Rat Glomerular Glycoprotein Composition and Metabolism in Aminonucleoside Nephrosis[1] (36737)

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The fundamental defect in human idiopathic nephrotic syndrome and aminonucleoside of puromycin (PA), (6 dimethyl-9-(3′-amino-3′-deoxy beta-D-ribofuranosyl-) purine, induced nephrotic syndrome in rats is presumed to be an increase in the permeability of the glomerular capillary filter (GCF) to plasma proteins. The GCF is composed of the glomerular basement membrane (GBM) and the contiguous glomerular epithelial cell membrane. GBM excludes large molecules such as ferritin from the urinary space (1) while the epithelial membrane excludes smaller molecules such as human myeloperoxidase (2). The precise chemical determinants of selective permeability of the GCF are unknown but are likely the sialoproteins of the epithelial cell membrane and GBM plus the highly cross-linked GBM collagen.

Previous studies have shown no significant changes in dilute acid solubility or amino acid composition of GBM from PA nephrotic rats; increased synthesis of GBM collagen was thought to be secondary to proteinuria (3). However, histochemical studies of the glomerulus in PA nephrotic rats have shown a striking decrease in staining for the glomerular polyanionic sialoprotein concomitant with the onset of significant proteinuria (4).

This study was designed to clarify and expand our histochemical observations by measuring the effect of PA on: (a) The carbohydrate composition of GBM (which consist of a glucose-galactose unit bound to hydroxylysine and a larger heterosaccharide unit linked to aspartic acid which contains glucosamine, galactose, mannose, fucose, and sialic acid) (5–7): (b) The glucosamine and sialic acid content of the whole glomerulus and glomerular cell membrane fractions, and (c) The glomerular metabolism of glucosamine and sialic acid.

Methods. Experimental design. The nephrotic syndrome was induced in 100 g, male Sprague-Dawley rats by either daily subcutaneous injections of 1.5 mg of PA for 10 days or by one intraperitoneal (ip) dose of PA[3] (15 mg). Animals were sacrificed 10–14 days after the start of PA injections at a time when marked proteinuria was present.

Another set of experiments was done to measure the effect of PA on glomerular sialoprotein metabolism. ^{14}C-1-D-glucosamine HCl (11.3 mCi/μmole)[4] was injected ip in a dose of 15 μCi/100 g as a precursor of *N*-acetyl glucosamine (GlcNAc) and sialic acid. In these experiments PA was always given ip. The isotope was given before and after PA as detailed below in Results, tables, and figures.

Twenty-four hour urinary excretion of protein was determined as previously described (3). Proteinuria was also estimated qualitatively by Albustix.[5]

Tissue preparation. Glomeruli were isolated using monel sieves, and GBM was prepared by sonicating glomeruli in 1.0 *M* sodium chloride and low speed (121*g*) centrifugation as described by Blau and Michael (3). Glomerular fractions were prepared from the supernatant remaining after the first 121*g* cen-

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trifugation. This material was centrifuged again at 121*g* for 15 min and any sediment discarded. The supernatant was centrifuged at 12,000*g* for 20 min at 4°. The 12,000*g* sediment was washed four times with distilled water, centrifuged each time at 12,000*g* for 5 min and lyophilized. The supernatant remaining was centrifuged at 188,000*g* for 60 min at 4°. The sediment was gently washed 4 times in distilled water, centrifuged each time at 188,000*g* and then lyophilized. The supernatant was dialyzed against distilled water for 4 days at 4° with frequent changes of water and lyophilized. Electron microscopy of these fractions carried out as previously described (5) revealed that the 121*g* sediment contained mostly GBM; smaller membrane vesicles were rarely seen but the spaces between GBM fragments often contained cellular debris. This debris may be derived from cells or from aggregated "fibrilles" of GBM. The 12,000*g* sediment and 188,000*g* sediment contained membranous vesicles but there was less debris in the latter than the former. Neither the 12,000*g* sediment nor the 188,000*g* sediment contained visible GBM fragments.

In each experiment GBM and glomerular fractions were isolated from the pooled kidneys of 25–50 rats and glomeruli from the pooled kidneys of 2–10 rats.

Biochemical methods. Sialic acid was measured by the thiobarbituric acid method of Warren (8) after hydrolysis in 0.1 *N* sulfuric acid at 80° for 1 hr. Under these conditions the recovery of sialic acid added to isolated glomerular preparations prior to hydrolysis was 90%. Preliminary experiments demonstrated that after GBM was hydrolyzed for 30, 60 or 120 min the maximal release of sialic acid occurred at 60 min. *N*-Acetyl neuraminic acid[6] was the standard.

Total hexosamine was measured by Ruinen, Scholten, and Mandema's (9) modification of the method of Cessi and Pilego. Hydrolysis took place in 1.5 ml of 6.0 *N* hydrochloric acid at 95° for 4 hr, under nitrogen. The total hydrolysate was analyzed after neutralization with sodium hydroxide. Recovery of standard D-glucosamine HCl[6] carried through the procedure was 92–98%.

The specific activity of sialic acid was measured after hydrolysis, as described above, by passing the filtered hydrolysate through a 2.5 × 1.5 cm column of Dowex 2x-8 (200–400 mesh) equilibrated with 0.1 *N* acetic acid. The column was rinsed with 15 ml of water, and the sialic acid eluted with 17 ml of 1.0 *M* acetate buffer (pH 4.60) (10). The buffer eluate was passed onto a 5 × 1.5 cm column of Dowex 50 WX-8 (100–200 mesh), equilibrated with 1.0 *N* hydrochloric acid, collected in boiling flasks, evaporated to dryness at 40° and made up with water. Aliquots were taken for counting in a Beckman LS-250 scintillation counter (11) and for the thiobarbituric color reaction. Since the recoveries of sialic acid carried through the entire procedure ranged from 65–90%, this method was used only for determination of specific activity.

The specific activity of *N*-acetyl glucosamine (GlcNAc) was determined by the method of Spiro and Spiro (12). The Morgan-Elson color reaction was used (13) and aliquots counted as above. GlcNAc was the standard. Recovery of glucosamine as GlcNAc ranged from 92 to 95%.

Hydroxyproline and hydroxylysine were measured as previously described after tissue hydrolysis in 6.0 *N* hydrochloric acid for 18 hr at 105°, under nitrogen (3). Glucose and galactose were determined by hydrolysis of tissue in 2.0 *N* hydrochloric acid for 2 hr at 100°. The hydrolysate was neutralized with 2.0 *N* NaOH, passed through a mixed column (0.9 × 9.0 cm) containing Dowex 50 WX-8 200–400 mesh (H^+ form) and Amberlite IR-45 (OH^- form), and eluted with water (14). The first 20 ml were collected, lyophilized, made up with water or buffer and aliquots used for glucose and galactose determination using specific enzyme coupled colorimetric reactions [glucose oxidase (15) and galactose oxidase (16)] or applied to a Technicon automatic carbohydrate analyzer for the determination of glucose, galactose and mannose. Both methods gave similar results for glucose and galactose content.

[6] Sigma Chemical Company, S. Louis, MO.

TABLE I. Sialic Acid and Hexosamine Concentration of Glomeruli and Glomerular Fractions.[a]

		Sialic acid			Hexosamine			Molar ratio[d]		
		No.[b]	μg/mg[c]	p	No.	μg/mg	p	No.	Ratio	p
Glomeruli	C	26	7.44 ±0.20		15	9.27 ±0.43		13	0.542 ±0.036	
	PA	12	6.73 ±0.66	<.01	8	9.27 ±0.36	>.5	8	0.520 ±0.024	>.1
GBM	C	20	8.74 ±0.46		10	12.8 ±0.68		10	0.417 ±0.023	
	PA	14	6.90 ±0.34	<.005	13	12.1 ±0.52	>.2	13	0.372 ±0.028	<.2
12,000g (sed.)	C	7	11.8 ±0.82		5	8.01 ±0.26		5	1.02 ±0.090	
	PA	7	6.38 ±0.55	<.005	5	8.17 ±0.53	>.5	5	0.609 ±0.072	<.005

[a] Sialic acid and hexosamine concentration of glomeruli, glomerular basement membrane (GBM) and 12,000g (sed.) of sonicated glomeruli after removal of GBM at 121g. In each experiment tissue was obtained from the pooled kidneys of 25 to 50 control and aminonucleoside (PA) treated rats 10 to 14 days after the first PA injection. All PA treated rats had heavy proteinuria at the time of sacrifice.

[b] Number of experiments.

[c] Expressed as mean ± SE.

[d] Molar ratio of sialic acid to hexosamine.

All results are reported as mean and standard error of the mean. p values were calculated using the Student's t test. Slopes were drawn by inspection and by the method of least squares. All determinations were done on dry, lyophilized material. Results are all reported as dry weight.

Results. Glomerular carbohydrate composition. Total sialic acid concentration of whole glomeruli, GBM, and 12,000g sediment from PA nephrotic rats was significantly lower than that of the respective control tissue (Table I). This decrease in sialic acid concentration was most evident in the 12,000g

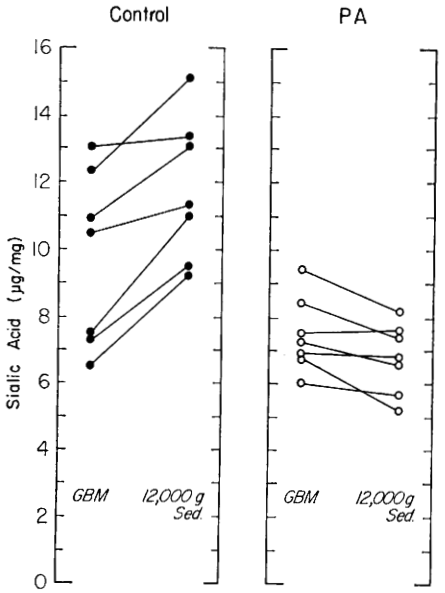


FIG. 1. Comparison of the sialic acid content of glomerular basement membrane (GBM) with that of the 12,000g sediment (sed.) in aminonucleoside (PA) nephrotic and control rats. Each line connects the value for sialic acid concentration of GBM to that of the 12,000g sed. obtained from the same pool of sonicated glomeruli from 25 to 50 rats. In control animals, the ratio of sialic acid concentration in GBM to that of the 12,000g sed. is always less than unity (0.817 ± 0.041) whereas in PA animals this ratio is greater than unity (1.11 ± 0.037) ($p < .005$). The sialic acid concentration in the 12,000g sed. from PA animals is significantly less than that of control animals (Table I). The sialic acid concentration of the GBM preparations from PA rats 7.44 ± 1.00 μg/mg is not significantly less than control values 9.76 ± .01 μg/mg ($p < .1$).

TABLE II. Glucose, Galactose, and Mannose Content of Glomerular Basement Membrane.[a]

	Control		PA		
	No. of expts.	μg/mg (mean ± SE)	No. of expts.	μg/mg (Mean ± SE)	p
Glucose	15	15.3 ±0.72	8	14.1 ±1.26	>.4
Galactose	15	16.2 ±0.90	8	14.4 ±1.26	>.2
Mannose	8	4.68 ±0.36	4	5.04 ±0.36	>.5
Glc/Gal[b]	15	0.942 ±0.042	8	0.981 ±0.051	>.5
Glc/OH–Ly[b]	7	0.824 ±0.036	4	0.754 ±0.047	>.5

[a] In each experiment glomerular basement membrane was obtained from the pooled kidneys of 25 to 50 rats. All aminonucleoside (PA) treated rats had heavy proteinuria at the time of sacrifice.

[b] Molar ratio.

sediment (with a range of 5.18 to 8.10 μg/mg in PA rats and 9.26 to 15.2 μg/mg in control animals). The differences in the sialic acid content of whole glomeruli is the most difficult to interpret because of considerable overlap between control and PA values. The mean sialic acid concentrations in glomeruli, GBM and 12,000*g* sediment from PA rats were similar. In control animals, however, the sialic acid content of GBM (8.74 ± 0.46 μg/mg) was significantly greater than that of glomeruli (7.44 ± 0.20 μg/mg); and the concentration of sialic acid in the 12,000*g* sediment (11.8 ± 0.82 μg/mg), was significantly greater than that of GBM ($p < .005$ for both). The concentration of sialic acid in the 12,000*g* sediment from glomeruli of nephrotic rats was similar or slightly lower than the concentration of sialic acid in the corresponding GBM, whereas the converse was found in control animals (Fig. 1). In the large number of experiments noted in Table I, there was a significant decrease in sialic acid concentration of GBM from PA rats when compared with control values; however, in the smaller number of experiments described in Fig. 1, no significant differences were seen—a finding probably related to the wide variability of the individual values of sialic acid. It is also possible that the decrease in sialic content of GBM could reflect contamination of GBM with 12,000*g* sediment membranes.

In contradistinction to the decrease in sialic acid content, there were no differences in the total hexosamine concentration of glomeruli, GBM, or 12,000*g* sediment from control and PA rats (Table I). The molar ratio of sialic acid to hexosamine in the 12,000*g* sediment from control rats was close to unity, 1.02 ± 0.09, while in PA nephrotic rats it was 0.609 ± 0.072 ($p < .005$). This ratio calculated for glomeruli and GBM was similar in control and PA treated rats ($p > .1$), a finding that appears to be a variance with the reduced values of sialic acid and probably reflects the wide variability in the individual sialic acid values (Table I). Four 12,000*g* sediment preparations from control rats had a mean hydroxyproline content of 3.05 μg/mg (range 2.35–4.56 μg/mg) while 4 preparations from PA rats had a mean hydroxyproline content of 4.07 μg/mg (range 2.56–5.75 μg/mg).

The glucose, galactose and mannose content of GBM from control and PA rats were not significantly different nor were there any apparent differences in the molar ratios of glucose to galactose or glucose to hydroxylysine in GBM (Table II).

Glomerular glycoprotein metabolism. ^{14}C-Glucosamine was given to control and PA rats by ip injection 2 days after a single ip injection of PA; increased urinary protein excretion developed in 25% of the rats between the third and fourth days after PA administration and in 100% by day 6. The animals were followed for 13 days after administration of the isotope and sacrificed at intervals from 6 hr to 13 days (Fig. 2A).

The specific activity of glomerular sialic acid from PA rats was greater than values obtained from control animals from days 1 to

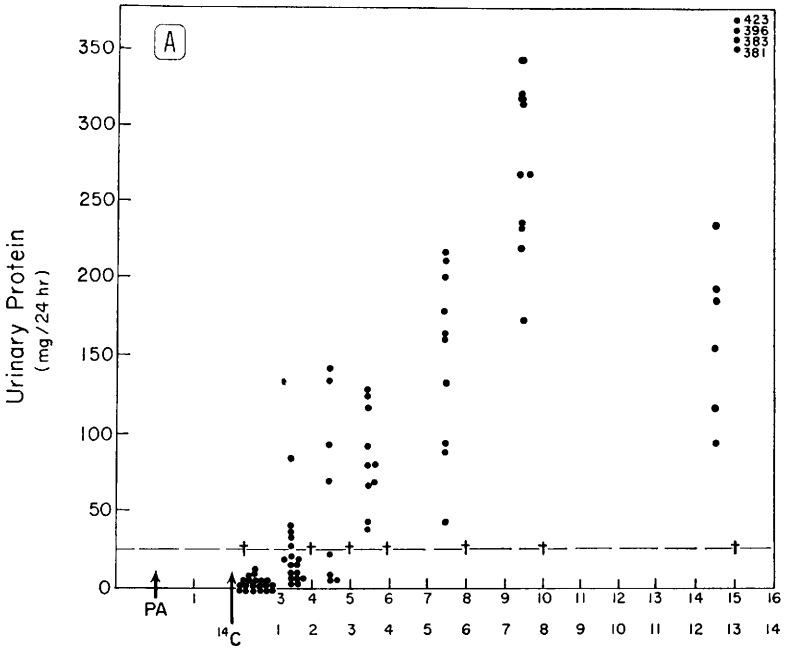


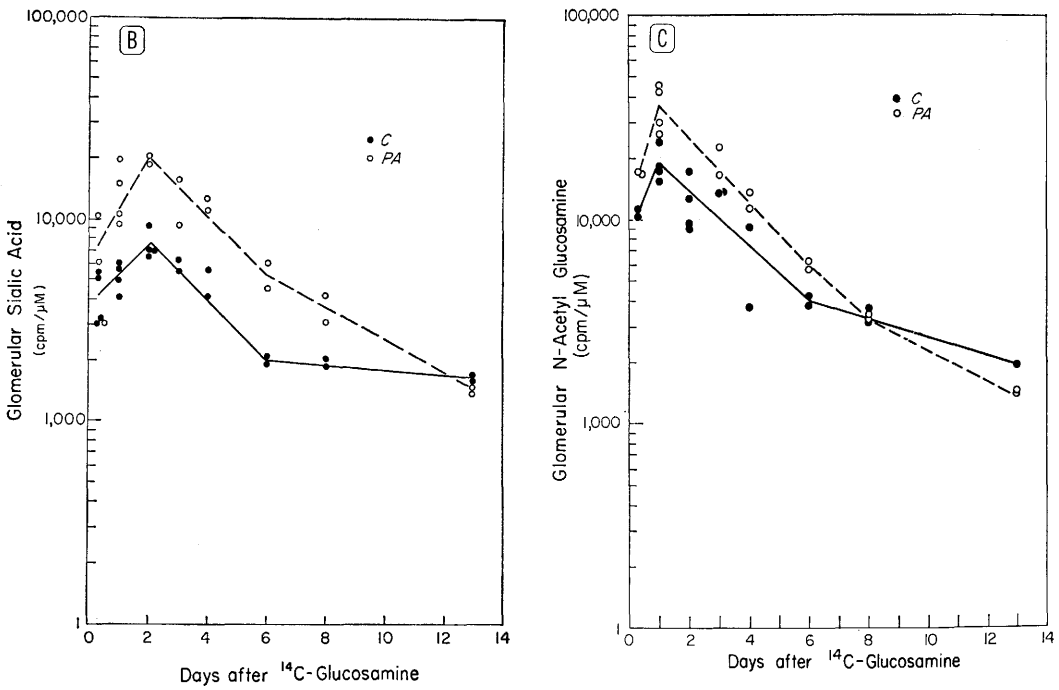
FIG. 2A. Experimental design and 24 hr urinary protein excretion. The top row of numbers on the abscissa refer to the number of days after an intraperitoneal injection of PA (in a dose of 15 mg/100 g). The bottom row of numbers refer to the number of days after the intraperitoneal administration of (^{14}C)-glucosamine (15 μCi/100 g). Animals were sacrificed at the intervals indicated by +. The upper limit of 24 hr urinary protein excretion in control rats is indicated by the broken line. The individual values for protein excretion of the PA rats are as indicated (•).

8 after glucosamine administration; there were no differences at 6 hr or 13 days (Fig. 2B). In control glomerular preparations the falloff in specific activity of sialic acid seemed to be biphasic with a rapid phase from days 2 to 6 with an estimated half-life of 2 days. In PA rats the falloff was not so clearly biphasic; the falloff during the first phase was similar to the control rats, but the half-life in the period from days 6 to 13 was 4 days compared to the estimated half-life of 20 days in the control rats.

The specific activity of glomerular GlcNAc from control and PA rats reached a maximum at day 1 and although the values for PA rats were greater than those from control rats until day 8 after administration of isotope, the rates of falloff appeared to be similar. The estimated half-life of the first phase of the slope was 2 days for control and PA values (Fig. 2C).

The specific activity ratios of sialic acid to GlcNAc were consistently greater in glomeruli from PA rats from days 3 to 13. In PA rats, this ratio approached unity at day 4 while this did not occur in control rats until day 13 (Fig. 3).

Since the previous experiment had shown an increase in specific activity of sialic acid of whole glomeruli in PA rats 3 days after administration of PA, an experiment was carried out to determine the effect of PA on sialoprotein metabolism of various glomerular fractions. ^{14}C-Glucosamine was given 2 days after administration of PA, as in the previous experiment, and the animals were sacrificed 24 hr later at a time when significant proteinuria was not present. The specific activity of sialic acid in all glomerular fractions from PA rats was two to five times greater than control values with the greatest increase occurring in the 188,000*g* sediment (Fig. 4). An increase was also observed in the specific activity of GlcNAc of all four glomerular fractions. Excepting the GBM fraction, the ratio of specific activity of sialic acid to spe-



(B). Specific activity of glomerular sialic acid. Each value was obtained from analysis of pooled glomeruli from the kidneys of 5 rats. Lines were drawn by inspection. (C). Specific activity of glomerular *N*-acetyl glucosamine. Each value was obtained from analysis of pooled glomeruli from the kidneys of 5 rats. Lines were drawn by inspection.

cific activity of GlcNAc was larger in glomerular fractions from PA rats than in control animals. In both groups of rats, only the GBM fraction had a ratio consistently greater than 1 (Table III).

The concentration of sialic acid in GBM (6.60 ± 0.50 μg/mg), and 12,000*g* sediment (7.78 ± 0.58 μg/mg) from these nonnephrotic rats was lower than values obtained from the respective control tissue (8.34 ± 0.50 μg/mg) and (10.5 ± 0.84 μg/mg) ($p < .05$ for both). In the 188,000*g* sediment from control and PA rats the concentration of sialic acid was similar 10.2 (± 0.50) and 8.08 (± 1.06) μg/mg ($0.5 < p < .1$).

Discussion. The precise mechanism of increased permeability of the GCF to plasma proteins in human nephrotic syndrome and that induced experimentally in animals is unknown. Data in the present study demonstrate a significant decrease in sialic acid concentration of glomeruli, GBM, and the 12,000*g* sediment of sonicated glomeruli from PA nephrotic rats—the only consistent biochemical change we have found to be associated with PA induced proteinuria.

The most significant decrease in sialic acid concentration was found in the 12,000*g* sediment of sonicated glomeruli from PA rats. Because the glomeruli were disrupted by ultrasonic forces, it is not possible to state with certainty the origin of the cell membrane components present in this fraction. Electron microscopy of sonicated membrane fractions revealed primarily GBM in the 121*g* sediment. With increasing gravity forces, membrane vesicles of smaller size were sedimented but no visible GBM fragments were detected in the 12,000*g* sediment. However, the demonstration of very low concentrations of hydroxyproline in this fraction suggests the presence of some collagen or GBM components. Michael, Blau, Vernier (4) have shown that colloidal iron and alcian blue staining for the negatively charged sialoprotein lining the epithelial cell adjacent to the

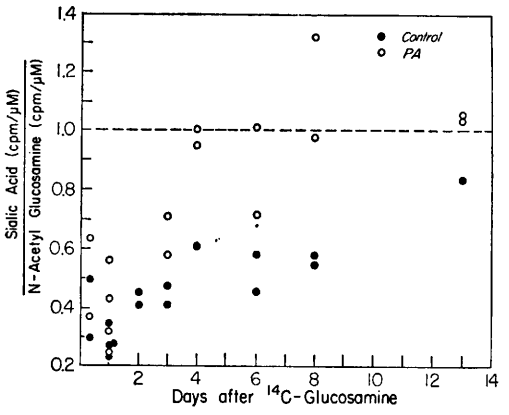


FIG. 3. Ratio of specific activity of sialic acid to specific activity of *N*-acetyl glucosamine. (^{14}C)-Glucosamine was given 2 days after a single intraperitoneal injection of PA and the animals sacrificed at the intervals noted above. Each point represents analysis of glomeruli obtained from the pooled kidneys of 5 rats.

GBM is markedly reduced in PA nephrosis. In addition, membrane fractions of sonicated glomeruli sedimented at 12,000 to 188,000*g* are colloidal iron positive (E. B. Blau and A. F. Michael, unpublished data), suggesting that these fractions contain surface membranes of the epithelial cell. It is possible that the highly significant decrease in sialic acid concentration in the 12,000*g* sediment is responsible for these histochemical observations. A decrease in the electrophoretic mobility of GBM from nephrotoxic nephrotic rats has been reported (17). This finding could represent a decrease in sialic acid concentration of the GBM since human white blood cells treated with neuraminidase have been shown to have decreased mobility in an electrophoretic field (18).

Fixed or protein bound polyanions are known to be essential in maintaining the ability of gel systems to exclude molecules (19, 20) and a decrease in glomerular membrane sialic acid would lower the net negative surface charge and thus hinder the membrane capacity to function as an effective selective filter.

The hexosamine and neutral sugar content of GBM and the hexosamine content of the 12,000*g* sediment from PA rats were similar to values found in control tissue. The molar ratios of glucose to galactose and glucose to hydroxylysine in GBM were similar in control and PA preparations and correlate well with previously reported data using GBM from other species (5–7). Our results are at variance with a recent report by Kefalides and Forsell-Knott (21) showing that GBM from nephrotic rats contains a glycopeptide with a ratio of glucose:galactose:hydroxylysine of 2:1:1.

We have found a concentration of sialic acid in GBM from control rats similar to that previously reported for GBM from other species and the molar ratio of sialic acid to hexosamine in GBM from control rats is similar to that reported by Spiro (6) and by Westberg and Michael (5) for bovine and human GBM, respectively. Colloidal iron does not significantly stain the GBM (4, 22, 23) and it has been suggested that the sialic acid found in GBM by biochemical methods represents contamination of GBM by other cellular components (24). The inability to stain the GBM with colloidal iron may result from the lower concentration of sialic acid in GBM than in sonicated cell membrane preparations or, more likely, that the sialic acid in this locus is sterically hindered from reacting.

Studies of glomerular glycoprotein metabolism were carried out by administration of ^{14}C-glucosamine as a precursor of GlcNAc and sialic acid. PA was given two days before administration of ^{14}C-glucosamine and the metabolism of glomerular sialic acid in PA rats differed from control animals in two ways. First the specific activity of sialic acid and GlcNAc from PA rats was greater than control values throughout most of the experiment; this increase in specific activity antedated the onset of significant proteinuria. Secondly, from 6 to 13 days after administration of isotope, the falloff of glomerular sialic acid from PA rats was increased ($t_{½}$ 4 days) over the rate of falloff observed in glomeruli from control rats ($t_{½}$ 20 days). This increase in specific activity of sialic acid and GlcNAc was seen before the onset of significant proteinuria in all glomerular frac-

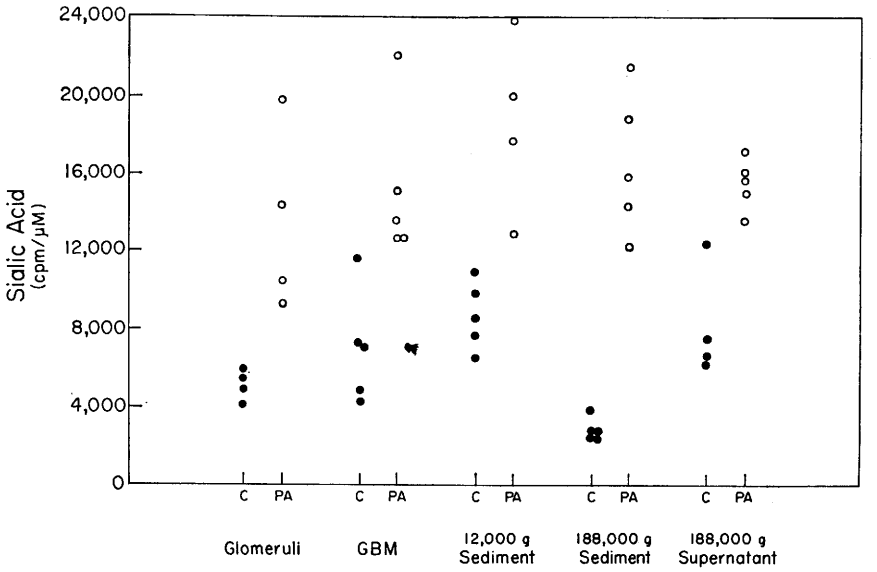


FIG. 4. Specific activity of sialic acid in glomeruli and fractions of sonicated glomeruli. (^{14}C)-Glucosamine was given 2 days intraperitoneal administration of PA and the animals sacrificed 24 hr later. Each point is described from analyses of pooled kidneys from 25 rats for glomerular fractions and 5 rats for whole glomeruli. See Table III for specific activity of *N*-acetyl glucosamine.

tions from PA rats—including GBM, membranous, and supernatant fractions.

A decrease in sialic acid pool size in the glomerular fractions from PA rats could account for the increase in specific activity since the sialic acid content of GBM and 12,000*g* sediment was significantly decreased although not nearly of the same order of magnitude as the change in specific activity. In addition, a marked increase in specific activity was seen in the 188,000*g* sediment unassociated with any significant change in sialic acid concentration. A more likely explanation of the data is that the synthetic rate

TABLE III. Specific Activity and Ratios of Sialic Acid and *N*-Acetyl Glucosamine (GlcNAc) in Glomerular Fractions from Control and PA Rats.[a]

Glomerular fractions		No. of expts.	GlcNAc[b] (mean and range)		Sp act sialic acid / sp act GlcNAc	
GBM	Control	4	4169	(3819–4665)	1.39	(1.08–1.69)
	PA	2	11,954	(10,795–13,114)	1.20	(1.15–1.26)
12,000*g* sed.	Control	4	12,118	(10,531–14,765)	0.771	(0.684–0.936)
	PA	3	20,058	(13,207–26,707)	0.917	(0.881–0.974)
188,000*g* sed.	Control	5	10,966	(8648–16,393)	0.281	(0.157–0.441)
	PA	3	26,019	(21,727–29,071)	0.557	(0.449–0.730)
188,000*g* super.	Control	3	12,916	(10,691–15,848)	0.527	(0.476–0.574)
	PA	1	19,128		0.780	

[a] (^{14}C)-Glucosamine was given 2 days after PA and the rats sacrificed 24 hr later. In each experiment glomerular basement membrane (GBM), 12,000*g* sediment (sed.), 188,000*g* supernatant (super.) were obtained from sonicated glomeruli using pooled kidneys of 25 rats. Values for SA of sialic acid are noted in Fig. 4.

[b] Cpm/μM.

of sialic acid is increased coupled with an increased rate of degradation. This explanation takes into account the increase in specific activity, the decrease in half-life and the decrease in the absolute amount of sialic acid. However, the exact mechanism by which PA induces changes in glomerular sialic acid content and metabolism remain unknown but it is likely that these changes are responsible for PA induced proteinuria.

Summary. In aminonucleoside nephrotic rats, the sialic acid concentration of glomerular membrane fractions were significantly less than values obtained from control preparations. No differences were detected in the concentrations of glucose, galactose, mannose, or hexosamine. The administration of ^{14}C-glucosamine as precursor of sialic acid, 2 days after giving aminonucleoside, was followed by a significantly greater increase in specific activity of glomerular sialic acid over the subsequent 1–8 days; the falloff in specific activity from days 6 to 13 had a half-life of 4 days compared with 20 days in control animals. The specific activity of sialic acid in glomerular basement and cellular membrane fractions 24 hr after administration of ^{14}C-glucosamine was 2–5 times greater in animals given aminonucleoside than in control rats. This increase in synthesis may be a consequence of increased degradation or turnover of glomerular membrane sialoprotein.

1. Farquhar, M. G., and Palade, G. E., J. Exp. Med. **114**, 699 (1969).

2. Graham, R. C., and Karnovsky, M. J., J. Exp. Med. **124**, 1123 (1966).

3. Blau, E. B., and Michael, A. F., J. Lab. Clin. Med. **77**, 97 (1971).

4. Michael, A. F., Blau, E. B., and Vernier, R. L., Lab. Invest. **23**, 649 (1970).

5. Westberg, N. G., and Michael, A. F., Biochemistry **9**, 3837 (1970).

6. Spiro, R. G., J. Biol. Chem. **242**, 1915 (1967).

7. Kefalides, N. A., and Winzler, R. J., Biochem. **5**, 702 (1966).

8. Warren, L., J. Biol. Chem. **234**, 1971 (1959).

9. Ruinen, L., Scholten, J. H., and Mandema, E., Clin. Chim. Acta **19**, 49 (1968).

10. Svennerholm, L., Acta Chem. Scand. **12**, 547 (1958).

11. Carter, G. W., and Van Dyke, K., Clin. Chem. **17**, 576 (1971).

12. Spiro, R. G., and Spiro, M. J., J. Biol. Chem. **241**, 1271 (1966).

13. Neuberger, A., and Marshall, R. D., *in* "Glycoproteins" (A. Gottschalk, ed.), p. 227. Elsevier, New York (1966).

14. Tengstrom, B., Scand. J. Clin. Lab. Invest. **18**, (Suppl. **92**, 104) (1966).

15. Hsia, D. Y., and Inouye, *in* "Inborn Errors of Metabolism," Part 2, p. 117. Year Book Med. Pub., Chicago (1966).

16. Hjelm, M., Clin. Chim. Acta **15**, 87 (1967).

17. Kalant, N., Misra, R. P., Manley, R. St. J., and Wilson, J., Nephron **3**, 167 (1966).

18. Lichtman, M. A., and Weid, R. I., Blood **35**, 12 (1970).

19. Laurent, T. C., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **25**, 1128 (1966).

20. Larsen, B., Nature (London) **215**, 641 (1967).

21. Kefalides, N. A., and Forsell-Knott, L., Biochim. Biophys. Acta **203**, 62 (1970).

22. Jones, D. B., Lab. Invest. **21**, 119 (1969).

23. Mohos, S. C., and Skoza, L., Science **164**, 1519 (1969).

24. Mohos, S. C., and Skoza, L., J. Cell Biol. **45**, 450 (1970).

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