Table 3. Strontium-90 and stable strontium concentration factors (CF) of aquatic plants from eutrophic and mesotrophic-eutrophic lakes during September 1966 and 1967.

Plant	Mean concentration factors	
	Wet weight	Ash weight
Chara sp.	276 ± 20*	2,867 ± 604
Equisetum sp.	75 ± 33	2,053 ± 601
Scirpus lacustris	24 ± 6	1,350 ± 740
Myriophyllum sp.	63 ± 19	3,294 ± 1,185
Stratiotes aloides	96 ± 35	3,677 ± 1,005
Elodea canadensis†		3,225 ± 2,098
Ratio $CF_{max} : CF_{min}$	>10	<3

* Ninety-five percent confidence limits. † Eutrophic lake only.

Table 4. Strontium-90 concentration factors for different lake substrates during September 1966.

Substrate	Concentration factors	
	Ash weight	Dry weight
Mesotrophic-eutrophic		
Silt	33–51	31–50
Sand with salt	28	26
Sand	11–14	10–14
Eutrophic		
Silt	54–90	50–83
Silt with sand	38	29
Dystrophic		
Peat	1,097–6,386	274–1,250
Peat with sand	2,536–12,691	1,725–2,067
Sand with peat	441–528	412–493
Sand with silt and peat	956	825

mixture of acetylene with air at the wavelength of strontium. Ninety-five percent confidence intervals for means were calculated where sample numbers permitted.

Concentration factors of ^{90}Sr for plankton of eutrophic (Table 1) and mesotrophic-eutrophic (*10*) lakes are similar, but are greatly different from those for plankton of a dystrophic lake (Table 2). This difference may be explained (*11*) in the following way: concentrations of carriers in samples from the dystrophic lake (1966—Ca, 3.6 to 6.1 mg/liter; 1967—Ca, 4.7 to 5.9 mg/liter, and Sr, 13.6 to 43 μg/liter) are much less than those for samples of the eutrophic lake (1966—Ca, 35.3 to 40.6 mg/liter, and Sr, 71 μg/liter; 1967—Ca, 43.8 to 46.3 mg/liter, and Sr, 67.6 to 80.6 μg/liter) and the mesotrophic-eutrophic lake (1966—Ca, 36.1 to 38.9 mg/liter).

The ^{90}Sr CF for water plants are also higher in the dystrophic lake than in the other lake types (Table 3). The ^{90}Sr CF's for water plants are less in spring than in autumn (3 to 7 times, calculating on the basis of wet weight). The ^{90}Sr CF are highest in the upper part of *Potamogeton lucens* (100 percent), average in the central part (50 percent), and least in the lower parts (including roots) (28 percent). These differences are noticed in autumn as well as in spring. The CF of stable Sr and ^{90}Sr are close—the mean ratio of Sr CF to ^{90}Sr CF in each sample of 9 species of plants equals to 1.1 ± 0.2. This means that complete exchange occurred between radioactive and stable Sr in lake plants.

Among lake substrates, sand substrates have the least ^{90}Sr CF (Table 4). The higher CF of sand substrates are a result of the admixture of silt and peat. The highest ^{90}Sr CF are of peat substrates from the dystrophic lake (Table 4). Concentration factors for ashed plankton in the eutrophic lake are significantly higher than those for ashed lake silt, but CF for ashed plankton and peat in the dystrophic lake are approximately equal.

Thus hydrobionts and substrates of a dystrophic lake are characterized by ^{90}Sr CF higher than those for mesotrophic-eutrophic and eutrophic lakes.

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Glomerular Sialoprotein

Abstract. *The high content of sialic acid in the glomerulus is associated with the cell membrane of epithelial cells lining the basement membrane. Whereas enzyme studies indicate that sialic acid is a determinant of the nephritogenic antigen, the physicochemical properties of this nephritogenic glycoprotein suggest that sialic acid may have an important role in the filtration mechanism.*

Although sialic acid is present in glomerular preparations (*1*), its exact localization has not been established and its significance has not been defined. We have noted that a high concentration of sialic acid is located in the cell membranes lining the basement membrane. This sialic acid can be neutralized by polycations and by antiserums to kidney tissue, and it can be removed by neuraminidase and trypsin; these reduce or abolish the nephritogenic antigenicity of the glomerulus.

The presence of acidic mucopolysaccharide in the glomerulus was shown (*2*) by the colloidal iron reaction, but the acidic component was not identified. Spiro (*1*) has provided evidence that "preparations of basement membrane" obtained by ultrasonic treatment of glomeruli contain two distinct carbohydrate units in approximately equal proportions: (i) a disaccharide unit containing glucose and galactose and (ii) an oligosaccharide consisting of galactose, mannose, hexosamine, fucose, and sialic acid. In view of the similarity of this oligosaccharide to that of the antigenic blood group substances

we have made a histochemical study of the glomerulus and directed it toward identification and localization of sialic acid. We used the colloidal iron technique in combination with neuraminidase treatment and subsequently examined the tissue by light and electron microscopy. Human, mouse, beef, rabbit, and guinea pig kidneys were used. At *p*H 2 the colloidal iron stain gives a positive reaction with sialic acid (*3*). Under these conditions a striking blue coloration of the glomeruli was found with light microscopy. For ultrastructural localization, paraffin-embedded sections (4 μ thick) which had been rehydrated, were placed on glass slides, treated with colloidal iron, and embedded in Epon. This technique allowed free access of colloidal iron to any part of the glomerulus. After polymerization, the blocks were removed from the slides by submerging them in liquid nitrogen. These blocks were cut into ultrathin sections (500 Å) and observed with an Elmiskop 1A before and after being stained by uranyl acetate. The colloidal iron deposit was heaviest on the epithelial cell membrane including the foot processes. The anchored portion of the foot processes also showed colloidal iron deposits, but of lesser concentration. On no occasion was any iron deposited in the basement membrane. The endothelial cells also showed localization of colloidal iron at their cell membranes but it was less intense than that in epithelial cells. The erythrocyte membranes were outlined by a discontinuous faint iron deposit. (Fig. 1, A and B). Ligand forming agents, such as adenosine triphosphate or potassium thiocyanate, in 0.1 percent concentrations, used in combination with the colloidal iron technique, enhanced the deposition of iron, though the basement membrane itself remained unstained.

Localization of the iron as judged by light and electron microscopy could be prevented by prior treatment of the sections with *Vibrio cholerae* neuraminidase (100 unit/ml) in 0.1*M* sodium acetate buffer, *p*H 5.5, containing 0.005*M* calcium acetate, for 5 hours at 37°C. This finding (Fig. 1, C and D) was unequivocal confirmation of the association of iron staining with sialic acid, in that the enzyme selectively removes this acid (*4*). The released sialic acid could be demonstrated in the soluble fraction of the neuraminidase digest by the thiobarbituric acid method (*5*) or by the resorcinol method (*6*). Only traces were found in the residue. The enzyme control was negative, and the heated enzyme or the buffer alone was unable to liberate sialic acid.

Digestion of the sections with trypsin (0.5 mg/ml) in 0.1*M* tris HCl buffer, *p*H 8.5, also prevented the staining by the above method. However, in the soluble trypsin digest sialic acid could be detected by the thiobarbituric acid method only after acid hydrolysis, an indication that trypsin splits off a soluble glycoprotein fragment containing sialic acid (*7*). The distinction between the effect of neurominidase and trypsin digestion is important for an understanding of the studies on neutralization by antiserum to kidney. Pure glomeruli (*8*) contained approximately 1 percent (dry weight) of sialic acid, an amount three to four times greater than the sialic acid content in the rest of the kidney. The soluble trypsin digest, while constituting only about 40 percent of the pure glomeruli, never-

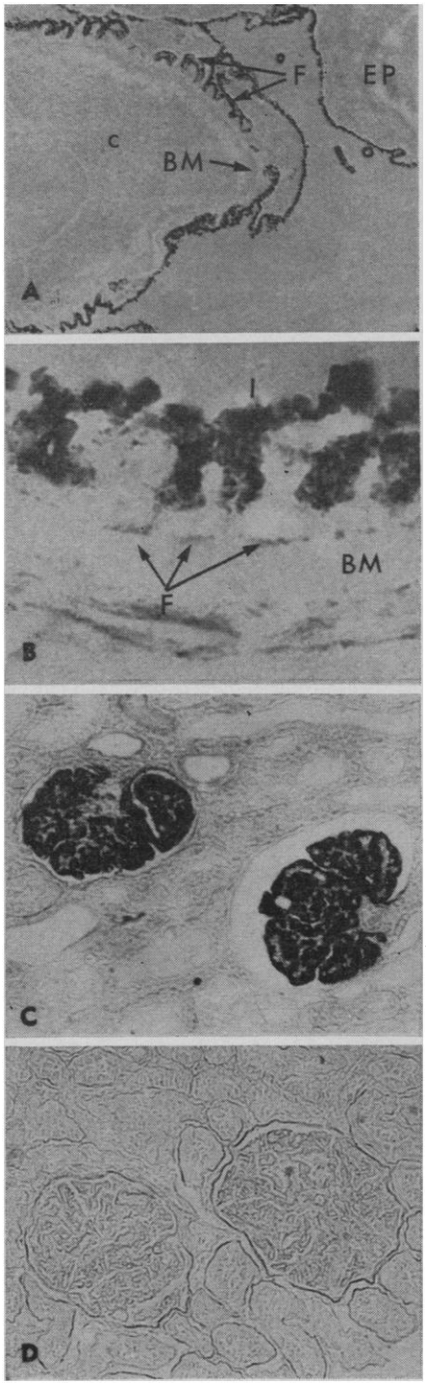

Fig. 1. (Upper two) Electron micrographs of a mouse glomerulus showing interaction of sialic acid and colloidal iron without counterstain. (A) Colloidal iron stain. There is a heavy iron deposit on epithelial cell membrane (*EP*) including foot processes (× 16,000). (B) Colloidal iron and potassium ferrocyanide reaction. There is a heavy crystalline deposit (*I*) (Prussian blue) along epithelial foot processes, a weak deposit on the anchored portion of foot processes (*F*) and an absence of deposit in the basement membrane (× 100,000). (Lower two) Prevention of sialic acid–colloidal iron interaction. (C) Colloidal iron-periodic acid Schiff stain of untreated beef kidney section. The deep stain of glomerulus is due to heavy deposits of Prussian blue (× 120). (D) Lack of stain after neuraminidase treatment (× 120).

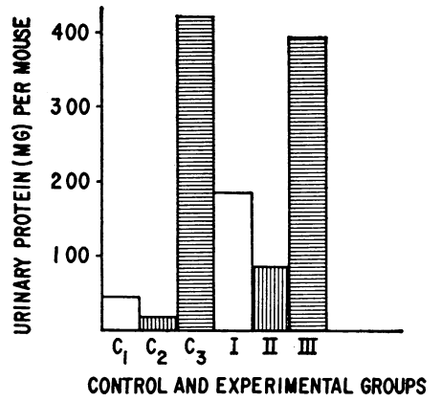

Fig. 2. Nephrotoxic effect of antiserum to kidney, absorbed with different glomerular preparations. Glomeruli digested with neuraminidase (I) or trypsin (III) change their capacity (C_2) to neutralize the antiserum; C_3 is the effect of the unabsorbed serum. Glomeruli treated with sodium acetate (C_1) served as the control for the neuraminidase-treated specimen. Column II reflects the neutralizing capacity of the trypsin-solubilized glomerular material. Bars indicate mean value of proteinuria per mouse in each test group of ten mice during the experimental period of 14 days. Reduction in bar length reflects antigenic activity of the absorbent. For absorption, 15 mg of lyophilized glomeruli or their equivalent preparation were used per milliliter of serum.

theless had twice as much sialic acid.

The capacity of compounds with high concentrations of basic amino acids (polycations) or of antiserum to kidney to interact with glomerular sialic acid was tested. The following were applied to paraffin embedded, rehydrated mouse kidney sections: protamine (composed of approximately 87 percent arginine) 25 mg/ml; histone, 2 mg/ml; polylysine, 10 mg/ml; undiluted rabbit antiserum to mouse kidney; 0.15*M* NaCl; bovine serum albumin; and normal rabbit serum. Colloidal iron deposition was prevented by the basic polyamino acids and by the antiserum to kidney, but not by the other, control solutions, an indication that polycations as well as specific antibodies can interfere with sialic acid activity.

The nephritogenic antigen can be solubilized and concentrated by trypsin digestion of whole glomeruli (*10*). Because our experiments showed that there was an increase of sialic acid in the soluble trypsin digests, the role of sialic acid in nephritogenic antigenicity was investigated. The degree of proteinuria produced in mice by antiserum to kidney, whether unabsorbed or absorbed with pure glomeruli or glomeruli treated with either neuraminidase or trypsin (*11*), was compared. Glomeruli treated with buffer alone were used as control. The degree of proteinuria produced in mice by rabbit antiserum to mouse kidney absorbed by the different glomerular preparations is shown in Fig. 2. Glomeruli treated with neuraminidase showed an approximate 50-percent reduction in the capacity for removing the nephrotoxic antibody from antiserum to kidney, indicating that sialic acid is an essential part of the nephritogenic antigen. Glomeruli treated with trypsin almost completely lost their neutralizing capacity when the sialic acid was removed, both being found in the soluble supernatant. The fact that the soluble trypsin digest contains complex protein fragments labeled with sialic acid (*7*) supports our contention that sialoprotein is the nephritogenic antigen.

Thus most of the sialic acid in the glomerulus is apparently in the cell membrane, particularly that of the epithelial cells, and not in the basement membrane proper. However, one cannot disregard the possibility that the basement membrane may contain sialic acid in an unreactive state or in a quantity less than that detectable by the colloidal iron reaction. Whether there is one or several discrete sialoproteins is not known. The localization of glomerular sialic acid at the cell membrane and the involvement of this acid in nephritogenic antigenicity indicates association of the cell membrane with this antigenicity. The participation of the cell membrane in nephritogenic antigenicity was considered earlier (*8*), by a quantitative comparison between the basement membrane and whole cells (rather than cell membranes). These results favored the basement membrane as being the site of the nephritogenic antigen. In our tests, the cell membrane preparations appeared nephritogenic (*7*). Recent work by Kefalides (*12*) on sonicated basement membrane preparations shows that the major component of the glomerular basement membrane is a collagen-like protein, free of heteropolysaccharides containing sialic acid. Whether the small quantities of heteropolysaccharide in this preparation represent contamination of the basement membrane by cell membrane or is an actual constituent of the basement membrane is not known.

One may wonder about the significance of the high concentration of this widely studied (*13*) substance in the glomerulus. A sialoprotein surface may result in an intense negative electrostatic field with a sieving effect capable of repelling similarly charged particles. In accord with the findings of Bungenberg de Jong (*14*), at physiological electrolyte concentrations the binding of monovalent cations by sialoproteins would increase their swelling pressure, making them impermeable for macromolecules. In contrast, the binding of polycations will lead to a decrease in swelling pressure and solubility, and consequently will increase the permeability for macromolecules (*15*). While this suggested function of the sialoproteins in glomerular filtration may constitute an asset, the antigenicity of this sialoprotein, the ready removal of the sialic acid by enzymes, and its easy binding by basic polyamino acids and specific antibodies makes it perhaps the most vulnerable component of the glomerulus with far-reaching implications in glomerular disease.

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