Renal processing of serum proteins in an albumin-deficient environment: an in vivo study of glomerulonephritis in the Nagase analbuminaemic rat

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Abstract

Background. Plasma albumin has been considered as important for governing glomerular permselectivity as well as being tubulotoxic in proteinuric states. The purpose of this study was to examine glomerular permselectivity and protein clearance of plasma albumin-deficient Nagase analbuminaemic rats (NAR) in normal and proteinuric states associated with puromycin aminonucleoside nephrosis (PAN) and anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) and to compare the results with those of previous studies using Sprague–Dawley rats.

Methods. Glomerular permselectivity was measured using tritium-labelled polydisperse Ficoll. In vivo fractional clearance (FC) of albumin, transferrin and immunoglobulin G was measured to include both intact and degraded forms of filtered material. Endogenous protein clearance was analysed using two-dimensional electrophoresis in combination with matrix-assisted laser desorption ionization (MALDI) mass spectrometry.

Results. FCs of proteins and Ficoll in control NAR were similar to those found in Sprague–Dawley rats. Despite the lack of serum albumin in NAR, proteinuria and morphological changes observed were also similar to those found in Sprague–Dawley rats, with total protein excretion increasing 6-fold in PAN rats and 4-fold in anti-GBM GN rats with respect to controls. Two-dimensional electrophoresis in combination with MALDI mass spectrometry identified the major proteins being excreted as transferrin and a group of mildly acidic proteins in the MW range 40–50 kDa, namely antithrombin III, kininogen, α-1-antiproteinase, haemopexin and vitamin D-binding protein.

Conclusions. Both diseases exhibited similar effects to those observed in Sprague–Dawley rats despite the lack of serum albumin, including inhibition of renal protein degradation. The net changes in protein FC, particularly in the range of radii of 36–55 Å, could not be accounted for by changes in size selectivity as Ficoll FC was little affected by the disease states. This emphasizes the need to reassess the relative importance of changes in renal tubular handling vs changes in glomerular capillary barrier in proteinuric states. These studies also demonstrate that albumin is not a critical factor in governing glomerular permselectivity or proteinuria.

Keywords: analbuminaemia; anti-GBM; glomerular permselectivity; glomerulonephritis; PAN; protein clearance

Introduction

Increased urinary albumin or protein excretion is a hallmark of glomerulonephritis (GN). Persistent proteinuria has been considered to mediate the progression of renal disease, due to the accumulation of filtered proteins in proximal tubular cells and pathological changes to the tubular interstitium. These ideas led to the conclusion that the alterations in GN were due to abnormal glomerular filtration and exposure of proximal tubular cells to overload proteinuria. Specific studies on overload proteinuria in vivo, generally induced with exogenous albumin administered over weeks, have been correlated with progressive tubular atrophy and interstitial fibrosis [1]. The causal factors
inducing toxicity have been ascribed to fatty-acid ligands of albumin [2] and albumin itself. Cell-culture studies have demonstrated that excess albumin concentrations may elicit release of inflammatory factors, although other studies have paradoxically found albumin to be beneficial to the survival of primary cultures of proximal tubular cells [3].

The present study investigates the renal processing of proteins by utilizing an environment that is free from albumin. The Nagase analbuminaemic rat (NAR) is a derivative of the Sprague–Dawley rat that does not produce endogenous serum albumin as a result of abnormal albumin pre-mRNA splicing [4]. The total plasma protein concentration in the NAR is normal, since other individual protein levels in the serum, such as α1-antitrypsin, α2-macroglobulin, α-X protein, transferrin and the immunoglobulins, are elevated [5]. Despite the absence of endogenous serum albumin, proteinuria in NAR has been observed in aging rats [6], after treatment with adriamycin [7] and in rats made nephrotic with Heymann nephritis antigen [8]. Some conflicting studies have been reported, namely Okuda et al. [9] and Abe et al. [10], where only a small increase in protein excretion is observed.

One of the major findings in Sprague–Dawley rats is that filtered proteins are considerably biochemically modified by lysosomal proteases before excretion, resulting in excretion of <10% intact protein and >90% protein-derived degradation products and not just intact unmodified protein [11]. It has been shown that this process is inhibited in Sprague–Dawley rats induced with GN.

This study examined the renal processing of serum proteins in terms of renal degradation and permeability in an albumin-deficient environment using NAR. This is also examined in NAR induced with models of proteinuria, namely puromycin aminonucleoside nephropathy (PAN) and anti-glomerular basement membrane GN (anti-GBM GN). PAN and anti-GBM GN are known to produce massive proteinuria in normal Sprague–Dawley rats [11]. Specifically, we examine the fractional clearance (FC) of carbon-14 labelled albumin, immunoglobulin G (IgG) and transferrin in control NAR as compared to NAR with PAN or anti-GBM GN. As the radioimmunoassay (RIA) method has been shown to detect only intact protein [11], radiolabelled proteins were used in addition to the RIA, as a comparative method of determining FC as radio labelling allows the detection of both intact and fragmented protein. The FC of Ficolls of equivalent radius to albumin (36 Å), transferrin (48 Å) and IgG (55 Å) were also measured to account for any changes in the permeability properties of the glomerular capillary wall (GCW).

Subjects and methods

Materials

Tritium-labelled water (0.25 mCi/g) and [14C]formaldehyde (56 mCi/mmol) was obtained from NEN Life Science Products, Inc. (Boston, MA, USA). Sodium boro-[^3H]hydride (11.7 Ci/mmol) was obtained from NEN Research Products, Du Pont (Wilmington, DE, USA). Bovine serum albumin (BSA), rabbit anti-rat transferrin, rabbit anti-rat albumin, [125I] (10 mCi) and bovine IgG were obtained from ICN Biomedicals Inc. (Aurora, OH, USA). Sheep anti-rabbit IgG (second antibody for RIA) was a gift from Mr David Casley (Department of Medicine, Austin & Repatriation Medical Centre, Heidelberg, Australia). Alzet osmotic pumps (model 2001) were obtained from Alza Pharmaceuticals (Palo Alto, CA, USA). Sephadex G-100, Sephadex G-25 in PD-10 columns, Sephadyl S-300 and blue dextran were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Glycerol was obtained from Ajax Chemicals (Auburn, NSW, Australia). Forthane was obtained from Abbott Laboratories (IL, USA). Polydisperse Ficoll 70, rat IgG, rat serum albumin (RSA), rat transferrin, rabbit anti-rat IgG (whole molecule, adsorbed with human IgG), sodium azide, sodium cyanoborohydride, thimerosal, puromycin aminonucleoside, 3,3-diaminobenzidine and benzoylated dialysis tubing (9 mm width, MW cut-off 2 kDa) were from Sigma Chemicals (St Louis, MO, USA).

Experimental design

Normal or diseased rats were constantly infused with radiolabelled proteins (albumin, transferrin or IgG) or polydisperse Ficoll for a 7 day period via mini-osmotic pumps. Blood and 24 h urine collections were made on day 7. Animals were killed 7 days after pump implantation and the radioactive material in blood, urine and one kidney examined to determine the FC of individual molecules. Ten- to 12-week-old NAR were obtained from the Biological Research Laboratory, Austin & Repatriation Medical Centre (Heidelberg, Victoria, Australia). Throughout the experimental period they were maintained as pairs, housed in rat boxes under a 12 h day/night cycle with free access to standard rat chow and water. Permission to perform all animal experiments was given by the Monash University Animal Ethics Committee.

Induction of PAN

Induction of PAN was performed on 20 rats as previously described [11]. Briefly, rats were immobilized by towel wrapping and an injection of 15 mg/100 g body weight of puromycin aminonucleoside made up as a 3.5% solution in phosphate-buffered saline (PBS) was administered via the tail vein. Age- and weight-matched controls were injected with an equivalent amount of saline. Osmotic pumps were implanted on day 2 after puromycin aminonucleoside administration and the animals were killed 7 days later. The kidneys were removed with one kidney being processed for disease pathology and the other kidney analysed for radioactivity.

Induction of anti-GBM GN

Passive accelerated anti-GBM GN was induced in 14 rats as previously described [12]. Briefly, the rats were immunized subcutaneously with 5 mg normal sheep IgG in
Freund’s complete adjuvant and 7 days later were injected intravenously with 5 ml/kg sheep anti-rat GBM serum. Osmotic pumps were implanted 3 h before anti-GBM serum administration. Animals were killed 7 days after anti-GBM serum injection, with the kidneys removed. One kidney was processed for disease pathology and the other was analysed for radioactivity. Controls were age- and weight-matched.

Microscopical analysis

Tissues for pathological assessment were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (2 μm) were stained with haematoxylin and eosin or periodic acid–Schiff (PAS) reagents.

Radiolabelling of macromolecules

RSA, transferrin and IgG were labelled with 14C using a reductive methylation technique modified from Eng [13] using [14C]formaldehyde. The specific activities achieved for [14C]RSA, [14C]transferrin and [14C]IgG were 2.39 × 10^6, 1.67 × 10^6 and 3.41 × 10^6 disintegrations per min (d.p.m.)/mg, respectively.

Polydisperse Ficoll 70 was tritiated according to van Damme et al. [14]. The specific activity of polydisperse Ficoll 70 was 2.2 × 10^6 d.p.m./mg.

All radiolabelled material was checked for integrity by size exclusion chromatography prior to administration.

In vivo FC studies of radiolabelled proteins and polydisperse Ficoll 70 using the osmotic pump method

Alzet osmotic pumps were filled with radiolabelled material ([14C]albumin and [1H]Ficoll, or [14C]transferrin or [14C]IgG) along with 0.02% w/v of thimerosal to inhibit bacterial growth. The Alzet osmotic pumps Model 2001 have a mean filling volume of 221 ± 8 μl, pumping rate of 0.95 ± 0.05 μl/h, length 3 cm, diameter 0.7 cm and empty weight 1.1 g. Once filled, the pumps were incubated for 1 h at 37°C. The concentrations of radioactivity initially in the pump filling volume of 221 ± 8 μl, pumping rate of 0.95 ± 0.05 μl/h, length 3 cm, diameter 0.7 cm and empty weight 1.1 g. Once filled, the pumps were incubated for 1 h at 37°C.

The concentrations of radioactivity initially in the pump were [14C]RSA ~6.38 × 10^4 d.p.m./ml, [14C]transferrin ~5.20 × 10^4 d.p.m./ml, [14C]IgG ~3.41 × 10^4 d.p.m./ml and [1H]Ficoll ~2.54 × 10^5 d.p.m./ml.

The rats were anaesthetized by inhalation with Forthane, an inhalant isofluorane anaesthetic, and the osmotic pumps implanted subcutaneously between the scapulae using sterile technique. The rats were implanted with one pump filled with [14C]albumin, [14C]transferrin or [14C]IgG and the albumin group were implanted with an additional pump filled with [1H]Ficoll. The rats were then maintained as pairs in rat boxes with free access to food and water at all times, being placed in metabolic cages on days 5 and 7 for 24 h urine collections. On day 7, the animals were culled by decapitation, a plasma sample taken and both kidneys removed for subsequent analysis. Both blood and urine samples were centrifuged for 10 min at 3000 r.p.m. in a KS-5200C Kubota bench top centrifuge (Kubota Corp., Tokyo, Japan) and then analysed for radioactivity. Glomerular filtration rate (GFR) was determined using the creatinine assay and urine flow rate (UFR) calculated from the volume of urine collected in a 24 h period.

Analysis of kidneys for radioactivity

One of the kidneys from each rat was weighed, minced and made up to 3 ml with 1.4 M NaOH in centrifuge tubes. The tubes were covered loosely and suspended in boiling water for 15–30 min to allow complete digestion. Four sample aliquots of 100 μl each were taken, then 50 μl hydrogen peroxide was added to decolourize the samples and the volume made up to 1 ml with 850 μl water. Four millilitres of scintillation fluid was added to the samples and they were rested in the dark overnight to reduce chemiluminescence. The samples were counted for radioactivity and the presence of the tracer in the kidneys, determined as d.p.m./g of tissue.

Column chromatography

Plasma and urine samples were analysed using a Sephadex G-100 column (column dimensions: 1.7 × 60 cm) or a Sephacryl S-300 column (column dimensions: 2.1 × 65 cm). The columns were run at 4°C with a flow rate of 20 ml/h in PBS containing 2 mg/ml BSA and 0.02% sodium azide, pH 7.4. The columns were calibrated using blue dextran and tritiated water to determine the void and total volume, respectively. Ninety-five fractions of ~1.71 ml (G-100) or 100 fractions of ~1.66 ml (S-300) were collected with recoveries between 95 and 105% obtained. Kav was determined with the formula:

$$
(V_e - V_o)/(V_t - V_o)
$$

where V_o is the void volume, V_e the elution volume and V_t the total volume of the column. The FC of [1H]Ficoll corresponding to these protein radii was then determined by examining the elution of urine and plasma samples containing [1H]Ficoll at these Kav values.

Albumin RIA

The RIA utilized [125I]-labelled RSA, prepared according to the chloramine-T method [15], rabbit antiserum (polyclonal) to rat albumin and sheep anti-rabbit antibodies. The urinary albumin concentration measured by this RIA had an interassay coefficient of variation of 7% at a concentration of 180 ng/ml. The detection limit of the assay was 31.2 ng/ml. The standard curve was prepared using an RSA standard (1 mg/ml) that was diluted to give a range of 4000–31.2 ng/ml.

IgG RIA

The RIA utilized [125I]-labelled rat serum IgG, prepared according to the chloramine-T method [15] using 10 μl of a 1 mg/ml IgG standard, rabbit antiserum (polyclonal) to rat IgG, sheep anti-rabbit antibodies and non-immune rabbit serum. The detection limit of the assay was 31.2 ng/ml. The standard curve was prepared using a rat serum IgG standard (1 mg/ml) that was diluted to give a range of 4000–31.2 ng/ml.

Transferrin RIA

The RIA utilized [125I]-labelled rat serum transferrin, prepared according to the chloramine-T method [15] using
10 μl of a 1 mg/ml transferrin standard, rabbit antiserum (polyclonal) to rat transferrin, sheep anti-rabbit antibodies and non-immune rabbit serum. The detection limit of the assay was 6 ng/ml. The standard curve was prepared using a rat serum IgG standard (1 mg/ml) that was diluted to give a range of 1600–6 ng/ml.

**Total protein assay**

Total urinary protein was determined using the biuret assay.

**Identification of major urine proteins**

Five of the major proteins in the NAR proteinuric urine were identified using matrix-assisted laser desorption ionization (MALDI) mass spectrometry (performed by the Australian Proteome Analysis Facility, Sydney, Australia). Urinary protein was separated using two-dimensional electrophoresis (first dimension pH 4–10 isoelectric focusing for 35 000 Vh, second dimension on an 8–18% T criterion format polyacrylamide slab gel) and visualized using a SYPRO Ruby fluorescent stain. A plasma sample from a control animal was also analysed and used as a reference. The key proteins present in the proteinuric urine sample were selected to undergo in gel tryptic digestion and subsequent MALDI peptide analysis.

**Counting of radioactivity**

Tritium and [14C] radioactivity was determined using a 1 : 3 sample to Optiphase scintillation fluid ratio and recorded on a Wallac 1409 liquid scintillation counter (Wallac, Finland).

**Statistical analysis**

All data are expressed as means ± SD, with n representing the number of determinations. Significance of results was determined using the Student’s t-test. A P-value of <0.05 was considered to be statistically significant.

**Results**

**Renal function and histology in NAR**

Control NAR had normal renal architecture by light microscopy (Figure 1A). Induction of PAN in NAR resulted in a decrease in the GFR and an increase in UFR (Table 1). Light microscopy identified mild glomerular hypercellularity and profound tubular changes, including prominent cast formation, dilation and some tubular atrophy. PAS-stained droplets were seen within the cytoplasm of some convoluted tubules. A diffuse interstitial mononuclear cell infiltration was also evident (Figure 1B).

Induction of accelerated anti-GBM disease did not affect the GFR or UFR (Table 1). Light microscopy at day 7 of the disease showed moderate glomerular hypercellularity, focal areas of glomerulosclerosis and PAS-stained material within some glomerular capillaries. Mononuclear cell infiltration was evident in the glomerulus, with a mild, patchy infiltrate in the interstitium, most notably around small vessels. There was significant tubular damage, including tubular atrophy and cast formation. PAS-stained droplets were seen within the cytoplasm of some convoluted tubules (Figure 1C).
Table 1. Physiological parameters of the control, anti-GBM GN and PAN experimental groups

<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min)</th>
<th>UFR (μl/min)</th>
<th>Plasma concentration (mg/ml)</th>
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<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (n=5)</td>
<td>2.5 ± 0.3</td>
<td>11.1 ± 2.9</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Transferrin (n=6)</td>
<td>1.8 ± 0.7</td>
<td>8.3 ± 8.7</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>Albumin (n=5)</td>
<td>1.8 ± 0.7</td>
<td>12.8 ± 2.9</td>
<td>0.016 ± 0.002</td>
</tr>
<tr>
<td>Anti-GBM GN</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IgG (n=4)</td>
<td>1.3 ± 0.2</td>
<td>10.4 ± 1.8</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Transferrin (n=4)</td>
<td>1.5 ± 0.4</td>
<td>12.0 ± 3.7</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>Albumin (n=6)</td>
<td>1.9 ± 0.2</td>
<td>12.0 ± 5.0</td>
<td>N/D</td>
</tr>
<tr>
<td>PAN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (n=6)</td>
<td>0.8 ± 0.1</td>
<td>24.5 ± 3.6</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Transferrin (n=6)</td>
<td>0.8 ± 0.3</td>
<td>26.5 ± 5.3a</td>
<td>0.55 ± 0.20</td>
</tr>
<tr>
<td>Albumin (n=6)</td>
<td>1.2 ± 0.3</td>
<td>35.7 ± 7.6e</td>
<td>0.0014 ± 0.0004c</td>
</tr>
</tbody>
</table>

Plasma concentration of proteins determined by RIA. Total plasma protein concentration in control NAR as measured by biuret was 70.48 ± 4.89 mg/ml (n=5). *P < 0.01, **P < 0.05, ***P < 0.001 compared with controls. N/D, not determined; n, number of rats used for infusion experiments.

Characteristics of protein excretion

Protein excretion in control animals did not differ significantly from pre-experimental values (75.9 ± 13.0 and 79.8 ± 18.8 mg/24 h, respectively). Proteinuria was evident in both disease states after 7 days (609.8 ± 140.4 mg/24 h in PAN and 371.0 ± 149.7 mg/24 h in anti-GBM GN).

The nature of the excreted protein from NAR was examined by two-dimensional electrophoresis and MALDI mass spectrometry. Figure 2a and b are two-dimensional electrophoresis profiles of plasma and urine samples from a NAR. The urine was collected from a NAR with PAN. The plasma is from a control NAR and is provided as a reference. Transferrin can be seen in both the plasma and urine at approximately pH 7.0. Five major proteins present in the urine (marked on Figure 2b) were analysed by mass spectrometry and identified as antithrombin III (48.9 kDa, pI 5.93, 26.1% protein sequence coverage), kininogen (45.7 kDa, pI 5.90, 17.2%), α-1-antiproteinase (42.7 kDa, pI 5.60, 52.5%), haemopexin (45.7 kDa, pI 5.90, 17.2%).
(48.9 kDa, pI 7.33, 28.4%) and vitamin D-binding protein (51.8 kDa, pI 5.41, 37.0%). MW and pI values are based on database information for the identifications made. The percentage value refers to the percentage of the total protein sequence covered by the peptides.

Neither serum nor urine samples from normal or diseased NAR contained rat serum albumin. Most of the plasma proteins that are excreted in the urine are in the MW range 40–50 kDa and have pI values between 4.9 and 6. These proteins also account for a large proportion of plasma proteins in control rats (Figure 2a). It should also be noted that some proteins can migrate differently on a two-dimensional gel than what is anticipated by theoretical MW and pI values. This is largely due to post-translational protein modifications, which can create different isoforms of the same proteins with different pI values. This may result in a series of spots with different pIs but the same molecular weight for a particular protein (Figure 2a and b).

**Integrity of excreted protein**

Size-exclusion chromatography of the urine (Figure 3) showed that all tracers were excreted as degraded fragments in control NAR. The profiles seen with NAR depict typical renal processing of filtered proteins seen in control Sprague–Dawley rats [11]. The radiolabelled proteinuria in the Nagase analbuminaemic rat

![Graph of size-exclusion chromatography](https://example.com/graph.png)

**Fig. 3.** Size-exclusion chromatography profiles of radiolabelled RSA (A and D), transferrin (B and E) and IgG (C and F) in the urine of control (closed circle) and diseased (open circle) rats. Profiles were obtained using a Sephadex G-100 column.
material remains intact in the plasma (data not shown), so the fragmentation of the tracers can be attributed to degradation by the kidney. We have previously demonstrated that degradation is not the result of enzymes in the urine or the result of extrarenal degradation before filtration [11]. Further, small fragments seen in the plasma at 7 days have very low clearance due to binding to plasma proteins [16] and would not be expected to contribute significantly to their appearance in urine.

With induction of either PAN or anti-GBM GN, the percentage of macromolecular-radiolabelled protein (we use the term ‘macromolecular’ to identify the protein as distinct from ‘intact’ to allow for the possibility that some minor alteration may have occurred to some of the protein during renal passage even though it elutes at essentially the same position on the size-exclusion column) being excreted increases significantly (Figure 3). This is particularly so for albumin with the percentage of macromolecular albumin being excreted increasing from ~3% in control NAR to 92–95% in anti-GBM and PAN, respectively, of the total albumin excreted. Transferrin and IgG were excreted in the diseased models as a combination of macromolecular and fragmented protein (50–54% and 75–86% in anti-GBM GN and PAN, respectively).

Protein FC

Table 2 shows the concentration of radiolabelled material in the kidneys on day 7 of the osmotic pump studies. For all of the tracers there is no more than a 2–3-fold increase in radioactivity from the plasma to the kidneys, which is representative of the normal flux through the kidney rather than accumulation of the tracer. In both control and diseased states the amount of tracer appearing in the urine was <1% of the infusion rate. For both transferrin and IgG, the amount of labelled protein being infused into the plasma was negligible when compared with the plasma protein concentration.

RIAs were performed, in conjunction with quantitation of the radiolabelled tracers, to determine the FC of each protein (Table 3). Each RIA was performed on a group of rats that was not infused with the given radiolabelled protein, so that it would not interfere with the protein concentration. The FCs measured by radioactivity in the control NAR were orders of magnitude greater than those measured by RIA. This is because the RIA can detect only intact protein and does not account for fragments in the urine [11].

Comparison of Ficoll and protein clearance

To determine whether the increases in FC of the proteins were the result of size-selective changes in the glomerular capillary wall, polydisperse [3H]Ficoll 70

### Table 2. The concentration of radiolabelled material in the plasma (d.p.m./ml) and kidney (d.p.m./g) on day 7 of the osmotic pump infusion and corresponding excretion rate

<table>
<thead>
<tr>
<th></th>
<th>Plasma (d.p.m./ml)/10⁴</th>
<th>Kidney (d.p.m./g)/10⁴</th>
<th>Urinary excretion (d.p.m./min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IgG (n = 5)</td>
<td>7.74 ± 4.95</td>
<td>3.01 ± 0.12</td>
<td>8.27 ± 2.01</td>
</tr>
<tr>
<td>Transferrin (n = 6)</td>
<td>1.07 ± 0.19</td>
<td>4.26 ± 0.38</td>
<td>9.72 ± 7.17</td>
</tr>
<tr>
<td>Albumin (n = 5)</td>
<td>8.46 ± 4.04</td>
<td>1.95 ± 0.11</td>
<td>2.03 ± 0.88</td>
</tr>
<tr>
<td>Polydisperse Ficoll 70 (n = 5)</td>
<td>7.92 ± 3.50</td>
<td>7.32 ± 1.88</td>
<td>157 ± 40</td>
</tr>
<tr>
<td><strong>Anti-GBM GN</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IgG (n = 4)</td>
<td>3.56 ± 4.87</td>
<td>2.54 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.47 ± 4.09</td>
</tr>
<tr>
<td>Transferrin (n = 4)</td>
<td>0.77 ± 0.49</td>
<td>3.93 ± 0.80</td>
<td>8.13 ± 2.84</td>
</tr>
<tr>
<td>Albumin (n = 6)</td>
<td>3.35 ± 2.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.75 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.5 ± 10.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Polydisperse Ficoll 70 (n = 6)</td>
<td>4.40 ± 1.13</td>
<td>8.83 ± 2.77</td>
<td>168 ± 64</td>
</tr>
<tr>
<td><strong>PAN</strong></td>
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<tr>
<td>IgG (n = 6)</td>
<td>1.00 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.82 ± 1.26</td>
<td>13.72 ± 2.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transferrin (n = 6)</td>
<td>0.80 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.09 ± 0.69</td>
<td>8.91 ± 1.80</td>
</tr>
<tr>
<td>Albumin (n = 6)</td>
<td>1.89 ± 2.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.04 ± 0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.5 ± 10.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Polydisperse Ficoll 70 (n = 8)</td>
<td>12.60 ± 2.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.33 ± 25.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.1 ± 12.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < 0.01, <sup>b</sup>P < 0.05 compared with controls. n, number of rats used for infusion experiments.

### Table 3. FCs of IgG, transferrin and albumin in anti-GBM GN, PAN and age-matched control rats as measured by both RIA and radioactivity on day 7 of the osmotic pump infusion

<table>
<thead>
<tr>
<th></th>
<th>RIA/10⁴</th>
<th>Radioactivity/10⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (n = 5)</td>
<td>0.014 ± 0.006</td>
<td>0.61 ± 0.32</td>
</tr>
<tr>
<td>Transferrin (n = 6)</td>
<td>0.0022 ± 0.0015</td>
<td>5.3 ± 2.0</td>
</tr>
<tr>
<td>Albumin (n = 5)</td>
<td>0.077 ± 0.061</td>
<td>0.38 ± 0.43</td>
</tr>
<tr>
<td><strong>Anti-GBM GN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (n = 4)</td>
<td>2.9 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.9 ± 6.8</td>
</tr>
<tr>
<td>Transferrin (n = 4)</td>
<td>2.0 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.3 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin (n = 6)</td>
<td>N/D</td>
<td>10.8 ± 7.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>PAN</strong></td>
<td></td>
<td></td>
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<tr>
<td>IgG (n = 6)</td>
<td>12.4 ± 5.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.4 ± 8.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transferrin (n = 6)</td>
<td>22.9 ± 18.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.2 ± 2.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin (n = 6)</td>
<td>42.9 ± 17.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.1 ± 21.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001 compared with controls. N/D, not determined; n, number of rats used for infusion experiments.
was introduced into the circulation of control, PAN and anti-GBM GN rats using osmotic pumps and the FC of 36, 48 and 55 Å Ficoll was determined (because these radii correspond with the radii of albumin, transferrin and IgG, respectively) using size-exclusion analysis on Sephacryl S-300. Figure 4 illustrates the changes in the FC of albumin, transferrin and IgG as determined by RIA and radioactivity and the FC of the corresponding [3H]Ficoll in controls, PAN and anti-GBM GN. It can be seen that whereas there was a considerable increase in the FC of albumin by RIA and radioactivity in PAN and anti-GBM GN, the increase in FC of 36 Å Ficoll in the diseased states was small and insignificant. By comparison, the increase of intact transferrin was 1000–2600-fold in PAN and anti-GBM GN, respectively, and that of IgG excretion 140–500-fold in proteinuric models. Albumin clearance in disease states was still significantly lower than the FC for 36 Å Ficoll.

Discussion

The equivalent levels of proteinuria observed in GN in NAR as compared with Sprague–Dawley rats [11] demonstrates that albumin is not required for induction of proteinuria or the maintenance of high levels of protein excretion. The results do not eliminate the possibility that flux of large quantities of intact protein (irrespective of the nature of the protein) distal to the GBM may be damaging to cells. The similarities of Sprague–Dawley rats [11] and NAR suggest that, irrespective of the nature of filtered protein, the same underlying mechanisms of filtration and cellular processing of filtered protein are involved. No significant increase in Ficoll clearance was observed in proteinuric NAR, although it cannot be ruled out that relatively minor changes in glomerular permselectivity occur. The moderate increases in FC of proteins and protein-derived material as measured by radioactivity in GN were observed in both NARs and Sprague–Dawley rats. Charge selectivity was demonstrated to be negligible in Sprague–Dawley rats [11] and evidence for the charge selectivity concept has now been seriously questioned [17]. In terms of cellular processing, extensive fragmentation has been identified for filtered and excreted albumin, transferrin and IgG in both control Sprague–Dawley rats and NAR. Both strains exhibit significant inhibition of the fragmentation process in GN. These results demonstrate that the intrinsic cellular machinery for processing proteins distal to the GBM is essentially the same in both strains. Previous studies have suggested that albumin may influence the permselectivity of the GCW. The lack of albumin in NAR did not influence size selectivity as there was no significant difference in the FC of Ficoll in the NAR compared with Sprague–Dawley rats [11]. Exogenous radioactive albumin FC, as measured by RIA, in NAR was 7.7 ± 6.1 x 10⁻⁵, which is considerably higher than that measured in Sprague–Dawley rats (4.4 ± 2.6 x 10⁻⁶). However, when all the albumin-derived material in urine is taken into account, the FC in NAR was lower (3.8 ± 4.3 x 10⁻⁴) than that in Sprague–Dawley rats (1.7 ± 0.4 x 10⁻³). Previous efforts to determine glomerular permeability of proteins in NAR have employed SDS–PAGE [18], which will only detect intact protein. Clearly, assays...
that only detect intact protein cannot yield true FC of proteins as they fail to detect fragments.

Emori et al. [5] have shown that to compensate for the lack of albumin in the plasma, other serum proteins are elevated. This group of plasma proteins constitutes a major fraction of the major proteins excreted in both PAN and GN. As we have demonstrated in this study, these proteins consist of transferrin and a group of relatively small, slightly acidic proteins with a molecular weight range of 40–50 kDa. The average FC of these proteins is 0.001. Studies on similar macromolecules, such as neutral and acidic horseradish peroxidase, yield FCs in the range of 0.01–0.07 [17]. This would suggest that filtered proteins in NAR are processed in a special manner. We have previously provided evidence that in Sprague–Dawley rats, filtered albumin is retrieved by cells distal to the GBM and returned to the blood supply intact [19]. Similar mechanisms may be operative for filtered plasma proteins in NAR.

Transferrin has been thought to play a role in tubulointerstitial injury in diabetic patients [20] and may have been the cause of tubulointerstitial injury observed in NAR in the absence of albumin. In addition, the other proteins identified in the proteinuric urine may have contributed to the nephrotoxic effect.

Studies of the renal handling of proteins in NAR offer an interesting comparison to renal handling in Sprague–Dawley rats and in humans. The major conclusions of this study are that GN is manifested in a very similar fashion in both NAR and Sprague–Dawley rats, despite the marked difference in plasma protein composition. GCW permeability also seems to be essentially independent of plasma protein composition. Post-glomerular tissue injury was observed in NAR, thus demonstrating that albumin is not crucial for glomerular permselectivity or eliciting tubular damage. The renal degradation pathway that operates on filtered proteins prior to excretion was evident in both rat species and was inhibited in renal disease. There is also evidence that, as for Sprague–Dawley rats where most of filtered albumin is retrieved, it is likely that a special mechanism for renal processing of the bulk of filtered plasma proteins in NAR may be operative.

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Conflict of interest statement. None declared.

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