Effect of Protein-Load Proteinuria on Glomerular Polyanion[1] (36891)

L. Paul Roy,[2,3] Robert L. Vernier, and Alfred F. Michael

Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455

The renal changes associated with parenteral protein-load proteinuria have been studied extensively (1–6). The proteinuria is considered to result from increased glomerular filtration of an increased load. This assumption is supported by the presence of albumin droplets in glomerular epithelial cells and a tendency for blunting and fusion of epithelial foot processes (1–3).

Glomerular epithelial cells are coated with polyanionic sialoprotein on their free surface down to the filtration slit membrane (7). We have shown a reduction in this polyanion in aminonucleoside nephrosis and suggested that the negative charge of this material may be of importance in the maintenance of epithelial foot processes and could possibly play a significant role in preserving relative glomerular impermeability to plasma proteins (7).

The purpose of this study was to evaluate the effects of acute and chronic protein-load proteinuria on this polyanionic epithelial sialoprotein.

Materials and Methods. All studies were performed on 200–250 g white female rats.

The protein load was delivered in two different ways in separate experiments. In the first experiment animals were anesthetized with intraperitoneal (ip) sodium pentobarbital 6 mg/100 g body weight and constant infusions were given via a catheter placed in the abdominal aorta, through an abdominal incision. The tip of the catheter was opposite the renal arteries. The distal aorta was ligated. The infusates were human serum albumin (obtained from the American National Red Cross) 15 g/100 ml in 10% mannitol, dextran (av. mol. wt. 77,500, Sigma Chemical Company) 15 g/100 ml in 10% mannitol or 10% mannitol in physiologic saline; 9 animals were in each group. A total of 15 ml was given over 90 min. Urine was collected from ureteric catheters. To prevent death from volume overload and maintain a high urine output, the animals received 1.0 ml 25% mannitol and 10 mg furosemide (Hoechst) by intravenous injection prior to commencement of the infusion.

In the second experiment, 15 animals were divided into 3 groups of 5 and given daily ip injections for 4 days. Each animal in the experimental group received 1.5 g human serum albumin in 6.0 ml physiological saline and animals in the control groups each received either 1.5 g dextran in 6.0 ml physiological saline or 6.0 ml physiological saline alone. Urine was collected in metabolic cages for 24 hr after the last injection for protein determination. All animals receiving dextran developed congestive cardiac failure. One was found dead on the third day and was excluded from the study. A second died just after the fourth injection and renal tissue was examined.

At the conclusion of each experiment the rats were killed by cervical dislocation. Renal tissue was obtained from all animals for light microscopy and from some animals for electron microscopy. Tissue was processed as previously described (7).

Glomerular polyanion was demonstrated histochemically with colloidal iron stain at pH 1.9 and 2.5 as previously described (7). Intensity of staining was based upon an arbitrary grading system of 0, 1, 2, or 3 in which

[1] This study was supported in part by grants from the National Institutes of Health (AM 12375, AI 10,704-13, HE05662) and the American Heart Association.

[2] Fellow of National Kidney Foundation, New York, New York and Postgraduate Medical Foundation, University of Sydney, Australia.

[3] Present address: Royal Alexandra Hospital for Children, Bridge Road, Camperdown, N.S.W., Australia 2050.

TABLE I. Results of Colloidal Iron (CI) Staining at pH 1.9 and 2.5 of Glomeruli from Animals Receiving 90 Min Infusions of Albumin, Dextran or Mannitol and Comparison with Urinary Protein Levels.

Infusion	No. of animals	CI score (out of 6) mean ± 1 SD	Urinary protein mg/10 min
Albumin	9	2.8 ± 0.83	6.3 –20
Dextran	9	3.9 ± 1.85	0.09– 1.50[a]
Mannitol	9	4.3 ± 1.32	0.05– 0.87[a]

[a] Urine specimen from one rat excluded, being heavily blood stained.
Student's t test: CI score.
Albumin vs. dextran, $0.025 < p < 0.05$.
Albumin vs. mannitol, $0.005 < p < 0.01$.

0 indicates no glomerular staining, 1 denotes barely detectable staining, 2—moderate staining, and 3—intense staining. The staining-intensity grades at both pH levels were added to give the colloidal iron score (out of 6).

Urine protein determinations were carried out on trichloracetic acid precipitates redissolved in 0.02 N NaOH (final concentration) using direct optical density measurements at 280 nm in a Beckman spectrophotometer for samples from the acute experiments and by the biuret reaction (8) for the 24 hr specimens. Bovine-serum albumin was the standard.

Results. The results from the experiments are shown in Table I and II. The intensity of staining of glomeruli from animals receiving intraarterial infusions of albumin was significantly less than that seen in animals from any of the control groups (Table I). In rats receiving daily intraperitoneal injections of albumin, there was a significant decrease in intensity of the colloidal iron stain when compared with dextran treated animals, but not with saline-treated controls (Table II and Fig. 1). However comparison of the score of the albumin-injected animals with the pooled score of the saline and dextran injected animals, revealed a significant difference ($p < 0.05$). Major, non-protein, colloid osmotic load did not reproducibly affect the intensity of colloidal iron stain.

Electron microscopic study of kidney biopsies from at least one animal in each group and 2 each in the daily albumin and daily saline injection groups revealed blunting and focal fusion of epithelial foot process in glo-

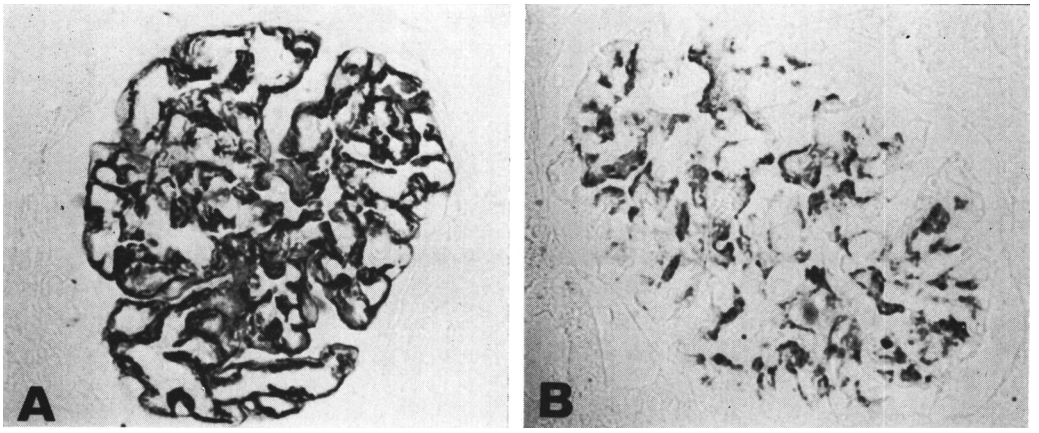

FIG. 1. Glomeruli stained with colloidal iron stain at pH 1.9 (A) dextran injections daily for 4 days—normal stain (3+). (B) albumin injections daily for 4 days—stain reduced in intensity (1+). (×480).

TABLE II. Results of Colloidal Iron (CI) Staining at pH 1.9 and 2.5 of Glomeruli from Animals Receiving Daily Injections of Albumin, Dextran, or Saline, for 5 Days and Comparison with Urinary Protein Excretion.

Intraperitoneal injection	No. of animals	Urinary protein (mg/24 hr)	CI score (out of 6)	CI score Mean ± 1 SD
Albumin	5	458	3	3.6 ± 1.15
		475	5	
		129	4	
		961	4	
		408	2	
Dextran	4	NE[a]	5	5.5 ± 0.60
		5.4	6	
		7.9	6	
		5.5	5	
Saline	5	4.6	5	4.8 ± 1.1
		1.5	5	
		3.9	3	
		12.5	5	
		5.3	6	

[a] NE = not estimated.
Student's t test:
Albumin vs. dextran, $0.005 < p < 0.01$.
Albumin vs. saline, $0.05 < p < 0.10$.

meruli from albumin treated animals alone (Fig. 2). Epithelial and endothelial cellular swelling was observed in glomeruli from all dextran-treated animals. Round, electron-dense inclusions were present in epithelial cells from albumin-treated animals. Similar, but vacuolated, inclusions were present in epithelial cells from dextran-treated animals.

Tissues from all animals showed the well-documented tubular changes observed with dextran or mannitol loading (9, 10).

Discussion. The precise mechanism of protein-load proteinuria is unknown. It is usually assumed to be a mass effect. The relative lack of quantitative data on reabsorption of protein renders correlations between degree of load and degree of proteinuria difficult. The present study indicates the extremely variable degree of proteinuria with similar loads. Proteinuria may persist for prolonged periods after cessation of protein loading in rats and glomerular sclerosis may occur. Immunologic mechanisms do not appear to be responsible for this phenomenon (11). The possibility also exists that protein-load proteinuria is due to a toxic effect of the heterologous protein. That proteinuria follows homologous protein infusions without any increase in apparent "pore size", as measured by dextran-clearance studies (12), suggests that mass effect at least plays a prominent role in protein-load proteinuria.

The results of these experiments indicate that the reduction in glomerular polyanion observed in proteinuric states is secondary to proteinuria. It is recognized that to prove conclusively that neither fusion of foot processes nor loss of polyanion are in any way responsible for increased permeability would require the demonstration of these phenomena in the absence of proteinuria. Our attempts to remove sialic acid from the polyanion by intraarterial infusion of neuraminidase have all resulted in acute shutdown of the perfused kidney.

There are 2 possible explanations for the apparent relationship between epithelial foot process fusion and reduction in glomerular polyanion. First, as previously suggested (7), foot processes may be maintained by the

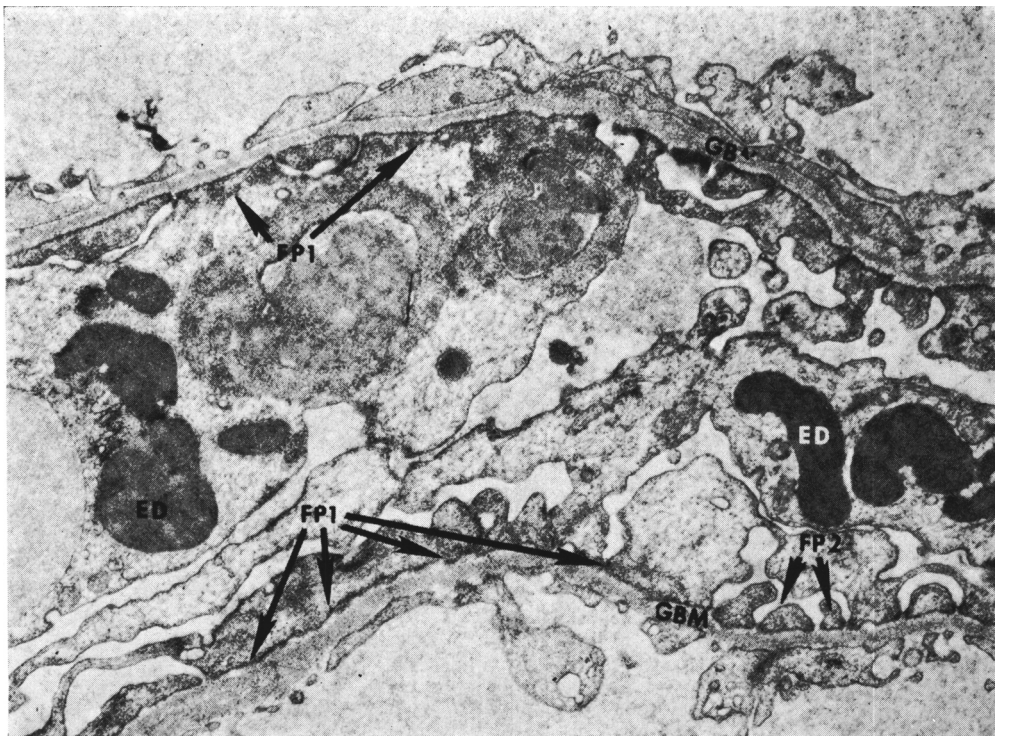

FIG. 2. Electron photomicrograph of portion of a glomerulus from an animal receiving daily albumin injections. Focal areas of foot process fusion (FP1) and area of normal foot processes are seen (FP2) (GBM = glomerular basement membrane, ED = electron dense bodies in epithelial cells). (×15,000).

negative surface charge due to the polyanion. Loss of the polyanion, as a result of proteinuria, would result in foot process fusion. This interpretation is favored indirectly by the very early (1½ hr) reduction in polyanion after the onset of proteinuria, suggesting that the polyanion may be lost first.

Alternatively, reduction in the total surface area of the epithelial cells subsequent to foot process fusion, together with an increased proportion of surface in contact with the glomerular basement membrane (GBM), would result in reduced polyanion histochemically. A decrease in the glomerular sialic acid content would be seen unless an increase of this substance, per unit surface area, had occurred.

The former hypothesis is favored by the early loss of polyanion, although these animals, with extremely heavy proteinuria, did not show the gross epithelial foot process fusion seen in animals with aminonucleoside nephrotic syndrome or in nephrotic syndrome in man. This mild foot process change was accompanied by an equally moderate reduction in polyanion suggesting that although the polyanion is "lost" secondary to proteinuria this apparent loss may be related directly to the degree of foot process fusion. The first explanation implies that the epithelial cell membrane of the podocyte adjacent to the slit pores is functionally separate from the remainder of the free surface; prior electron microscopic studies of glomeruli from aminonucleoside nephrotic syndrome show normal colloidal iron stain of the remaining free epithelial surface (7).

Summary. Protein-load proteinuria was induced in rats by either intraarterial infusions lasting 1½ hr or by 4 daily, intraperitoneal injections. Control rats were given medium molecular-weight dextran to assess the effect of colloid osmotic load. Glomerular polyanion was reduced histochemically in animals with

proteinuria. Reduction of glomerular polyanion and epithelial foot process fusion both may be secondary to proteinuria. Eidence that the negatively charged sialoprotein may be responsible for maintenance of epithelial foot processes is discussed.

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Received June 29, 1972. P.S.E.B.M., 1972, Vol. 141.