

# *Acta Medica Okayama*

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*Volume 43, Issue 6*

1989

*Article 8*

DECEMBER 1989

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## Human liver ferritin as a new tracer for studying glomerular permeability.

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## Abstract

Sprague-Dawley rats, 6 with aminonucleoside nephrosis and 6 controls, were intravenously injected with human liver ferritin isolated from post mortem liver, and their 24-h urine samples were examined for human ferritin by immunoradiometric assay. In rats with aminonucleoside nephrosis, the amount of excreted ferritin in urine was forty times greater than in control rats. Much more monomeric ferritin was excreted than that of polymeric ferritin. We are the first to have utilized human liver ferritin as a tracer to measure a minor amount of ferritin by a commercially available kit. Our present study seems to indicate a critical role for glomerular basement membrane as a size barrier.

**KEYWORDS:** glomerular permeability, size barrier, human liver ferritin, immunoradiometric-assay

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\*PMID: 2624144 [PubMed - indexed for MEDLINE]

## Human Liver Ferritin as a New Tracer for Studying Glomerular Permeability

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Sprague-Dawley rats, 6 with aminonucleoside nephrosis and 6 controls, were intravenously injected with human liver ferritin isolated from post mortem liver, and their 24-h urine samples were examined for human ferritin by immunoradiometric assay. In rats with aminonucleoside nephrosis, the amount of excreted ferritin in urine was forty times greater than in control rats. Much more monomeric ferritin was excreted than that of polymeric ferritin. We are the first to have utilized human liver ferritin as a tracer to measure a minor amount of ferritin by a commercially available kit. Our present study seems to indicate a critical role for glomerular basement membrane as a size barrier.

**Key words :** glomerular permeability, size barrier, human liver ferritin, immunoradiometric assay

Horse spleen ferritin has been utilized as a tracer in the study of glomerular permeability (1-3). Ferritin is advantageous over other tracers such as dextran (4, 5), because of its solidity and electron opacity. Furthermore, the size of ferritin can be varied by using naturally-occurring size isomers (6, 7). Recently a method to measure a minor amount of human ferritin by radioimmunoassay has been developed and has been of clinical use (8). This method allowed us to utilize human ferritin to study the changes in glomerular permeability in experimental glomerulonephritis.

Human liver ferritin was isolated from a post mortem sample by the method of Cham *et al.* (9). In brief, liver tissue was cut into small pieces and homogenized with 0.05M phosphate buffer saline (PBS) in a blender and then with Polytron (Kinematica, Switzerland). The material was

centrifuged at  $2,000 \times g$  for 15min and the supernatant was diluted with methanol to a final concentration of 40%, v/v. This preparation was heated at 75°C for 10min and then immediately cooled in ice. After centrifugation at  $2,000 \times g$  for 15min, the yellowish solution was filtrated, dialized against PBS and concentrated. Ferritin isolated in this way was separated into monomeric and polymer-rich ferritin on a Sepharose 6B (Pharmacia-LKB Biotechnology, Uppsala, Sweden) column (10). Human ferritin in each fraction was measured by immunoradiometric assay (ferritin kit Daiichi, Daiichi Pure Chemical Co., Ltd., Tokyo, Japan).

Six female Sprague-Dawley rats, weighing 40-50g were intravenously administered 15mg of puromycin aminonucleoside (PAN) per 100g of body weight and studied 14 days later when massive proteinuria was detected. These rats were injected intravenously with 0.1-1.5mg of

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isolated human liver ferritin in 1 ml saline, 4 rats with monomeric ferritin and 2 with polymer-rich ferritin. Four control rats were injected with monomeric ferritin, and 2 were injected with polymer-rich ferritin. The rats were kept in metabolic cages with free access to water, and 24-h urine samples were collected. Blood samples were taken after 24h from the femoral vein. Urinary protein was determined by fluorometry with a pyrogallol red-molybdate complex. Serum and urinary levels of human liver ferritin were measured by the immunoradiometric assay.

Urinary protein was significantly higher in rats injected with PAN ( $50.63 \pm 10.58$  mg/day, mean  $\pm$  SEM) than in control rats ( $2.28 \pm 0.48$  mg/day). In both nephrotic and control rats, urinary excretion of human liver ferritin increased with increases in the amount of ferritin injected, and the ratio of 24-h urinary ferritin to the amount of ferritin injected remained constant (Table 1). Urinary excretion of monomeric ferritin was nearly forty times greater in nephrotic rats than in control rats. Polymer-rich ferritin also was excreted in the urine more by nephrotic rats than by control rats, although much lower in polymer-rich ferritin than in monomeric ferritin (Fig. 1). The difference between monomer and polymer probably shows that monomer was more permeable through the glomerular basement membrane (GBM) than polymer. However, it must be considered that polymeric ferritin might be captur-

ed by other organs before reaching kidney. On this point, we are now under examination.

A simple and rapid two-step isolation method for human liver ferritin was used in this study, and the yield was found to be 40 %, which is as good as the yield reported for other methods (9).

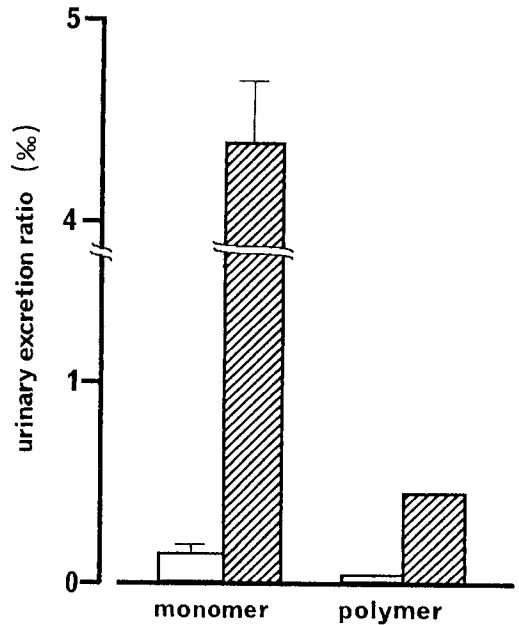


Fig. 1 The ratio of human liver ferritin excreted in urine to the ferritin injected in rats with aminonucleoside nephrosis (▨) and in control rats (□). The results are presented as the mean  $\pm$  SEM of 4 rats administered monomeric ferritin, and as the mean only of 2 rats administered polymer-rich ferritin.

Table 1 Urinary excretion of human liver ferritin for 24h after injection with various amounts of ferritin in rats with aminonucleoside nephrosis and control rats

Ferritin injected (mg)	Aminonucleoside nephrosis			Control	
	Ferritin excreted ( $\mu$ g/24h)	UER <sup>a</sup> (%)		Ferritin excreted ( $\mu$ g/24h)	UER <sup>a</sup> (%)
Monomer	1.5	7.170	4.780	0.079	0.053
	1.0	4.784	4.784	0.133	0.133
	0.3	1.342	4.467	0.046	0.153
	0.1	0.321	3.210	0.011	0.108
Polymer	0.3	0.176	0.587	0.004	0.013
	0.1	0.025	0.248	0.000	0.000

a : Urinary excretion ratio, the ratio of human liver ferritin excreted in urine for 24h to the injected amount.

Isolated human liver ferritin was measurable by a kit utilizing a two-site immunoradiometric assay (8), so that a minor amount of urinary ferritin could be detected.

From the results of our urinary excretion ratio of injected human liver ferritin, glomerular permeability, especially as a size barrier, has presumably been changed by PAN nephrosis. In connection with this point, we have reported the changes in the ultrastructure of GBM in rats with PAN nephrosis (10). We were the first to demonstrate the GBM to be a regular meshwork composed of fine fibrils by the observation of isolated GBM after negative staining (11-13). The size of pores was uniform and approximately 3 to 4 nm in diameter in control rats, whereas the size of pores was enlarged and elongated 3 to 12 nm in diameter in PAN nephrosis rats (10). As the size of the albumin molecule is 4 by 14 nm, we presumed that proteinuria was due to the enlargement of the pores in GBM.

Monomeric ferritin is a particle approximately 12 nm in diameter, so even ferritin probably can penetrate through nephrotic GBM. In view of our previous and present study, the GBM seems to play a critical role as a size barrier in glomerular filtration, although further study associated with charge interaction is necessary.

**Acknowledgments.** This research was supported in part by a Research Grant for Progressive Glomerular Lesions from the Intractable Disease Division, Public Health Bureau, Ministry of Health and Welfare of Japan. The authors are grateful to Mr. Takao Akiyama and Miss Yoko Tahara for their assistance with the measurement of ferritin.

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Received July 10, 1989 ; accepted October 3, 1989