Acta Medica Okayama

Volume 34, *Issue* 1 1980 *Article* 9

FEBRUARY 1980

Changes in the molecular sieve of glomerular basement membrane in rats with aminonucleoside nephrosis.

Yasumasa Takaya[*] Zensuke Ota[†] Hirofumi Makino[‡]

Keiko Kida[**] Akira Miyoshi[††] Makoto Hiramatsu[‡‡]

Kayo Takahashi[§] Tadashi Ofuji[¶]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,
[§]Okayama University,
[¶]Okayama University,

Changes in the molecular sieve of glomerular basement membrane in rats with aminonucleoside nephrosis.*

Yasumasa Takaya, Zensuke Ota, Hirofumi Makino, Keiko Kida, Akira Miyoshi, Makoto Hiramatsu, Kayo Takahashi, and Tadashi Ofuji

Abstract

Isolated and purified glomerular basement membranes (GBM) of normal and aminonucleoside (PAN) nephrosis rats were observed by electron microscopy after negative staining. Although GBM of normal rats appeared as a molecular sieve with uniform pores, GBM of nephrotic rats showed enlargement and elongation of the pores. For an average of fifty pores, the long dimension was 40.4+/-10.7 A and the short dimension 13.8+/-3.6 A in nephrosis whereas the long dimension was 12.3+/-2.5 A and the short dimension 8.4+/-1.0 A in normal rats. Changes in the pores in GBM were thought to result in increased permeability of serum protein and hence proteinuria.

KEYWORDS: gromerular basement membranc, aminonucleoside nephrosis, negative staining.

---

*PMID: 6446842 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **34**, (1), 67—70 (1980)

BRIEF NOTE

CHANGES IN THE MOLECULAR SIEVE OF GLOMERULAR BASEMENT MEMBRANE IN RATS WITH AMINONUCLEOSIDE NEPHROSIS

Yasumasa TAKAYA, Zensuke OTA, Hirofumi MAKINO, Keiko KIDA, Akira MIYOSHI, Makoto HIRAMATSU, Kayo TAKAHASHI and Tadashi OFUJI

Third Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan (Director: Prof. T. Ofuji)

Received November 17, 1979

Abstract. Isolated and purified glomerular basement membranes (GBM) of normal and aminonucleoside (PAN) nephrosis rats were observed by electron microscopy after negative staining. Although GBM of normal rats appeared as a molecular sieve with uniform pores, GBM of nephrotic rats showed enlargement and elongation of the pores. For an average of fifty pores, the long dimension was 40.4 ± 10.7 Å and the short dimension 13.8 ± 3.6 Å in nephrosis whereas the long dimension was 12.3 ± 2.5 Å and the short dimension 8.4 ± 1.0 Å in normal rats. Changes in the pores in GBM were thought to result in increased permeability of serum protein and hence proteinuria.

Key words: glomerular basement membrane, aminonucleoside nephrosis, negative staining.

The ultrastructure of the glomerular basement membranes (GBM) of the kidney has been shown to be a fine meshwork (*i. e.* a molecular sieve) by electron microscopy using negative staining. The pores are smaller than the size of albumin molecules (1). Therefore, the GBM is considered to play an important part in glomerular permeability as a main filtration barrier (2–4). The purpose of this study was to demonstrate the ultrastructural changes of the molecular sieve in nephrotic rats with severe proteinuria.

Nephrosis thought to resemble human lipoid nephrosis was produced in rats by daily injections of Puromycin aminonucleoside (PAN) (5, 6). We used 26 male Sprague-Dawley rats weighing approximately 100 g. The rats were kept in metabolic cages for 15 days with free access to standard feed and water. Fifteen experimental rats received daily subcutaneous injections of 1.5 mg per 100 g body weight of PAN as a 0.5% aqueous solution for 14 days. Eleven

control rats received injections of distilled water. Urines were tested for protein semiquantitatively by the Combistix method every day and the animals were weighed regularly.

PAN injections produced nephrotic rats with remarkable uniformity. Proteinuria appeared at about the 6th day of PAN injections, and ascites at about the 9th day. All animals were killed by exanguination from the heart on the 15th day. Total and fractional proteins, cholesterol and urea nitrogen in sera were measured. Serum albumin was markedly decreased, and serum cholesterol was increased, in nephrotic rats.

The kidneys were examined by light microscopy and electron microscopy of ultrathin sections. Glomeruli from nephrotic rats appeared almost normal by light microscopy. Although extensive fusion of the foot process of podocytes was observed on ultrathin sections, the GBM appeared normal.

GBM were isolated and purified according to a modification of Spiro's method (7). Successful purification was confirmed both by phase contrast microscopy with which GBM were seen as smooth, sharply refractive membranous fragments, and by negative staining which showed characteristic findings. GBM were studied with an electron microscope, Hitachi H 700 H type, using negative staining with 1.0% phosphotungustic acid, pH 7.3.

In lower magnification, isolated GBM showed fragments with linear contour, angular ends and spongy surface characteristic of GBM: this feature confirmed that the specimen under the observation was GBM (1, 3). In higher magnification, GBM showed a molecular sieve composed of fine strands and pores. In GBM of normal rats, strands were almost equal in width and three dimensionally continuous, measuring 11.3 Å in width. Pores were round or oval, measuring 12.3 ± 2.5 Å in long dimension and 8.4 ± 1.0 Å in short dimension for an average of fifty. In nephrotic rats, GBM showed the same basic structure except for the size and shape of pores. Although some of the pores remained similar in size to the normal, many of them were enlarged and elongated. For an average of fifty pores, the long dimension was 40.4 ± 10.7 Å and the short dimension 13.8 ± 3.6 Å in nephrosis. The largest long dimension measured was more than 100 Å and the largest short dimension more than 50 Å (Fig. 1).

In the nephrotic syndrome, increased permeability of the glomerulus is accepted as the basic functional defect. Although there have been many studies on glomerular permeability using various kinds of tracers and electron microscopes (8–11), neither the normal ultrastructure nor changes in nephrosis of the GBM have been clarified. We are the first to demonstrate that the GBM in man, cows and rats is a molecular sieve composed of fine strands and pores, and thus the main filtration barrier in glomeruli (1–4).

The size of albumin molecule is stated to be 150×38 Å in man and 129×39

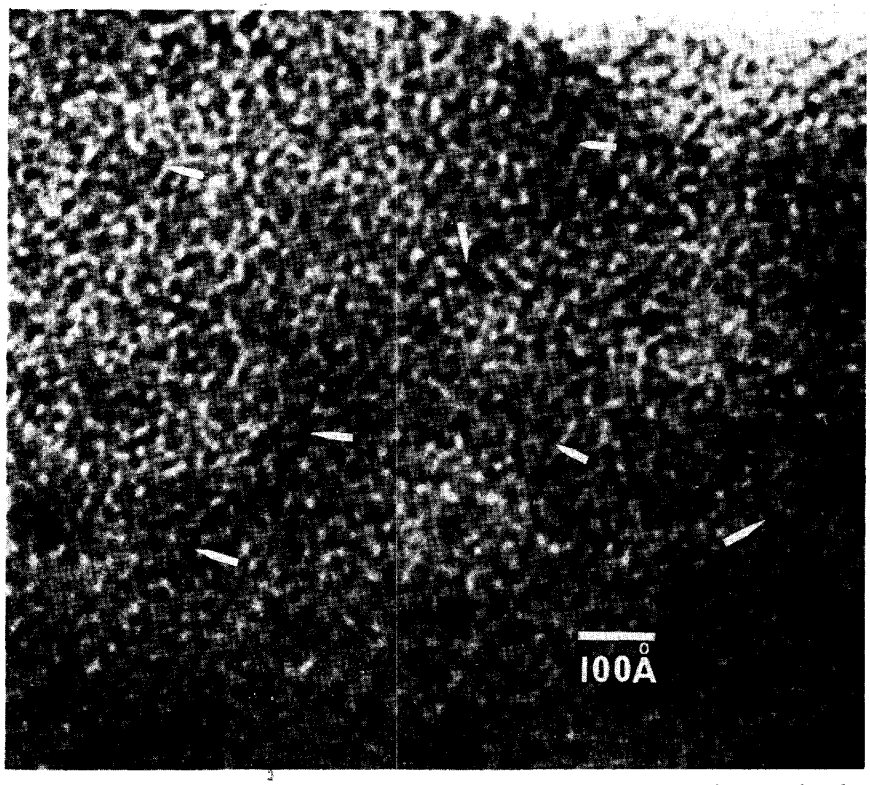

Fig. 1. The glomerular basement membrane of nephrotic rats showing a molecular sieve composed of fine strands and pores. Many of the pores are enlarged and elongated. ×1,000,000

Å in cattle, and it is probably of this order in rats since the molecular weights of albumin are similar regardless of the species. Enlargement and elongation of pores in nephrosis revealed in the present study appear to allow albumin molecules to pass through the GBM very easily. Hence, it is concluded that the pathogenesis of proteinuria in aminonucleoside nephrosis is change in the size of the pores of the molecular sieve in the GBM.

Acknowledgment. This study was supported in part by a Research Grant for Glomerular Lesions from the Intractable Diseases Division, Public Health Bureau, Ministry of Health and Welfare, Japan. We thank Mr. Nobuo Hayashi and Mr. Noboru Kishimoto for technical assistance in electron microscopy.

REFERENCES

1. Ota, Z., Makino, H., Miyoshi, A., Hiramatsu, M., Takahashi, K. and Ofuji, T.: Electron microscopic demonstration of meshwork structure in human and bovine glomerular basement membrane. *Acta Med. Okayama* **31**, 339-342, 1977.
2. Ota, Z., Ofuji, T., Takahashi, K., Hiramatsu, M., Miyoshi, A., Makino, H., Takaya, Y. and

Kida, K.: Ultastructure of human and bovine glomerular basement membranes —demonstration of meshwork structure. — In *Annual Report of Group Study for Renal Glomerular Lesion*, sponsored by Japanese Ministry of Welfare, pp. 194-201, 1978 (in Japanese).

3. Ota, Z., Makino, H., Miyoshi, A., Hiramatsu, M., Takahashi, K. and Ofuji, T.: Molecular sieve in glomerular basement membrane as revealed by electron microscopy. *J. Electron Microsc.* **28**, 20-28. 1979.
4. Ota, Z.: Ultastructure of glomerular basement membrane as revealed by electron microscopy.: *Jpn. J. Nephrol.* **21**, 328-335, 1979 (in Japanese).
5. Frenk, S., Antonowicz, I., Craig, J. M. and Metcoff, J.: Experimental nephrotic syndrome induced in rats by aminonucleoside. Renal lesions and body electrolyte composition. *Proc. Soc. Exp. Biol. Med.* **89**, 424-427, 1955.
6. Fiegelson, E. B., Drake, J. W. and Recant, L.: Experimental aminonucleoside nephrosis in rats. *J. Lab. Clin. Med.* **50**, 437-446, 1957.
7. Spiro, R. G.: Studies on the renal glomerular basement membrane. *J. Biol. Chem.* **242**, 1915-1922, 1967.
8. Caulfield, J. P. and Farquhar, M. G.: The permeability of glomerular capillaries of aminonucleoside nephrotic rats to graded dextrans. *J. Exp. Med.* **142**, 61-83, 1975.
9. Rennke, H. G., Cotran, R. S. and Venkatachalam, M. A.: Role of molecular charge in glomerular permeability. Tracer studies with cationized ferritins. *J. Cell Biol.* **67**, 638-646, 1975.
10. Farquhar, M. G. and Palade, G. E.: Glomerular permeability. II. Ferritin transfer across the glomerular capillary wall in nephrotic rats. *J. Exp. Med.* **114**, 699-715, 1961.
11. Ryan, G. B., Rodewald, R. and Karnovsky, M. J.: An ultrastructural study of the glomerular slit diaphragm in aminonucleoside nephrosis. *Lab. Invest* **33**, 461-468, 1975.