicate that acute sodium depletion by peritoneal

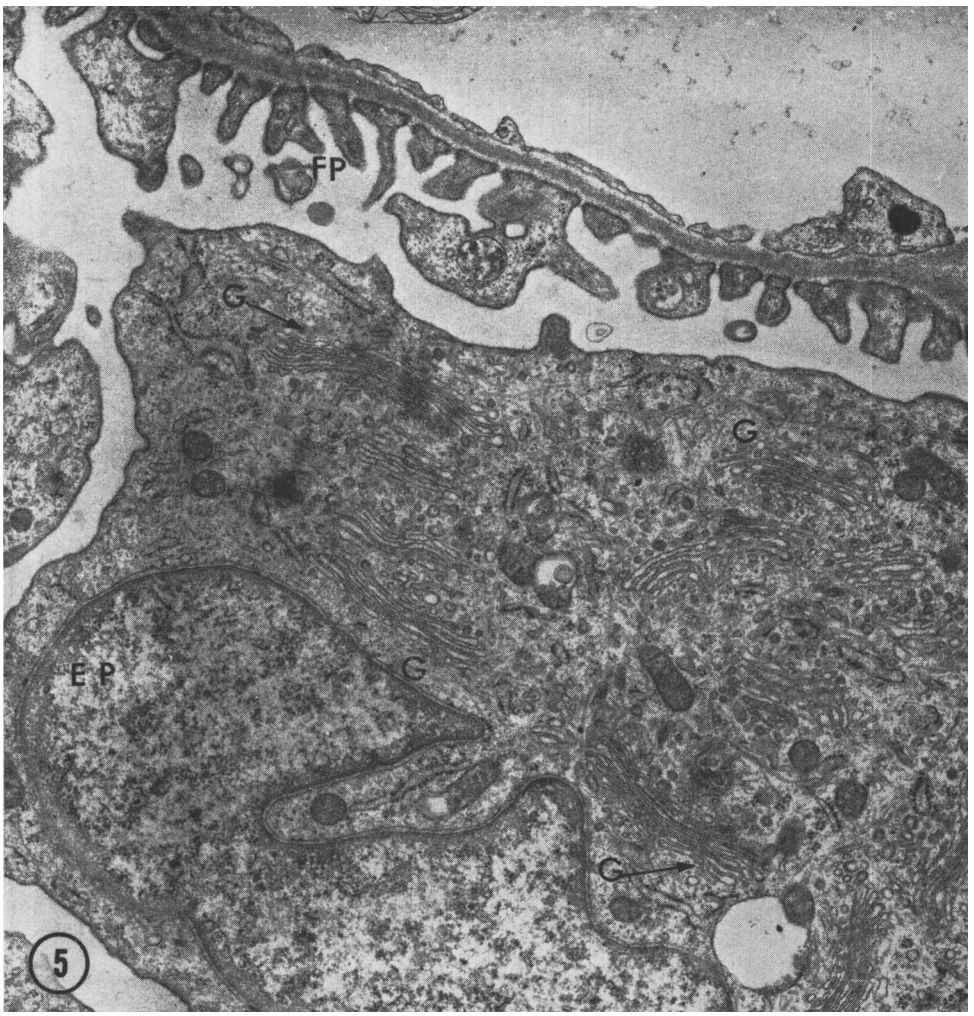

FIG. 5. Visceral epithelial cell (EP) from a sodium depleted rat. The Golgi zones (G) are very prominent and extend throughout the entire cell cytoplasm. The foot processes (FP) are normal; ×10,300.

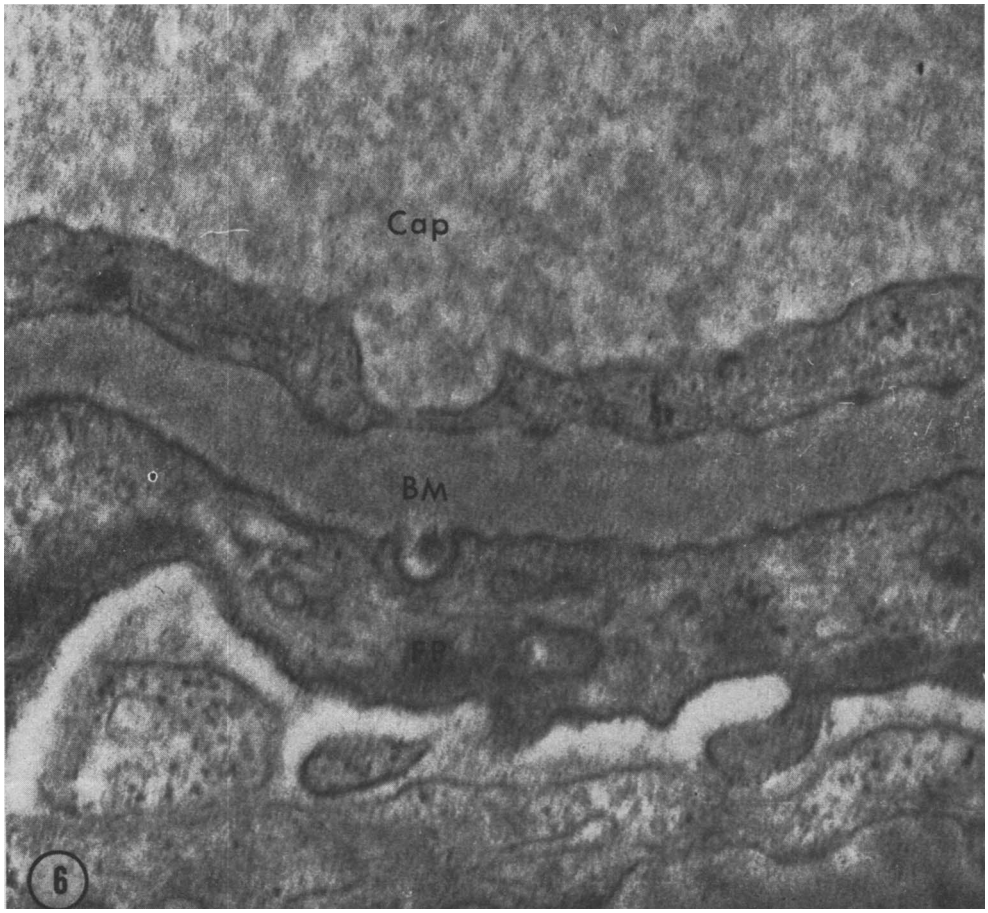

FIG. 6. Detail of a peripheral glomerular capillary loop (Cap) in a sodium-depleted rat. The basement membrane (BM) is thin and uniform. There is a complete fusion of the foot processes (FP); ×28,000.

dialysis resulted in an increased rate of urinary protein excretion. The control group was identical to the experimental group in every detail except for the sodium content and the osmotic activity of the dialyzing fluid; the control dialyzing fluid was hypertonic whereas the experimental dialyzing fluid was hypotonic. The difference in the quantitative urine protein excretion following the two types of dialysis was significant. The individual difference was also marked with 0 out of 33 control dialyses versus 19 out of 37 sodium depleting dialyses being followed by a greater than threefold increase in the 24-hr urinary protein excretion. The data presented, however, provide no direct evidence that an increased endogenous production of renin is the mechanism whereby sodium depletion results in proteinuria. The data also do not differentiate whether the proteinuria is due to sodium depletion *per se* or is the result of changes in tonicity brought about by dialysis with a hypotonic solution. The marked decrease in urine sodium excretion following the sodium depleting dialyses may be due to an increased secretion of renin or, more likely, aldosterone. Evidence indicates that in the rat, sodium depletion results in an increased production of aldosterone which may be independent of the concurrent increase in renin production (5, 12). The increased urine acidity following dialysis

might have explained the increased urinary protein excretion (10), but there was no difference in urine pH between the sodium-depleted and the nondepleted group. This experimental model, may, however, be the basis for future experiments to establish whether increased endogenous renin production is related to an increased urinary protein excretion.

The electron microscopic findings showed a good correlation with the presence of proteinuria. The nondepleted rats and the sodium-depleted rats without an elevated urinary protein excretion showed no changes from the normal unmanipulated rats which were studied. The changes in the sodium-depleted rats with an increased urinary protein excretion were primarily in the visceral epithelial cells. These changes were similar to those described by Fisher and Masson (9) and Deodhar *et al.* (8) in animals who developed proteinuria following injection of exogenous renin. This, however, does not necessarily mean that the mechanism of the proteinuria in this experiment was due to an increased production of endogenous renin, since many other stimuli could have produced the same morphologic response. Many workers are of the opinion that these changes are nonspecific and secondary to an increased transfer of protein through the capillary basement membrane no matter what the cause (7). The degree of changes observed in this experiment correlated roughly with the quantitative level of protein excretion, *i.e.*, those with the highest absolute level of urinary excretion showed the most marked changes and vice versa.

The presence of colloidal carbon in the visceral epithelial cells only in the sodium-depleted rats suggests that there is an increased passage of large molecules across the glomerular capillary wall. This finding is similar to the data on saccharated ferric oxide reported by Deodhar *et al.* (8). Unfortunately, observations on the passage across the glomerular capillary wall of large exogenous molecular tracer substances do not necessarily reflect the glomerular passage of endogenous large protein molecules (8).

The correlation of the morphologic appearance of the JGA and renin production reported in the literature (16) might have provided some indirect evidence of increased renin production if similar changes were observed in this experiment. The increased granularity of the JGA seen in this experiment by electron microscopy was not associated with increased cellularity and was seen only in a few animals.

Study of the JGA by light microscopy revealed no differences between the sodium-depleted and the nondepleted animals. This was not unexpected since the experimental studies correlating increased JGA granularity with sodium depletion and increased renin levels were done in chronic experiments using dietary salt restriction (10, 16). The acuteness of this experiment may not allow time for the development of the classical changes in the JGA following depletion. The electron microscopic study of the JGA is of equivocal value as a measure of renin production since (i) the sample size is much too small to be representative, (ii) there are no data to correlate the electron microscopic appearance in acute sodium depletion with actual renin production.

Summary. Female Wistar rats were submitted to peritoneal dialysis with either a balanced ionic solution (control group), or a solution which was identical except that it was deficient in sodium (sodium depleted). The 24-hr urine protein excretion on the day before dialysis was compared to that on the day after dialysis. Kidney specimens were taken for morphologic study by light and electron microscopy at the end of urine collections to correlate any structural changes with the degree of protein excretion.

Nineteen out of 37 sodium depleting dialyses were followed by a threefold, or greater, increase in urine protein excretion while 0 out of 33 control dialyses were followed by an equivalent increase. Quantitatively there was a highly significant increase ($p < 0.001$) in urinary protein excretion following the sodium depleting dialyses as compared to the control dialyses.

Study of the kidneys by electron microsco-

py showed definite ultrastructural changes in those animals which showed an increase in urinary protein excretion while no changes were seen either in animals submitted to control dialysis or in sodium-depleted animals which failed to show an increase in urinary protein excretion.

The results confirm that sodium depletion can induce proteinuria in the rat. The ultrastructural changes in the glomeruli are interpreted as secondary to proteinuria.

1. Addis, T., Barrett, E., Boyd, R. I., and Ureen, H. J., J. Exp. Med. **89**, 131 (1949).

2. Brozovic, L., Castenfors, J., and Piscator, M., Acta. Physiol. Scand. **70**, 143 (1967).

3. Brown, J. J., Davies, D. L., Lever, A. F., and Robertson, J. I. S., Can. Med. Ass. J. **90**, 201 (1964).

4. Brubacker, E. S., and Vander, A. J., Amer. J. Physiol. **214**, 15 (1968).

5. Cade, R., and Pererich, T., Amer. J. Physiol. **208**, 1026 (1964).

6. Castenfors, J., Mossfeldt, F., and Piscator, M., Acta. Physiol. Scand. **70**, 194 (1967).

7. Churg, J., Mautner, W., Grishman, E., and Eisner, G. M., Arch. Intern. Med. **109**, 97 (1962).

8. Deodhar, S. D., Cuppage, F. E., and Gableman, E., J. Exp. Med. **120**, 677 (1964).

9. Fisher, E, R., and Masson, G. M. C., Arch. Pathol. **71**, 26 (1961).

10. Gardner, K. D., J. Clin. Invest. **40**, 525 (1961).

11. Lippman, R. W., Ureen, H. J., and Oliver, J., J. Exp. Med. **93**, 605 (1951).

12. Marieb, N. J., and Mulrow, P. J., Endocrinology **76**, 657 (1965).

13. Masson, G. M. C., Del Greco, F., Corcoran, A. C., and Page, I. H., Proc. Soc. Exp. Biol. Med. **83**, 631 (1953).

14. Natelson, S., "Microtechniques of Clinical Chemistry," 2nd ed. Thomas, Springfield, Ill. (1961).

15. Pickering, G. W., and Prinzmetal, M., J. Physiol. **98**, 314 (1940).

16. Pitcock, J. A., Hartroft, P. M., and Newmark, L. N., Proc. Soc. Exp. Biol. Med. **100**, 868 (1959).

17. Robinson, R. R., Lecocq, F. R., Phillippi, P. J., and Glenn, W. G., J. Clin. Invest. **42**, 100 (1963).

18. Tobian, L., and Nason, P., J. Lab. Clin. Med. **67**, 224 (1967).

19. Turgeon, C., and Sommers, S. C., Amer. J. Pathol. **38**, 227 (1961).

20. Veyrat, R., de Champlain, J., Boucher, R., and Genest, J., Can. Med. Ass. J. **90**, 215 (1964).

Received Apr. 22, 1970. P.S.E.B.M., 1970, Vol. 135.