Determination of Glomerular Size-Selectivity in the Normal Rat With Ficoll

James D. Oliver III, Sharon Anderson, Julia L. Troy, Barry M. Brenner, and William M. Deen

ABSTRACT

Diffusion studies in vitro indicate that Ficoll behaves more like an ideal spherical molecule than does dextran, suggesting that Ficoll would be a better probe of glomerular pore size than the commonly used dextran. To examine the differences between these macromolecules in vivo, the fractional clearances of dextran and dextran were measured over a wide range of molecular sizes (Stokes-Einstein radius, r, from 19 to 65 Å) in normal euvolemic Munich-Wistar rats. Whole-kidney and single-nephron hemodynamic conditions were characterized through a combination of clearance and micropuncture measurements. The fractional clearance, or sieving coefficient (\(\theta\)), for dextran significantly exceeded that of Ficoll at all molecular sizes examined, \(\theta\) for dextran being ~10 times that for Ficoll for \(r_\theta > 30\) Å. Thus, the results with Ficoll imply a more size-restrictive barrier than do the results with dextran. The values of \(\theta\) for Ficoll approximated previously reported values for uncharged globular proteins. Although \(\theta\) for Ficoll at \(r_\theta = 35\) Å was much smaller than the corresponding value for dextran, it was still ~30 times greater than typical values of the filtrate-to-plasma concentration ratio for serum albumin (a polyanion) in the rat, in agreement with the concept that glomerular charge-selectivity normally plays an important role in the prevention of proteinuria. Three membrane-pore models were compared in their ability to represent the dextran and Ficoll sieving data. A lognormal pore-size distribution in parallel with a nonselective "shunt" pathway was found to be more effective than either an isoporous membrane with a shunt or a purely lognormal distribution. On the basis of these laboratory results and computations, Ficoll may be preferred over dextran in future studies of glomerular size-selectivity.

Key Words: Dextran, Ficoll, permselectivity, proteinuria, membrane pore size

Dextran is the test macromolecule most frequently used in studies of glomerular permselectivity. Unlike proteins, dextran is not reabsorbed (or secreted) by the tubules (1), so that its sieving coefficient, or filtrate-to-plasma concentration ratio (\(\theta\)), can be determined from its urinary clearance relative to that of inulin. Infusing a polydisperse dextran mixture and using gel chromatography to fractionate plasma and urine samples according to dextran size allow one to determine simultaneously the sieving coefficients of chemically identical molecules of widely varying molecular radii. Dextran has therefore been adopted in animal and clinical studies as a probe of the size-selective properties of the glomerular barrier.

Studies using a restricted range of dextran sizes (Stokes-Einstein radius, \(r_\theta\) up to ~45 Å) have generally suggested that the normal glomerular wall has an effective pore radius of 50 to 55 Å in rats (1,2) and humans (3). More recent studies with \(r_\theta\) up to at least 60 Å have shown that measurable amounts of these large dextrans cross the glomerular capillary walls, which suggests that even in healthy individuals, there are some pores of much larger size than was previously assumed (4-6). This finding has been modeled by postulating that the filtration barrier contains a relatively small number of nonselective "shunts" in addition to the ~50 to 55 Å pores (4) or by assuming that it possesses some continuous (e.g., lognormal) distribution of pore sizes (4,6). In experimental forms of proteinuria (6) and in nephrotic humans (4,5), a frequent finding is a selective increase in the sieving coefficients of the largest dextran molecules studied. Such increases suggest that an increased number of shunts or a shift in the pore-size distribution toward larger pores is an important underlying defect in proteinuric conditions.

Although it seems clear that defects in glomerular
size-selectivity contribute to proteinuria, one aspect of the findings with dextran has remained poorly understood. Namely, the sieving coefficients for dextran in normal individuals tend to be unexpectedly large, given the absence of proteinuria. Consequently, although the pore-size parameters derived from dextran sieving data for nephrotic humans are often able to account quantitatively for the observed levels of proteinuria, the pore-size parameters in healthy controls fail to explain the absence of appreciable protein excretion (4,5). Because albumin has a net negative charge under physiologic conditions, the presence of, at most, trace levels of albumin excretion in healthy individuals might be explained largely by the charge-selectivity of the normal glomerular capillary wall. Indeed, studies with a variety of test macromolecules have shown that the transmural passage of polyanions is restricted relative to neutral molecules of similar size and structure (2,7,8). However, the charge-selectivity of the filtration barrier does not explain the virtual absence in normal urine of immunoglobulin G (IgG), which is nearly neutral. Protein reabsorption by the tubules certainly contributes to the discrepancy between the urinary clearances of dextran and protein in normal individuals, but the reported values of fractional protein reabsorption of ~90% (9,10) would account for only a 10-fold difference between dextran and proteins such as IgG. Therefore, some factor in addition to charge-selectivity and protein reabsorption is needed to explain the differing urinary excretion rates of dextran and proteins of similar radii.

There is evidence that, in addition to the effects of molecular size and charge, differences in molecular configuration may have an important influence on the relative filtration rates of proteins and various exogenous macromolecules. The sieving coefficient of an electrically neutral globular protein, horseradish peroxidase, has been found to be approximately one seventh that of dextran of the same molecular radius (r, = 30 Å) (11). When compared using the same values of r, the sieving coefficient of dextran roughly equals that of polyvinylpyrrolidone (12) but significantly exceeds that of polyethylene glycol (PEG) (13) or Ficoll (14), a cross-linked copolymer of sucrose and epichlorohydrin. Of interest is the fact that the relative ranking of filtration rates in vivo is mimicked by the diffusion rates of these polymers through synthetic porous membranes; that is, dextran ~ polyvinylpyrrolidone > Ficoll > PEG (15,16). Thus, the transport rates of various neutral macromolecules differ appreciably and the relative rates seem more dependent on the type of permeating molecule than on the chemical composition of the barrier.

Particularly noteworthy is the fact that in track-etch polycarbonate membranes of known pore size, the measured diffusivity for Ficoll has been found to closely match the theoretical predictions for a neutral, solid sphere, whereas that for dextran is much larger (15,16). These findings and the aforementioned similarities among transport rates in vivo and in vitro suggest that Ficoll should provide accurate absolute values of effective pore size, values that might be extrapolated reliably to proteins. These results also imply that using dextran together with the customary solid sphere theory will lead to systematic overestimates of glomerular pore size. In other words, Ficoll is probably a more accurate marker for the filtration of neutral, globular proteins than is dextran. As with dextran, Ficoll is not secreted or reabsorbed by the tubules (14) and it can be prepared in a broad range of sizes.

The study presented here was undertaken to compare the filtration characteristics of Ficoll in normal rats with those of dextran. There has been only one previous physiologic investigation with Ficoll (14), and the study presented here extends and improves upon that in several respects. A much wider range of molecular radii was examined here, and the gel chromatographic procedures were refined with data on the molecular radius and elution volume of Ficoll and dextran standards. An additional improvement is that both single-nephron and whole-kidney hemodynamic quantities were measured, whereas neither was reported in the previous study. Finally, several kinds of pore-size distributions are compared in their ability to describe the results presented here, whereas pore-size calculations were not performed in the previous study.

**METHODS**

**Preparation of Radiolabeled Polymers**

Tritiated dextran (*#TRA 382; average molecular weight, 70,000) was obtained from Amersham Corp. (Arlington Heights, IL). Ficoll was tritiated by the following modified version of the protocol of Bohrer et al. (14). One gram of Ficoll 70 (Pharmacia Fine Chemicals, Piscataway, NJ) was dissolved in 5 mL of water and was oxidized by the addition of 0.525 g of NaIO4 and reaction in the dark for 18 h. The solution was then desalted by elution through disposable PD-10 gel chromatography columns (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). The pH was adjusted to 8.0 by the addition of NH4OH. One hundred millicuries of tritiated sodium borohydride (Dupont, NEN Research Products, Boston, MA) was added to the oxidized Ficoll in a fume hood. After 4 h, excess (0.2 g) unlabeled NaBH4 was added and the reaction was allowed to proceed for an additional hour. A few drops of acetic acid were added to remove residual borohydride, and then the unreacted label was removed by elution with phosphate-buffered saline (pH
was approximately 10 mL in volume.

Animal Studies
Sixteen healthy male Munich-Wistar rats with weights ranging from 240 to 306 g were used in these studies. All were fed *ad libitum* standard rat chow (Wayne Rodent Blox; Allied Mills, Chicago, IL) containing ~24% protein by weight and were allowed free access to water. Sieving measurements were performed in each animal with either dextran (seven animals) or Ficoll (nine animals) as the infused tracer. Micropuncture measurements of single-nephron pressures and flows were performed immediately before the sieving studies in eight of the animals (two with dextran, six with Ficoll).

Surgical Preparation
The rats were anesthetized with Inactin (100 mg/kg ip) (Andrew Lockwood & Associates, Sturtevant, WI) and placed on a temperature-regulated table. The left femoral artery (FA) was catheterized with a polyethylene tube (PE-50; Clay Adams, Parsippany, NJ), and a baseline collection of blood was obtained for hematocrit and insulin “blank” measurements. The FA catheter was used for subsequent blood sampling and for the estimation of mean arterial pressure (MAP) via an electronic transducer connected to a recorder. After tracheostomy, PE-50 catheters were inserted into the left and right jugular veins for infusions of inulin, *para*-aminohippurate (PAH), plasma, and the radiolabeled macromolecules. The left kidney was exposed and suspended on a lucite holder, and its surface was illuminated and bathed with isotonic saline. The left ureter was catheterized (PE-10; Clay Adams) for urine collections.

Euvolemia was maintained by the following protocol: Isotonic rat plasma was infused at 0.1 mL/min to a volume equal to 1% of the body weight, followed by a sustained infusion rate of 1.6 mL/kg/h for the duration of the experiment. An iv infusion of 10% inulin and 0.8% PAH in 0.9% NaCl at 1.2 mL/h was started 1 h before the measurements and was continued through the experiment.

Whole Kidney Hemodynamic Measurements
FA blood samples were obtained for the determination of hematocrit and plasma concentrations of protein (CA), inulin, and PAH. Timed urine collections were made for the determination of flow rate and inulin and PAH concentrations. These measurements permitted the calculation of GFR (from inulin clearance), RPF (from PAH clearance), and whole-kidney filtration fraction (FF).

Micropuncture Measurements
In the animals subjected to micropuncture, timed samples of fluid were collected from superficial proximal tubules for the determination of flow rate and inulin concentration for the calculation of single-nephron GFR (SNGFR). Blood samples from efferent arterioles were obtained for the determination of the efferent protein concentration (CE). Single-nephron-filtration fraction (SNFF) and glomerular plasma flow rate (Qg) were calculated from CA, CE, and SNGFR.

Fractional Clearance Measurements
Immediately after the micropuncture measurements had been completed, a 0.4-mL bolus of isotonic saline solution containing either titrated dextran or Ficoll of broad molecular size distribution (dextran concentration, <100 mg/dL; SA, ~50 μCi/mL; Ficoll concentration, <700 mg/dL; SA, ~110 μCi/mL) was infused iv over 1.5 min, followed immediately by a constant infusion of the same solution at the rate of 1.2 mL/h. Ten to 15 min after the priming bolus, a continuous FA blood collection was begun at a rate of 24 μL/min for 15 min. Urine collections were initiated and terminated 1.5 min after the arterial blood collections to allow for the transit time from Bowman’s space to the tip of the ureteral catheter.

The FA sample was spun at 3,000 rpm in a refrigerated centrifuge (Sorvall Model RT6000B; DuPont, Wilmington, DE) to remove cells, yielding a supernatant plasma volume of approximately 150 μL. Duplicate aliquots of 25 to 75 μL of FA plasma or 50 to 100 μL of urine were added to 1 mL of 2 mg% blue dextran (Sigma Chemical Co., St. Louis, MO). These samples were then fractionated and counted as described below.

Analytical Methods
The volume of fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary of known internal diameter. Tubule fluid inulin concentration was measured by the micro-fluorescence method of Vurek and Pegram (17). Inulin concentrations in plasma and urine were measured by the macroanvthrone method of *Führ* et al. (18). Protein concentrations were determined by the fluorometric method of Viets et al. (19). PAH concentrations were measured by the method of Chasis et al. (20).
Labeled plasma or urine samples were drawn into a syringe, and the empty vial was rinsed twice with a volume of 0.5 mL of distilled water. The rinsings were added to the sample. The total 2-mL volume was fractionated on a Sephacryl S-300 HR column (Pharmacia) that had been calibrated with dextran and Ficoll standards as described in the Appendix. The eluent buffer was 0.05 M ammonium acetate at pH 7. The samples were fractionated into 3-mL aliquots, and 2 mL from each aliquot was mixed with 4 mL of scintillation fluid (Ultima Gold; Packard Instrument Co., Downers Grove, IL). Activity was measured in a scintillation counter (Tri-Carb Model 4530; Packard Instrument Co.). On the basis of an analysis of the minimum significant activity above background, rates below 200 cpm were rejected.

**Calculation of Sieving Coefficients**

The sieving coefficient (θ) for a given molecule is the ratio of its concentration in Bowman's space to its concentration in arterial plasma water. Sieving coefficients for dextran or Ficoll were calculated from fractional clearances:

\[
\theta = \frac{(U/P)_{kpm}}{(U/P)_{muin}} \times f \quad \text{Equation 1}
\]

\[
f = -0.007895C_A + 0.9916 \quad \text{Equation 2}
\]

where \((U/P)_{kpm}\) is the urine-to-plasma ratio of scintillation counts per unit volume of sample, \((U/P)_{muin}\) is the urine-to-plasma ratio of inulin concentration, and \(f\) is a volume correction factor that accounts for the fact that the inulin plasma concentrations were measured in protein-free plasma water, whereas the plasma samples subjected to scintillation counting contained protein. A typical value for \(f\) was 0.95. Finally, \(\theta\) was calculated at integer values of \(r\) from the raw sieving curve by the interpolation scheme of Akima (21).

**Analysis of Data**

The ultrafiltration data were interpreted in terms of specific membrane properties by use of a mathematical model described previously (4). Briefly, this model treats the glomerular tuft as consisting of a number of identical capillaries in parallel and uses steady-state differential mass balance equations to describe the variations in plasma flow rate and solute concentration with axial position along a capillary. Two solutes are considered in the model—total plasma protein and an uncharged test macromolecule of arbitrary size present at tracer concentrations. The sieving characteristics of the capillary wall and test molecule are described by the theory of hindered transport for ideal, spherical molecules passing through long, cylindrical membrane pores. The governing equations were formulated to accommodate any hypothetical pore-size distribution, and several alternative distributions were considered in analyzing the group mean Ficoll or dextran sieving data (see below). The ultrafiltration coefficient for water \((K_i)\) was calculated either on a single-nephron basis (with SNGFR, \(Q_A\), \(C_A\), and \(\Delta P\)) or on a whole-kidney basis (with GFR and RPF in place of SNGFR and \(Q_A\)), again with group mean input values (22).

The simplest pore-size distribution used here envisons the glomerular barrier as containing many identical pores of radius \(r_0\), along with a few much larger pores that act as nonselective leaks or "shunts" for all molecular sizes of interest (4). We term this the "isoporous plus shunt" model. The relative prominence of the shunt pathway is described by a parameter \(\omega_o\), which is the fraction of the filtrate volume that would pass through the shunts in the hypothetical situation of zero colloid osmotic pressure. An alternative model considers the pore radius \(r\) to obey a lognormal probability distribution, where the probability density function \(g(r)\) is given by the following equation (4,6):

\[
g(r) = \frac{1}{\sqrt{2\pi} r \ln s} \exp \left( -\frac{1}{2} \left( \ln r - \ln u \right)^2 \right) \quad \text{Equation 3}
\]

The two parameters appearing in this model (replacing \(r_0\) and \(\omega_o\)) are \(u\) and \(s\). The probability density function has its maximum at a pore radius \(r = u\), whereas the breadth of the distribution is determined by \(s\). A three-parameter model, not previously used, was obtained by adding a shunt to the lognormal distribution. This "lognormal plus shunt" model has parameters \(u\), \(s\), and \(\omega_o\).

In fitting membrane parameters for the various heteroporous models to the dextran or Ficoll data, we used an approximate method for integrating the mass balance equations (4). Powell's method (23) was used to minimize the value of \(\chi^2\), defined as

\[
\chi^2 = \sum_{i=1}^{m} \left( \frac{\theta_{i,exp} - \theta_{i,calc}}{\sigma_i} \right)^2 \quad \text{Equation 4}
\]

where \(m\) is the number of datum points in the sieving curve, \(\theta_{i,exp}\) and \(\theta_{i,calc}\) are the experimental and calculated sieving coefficients, respectively, for molecular size \(i\), and \(\sigma_i\) is the standard error of \(\theta_{i,exp}\).

Standard errors \(\sigma_j\) for each fitted parameter \(a_j\) were estimated by (23)

\[
\sigma_j = \sqrt{C_{jj}} \quad \text{Equation 5}
\]

where \(C_{jj}\) are the diagonal elements of the covariance matrix.
Determination of Size-Selectivity With Ficoll

matrix C of the fit with respect to the parameter vector a:

\[ C = \alpha^{-1} \]

\[ a_{ik} = \sum_{j=1}^{n} \frac{\partial \theta_{ij}}{\partial a_k} \left( \frac{\partial \theta_{ij}}{\partial a_l} \right) \]

The partial derivatives were calculated numerically by use of a finite-difference scheme. This method assumes that \( \theta_{ij} \) depends linearly on the fitted parameters near the minimum in \( \chi^2 \) and tends to underestimate the true value of the standard error for what is actually a nonlinear model.

Statistical comparisons were made by use of the unpaired two-sample t test.

RESULTS

Hemodynamic Data

The mean values of various systemic and whole-kidney quantities are shown in Table 1 for the rats given dextran, the rats given Ficoll, and the two groups combined. There were no statistically significant differences in body weight, aP, systemic hematocrit, GFR, FF, or Cx between the dextran and Ficoll groups. There was a modest difference (20%) in RPF between the two groups, which did achieve statistical significance. As will be discussed, a difference in RPF of this magnitude is predicted to have little effect on the measured dextran or Ficoll sieving curves.

The single-nephron quantities obtained in the eight rats that underwent micropuncture are summarized in Table 2. The value of the single-nephron glomerular ultrafiltration coefficient (Kr) computed from the data in Table 2 was 4.25 nL/min/mm Hg. All of these values are within typical ranges for normal euolemic Munich-Wistar rats in this laboratory. Because of this and the aforementioned similarities in whole-kidney hemodynamic quantities, the data in Table 2 were taken as representative of the entire group of rats studied (including those that did not undergo micropuncture).

Fractional Clearance Data and Pore-Size Parameters

The sieving coefficients (fractional clearances) measured for dextran and Ficoll are shown in Table 3 and Figure 1. As the Stokes-Einstein radius (rs) increased from 19 to 65 Å, \( \theta \) for dextran decreased from \( 7.1 \times 10^{-3} \) to \( 7.7 \times 10^{-3} \); the corresponding range of \( \theta \) for Ficoll was \( 3.3 \times 10^{-4} \) to \( 7.1 \times 10^{-4} \). At any given value of rs, \( \theta \) for dextran greatly exceeded that for Ficoll, with a dextran-to-Ficoll ratio of \( \approx 10 \) for \( rs > 30 \) Å. The differences between dextran and Ficoll were highly significant (\( P < 0.01 \)) for all molecular radii examined.

The membrane-pore parameters derived from the dextran and Ficoll data are shown in Table 4. The calculations used the single-nephron hemodynamic quantities given in Table 2, as well as the full range of sieving data (19 \( \leq rs \leq 65 \) Å). The "goodness of fit" of the various pore-size distributions may be judged from the values of \( \chi^2 \) given in Table 4 or from the calculated sieving curves shown in Figure 1. Based either on the relatively large values of \( \chi^2 \) or the visual appearance of the sieving curves, the simple isoporous plus shunt model provided a relatively poor fit to either sieving curve, although much better for dextran than for Ficoll. The values of rs and \( \omega_0 \) for Ficoll were much lower than those for dextran, reflecting the smaller sieving coefficients for Ficoll. The lognormal pore-size distribution, which also contains two parameters, performed better than the isoporous plus shunt model in either case. However, the lognormal distribution became noticeably less accurate at large or small rs than at intermediate rs (Figure 1).

Additional improvements in goodness of fit were obtained with the lognormal plus shunt model. Although any lognormal distribution theoretically includes some shuntlike pores of infinite size, for the parameter values in Table 4 (\( u = 20.8 \) Å and \( s = 1.40 \) for dextran; \( u = 11.4 \) Å and \( s = 1.46 \) for Ficoll), the contributions to \( \theta \) of pores over 100 Å were calculated to be negligible. Thus, the addition of a shunt to the lognormal distribution did not lead to any appreciable

### TABLE 1. Whole-kidney hemodynamics\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Dextran (N=7)</th>
<th>Ficoll (N=9)</th>
<th>Total (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>257±5</td>
<td>273±9</td>
<td>266±6</td>
</tr>
<tr>
<td>aP (mm Hg)</td>
<td>104±3</td>
<td>112±3</td>
<td>109±2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.9±0.5</td>
<td>47.3±3.5</td>
<td>46.7±0.5</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>1.1±0.04</td>
<td>1.0±0.04</td>
<td>1.1±0.03</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>4.8±0.2</td>
<td>4.0±0.2b</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>FF</td>
<td>0.24±0.02</td>
<td>0.24±0.01</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>Cx (g/dL)</td>
<td>5.1±0.1</td>
<td>5.2±0.1</td>
<td>5.1±0.1</td>
</tr>
</tbody>
</table>

\(^a\) All values shown are mean ± SE.

\(^b\) P < 0.05; Ficoll versus dextran.

### TABLE 2. Single-nephron pressures and flows\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>SNGFR (nl/min)</th>
<th>QA (nl/min)</th>
<th>SNFF</th>
<th>AP (mm Hg)</th>
<th>Cx (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>49.8±3.2</td>
<td>172±12</td>
<td>0.29±0.01</td>
<td>33.8±1.5</td>
<td>5.2±0.1</td>
</tr>
<tr>
<td>Ficoll</td>
<td>49.8±3.2</td>
<td>172±12</td>
<td>0.29±0.01</td>
<td>33.8±1.5</td>
<td>5.2±0.1</td>
</tr>
</tbody>
</table>

\(^a\) All values shown are mean ± SE for eight rats.
double counting of very large pores. The values of $\omega_0$ obtained from the lognormal plus shunt model were fairly similar to those from the isoporous plus shunt case. With either type of shunt model, the value of $\omega_0$ calculated for Ficoll was $\sim 1/10$ that for dextran.

**Sensitivity of Calculations to Hemodynamic Inputs**

To determine the sensitivity of the pore-size parameters to the hemodynamic inputs used in the calculations, we used four different sets of inputs. The single-nephron values shown in Table 2, which were used to obtain the parameter values in Table 4 and the theoretical curves in Figure 1, are denoted as Case 1. Cases 2 through 4 were based on various choices for the pertinent whole-kidney quantities (GFR, RPF, and $C_A$). Case 2 used the whole-kidney data averaged for all animals studied (right-hand column of Table 1). Cases 3 and 4 differed from Case 2 only in the assumed value of RPF. The average RPF for the dextran group (4.8 mL/min) was used in Case 3, whereas the average RPF for the Ficoll group (4.0 mL/min) was used in Case 4. In each case, the value of $\Delta P$ used was that obtained from micropuncture.

The range of each membrane-pore parameter obtained from the four cases is shown in Figure 2. In general, changing the hemodynamic inputs resulted in only minor variations in the computed pore-size parameters. (Because the results for the four cases were so similar, only the maximal range of each parameter is shown in Figure 2, rather than the individual values.) The similarity of the results for Case 1 to those for Cases 2 through 4 indicates that the choice of single-nephron versus whole-kidney hemodynamic inputs does not affect the trends observed in the pore-size parameters. Likewise, the similarity between Cases 3 and 4 demonstrates that the measured difference in RPF between the dextran and Ficoll groups also has a negligible effect on the computed pore sizes.

The possible significance of the measured difference in RPF between the dextran and Ficoll groups was also considered in terms of its expected effect on the sieving curves. To do this, we chose as a reference condition the theoretical sieving curves obtained by fitting the dextran or Ficoll data (with single-nephron hemodynamic inputs) with the lognormal plus shunt model. We then varied $Q_A$ by $\pm 10\%$ from the reference value of 172 nL/min for both dextran and Ficoll. The resulting set of sieving curves encompasses a variety of situations, including up to a 20% difference in

### Table 3. Sieving coefficients ($i_i$) for dextran and Ficoll

<table>
<thead>
<tr>
<th>$i_i$ (Å)</th>
<th>Dextran ($N = 7$)</th>
<th>Ficoll ($N = 9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>$7.06 \times 10^{-1} \pm 8.72 \times 10^{-2}$</td>
<td>$3.28 \times 10^{-1} \pm 3.68 \times 10^{-2}$</td>
</tr>
<tr>
<td>21</td>
<td>$5.74 \times 10^{-1} \pm 7.65 \times 10^{-2}$</td>
<td>$2.01 \times 10^{-1} \pm 2.66 \times 10^{-2}$</td>
</tr>
<tr>
<td>23</td>
<td>$4.81 \times 10^{-1} \pm 8.02 \times 10^{-2}$</td>
<td>$1.20 \times 10^{-1} \pm 1.80 \times 10^{-2}$</td>
</tr>
<tr>
<td>25</td>
<td>$3.72 \times 10^{-1} \pm 6.94 \times 10^{-2}$</td>
<td>$7.39 \times 10^{-2} \pm 1.25 \times 10^{-2}$</td>
</tr>
<tr>
<td>27</td>
<td>$2.84 \times 10^{-1} \pm 5.57 \times 10^{-2}$</td>
<td>$4.09 \times 10^{-2} \pm 8.09 \times 10^{-3}$</td>
</tr>
<tr>
<td>29</td>
<td>$2.16 \times 10^{-1} \pm 4.26 \times 10^{-2}$</td>
<td>$2.65 \times 10^{-2} \pm 4.61 \times 10^{-3}$</td>
</tr>
<tr>
<td>31</td>
<td>$1.64 \times 10^{-1} \pm 3.36 \times 10^{-2}$</td>
<td>$1.65 \times 10^{-2} \pm 3.05 \times 10^{-3}$</td>
</tr>
<tr>
<td>33</td>
<td>$1.35 \times 10^{-1} \pm 3.34 \times 10^{-2}$</td>
<td>$1.13 \times 10^{-2} \pm 2.49 \times 10^{-3}$</td>
</tr>
<tr>
<td>35</td>
<td>$8.94 \times 10^{-2} \pm 1.89 \times 10^{-2}$</td>
<td>$8.15 \times 10^{-2} \pm 1.05 \times 10^{-2}$</td>
</tr>
<tr>
<td>37</td>
<td>$6.99 \times 10^{-2} \pm 1.45 \times 10^{-2}$</td>
<td>$6.04 \times 10^{-2} \pm 1.35 \times 10^{-2}$</td>
</tr>
<tr>
<td>39</td>
<td>$5.26 \times 10^{-2} \pm 1.05 \times 10^{-2}$</td>
<td>$4.79 \times 10^{-2} \pm 1.10 \times 10^{-2}$</td>
</tr>
<tr>
<td>41</td>
<td>$4.18 \times 10^{-2} \pm 8.11 \times 10^{-3}$</td>
<td>$3.76 \times 10^{-2} \pm 9.06 \times 10^{-4}$</td>
</tr>
<tr>
<td>43</td>
<td>$3.30 \times 10^{-2} \pm 6.12 \times 10^{-3}$</td>
<td>$3.32 \times 10^{-2} \pm 8.50 \times 10^{-4}$</td>
</tr>
<tr>
<td>45</td>
<td>$2.34 \times 10^{-2} \pm 4.03 \times 10^{-3}$</td>
<td>$2.57 \times 10^{-2} \pm 6.79 \times 10^{-4}$</td>
</tr>
<tr>
<td>47</td>
<td>$2.14 \times 10^{-2} \pm 3.56 \times 10^{-3}$</td>
<td>$2.23 \times 10^{-2} \pm 5.96 \times 10^{-4}$</td>
</tr>
<tr>
<td>49</td>
<td>$1.80 \times 10^{-2} \pm 2.95 \times 10^{-3}$</td>
<td>$1.94 \times 10^{-2} \pm 5.27 \times 10^{-4}$</td>
</tr>
<tr>
<td>51</td>
<td>$1.55 \times 10^{-2} \pm 2.43 \times 10^{-3}$</td>
<td>$1.66 \times 10^{-2} \pm 4.53 \times 10^{-4}$</td>
</tr>
<tr>
<td>53</td>
<td>$1.36 \times 10^{-2} \pm 2.06 \times 10^{-3}$</td>
<td>$1.41 \times 10^{-2} \pm 3.74 \times 10^{-4}$</td>
</tr>
<tr>
<td>55</td>
<td>$1.18 \times 10^{-2} \pm 1.78 \times 10^{-3}$</td>
<td>$1.23 \times 10^{-2} \pm 3.30 \times 10^{-4}$</td>
</tr>
<tr>
<td>57</td>
<td>$1.05 \times 10^{-2} \pm 1.61 \times 10^{-3}$</td>
<td>$1.10 \times 10^{-2} \pm 3.11 \times 10^{-4}$</td>
</tr>
<tr>
<td>59</td>
<td>$9.46 \times 10^{-3} \pm 1.54 \times 10^{-3}$</td>
<td>$9.75 \times 10^{-3} \pm 2.02 \times 10^{-4}$</td>
</tr>
<tr>
<td>61</td>
<td>$9.00 \times 10^{-3} \pm 1.48 \times 10^{-3}$</td>
<td>$8.68 \times 10^{-3} \pm 2.27 \times 10^{-4}$</td>
</tr>
<tr>
<td>63</td>
<td>$8.34 \times 10^{-3} \pm 1.41 \times 10^{-3}$</td>
<td>$7.83 \times 10^{-3} \pm 2.04 \times 10^{-4}$</td>
</tr>
<tr>
<td>65</td>
<td>$7.71 \times 10^{-3} \pm 1.33 \times 10^{-3}$</td>
<td>$7.10 \times 10^{-3} \pm 1.72 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

* All values are given as mean ± SE.
Determination of Size-Selectivity With Ficoll

Figure 1. Sieving coefficient ($\theta$) of dextran and Ficoll as a function of molecular radius ($r_\text{s}$). The experimental values are shown individually as mean ± SE, whereas the curves represent the best fits to the data obtained with the three types of pore-size distributions.

TABLE 4. Membrane pore-size parameters for dextran and Ficoll

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Dextran</th>
<th>Ficoll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoporous Plus Shunt</td>
<td>$r_0$ (Å)</td>
<td>$44.3 \pm 1.1$</td>
<td>$29.1 \pm 0.8$</td>
</tr>
<tr>
<td></td>
<td>$\omega_0$</td>
<td>$5.02 \times 10^{-3} \pm 5.51 \times 10^{-4}$</td>
<td>$5.26 \times 10^{-4} \pm 1.20 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>$\omega_0$</td>
<td>103</td>
<td>208</td>
</tr>
<tr>
<td>Lognormal</td>
<td>$u$</td>
<td>$15.4 \pm 1.7$</td>
<td>$6.19 \pm 1.53$</td>
</tr>
<tr>
<td></td>
<td>$s$</td>
<td>$1.52 \pm 0.03$</td>
<td>$1.64 \pm 0.06$</td>
</tr>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>35.4</td>
<td>64.6</td>
</tr>
<tr>
<td>Lognormal Plus Shunt</td>
<td>$u$</td>
<td>$20.8 \pm 0.4$</td>
<td>$11.4 \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>$s$</td>
<td>$1.40 \pm 0.01$</td>
<td>$1.46 \pm 0.01$</td>
</tr>
<tr>
<td></td>
<td>$\omega_0$</td>
<td>$3.04 \times 10^{-3} \pm 9.75 \times 10^{-6}$</td>
<td>$3.82 \times 10^{-4} \pm 3.47 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>1.06</td>
<td>12.8</td>
</tr>
</tbody>
</table>

DISCUSSION

This study confirms the finding of Bohrer et al. (14) that, for a given value of $r_\text{s}$, dextran passes across the glomerular capillary wall more readily than does Ficoll. Although qualitatively similar to the earlier results, these data show a significantly greater separation between the sieving curves for dextran and Ficoll. One factor that may have contributed to these plasma flow rates between the dextran and Ficoll groups. As shown in Figure 3, altering $Q_\text{a}$ had relatively minor effects on the computed sieving curves and the range of sieving coefficients for dextran remained very distinct from that for Ficoll. This demonstrates that variations in the plasma flow rate of this magnitude are incapable of explaining the large differences in the observed sieving behavior of the two polymers.
is a difference in the choice of hemodynamic inputs in the model. The ranges shown for dextran (D) and Ficoll (F) encompass various values of D and F previously for normal rats obtained by us are comparable to those reported previously, 

- Figure 2. Sensitivity of the membrane-pore parameters to the choice of hemodynamic inputs in the model. The ranges shown for dextran (D) and Ficoll (F) encompass various choices of single-nephron or whole-kidney data, as described in the text by Cases 1 through 4.

quantitative differences is a difference in experimental design. The study by Bohrer et al. (14) involved two experimental periods in each animal, one with dextran and one with Ficoll. The order of the dextran and Ficoll infusions was varied, and an effort was made to wait long enough between the experimental periods to allow the first polymer to be cleared completely. Nonetheless, a small amount of the tritiated polymer from the first period may have been present during the second. This would tend to minimize the observed differences between dextran and Ficoll, especially for the largest molecules, which are cleared most slowly. To avoid this possible problem in the study presented here, we deliberately chose to study dextran and Ficoll in separate groups of animals. This was especially important because our study included molecular radii up to 65 Å, whereas that of Bohrer et al. (14) did not go beyond 44 Å.

For dextran radii up to ~40 to 50 Å, the values of $\theta$ obtained by us are comparable to those reported previously for normal rats (1,2,6,14,24–28). However, for larger dextran sizes, the values of $\theta$ presented here begin to exceed those in other studies (6,24–28), reaching more than a 10-fold difference at $r_*$ = 60 Å. The reasons for this discrepancy are not clear, but a contributing factor may have been differences in gel column calibration. As discussed in the Appendix, the customary use of protein standards for column calibration may lead to errors in the molecular radius of dextran (or other nonprotein polymers such as Ficoll), the magnitude of the error being dependent on the column packing material. To avoid this source of error, we used nearly monodisperse samples of dextran or Ficoll for column calibration, with $r_*$ determined for each standard by quasielastic light scattering. Because different column packings were used in the various studies cited above, the correction factors derived in the Appendix are not applicable and we are unable to estimate the extent to which this factor may have contributed to the discrepancies in $\theta$ for dextran. Of course, physiologic differences among the groups of animals studied or other technical differences may also have contributed.

The diffusion rates of Ficoll through synthetic membranes of known pore radius suggest that Ficoll molecules behave as ideal, neutral, rigid spheres (15,16). Given that Ficoll is uncharged, that its cross-linked structure should confer rigidity, and that sedimentation and viscosity data suggest that it is approximately spherical (14), this behavior is not unexpected. In contrast, the diffusion rates of dextran through the same synthetic membranes have been found to greatly exceed theoretical predictions for neutral spheres. There appears to be no simple explanation for the behavior of dextran. The most obvious difference between dextran and Ficoll is that, whereas Ficoll is cross-linked and approximately spherical, dextran is roughly linear, existing in solution as an approximately random coil. However, the available theory for neutral random coils (29) suggests that the observable rate of transmembrane diffusion should be less rapid, not more rapid, than that of a neutral sphere of equivalent $r_*$. Consistent with this prediction is the finding that the effective diffusivity of PEG is track-etch polycarbonate membranes is lower than that of Ficoll (16). The anomalous enhancement of dextran diffusion rates might be explained by weak attractive interactions between dextran and the membrane material, which would tend to elevate dextran concentrations in membrane pores (16,30). Whatever the explanation, the relative rates of dextran and Ficoll diffusion through synthetic membranes mimic the behavior of these polymers in the glomerulus.

The structure of Ficoll and its diffusion behavior in vitro suggest that it may be a much better marker than dextran for the glomerular filtration of neutral, globular proteins. Supporting this is our finding that the ratio of $\theta$ for Ficoll to $\theta$ for dextran at $r_*$ = 30 Å is ~0.11. This value is very similar to the analogous sieving coefficient ratio for a neutral globular protein of ~30 Å radius (horseradish peroxidase) and dextran (11). With regard to IgG, a group of molecules with $r_*$ ≈ 55 Å, and little net charge on average, less precise
comparisons are possible because \( \theta \) for IgG has not been determined simultaneously with that for Ficoll or dextran. Our value of \( \theta \) for Ficoll at \( r_s = 55 \text{Å} (1.23 \times 10^{-9}) \) is roughly comparable to that reported for IgG in rats \((5 \times 10^{-8})\), the latter value being based on tubule fluid samples \((9)\). Thus, the available evidence \textit{in vivo} supports the view that Ficoll is a good marker for the filtration of neutral proteins. The aforementioned value of \( \theta \) for Ficoll at \( r_s = 55 \text{Å} \) provides a much more satisfactory explanation for the absence of immunoglobulinuria than does the 10-fold higher value of \( \theta \) for dextran.

The analysis of tubule fluid samples obtained by micropuncture in rats \((9,10)\) suggests that \( \theta \) for serum albumin is typically \( ~3 \times 10^{-4} \). This is \(~30 \) times lower than the present value of \( \theta \) for Ficoll at this molecular size \((r_s = 35 \text{Å})\) and \(~300 \) times lower than the value of \( \theta \) for dextran. The fact that this negatively charged protein is filtered much less readily than Ficoll is consistent with the concept that the normal absence of albuminuria is dependent in part on the charge-selectivity of the glomerular barrier. However, the results presented here imply that the concentration of fixed negative charges in the glomerular capillary wall does not need to be as large as that previously reported \((31)\). That is, attempting to explain the 300-fold difference between albumin and dextran on the basis of charge alone requires that one postulate many more fixed charges than are needed to account for the 30-fold difference between albumin and Ficoll. Although it remains likely that a loss of charge-selectivity would contribute significantly to albuminuria, the results for Ficoll set a new and lower bound on the potential contribution of charge defects to proteinuria.

We previously compared the ability of a variety of theoretical pore-size distributions to represent fractional clearance data for dextran in normal and nephrotic humans \((4)\). In those comparisons, the isoporous plus shunt model was somewhat superior to the lognormal distribution. For that reason and because the values of \( r_0 \) and \( s \) have a simpler interpretation than those of \( u \) and \( s \), we have preferred the isoporous plus shunt model in subsequent studies with dextran. However, the curve-fitting results in Table 4 and Figure 1 indicate that for the dextran or Ficoll data presented here, the lognormal distribution is clearly superior to that for the isoporous plus shunt model. The same conclusion was reached by Remuzzi \textit{et al.} \((6)\), on the basis of fits to dextran data in rats.
It is less easy to judge whether the lognormal plus shunt model, introduced here, offers a significant improvement over the lognormal distribution. The lognormal plus shunt model has more degrees of freedom (three parameters versus two for the other models) and should yield smaller values of the error measure, \( \chi^2 \), on that basis alone. We compared the performance of the lognormal and lognormal plus shunt models using an F test, which makes allowances for differences in the number of degrees of freedom (32). By that measure, the lognormal plus shunt model does provide a significant improvement over the lognormal distribution for either test macromolecule.

Another way to judge the performance of the models is to consider the values of \( \chi^2 \) in relation to the number of datum points per sieving curve, m. On the basis of the definition of \( \chi^2 \) (Equation 4), if the computed sieving coefficients differed from the measured values by exactly 1 SE at all molecular sizes, then \( \chi^2 = m \). If one accepts that fitting the experimental sieving curves more accurately than this is probably not meaningful, then one should discount the importance of achieving values of \( \chi^2 < m \). In this study, \( m = 24 \) and the lognormal plus shunt model reduced \( \chi^2 \) from 35.4 to 1.05 for dextran and from 64.6 to 12.5 for Ficoll, relative to the lognormal model (Table 4). Thus, by this measure, the improvement obtained with the lognormal plus shunt model for dextran is probably unimportant. However, the improvement seen for Ficoll is more significant. We therefore conclude that the lognormal plus shunt model merits further examination in future studies, at least with Ficoll.

The alternative theoretical models for the glomerular barrier considered here are highly idealized in that they are all based on the concept of the hindered transport of neutral spheres through cylindrical membrane pores. We have discussed above the evidence from studies in vitro that Ficoll does in fact closely resemble an ideal, neutral sphere. It seems likely then that the opportunity for future advances in the description of Ficoll movement through the glomerular capillary wall lies much more in a closer representation of the actual ultrastructure of the barrier than in some improvement in the representation of the permeating macromolecule. The situation with dextran is much more complicated in that currently available models are unable to predict its transport rates even in synthetic membranes with well-defined pores of known size. Because Ficoll behaves much like an ideal sphere and because it possesses the other desirable characteristics for an in vivo tracer, which have led to the widespread use of dextran, we recommend that Ficoll be preferred over dextran in future studies of glomerular size-selectivity.

**APPENDIX**

**Selection of Calibration Standards**

Obtaining reliable sieving curves requires that care be exercised in calibrating the gel permeation chromatography (GPC) columns. The most direct way to determine correct molecular sizes is to calibrate the GPC columns with narrow molecular weight-range fractions of the polymer of interest (e.g., dextran). Because these are often not readily available, it has been common practice in sieving studies to use GPC columns that have been calibrated with various globular proteins. However, studies by Jørgensen and Møller (13) with Sephadex columns and by Squire (33) and Frigon et al. (34) with silica gel (TSK-SW) columns indicate that significant discrepancies may exist between the true Stokes-Einstein radius \( r_s \) of a dextran molecule and the value estimated from GPC with protein calibration. The magnitude of this discrepancy is highly dependent upon the column packing material, but it is typically 5 to 10 Å. We report here a comparison of calibration results obtained with proteins or with nearly monodisperse dextran or Ficoll standards with Sephacryl S-300 HR columns. The \( r_s \) values of the various dextran and Ficoll samples were measured by quasielastic light scattering, and correlations of \( r_s \) versus molecular weight are given here for both polymers. We also derive correction factors that may be used in conjunction with protein calibrations of this type of column.

**Molecular Radius of Dextran and Ficoll Standards**

Ten relatively monodisperse samples of dextran and six of Ficoll were obtained courtesy of Dr. K. Granath of Pharmacia (Uppsala, Sweden). The weight-average molecular weights \( (M_w) \) and number-average molecular weights \( (M_n) \), as supplied by Dr. Granath, are given in Table 5. The polydispersity index \( (M_w/M_n) \) for all samples was <1.15.

The diffusion coefficients \( (D) \) for the dextran and Ficoll standards were measured by quasielastic light scattering. Polymer samples (2 or 5 mg/mL) were prepared by dissolving dextran or Ficoll in high-purity water (MilliQ; Millipore Corporation, Bedford, MA) and filtering the solution five times through 0.22-μm-pore-diameter filters (Millipore; Millipore). The light scattering apparatus consisted of a 2-W argon-ion laser (Model 95-2 Ion Laser; Lexel, Fremont, CA), goniometer (Model BI-2000 SM; Brookhaven Instruments Corp., Holtsville, NY) and 136-channel digital correlator (Model BI-2030 AT; Brookhaven Instruments). The sample temperature was maintained at 25°C by a circulating water bath (System II Liquid/Liquid Recirculator, Neslab; Newington, NH). The wavelength of the measured light was 488 nm.
TABLE 5. Polymer samples used for GPC calibration

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Sample ID</th>
<th>M&lt;sub&gt;w&lt;/sub&gt;</th>
<th>M&lt;sub&gt;n&lt;/sub&gt;</th>
<th>M&lt;sub&gt;w&lt;/sub&gt;/M&lt;sub&gt;n&lt;/sub&gt;</th>
<th>r&lt;sub&gt;s&lt;/sub&gt; (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>FDR 7314 + 403 Fr 4</td>
<td>87,500</td>
<td>76,700</td>
<td>1.14</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>70 Fr i-1</td>
<td>72,100</td>
<td>62,600</td>
<td>1.15</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>FDR 7314 + 403 Fr 8</td>
<td>53,300</td>
<td>48,100</td>
<td>1.11</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>FDR 7314 Fr 22</td>
<td>41,200</td>
<td>38,000</td>
<td>1.08</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>RMI T6550 Fr 3</td>
<td>40,400</td>
<td>38,900</td>
<td>1.04</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>FDR 7314 Fr 32-36</td>
<td>25,600</td>
<td>24,200</td>
<td>1.06</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>FDR 7314 Fr 32</td>
<td>25,600</td>
<td>24,200</td>
<td>1.06</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>T5558 + 7650 Fr 2</td>
<td>10,000</td>
<td>9,200</td>
<td>1.09</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>PD T5558 Fr 10</td>
<td>9,860</td>
<td>9,430</td>
<td>1.05</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>T5558 + 7650 Fr 5</td>
<td>4,300</td>
<td>3,880</td>
<td>1.11</td>
<td>20</td>
</tr>
<tr>
<td>Ficoll</td>
<td>T1800 Fr 20</td>
<td>132,000</td>
<td></td>
<td></td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>T2580-IWB Fr 2</td>
<td>71,800</td>
<td>64,600</td>
<td>1.11</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>T2580-IWB Fr 4</td>
<td>58,700</td>
<td>53,000</td>
<td>1.11</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>T2580-IWB Fr 8</td>
<td>37,500</td>
<td>34,500</td>
<td>1.09</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>T2580-IWB Fr 11</td>
<td>21,800</td>
<td>20,300</td>
<td>1.07</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>T2580-IWB Fr 12</td>
<td>17,500</td>
<td>16,200</td>
<td>1.08</td>
<td>30</td>
</tr>
</tbody>
</table>

Sample ID, M<sub>w</sub>, and M<sub>n</sub> are as provided by Dr. K. Granath of Pharmacia. Values of r<sub>s</sub> were determined by quasielastic light scattering.

Figure 4. Stokes-Einstein radii (r<sub>s</sub>) of dextran and Ficoll as a function of weight - average molecular weight (M<sub>w</sub>). M<sub>w</sub>. The results presented here for dextran are compared with those from the literature (13,36,37). The curves are the correlations given by Equations 9 and 10.

the aperture was 200 nm, and the scattering angle was 135 degrees. Sampling time varied from 0.1 to 0.5 µs, depending on molecular weight, with a total counting time of 1 min. Diffusion coefficients were fitted to the autocorrelation function by a second-order cumulant method (35).
The Stokes-Einstein radius \( (r_s) \) is related to \( D \) according to

\[
\frac{kT}{6\pi\mu r_s} \qquad \text{Equation 8}
\]

where \( k \) is Boltzmann's constant, \( T \) is absolute temperature, and \( \mu \) is the viscosity of water. The values of \( r_s \) obtained by quasielastic light scattering are shown in Table 5 and are plotted as a function of \( M_\text{w} \) in Figure 4. The dependence of \( r_s \) on \( M_\text{w} \) is described to within 9% by the correlations

\[
r_s = 0.488 M_\text{w}^{0.437} \text{ dextran} \quad \text{Equation 9}
\]

\[
r_s = 0.421 M_\text{w}^{0.437} \text{ Ficoll} \quad \text{Equation 10}
\]

where \( r_s \) is given in Å.

Also shown in Figure 4 are \( r_s \) values reported previously for dextran by three different methods. The data of Granath (36) were obtained by measurements in a diffusion cell, Callaghan and Pinder (37) used pulsed field gradient nuclear magnetic resonance, and Jørgensen and Møller (13) used analytical centrifugation. Those first two studies used commercial dextran fractions having significant polydispersity \( (M_\text{w}/M_\text{N} > 1.3) \), whereas Jørgensen and Møller used narrow fractions comparable to those used here. Our results for dextran coincide fairly closely with those of Callaghan and Pinder (37) and Jørgensen and Møller (13), whereas the \( r_s \) values of Granath (36) are larger at any given value of \( M_\text{w} \). For the same \( M_\text{w} \), the Stokes-Einstein radius of Ficoll tends to be somewhat lower than that for dextran. There appear to be no previously published data for \( r_s \) versus \( M_\text{w} \) for Ficoll.

**Column Calibration**

A set of GPC columns of 2.6 cm in diameter (Model C 26/100; Pharmacia) was packed with Sephacryl S-300 HR (Pharmacia). The packed column height was 56 cm, and a buffer flow rate of 3.8 to 4.0 mL/min was maintained by a peristaltic pump (2232 Microperpex S; LKB, Bromma, Sweden). The eluent buffer was 0.05 M ammonium acetate at pH 7. The void volume \( (V_0) \) of the columns was determined from the elution of blue dextran (Sigma). Continuous dextran and Ficoll elution curves were determined by differential refractometry (Series R-400 Differential Refractometer; Waters Associates, Milford, MA). The

---

![Figure 5. Fractional volume parameter \( (K_{AV}) \) as a function of molecular radius \( (r_s) \) for dextran, Ficoll, and various globular proteins on Sephacryl S-300 HR columns. The datum points are shown by the individual symbols, whereas lines represent the correlations given by Equation 12 and Table 6.](image-url)
elution volumes of colored proteins were measured by the collection of samples in an automatic fraction collector (2070 Ultrorac II, LKB) and by the reading of light absorbance at 415 nm (DU-50 Spectrophotometer; Beckman Instruments, Fullerton, CA). The proteins used were ferritin (horse spleen), horseradish peroxidase, myoglobin (horse heart), and cytochrome c (horse heart) (Sigma). On the basis of published diffusivities (38-41), their molecular radii were taken to be 59.4, 30.4, 19.0, and 16.5 Å, respectively.

The GPC elution data were correlated with the customary fractional volume $K_0$, defined as

$$K_0 = \frac{V_e - V_0}{V_t - V_0}$$  
Equation 11

where $V_e$ is the sample elution volume and $V_t$ is the total packed volume of the column. The calibration results for the Sephacryl columns are shown in Figure 5 as semilog plots of $K_0$ versus $r_5$. Unlike those on the columns used by Jørgensen and Møller (13) and Frigon et al. (34), the differences between protein and polysaccharide elution volumes on Sephacryl S-300 HR columns are fairly small.

The best-fit lines for the data in Figure 5 are of the form

$$K_0 = -a \ln r_5 + b$$  
Equation 12

With $r_5$ expressed in Å, the values of the constants $a$ and $b$ for the various polymers are given in Table 6. These results may be used to derive correction factors for $r_5$ of dextran or Ficoll that compensate for the use of proteins as the calibration standard. For a dextran or Ficoll sample of a given $K_0$, we would infer a radius $\tilde{r}_5$ from the protein calibration curve, that will differ in general from the true Stokes-Einstein radius $r_5$. Correction factors, defined as $\Delta = r_5 - \tilde{r}_5$, are shown in Figure 6 for dextran and Ficoll. As shown, Sephacryl S-300 HR columns calibrated with proteins would yield slight underestimates of $r_5$ for both dextran and Ficoll ($\Delta > 0$).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$a$</th>
<th>$b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>0.3150</td>
<td>1.522</td>
</tr>
<tr>
<td>Ficoll</td>
<td>0.2924</td>
<td>1.438</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.3048</td>
<td>1.458</td>
</tr>
</tbody>
</table>

**Table 6. Constants in correlations of $K_0$ versus $r_5$ (Equation 12)**

![Figure 6](image-url)
ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of S.J. Downes and M. Lee. This work was supported by grants from the NIH (DK 41641. DK P01-40839, and DK 30410) and the Johnson & Johnson HST Research Fund.

REFERENCES

32. Motulsky HJ, Ransnas LA: Fitting curves to


