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Aminonucleoside Nephrosis in PRO/Re Mice[1] (39802)

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The administration of 6-dimethylamino-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine, the aminonucleoside of puromycin (PAN), to rats and monkeys results in biochemical and renal glomerular changes closely resembling those seen in the nephrotic syndrome in man (1–3). In contrast, tangible effects have not been observed in other species such as guinea pigs, dogs, man, mice, and rabbits following treatment with PAN in its established dosages and routes of administration (4–7). We are reporting the induction of renal disease by PAN in the hyperprolinemic PRO/Re strain of mice. The latter is an inbred strain and is a crossbreed between C57BL/6J and 129ReJ (8). The mice have a deficiency of proline oxidase and are an established experimental model for Type I hyperprolinemia in man (9, 10).

Materials and methods. Twelve-week-old female PRO/Re mice (four animals) weighing 20–25 g were given PAN (2 mg/100 g body weight) subcutaneously daily for 21 days. Control groups of animals in batches of four received daily injections of 0.5 ml of physiological saline. Parental (C57BL/6J and 129ReJ) and nonparental (AKR/J, BDP/J, CD1, and C58/J) strains of mice were similarly grouped into experimental and control sets. All the above experiments were done in duplicate.

Urinary protein concentrations were determined daily. After 3 weeks, the animals were anesthetized and blood was drawn from the abdominal aorta. Plasma protein fractions, cholesterol, and urea nitrogen were determined by following the methods of Lowry (11), Abell *et al.* (12), and Fawcett and Scott (13), respectively. The thoracic aorta was catheterized. The kidneys were flushed with Hank's balanced salt solution (290 mOsm/liter) for 1 min and perfused with 2.5% buffered glutaraldehyde (395 mOsm/liter) at a constant pressure of 120 mm Hg for 30 min at 4°. For scanning electron microscopy the kidneys were bisected and further fixed in the same fixative for 4 days. These were then washed with phosphate buffer and then dehydrated through a graduated series of acetone/water and dried in a Bomar critical-point dryer. The specimen was coated with carbon and recoated with gold–palladium alloy to a thickness of approximately 200 Å. A Cambridge stereoscan Mark IIA scanning electron microscope was used to examine the mouse renal glomerulus at an accelerating voltage of 20 kV for all specimens. Usually 18 to 20 glomeruli per mouse were examined. For transmission electron microscopy the renal tissue blocks were postfixed in buffered 2% osmium tetroxide for 1 hr, dehydrated, embedded in EPON, thin-sectioned, stained with uranyl acetate and lead citrate, and examined by light microscopy after treatment with various stains, e.g., hematoxylin–eosin, Masson's trichrome, periodic acid–Schiff, silver methenamine, and Lendrum's method for fibrin. In a separate group of experiments, the kidneys were removed under ether inhalation anesthesia, bisected, and one-half was immediately frozen in liquid nitrogen and saved for immunofluorescence microscopy. The sections were washed with phosphate-buffered saline (PBS) and treated for 30 min at 37° with fluorescein-conjugated IgG fractions of rabbit anti-mouse total globulins, transferrin, complement, albumin, and fibrinogen. The sections were washed with PBS and studied with a Zeiss fluorescence microscope.

Results. Biochemical and renal glomerular alterations were observed only in PRO/Re mice treated with PAN. The biochemical changes included hypoalbuminemia, hyper-

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alphaglobulinemia (Table I); hypercholesterolemia, azotemia (Table II); and proteinuria in excess of 50 mg/24 hr/100 g body weight of mouse (Fig. 1). Excess urinary proteins were composed mainly of albumin and α-globulin fractions. In essence, the changes were similar in nature to those observed in the nephrotic syndrome in man and in experimental nephrosis in rats and monkeys (1-3).

The glomeruli were hypercellular with increased mesangial matrices and occasional necrotic foci. The lumens of glomerular capillaries were obliterated either by eosinophilic hyaline-like material or partly by the expanded mesangium. This material gave the initial impression that it might be amyloid; however, Lendrum and immunofluorescent stains demonstrated its fibrinous nature.

TABLE I. PLASMA PROTEINS (g/100 ml).

	PRO/Re			C57BL/6J[b]				129ReJ[b]			
Strain[a]	Test[c]	Control[d]	P_1[e]	Test	Control	P_1	P_2[f]	Test	Control	P_1	P_2
Total protein	3.09 ± 0.52	6.60 ± 0.84	<0.001	5.62 ± 0.72	6.63 ± 0.40	NS[g]	NS	5.47 ± 0.18	5.98 ± 0.48	NS	NS
Albumin	1.20 ± 0.52	3.72 ± 0.26	<0.001	3.38 ± 0.67	4.22 ± 0.42	NS[g]	NS	3.46 ± 0.30	3.85 ± 0.45	NS	NS
α^1	1.11 ± 0.19	0.48 ± 0.19	<0.01	0.61 ± 0.21	0.77 ± 0.21	NS	NS	0.54 ± 0.07	0.52 ± 0.05	NS	NS
α^2	0.43 ± 0.13	0.50 ± 0.34	NS	0.40 ± 0.07	0.54 ± 0.53	NS	NS	0.31 ± 0.07	0.64 ± 0.33	NS	NS
β	0.30 ± 0.16	1.42 ± 0.63	<0.02	0.49 ± 0.08	0.54 ± 0.11	NS	<0.05	0.45 ± 0.21	0.57 ± 0.19	NS	<0.05
γ	0.05 ± 0.01	0.31 ± 0.16	<0.05	0.73 ± 0.20	0.48 ± 0.24	NS	NS	0.70 ± 0.18	0.50 ± 0.38	NS	NS

[a] The results obtained with C75BL/6J and 129ReJ were similar for test and control strains of AKR/J, BDP/J, C58/J, and CD1 mice when injected with puromycin and physiological saline, respectively.
[b] Each strain included two groups of animals, test and control.
[c] Test animals (four mice) received aminonucleoside of puromycin (2 mg/100 g body weight daily sc for 21 days).
[d] Control animals (four mice) received equivalent volume of normal saline sc for 21 days.
[e] P_1 value denotes the statistical difference between the individual test and control strains of mice.
[f] P_2 value denotes the statistical difference between different control strains of mice.
[g] NS, not significant.

TABLE II. PLASMA UREA NITROGEN AND PLASMA CHOLESTEROL (mg/100 ml).

Strain[a]	Test[b]	Control[c]	P_1[d]	P_2[e]
Urea nitrogen				
PRO/Re	212.50 ± 41.13	27.12 ± 1.79	<0.001	
C57BL/6J	29.87 ± 2.99	26.62 ± 1.25	NS[f]	NS
129ReJ	30.62 ± 2.25	30.50 ± 0.81	NS	NS
CD1	26.75 ± 2.17	30.12 ± 2.21	NS	NS
AKR/J	29.87 ± 2.98	31.25 ± 1.70	NS	NS
BDP/J	27.12 ± 1.88	29.00 ± 3.52	NS	NS
C58/J	29.16 ± 0.57	27.62 ± 1.31	NS	NS
Cholesterol				
PRO/Re	310.00 ± 23.80	71.25 ± 11.08	<0.001	
C57BL/6J	80.00 ± 9.12	73.75 ± 12.50	NS	NS
129ReJ	71.25 ± 4.78	82.50 ± 6.45	NS	NS
CD1	63.75 ± 2.50	75.00 ± 9.12	NS	NS
AKR/J	75.00 ± 9.12	82.50 ± 6.45	NS	NS
BDP/J	82.50 ± 6.45	78.75 ± 11.08	NS	NS
C58/J	76.25 ± 8.53	86.25 ± 2.50	NS	NS

[a] Each strain included two groups of animals, test and control.
[b] Test animals (four mice) received aminonucleoside of puromycin (2 mg/100 g body weight daily, sc for 21 days).
[c] Control animals (four mice) received equal volume of normal saline sc for 21 days.
[d] P_1 value denotes the statistical difference between individual test and control strains of mice.
[e] P_2 value denotes the statistical difference between different control strains of mice.
[f] NS, not significant.

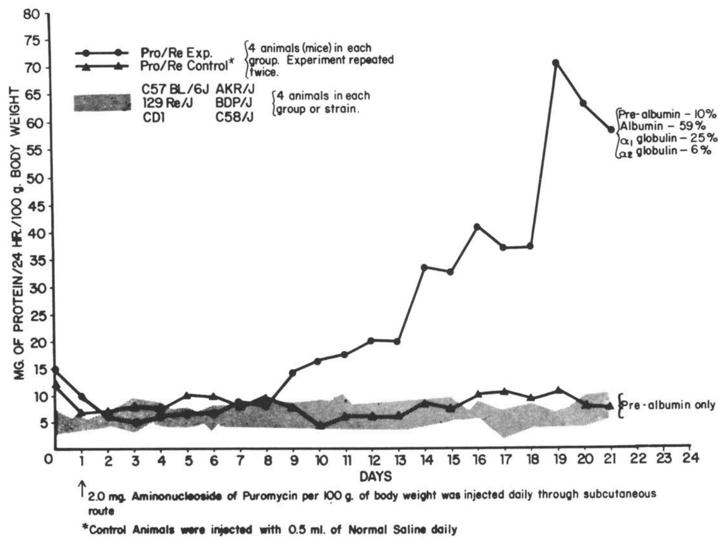

FIG. 1. Significant proteinuria is observed only in PRO/Re mice treated with PAN. The urinary proteins are composed mainly of albumin and α-globulins.

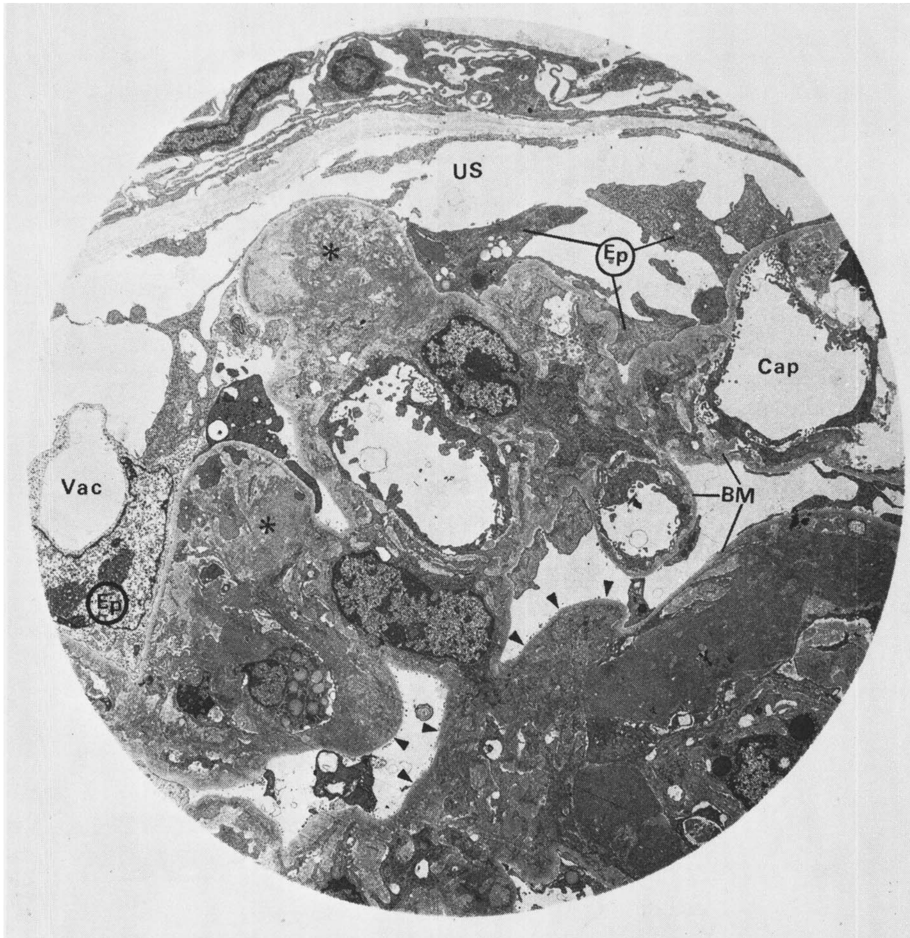

FIG. 2. Transmission electron micrograph of the glomerulus from the nephrotic PRO/Re mouse with subendothelial and endocapillary deposits (asterisks); a large vacuole (Vac) in the visceral epithelium (Ep); bare areas of the basement membrane (arrow heads). Capillary lumen (Cap); glomerular basement membrane (BM); urinary space (US). × 2000.

Ultrastructurally (Figs. 2 and 3), the visceral epithelial cells were swollen and revealed extensive approximation of foot processes. There was increased complexity of the cytoplasm with many intracytoplasmic vacuoles and percellular channel formation. Numerous osmiophilic droplets, an enormous increase in intracytoplasmic microfilaments, rough endoplasmic reticulum, and ribosomes were observed. Myelin figures were seen as well. Similar subcellular alterations were recognized in the parietal epithelium. Extensive but patchy subendothelial deposition of moderately electron-dense material, very often forming micronodular masses, was observed. The electron-dense material was either granular or amorphous. Many fibrils exhibiting an average axial periodicity of 220 Å with their approximate width of 140 Å (fibrin) were seen embedded in the substance of this material. Similar material was discovered in the mesangium and capillary lumens. The basement membrane was irregularly thickened, occasionally laminated, split, and had lipid droplets in it. Many segments of the basement membrane appeared bare and exposed to the urinary space. There was moderate increase in the rough endoplasmic reticulum of the mesangial and endothelial cells. The cytoplasmic processes of the mesangial cells were seen embedded in the rarefied suben-

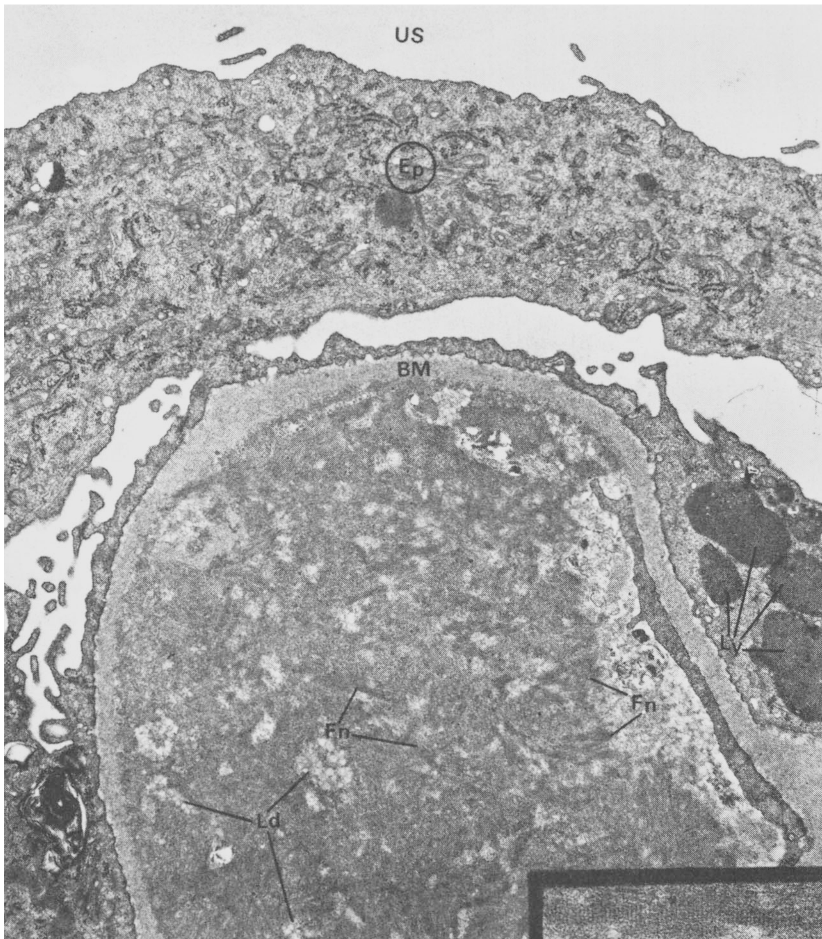

FIG. 3. Transmission electron micrograph of the glomerulus from the nephrotic PRO/Re mouse. The lumen of peripheral capillary loop is obliterated by deposition of moderately electron-dense material with entrapped fibrin (Fn) and lipid droplets (Ld). Many lysosomal bodies (Ly) are present in the cytoplasm of the visceral epithelial cell (Ep). × 8000. Inset showing higher magnification (× 18,000) of the fibrils of fibrin.

dothelial space and in the substance of electron-dense material. The tubular epithelium contained many osmiophilic lysosomal bodies and intracytoplasmic vacuoles. There was retraction of their interdigitating lateral processes.

On the other hand, in the parental and nonparental strains of mice treated with PAN, little more than minimal approximation of visceral epithelial foot processes was observed.

Stereoscopically (Figs. 4 and 5), the visceral epithelial cytoplasmic processes including first, second, and third orders were swollen. The first-order processes were drawn closer and seemed to be separated by irregular crevices. The terminal pedicles (foot processes) were approximated. In some glomeruli the podocytes (visceral epithelial cells) had smooth glazed appearances and cytoplasmic processes were no longer recognizable. A few glomeruli had bared areas of basement membrane with loss of podocytes and their cytoplasmic processes in these regions. There was marked reduction in the number of microvilli and cilia on the surface of visceral as well as parietal epithelial cells. The endothelium of glomerular capillary loops revealed focal loss of its network of pores and ridges.

Discussion. Type I hyperprolinemia in man is inherited as a recessive trait. It is sometimes associated with hereditary nephropathy which is inherited as a dominant trait (14). Hyperprolinemic PRO/Re mice with the same enzymatic defect as in the

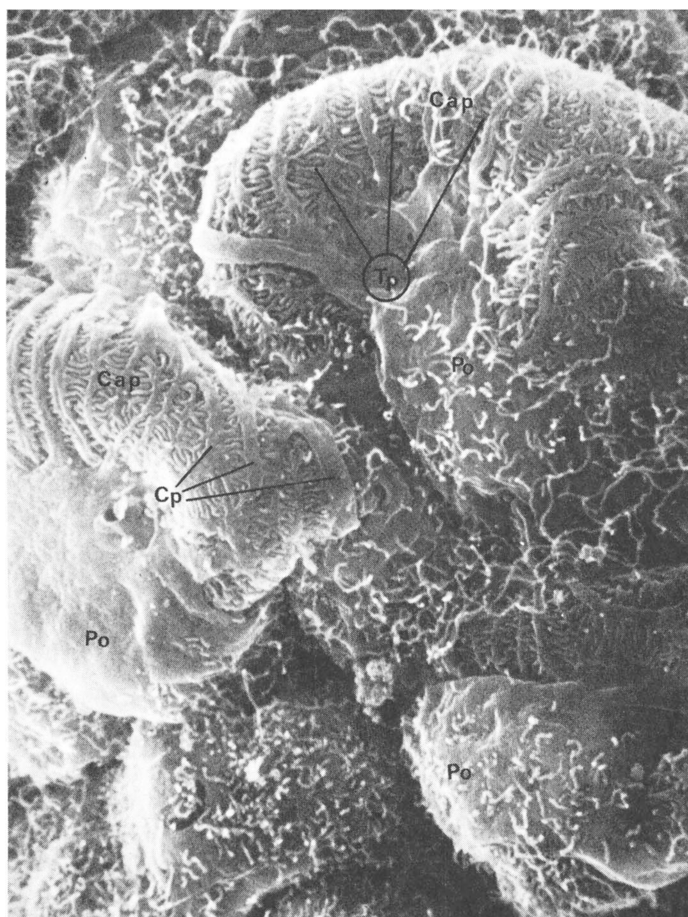

FIG. 4. Scanning electron micrograph of the glomerulus from an intact PRO/Re mouse; the inflated winding capillary loops (Cap) are covered by podocytes (Po) with their cytoplasmic processes (Cp) and terminal pedicles (Tp). The surfaces of the podocytes have numerous microvilli. × 3200.

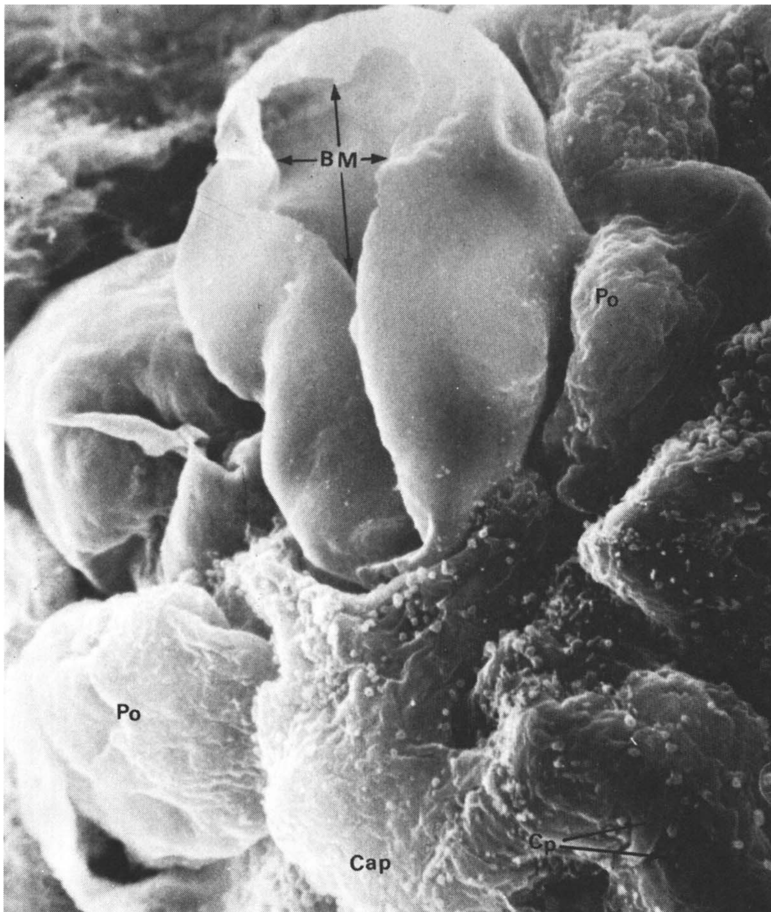

FIG. 5. Scanning electron micrograph of the glomerulus from the nephrotic PRO/Re mouse. In certain regions the podocytes (Po) have been sloughed from the capillary loops (Cap) exposing the surface of the glomerular basement membrane (BM). Terminal pedicles are no longer discernible. A few approximated cytoplasmic processes can be seen. There is a marked reduction in the number of microvilli. × 3200.

human Type I have not exhibited any distinctive renal abnormalities under basal conditions. However, it is of interest that when treated with PAN they develop nephrosis with striking renal changes. The parental strains of PRO/Re mice as well as nonparental strains are not responsive to PAN.

Numerous metabolic studies have been performed in an attempt to understand the basis for the nephrotoxicity of PAN in certain mammalian species and not in others. The results have not been conclusive (5, 6, 15, 16). PAN is demethylated *in vitro* to a monomethyl nucleoside by liver microsomal enzymes and is subsequently phosphorylated to its 5′-nucleotide (17). The demethylated and phosphorylated products of PAN have the same toxicity as PAN (5). In contrast to liver cells, PAN remains unchanged when incubated with renal cells (18). However, when renal cells are incubated with monomethyl nucleoside, they are able to phosphorylate and synthesize the 5′-nucleotide (18). Since the site of action of PAN has been localized in the kidney by renal transplantation (19), and PAN and its monomethyl nucleoside are rapidly excreted in the urine (5), it is therefore conceivable that in susceptible animals the 5′-nucleotide may be synthesized in greater amounts in the kidney and also retained for longer periods so as to induce nephrosis. This may be the case in susceptible PRO/Re strain mice. Alternatively, it may be that PRO/Re mice

lack an enzyme, possibly sharing the same genetic locus with proline oxidase, which in other strains of mice inactivates or catabolizes the active metabolites of PAN in the kidney.

The morphological changes induced by PAN in this strain resemble the changes observed in rats and monkeys (20–24). In addition, there are other distinctive features. In general, the lesions are more severe than those observed in other species. There is extensive but focal necrosis with sloughing and detachment of the visceral epithelial cells leaving behind bare areas of the glomerular basement membrane. Above all there is extensive fibrin deposition subendothelially with obliterative changes of glomerular capillaries. Renal lesions of this type bear some resemblance to certain thrombotic states such as thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome in man (25).

Summary. Administration of aminonucleoside of puromycin to PRO/Re strain mice resulted in biochemical and renal glomerular alterations. The biochemical changes included hypoalbuminemia, hyperalphaglobulinemia, hypercholesterolemia, and azotemia. Morphologically, there was approximation of visceral epithelial foot processes, intracytoplasmic vacuolization or percellular channel formation, and immense deposition of moderately electron-dense material in the subendothelial regions and lumens of glomerular capillaries. Attempts to induce similar changes in either of the parental and in other nonparental strains of mice of diverse genetic origins were unsuccessful.

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