PERMSELECTIVITY OF THE GLOMERULAR CAPILLARY WALL TO MACROMOLECULES

II. EXPERIMENTAL STUDIES IN RATS USING NEUTRAL DEXTRAN

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ABSTRACT To determine the permselectivity characteristics of the glomerular capillary wall, known molecular size fractions of [^{3}H]dextran, prepared by gel chromatography, were infused into normally hydrated Wistar rats, thus permitting simultaneous measurement of Bowman's space/plasma water (BS/P) and urine/plasma water (U/P) concentration ratios, along with glomerular pressures and flows. Since $(BS/P)_{inulin}$ = 1.01 ± 0.01 SE (n = 34, radius = ~14 Å) and since $(BS/P)_{dextran}/(BS/P)_{inulin}$ equaled $(U/P)_{dextran}/(U/P)_{inulin}$ for dextrans ranging in molecular radius from 21 to 35 Å, these findings validate that dextrans are neither secreted nor reabsorbed. For dextran radii of 20, 24, 28, 32, 36, 40, and 44 Å, $(U/P)_{dextran}/(U/P)_{inulin}$ averaged 0.99, 0.92, 0.69, 0.42, 0.19, 0.06, and 0.01, respectively. In accord with theoretical predictions that these fractional dextran clearances should vary appreciably with changes in glomerular transcapillary pressures and flows, an increase in glomerular plasma flow rate, induced in these same rats by plasma volume expansion, resulted in a highly significant lowering of fractional clearance of all but the smallest and largest dextrans studied. These findings emphasize that fractional solute clearances alone are inadequate to describe the permselective properties of the glomerular capillary wall unless glomerular pressures and flows are also known. This sensitivity of fractional dextran clearance to changes in plasma flow indicates that dextrans are transported across the capillary not only by bulk flow but also to an important extent by diffusion.

INTRODUCTION

In mammals, normally about one-fifth to one-third of the large volume of plasma entering the renal glomerulus passes through the walls of the glomerular capillaries, driven by an imbalance of transcapillary hydraulic and colloid osmotic pressures which has recently been shown experimentally to be quite small (1–10). Using micropuncture techniques, the composition of the fluid passing through the capillary walls is found to conform to that of a nearly ideal ultrafiltrate, closely resembling plasma water with respect to low molecular weight solute concentrations (11). For solutes with

molecular weights greater than approximately 5,000, however, transport becomes restricted, the extent of restriction being almost complete for molecules the size of serum albumin or above. To a large extent, our current understanding of the mechanisms governing this permselectivity of the glomerular capillary wall derives from differential solute clearance studies, in which the urinary excretion of some test macromolecule (such as polyvinylpyrrolidone [PVP], dextran, or protein) is compared to that of a reference solute assumed to appear in Bowman's space in the same concentration as in plasma water (12–15).

Previous studies (12, 14–18) have pointed out that fractional clearance values obtained for these macromolecules will be influenced not only by the properties of the capillary wall, but also by the glomerular filtration rate. Thus, as described in detail in the companion study (19), variations in the transcapillary hydraulic and oncotic pressure differences and the glomerular capillary plasma flow rate are expected to alter these fractional clearances. Only recently has it become possible to measure the pertinent transcapillary forces and flows, owing to the discovery of a unique strain of Wistar rats with accessible surface glomeruli and to the more or less simultaneous development of appropriate micropuncture and microanalytical techniques (1–9). Accordingly, the present experimental study was undertaken to examine the transport of macromolecules of well-defined molecular size across glomerular capillaries in these unique rats, and to determine the extent to which the transport of these solutes varies in response to measured alterations in the determinants of glomerular filtration rate. As test macromolecules tritiated, electrically neutral dextrans (selected because dextrans are known to be biologically and chemically inert) of high specific activity were employed, with molecular size determined by standard quantitative gel chromatographic techniques. Since inulin was found to appear in Bowman's space in the same concentration as in plasma water, this substance served as a reference marker against which the transport of dextrans is compared.

MATERIALS AND METHODS

Animal Studies

Experiments were performed on 30 Munich Wistar rats ranging in body weight from 173–274 g, and allowed free access to food and water. They were anesthetized with Inactin (100 mg/kg body wt) and prepared for micropuncture as described previously (1–9).

Studies with Dextrans of Narrow Molecular Size Distribution. Studies were undertaken initially to determine whether fractional dextran clearances obtained for the kidney as a whole (estimated from comparison of the urinary clearance of various sized dextrans to that of inulin) can be equated with clearances of these substances across single accessible surface glomeruli. Since Berglund et al. (20) have recently suggested that inulin, mol wt ~ 5,200, is transported across the walls of glomerular capillaries less readily than is polyethylene glycol, mol wt 1,000 (PEG-1000), experiments were first performed in 14 rats to examine directly the Bowman's space to plasma water[1] ratios for nonisotopic as well as [^{14}C]- and [methoxy-^{3}H]inulins, and [^{3}H]PEG-1000.

[1] Throughout this paper, concentrations of all solutes in plasma are expressed in terms of mass per unit volume of plasma water.

Having established that inulin indeed appears in Bowman's space in the same concentration as in plasma water, experiments examining the validity of equating fractional dextran clearances for a single glomerulus with those for the kidney as a whole were then performed as follows. Tritiated dextrans of narrow molecular size distribution, prepared in the manner described below, and characterized with respect to average Stokes-Einstein radius, were used as test solutes in nine rats. An 0.4 ml priming infusion, containing nonisotopic inulin (6 g/100 ml) and tritiated dextran (<200 mg/100 ml, activity = 0.4 mCi/ml), was injected into the left jugular vein 30 min prior to micropuncture, followed immediately by continuous infusion of the same solution at the rate of 1.2 ml/h. This infusion was continued throughout the duration of each experiment.

During this normal hydropenic period, 15-min urine samples were collected from a catheter in the left ureter for measurement of urine flow rate and inulin and dextran concentrations. During each urine collection period, two or three samples of fluid (30–50 nl each) from Bowman's space of superficially accessible glomeruli were also collected for determination of inulin and dextran concentrations. At the midpoint of each urine collection period, 100 μl of blood was withdrawn from the femoral artery for determination of dextran, inulin, and protein concentrations and measurements of arterial hematocrit. Concentrations measured in femoral arterial plasma are taken as representative of concentrations in afferent arteriolar plasma.

Following measurements in normal hydropenia, four of these rats received an intravenous infusion of homologous rat plasma given at the rate of 0.1 ml/min until a total volume equal to 5% body weight had been given. After volume expansion, priming and sustaining infusions of the inulin-tritiated dextran solution were readministered intravenously, following which measurements made during normal hydropenia were repeated.

Studies with Dextrans of Wide Molecular Size Distribution. Having established in the above studies that fractional urinary dextran clearances are the same as fractional dextran clearances measured for single accessible glomeruli in the same kidney (i.e. dextrans are neither secreted nor reabsorbed), justification is provided for relying on urinary clearances to assess the permselectivity characteristics for all glomeruli in a single kidney, now using an homologous series of dextrans of widely varying molecular size. These experiments were performed in seven normal hydropenic rats, in which 0.4 ml of a solution of nonisotopic inulin in isotonic saline (10 g/100 ml) was infused intravenously 45 min prior to micropuncture, followed immediately by a constant infusion of the same solution at the rate of 1.2 ml/h. 0.4 ml of an isotonic saline solution containing tritiated dextran of wide molecular size distribution (dextran concentration < 200 mg/100 ml, activity = 0.25 mCi/ml, see below for details of preparation) was infused intravenously,[2] followed immediately by a constant infusion of the same solution at the rate of 1.2 ml/h. Approximately 2–3 min after completion of the priming injection, a continuous collection[3] of blood from the femoral artery was begun at a constant rate (1.2 ml/h), using a continuous withdrawal pump (model 941, Harvard Apparatus Co., Willis, Mass.). To determine the transit time (τ) for tubule fluid to travel from Bowman's space to the tip of the ureteral catheter, a bolus of Lissamine green dye was injected intravenously (21). Urine collec-

[2]With the infusate volume and dextran concentration employed in these studies, plasma dextran concentration was less than ~10 mg/100 ml, or less than $\sim 5 \times 10^{-9}$ mol/cm^3 assuming a number-average molecular weight of $\sim 10^4$ g/mol. This concentration can be used to estimate the osmotic pressure due to the dextran, given by $\sigma RT\Delta C_s$, where σ is the reflection coefficient, R and T are the gas constant and absolute temperature, respectively, and ΔC_s is the transmembrane dextran concentration difference. Assuming as an extreme case that $\sigma = 1$ and $\Delta C_s = 5 \times 10^{-9}$ mol/cm^3, $\sigma RT\Delta C_s < 0.2$ mm Hg. This confirms the assumption made in the companion study (19) that the osmotic contribution of dextran to volume flow is negligible.

[3]Continuous collection of blood provides a more accurate assessment of the time-averaged solute concentration than do the more commonly employed techniques based on a series of collections made intermittently during the course of an experiment.

tion was initiated τ min (approximately 2–3 min) following initiation of the continuous femoral arterial blood collection and terminated τ min after the end of the blood collection period. 40–100 μl of the femoral arterial blood plasma and 15–100 μl of the urine collected in a given period were each mixed with 1 ml of distilled water and 2 mg of blue dextran and chromatographed on Sephadex G100. Additional aliquots of urine and blood from each period were used for subsequent determinations of inulin concentration, and, in the case of femoral arterial blood, total protein concentration as well.

During this normal hydropenic period, two to three exactly timed (1–2 min) samples of fluid were collected from surface proximal convoluted tubules for determination of flow rate and inulin concentration, and calculation of single nephron glomerular filtration rate, SNGFR. In addition, samples of blood (50–150 nl each) were collected from two to three surface efferent arterioles for determination of total protein concentration. Total protein concentrations measured in femoral arterial plasma are taken as representative of concentrations in afferent arteriolar plasma. These estimates of afferent (C_{PA}) and efferent (C_{PE}) arteriolar protein concentration permit calculation of single nephron filtration fraction, SNFF, and initial glomerular capillary plasma flow rate, Q_A (see equations below). Mean arterial pressure, $\overline{AP}$, was monitored using an electronic transducer (model P23Db, Statham Instruments, Los Angeles, Calif.) connected to a direct-writing recorder (model 7702B, Hewlett-Packard Co., Palo Alto, Calif). Hydraulic pressure measurements were obtained in surface glomerular capillaries, $\overline{P}_{GC}$, proximal tubules, P_T, and third-order branch peritubular capillaries, P_C, using continuous-recording, servo-null micropipette transducer techniques (22).

Following measurements in normal hydropenia, each of these seven rats was volume expanded with homologous rat plasma, as described above. Priming and sustaining infusions of inulin and tritiated dextrans were again administered, as in normal hydropenia, following which fractional dextran clearances and micropuncture measurements were repeated.

Analytical Determinations

The volumes of fluid obtained from proximal tubules and Bowman's spaces were estimated from the length of the fluid column in a constant-bore capillary tube of known internal diameter. The concentration of nonisotopic inulin in tubule fluid was measured, usually in duplicate, by the microfluorescence method of Vurek and Pegram (23). Nonisotopic inulin concentrations in plasma and urine were determined by the macroanthrone method of Führ et al. (24). Protein concentrations in efferent arteriolar and femoral arterial blood plasmas were determined usually in duplicate, with an ultramicrocolorimeter (American Instrument Co., Silver Spring, Md.) using a microadaptation (25) of the technique of Lowry et al. (26). Unlabeled dextran concentrations were measured by the Molish reaction (1 ml of sample + 1 ml of 5% phenol + 51 ml of concentrated H_2SO_4; optical density of the resultant colored solution was then determined colorimetrically at 490 nm). The specific activities of [^{14}C]inulin, tritiated inulins, dextrans, and PEG-1000, were determined using a liquid scintillation spectrometer (model 2425 Tri-Carb, Packard Instrument Co., Downers Grove, Ill.). Scintillation was produced by mixing 2 ml of aqueous sample with 7 ml of Aquasol (New England Nuclear, Pilot Chemicals Division, Boston, Mass.) and shaking to form a stiff gel.

Chromatographic Procedures

Sephadex G100 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) was used with an 0.05 N solution of ammonium acetate as buffer. Chromatographic columns 2.5 cm ID × 50 cm, 2.5 cm ID × 100 cm (Medical Research Apparatus, Boston, Mass.), and 1.5 cm ID × 50 cm (Arthur H. Thomas Co., Philadelphia, Pa.) were used. Eluted fractions were collected with an automatic fractionator (model 700 Ultrorac, LKB Instruments Inc., Rockville, Md.).

TABLE I

CHROMATOGRAPHIC DATA ON SEPHADEX G100

Substance	a	K_{AV}
	Å	
Present study		
Dextran T10	22.0	0.45
T20	34.0	0.19
Cytochrome C	16.4	0.64
Myoglobin	19.8	0.55
Horseradish peroxidase	30.2	0.29
Data from Laurent and Killander (27)		
Ribonuclease	19.2	0.58
Trypsin	19.4	0.51
Chymotrypsinogen	22.4	0.45
Cytochrome C (dimer)	23.4	0.42
Ovalbumin	27.3	0.29
		0.22
Hemoglobin	31.3	0.28
Serum albumin	35.5	0.19
Transferrin	38.1	0.10
Serum albumin (dimer)	43.5	0.06

Blue dextran (2 mg/ml) was used to determine the void volume (V_0); the fractional volume available to the solute (K_{AV}) was then calculated:

$$K_{AV} = (V_e - V_0)/(V_t - V_0) \quad (1)$$

where V_e is the elution volume of the solute and V_t is the total volume of the gel column.

Calibration of each column containing Sephadex G100 was performed using dextran T10 and T20 fractions (Pharmacia) and cytochrome C, horseradish peroxidase and myoglobin (Sigma Chemical Co., St. Louis, Mo.). Results from these calibrations are shown in Table I, together with data from Laurent and Killander (27). Stokes-Einstein radii (a) for dextrans prepared in the present study were calculated using data of Granath and Kvist (28), which showed a linear relationship between a and weight-average molecular weight ($\bar{M}w$) on a log-log plot. This relationship is described by the equation: $\log a = 0.472 \log \bar{M}w - 1.196$. K_{AV} values obtained for test macromolecules employed in the present study, together with values reported by others (27), yielded a linear relationship when plotted against log a, as shown in Fig. 1. Thus, Stokes-Einstein radii for test dextrans were determined from K_{AV} values, using Fig. 1.

Preparation of Tritiated Dextrans

Dextrans, consisting of a backbone of repeating *D*-glucose units, were cleaved oxidatively at the 2,3 and 3,4 carbon bonds by periodate ion, yielding formic acid (Fig. 2, step I). The ketone groups so formed then were reduced with tritiated borohydride, thereby introducing the tritium label into the dextran molecule (Fig. 2, step II). Dextran oxidation was carried out for 8 h in the dark by mixing dextrans (Pharmacia) and sodium periodate in water. Reduction of the oxidized product was then carried out with tritiated sodium borohydride (New England Nuclear, activity = 4.35 mCi/mg) in water at pH 8.0 for 4 h. Dextrans of narrow molecular size distribution[4] were prepared by repeated fractionation of dextran T10, T20, or T40. Dex-

[4]The ratio of weight-average to number-average molecular weight for the narrow dextran fractions was less than 1.3.

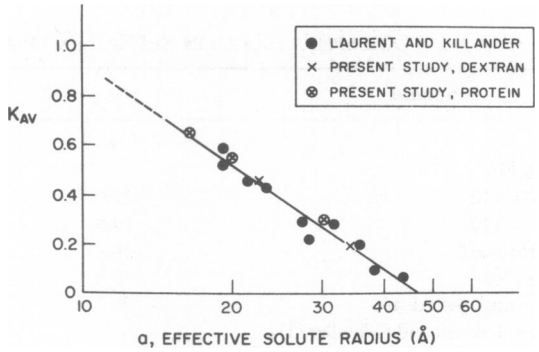

FIGURE 1 Relationship between K_{AV} and effective solute radius (gel chromatography data on Sephadex G100). Data of Laurent and Killander (27) are plotted together with values obtained for dextran and protein in the present study.

trans of wide molecular size distribution were prepared by mixing varying amounts of dextran T10, T20, and T40, followed by introduction of the tritium label via the oxidation-reduction reactions described above.

Calculations

Single nephron glomerular filtration rate:

$$\text{SNGFR} = (TF/P)_{IN} \cdot V_{TF}, \tag{2}$$

where $(TF/P)_{IN}$ and V_{TF} refer to transtubular inulin concentration ratio and tubule fluid flow rate, respectively.

Single nephron filtration fraction:

$$\text{SNFF} = 1 - (C_{PA}/C_{PE}) \tag{3}$$

Initial glomerular plasma flow rate:

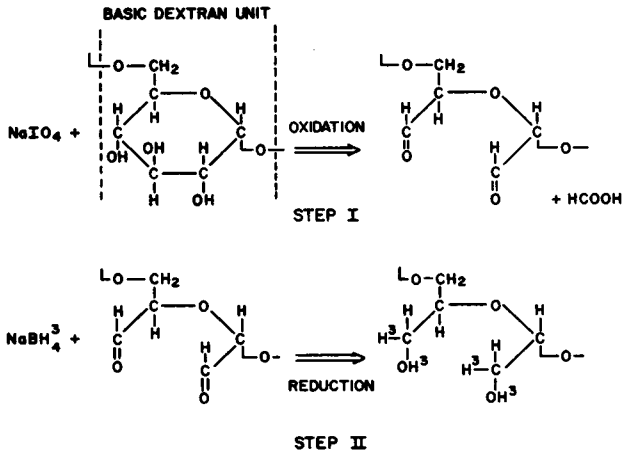

FIGURE 2 Oxidation and reduction reactions employed in the preparation of tritiated dextrans.

$$Q_A = \text{SNGFR/SNFF}. \quad (4)$$

Mean glomerular transcapillary hydraulic pressure difference:

$$\overline{\Delta P} = \bar{P}_{GC} - P_T. \quad (5)$$

Estimates of colloid osmotic pressure (π) of plasma entering and leaving glomerular capillaries can be obtained from measurements of protein concentrations (C_P) in femoral arterial and efferent arteriolar blood plasma using the equation

$$\pi = 1.63C_P + 0.294C_P^2, \quad (6)$$

where $4 \leq C_P \leq 10$ g/100 ml. This equation agrees to within 0.4 mm Hg of the more commonly employed empirical equation derived by Landis and Pappenheimer (29). Eq. 6 assumes an albumin/globulin ratio of 1.0, the ratio found in normal rats in this laboratory.

The ultrafiltration coefficient, K_f, and the other membrane parameters r_o and S'/l (for the model based on pore theory) and ωS and σ (for the model based on the Kedem-Katchalsky equations) were obtained by solving differential equations which give the rate of change of protein and test solute concentrations with distance along an idealized glomerular capillary. These equations are derived and discussed in detail in the preceding report (19).

RESULTS

The results of preliminary experiments demonstrating that inulin appears in glomerular ultrafiltrate in the same concentration as in plasma water are summarized in Table II. The ratio of inulin concentration in Bowman's space to that in plasma water $(BS/P)_{inulin}$, was measured in 34 nephrons of 14 rats. Ratios averaged 1.02 ± 0.02 SE (n = 22) for nonisotopic inulin, 1.00 ± 0.02 for [^{14}C]inulin (n = 10), and 1.03 for [methoxy-^{3}H]inulin (n = 2). These findings confirm the conclusion of others (11) that inulin is an ideal marker of glomerular ultrafiltration and refute the thesis of Berglund

TABLE II

GLOMERULAR TRANSCAPILLARY CONCENTRATION RATIOS FOR SMALL SOLUTES

Test solute	Molecular weight	Effective solute radius	(BS/P)*
		Å	
Inulin	5,200	~14	1.02 ± 0.02 SE (22)‡
[^{14}C]inulin	5,200	~14	1.00 ± 0.02 (10)
[methoxy-^{3}H]inulin	5,200	~14	1.03 (2)
[^{3}H]polyethylene glycol	1,000	7	1.01 ± 0.01 (9)

*Bowman's space to plasma water concentration ratio.

‡Number of observations.

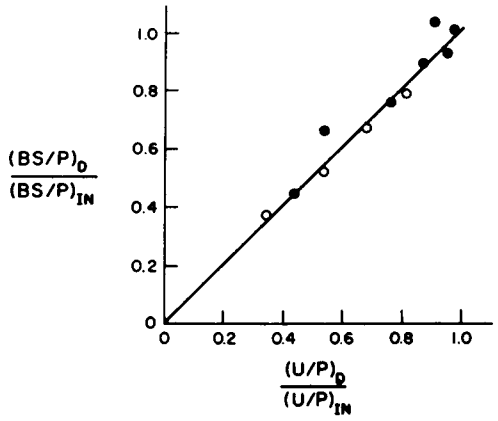

FIGURE 3 Comparison of $(BS/P)_{dextran}/(BS/P)_{inulin}$ ratios with $(U/P)_{dextran}/(U/P)_{inulin}$ ratios obtained in the same rats. The line of identity is indicated. Solid circles denote measurements obtained during normal hydropenia; open circles represent values obtained during plasma volume expansion.

et al. (20) that PEG-1000, but not inulin, is a valid marker for determining glomerular filtration rate. As given in Table II, the mean BS/P ratio for PEG-1000 is also unity.

Studies with Dextrans of Narrow Molecular Size Distribution

Fig. 3 summarizes data comparing $(BS/P)_{dextran}$ ratios, normalized to the simultaneous $(BS/P)_{inulin}$ ratios, with final urine/plasma $(U/P)_{dextran}$ ratios, again normalized to the simultaneous $(U/P)_{inulin}$ ratios. As can be seen, fractional dextran clearance ratios obtained for single superficial glomeruli, plotted on the ordinate of Fig. 3, were essentially the same as ratios measured for the kidney as a whole, plotted on the abscissa, for dextrans ranging in molecular radius from 20 to 35 Å. This equality was found to hold after (open circles), as well as before (solid circles), plasma loading. For all paired measurements, the ratio of $(BS/P)_{dextran}/(BS/P)_{inulin}$ to $(U/P)_{dextran}/(U/P)_{inulin}$ averaged 1.02 ± 0.02, a value not significantly different from unity ($P > 0.2$).[5] These findings demonstrate that dextrans are neither secreted nor reabsorbed by the renal tubules and also suggest that fractional dextran clearances are homogeneous from glomerulus to glomerulus within a single kidney.

Studies with Dextrans of Wide Molecular Size Distributions

This evidence that fractional dextran clearances for the kidney as a whole provide an accurate measure of dextran permeation across capillaries of a single glomerulus makes it possible to characterize the glomerular transport of dextrans of widely varying molecular size in a single animal, since sufficient quantities of blood and urine (see Methods) can be collected to permit chromatographic separation of the polydisperse dextran into

[5] It should be noted that the ratio $(BS/P)_{dextran}/(BS/P)_{inulin}$ and the ratio $(U/P)_{dextran}/(U/P)_{inulin}$ are equivalent to the quantity θ_{SB} in the companion study (19).

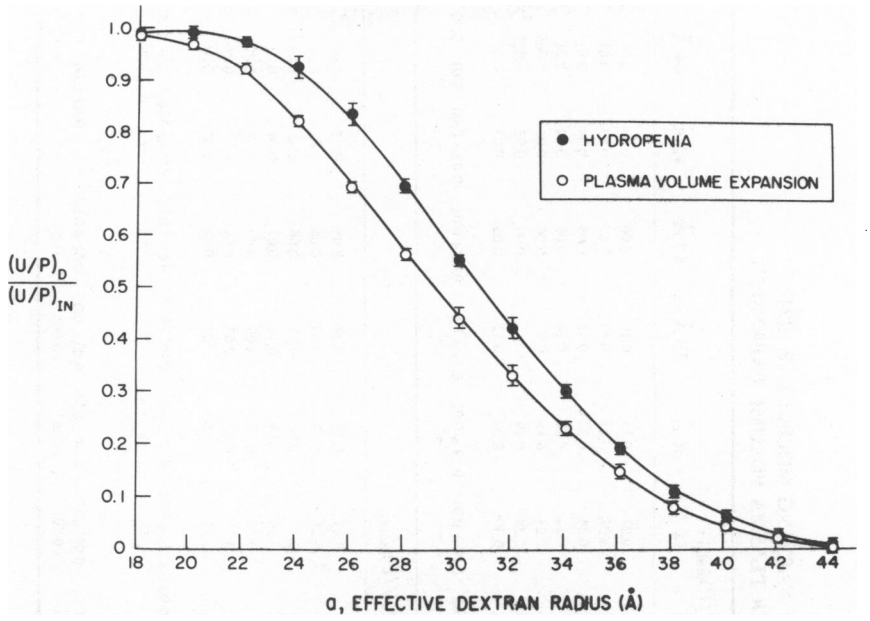

FIGURE 4 Comparison of fractional dextran clearances plotted as a function of effective dextran radius for rats studied before and after plasma volume expansion. Values are expressed as means ± 1 SE.

constituent narrow molecular size fractions. Thus, fractional dextran clearance profiles can be constructed for each rat, based on simultaneous clearances of dextran populations ranging in molecular radius from roughly 18 to 44 Å.

The relationship between the fractional clearance of dextran, given by the ratio $(U/P)_{dextran}/(U/P)_{inulin}$, and effective dextran radius in the normal hydropenic rat is given in Fig. 4 (solid circles). Values are expressed as means ± 1 SE. As can be seen, measurable restriction to dextran transport does not occur until the effective radius equals approximately 21 Å. Thus, inulin and PEG-1000, with effective radii of ~14 Å and 7 Å, respectively, are freely permeable (Table II). For dextran radii exceeding approximately 22 Å, fractional clearance decreases progressively with increasing size, approaching zero at 44 Å. Fractional clearances for dextrans of varying molecular size obtained from individual rats are shown in the left-hand portion of Table III. Remarkably little variation from rat to rat was observed.

A summary of a number of pertinent whole kidney and single nephron measurements for each of these seven rats is shown in the left-hand portion of Table IV. Values for $\overline{\Delta P}$, $\overline{P}_{GC}$, P_T, and P_C are typical of values for normal hydropenic rats reported from this laboratory previously (1–3, 5, 7–9), as are values for C_{PA}, C_{PE}, SNGFR, and Q_A. Achievement of filtration pressure equilibrium is indicated by the finding that $\pi_E/\overline{\Delta P}$ averaged 0.97 ± 0.03, a value not significantly different from one ($P > 0.5$).

From the theoretical analysis provided in the companion study (19), one would pre-

TABLE III

$(U/P)_{DEXTRAN}/(U/P)_{INULIN}$ RATIOS FOR DEXTRANS OF VARYING MOLECULAR SIZE MEASURED DURING NORMAL HYDROPENIA AND AFTER PLASMA VOLUME EXPANSION

Rat no.	18 Å	20 Å	22 Å	24 Å	26 Å	28 Å	30 Å	32 Å	34 Å	36 Å	38 Å	40 Å	42 Å	44 Å
	Normal hydropenia, $(U/P)_{dextran}/(U/P)_{inulin}$													
24	1.00	1.00	0.99	0.94	0.84	0.69	0.54	0.39	0.27	0.16	0.10	0.06	0.03	0.01
25	1.00	1.00	1.00	0.95	0.87	0.74	0.60	0.46	0.32	0.21	0.13	0.07	0.03	0.01
26	1.00	1.00	0.99	0.93	0.85	0.73	0.60	0.47	0.34	0.22	0.12	0.08	0.04	0.01
27	0.97	0.97	0.96	0.89	0.80	0.68	0.57	0.45	0.33	0.22	0.14	0.08	0.04	0.01
28	1.00	1.00	1.00	1.00	0.86	0.68	0.51	0.35	0.23	0.15	0.07	0.04	0.01	0.005
29	0.98	0.96	0.89	0.81	0.73	0.62	0.51	0.39	0.28	0.19	0.10	0.06	0.03	0.005
30	1.00	1.00	1.00	0.95	0.86	0.71	0.55	0.42	0.30	0.20	0.12	0.06	0.03	0.01
Mean ± 1 SE	0.99 ± 0.01	0.99 ± 0.01	0.97 ± 0.01	0.92 ± 0.02	0.83 ± 0.02	0.69 ± 0.01	0.55 ± 0.01	0.42 ± 0.02	0.30 ± 0.01	0.19 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.03 ± 0.00	0.01 ± 0.00
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	Plasma volume expansion, $(U/P)_{dextran}/(U/P)_{inulin}$													
24	1.00	0.97	0.92	0.83	0.71	0.59	0.47	0.34	0.23	0.15	0.09	0.05	0.03	0.01
25	1.00	0.95	0.91	0.81	0.68	0.57	0.45	0.35	0.24	0.15	0.09	0.05	0.03	0.01
26	1.00	0.97	0.93	0.83	0.72	0.58	0.48	0.38	0.27	0.17	0.09	0.06	0.03	0.01
27	0.97	0.92	0.89	0.82	0.71	0.60	0.50	0.39	0.29	0.18	0.11	0.07	0.04	0.01
28	1.00	1.00	0.96	0.83	0.68	0.52	0.37	0.25	0.17	0.10	0.05	0.03	0.01	0.005
29	0.98	0.92	0.86	0.76	0.63	0.53	0.43	0.32	0.22	0.13	0.07	0.04	0.02	0.01
30	1.00	1.00	0.94	0.83	0.68	0.55	0.41	0.30	0.21	0.15	0.08	0.04	0.02	0.01
Mean ± 1 SE	0.99 ± 0.00	0.96 ± 0.01	0.92 ± 0.01	0.82 ± 0.01	0.69 ± 0.01	0.56 ± 0.01	0.44 ± 0.02	0.33 ± 0.02	0.23 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.03 ± 0.00	0.01 ± 0.00
n	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Paired difference from hydropenia ± 1 SE	0.00 ± 0.00	−0.03 ± 0.01	−0.06 ± 0.01	−0.11 ± 0.01	−0.14 ± 0.01	−0.13 ± 0.01	−0.11 ± 0.01	−0.08 ± 0.01	−0.06 ± 0.01	−0.04 ± 0.01	−0.03 ± 0.00	−0.02 ± 0.00	−0.01 ± 0.00	0.00 ± 0.00
P value	>0.5	<0.025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.5	>0.5

TABLE IV

SUMMARY OF SEVERAL MEASURES OF WHOLE KIDNEY AND SINGLE NEPHRON FUNCTION IN RATS DURING NORMAL HYDROPENIA AND AFTER PLASMA VOLUME EXPANSION

Rat no.	Body wt.	Kidney wt.	$\overline{AP}$	P_{GC}	P_T	P_C	C_{PA}	C_{PE}	π_A	π_E	$\pi_E/\Delta P$	SNGFR	SNFF	Q_A
			Normal hydropenia											
	g	*g*	*mm Hg*		*mm Hg*		*g/100 ml*		*mm Hg*			*nl/min*		*nl/min*
24	253	1.18	105	46	14	9	4.9	7.8	15.2	30.4	0.97	38.1	0.37	103.0
25	202	0.67	125	50	15	10	4.5	8.0	13.5	31.6	0.90	25.1	0.44	57.2
26	227	0.67	120	50	12	10	4.9	8.1	15.2	32.3	0.86	26.5	0.41	66.2
27	230	0.92	125	50	15	12	4.8	8.9	14.8	37.7	1.08	28.0	0.46	61.6
28	199	0.82	130	44	13	9	5.5	7.9	17.9	31.0	1.00	23.2	0.33	77.5
29	194	0.72	125	47	12	7	4.6	8.6	13.9	35.6	1.02	25.9	0.48	55.2
30	203	—	145	44	11	7	4.8	7.9	14.8	31.0	0.94	26.5	0.39	67.8
Mean ±1 SE	215 ± 8	0.83 ± 0.1	125 ± 4	47 ± 1	13 ± 0.6	9 ± 0.7	4.9 ± 0.1	8.2 ± 0.2	15 ± 0.5	33 ± 1.0	0.97 ± 0.03	27.6 ± 1.8	0.41 ± 0.02	70.8 ± 6
n	7	6	7	7	7	7	7	7	7	7	7	7	7	7
			Plasma volume expansion											
24			95	63	22	15	5.2	7.6	16.5	29.2	0.71	59.9	0.32	187.2
25			110	63	20	14	5.7	8.4	18.8	34.2	0.80	45.7	0.32	142.8
26			110	52	15	13	5.0	7.8	15.6	30.4	0.82	42.3	0.36	117.5
27			120	52	14	12	6.0	6.8	20.3	24.5	0.64	63.2	0.12	526.7
28			110	44	13	13	5.9	8.1	19.8	32.3	1.04	53.4	0.31	197.8
29			110	49	15	11	5.5	7.7	17.9	29.8	0.88	35.8	0.38	123.4
30			130	55	16	15	5.9	7.6	19.8	29.2	0.79	58.1	0.21	264.1
Mean ±1 SE			112 ± 4	54 ± 3	16 ± 1	13 ± 0.6	5.6 ± 0.1	7.7 ± 0.2	18.4 ± 0.7	29.9 ± 1.1	0.81 ± 0.05	51.2 ± 3.8	0.29 ± 0.03	223 ± 54
n			7	7	7	7	7	7	7	7	7	7	7	7
Paired change from hydropenic period ±1 SE			−13 ± 2	+7 ± 3	+3 ± 1	+4 ± 1	+0.7 ± 0.2	−0.4 ± 3	+3 ± 1	−3 ± 2	−1.6 ± 0.06	+23.6 ± 3	−0.12 ± 0.04	+153 ± 55
P value			<0.001	<0.05	<0.05	<0.005	<0.01	>0.1	<0.025	>0.1	<0.05	<0.001	<0.025	<0.05

dict that if dextrans are transported across the glomerular capillary wall to a significant extent by diffusion, fractional dextran clearances will decrease with increasing glomerular plasma flow rate, Q_A. On the other hand, if dextrans are transported almost entirely by bulk flow, fractional dextran clearances would be expected to be insensitive to changes in Q_A. To examine these possibilities experimentally, measurements of fractional dextran clearances were repeated in these seven rats following volume expansion with isoncotic plasma, a maneuver previously shown by us (2–4, 7) to result in substantial increases in Q_A. Indeed, as shown in the right-hand portion of Table IV, plasma loading achieved the desired effect of producing large increases in Q_A, as well as less dramatic but statistically significant changes in most of the other quantities studied. Similar changes in these measures of single nephron function following 5% plasma loading have been observed by us previously (4). As shown by the open circles in Fig. 4, and by the individual animal data in the right-hand portion of Table III, alterations in glomerular pressures and flows induced by plasma loading resulted in highly significant reductions in the fractional clearance of all but the smallest and largest dextrans studied.

As shown in the right-hand portion of Table IV, since filtration pressure equilibrium was not obtained in six of seven rats following plasma volume expansion ($\pi_E/\overline{\Delta P} < 1$), values of the glomerular ultrafiltration coefficient, K_f, could be calculated (30). Values of K_f[0.05–0.07 nl/(s·mm Hg)] were within the range reported by us previously for Munich Wistar rats (4). In further agreement with results reported previously for the normal Wistar rat, K_f calculated from the mean values of $\overline{\Delta P}$, C_{PA}, C_{PE}, and Q_A in Table IV was found to be 0.071 nl/(s·mm Hg). This value has been employed in obtaining estimates of the membrane parameters r_o and S'/l (for the model based on pore theory) and ωS and σ (for the model based on the Kedem-Katchalsky equations).

Membrane Parameters Derived from Pore Theory. As discussed in the preceding report (19), the membrane parameters K_f, r_o, and S'/l are related as follows:

$$K_f = (S'/l)(r_o^2/8\eta) \tag{7}$$

where η, the viscosity of water at 37°C has a value of 0.007 P. Thus, given Eq. 7 and the experimentally determined value of K_f, specification of r_o also provides a value of S'/l. To determine r_o from mean values of $\overline{\Delta P}$, Q_A, C_{PA}, K_f, and the mean values of the $(U/P)_{\text{dextran}}/(U/P)_{\text{inulin}}$ ratios for various effective dextran radii, Eqs. 14 and 20 in ref. 19 are solved iteratively. In this computation r_o is varied until $|[\theta_{SB}]_{\text{calc}} - [\theta_{SB}]_{\text{exp}}| < 0.001$ for each dextran radius, where $[\theta_{SB}]_{\text{calc}}$ and $[\theta_{SB}]_{\text{exp}}$ are the calculated and measured values, respectively, of the $(U/P)_{\text{dextran}}/(U/P)_{\text{inulin}}$ ratio. Values of r_o computed in this manner for dextrans of varying molecular size are shown in Fig. 5 A. Curves are shown for rats studied before and after plasma volume expansion. Vertical bars denote the sensitivity of the calculated values of r_o to ± 1 SE variations in the measured inputs. For values of the effective dextran radius greater than ~28 Å, r_o is found to be relatively independent of dextran size, averaging approximately 52 Å both in normal hydropenia and after plasma volume expansion. For progres-

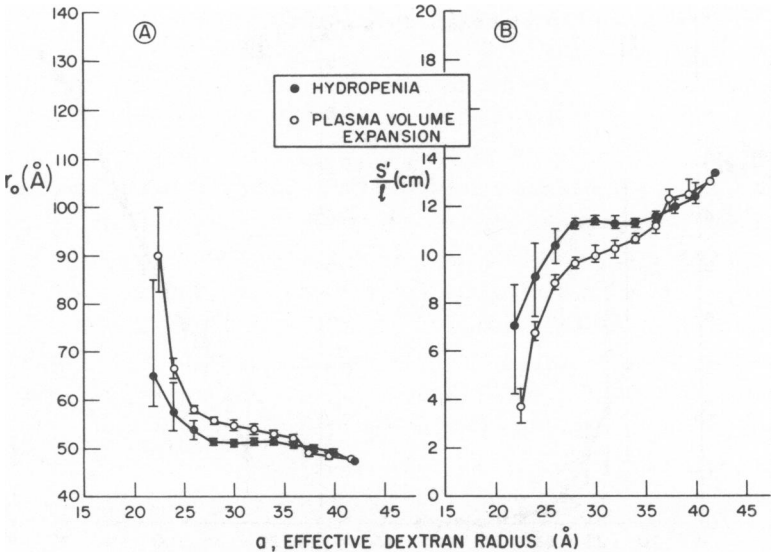

FIGURE 5 (*A*) The relationship between r_o, the pore radius, and effective dextran radius, a, for rats studied before and after plasma volume expansion. Vertical bars denote the sensitivity of the calculated value of r_o to ± 1 SE variations in the measured inputs. (*B*) The relationship between S'/l, ratio of the total pore area to pore length, and effective dextran radius, a, for rats studied before and after plasma volume expansion. The significance of the vertical bars is as defined above.

sively lower values of a, however, r_o is no longer constant but rather increases markedly with decreasing dextran size. Since S'/l is proportional to r_o^{-2} (Eq. 7), it follows that as dextran radius falls, S'/l must necessarily decrease even more dramatically than r_o rises, as shown in Fig. 5 B.

Membrane Parameters Derived from the Kedem-Katchalsky Equations. In addition to K_f, the membrane parameters required to characterize the glomerular capillary wall according to the Kedem-Katchalsky equations are ωS, the product of solute permeability (ω) and total capillary surface area (S), and σ, the reflection coefficient. The two unknown parameters (ωS and σ) are calculated using mean values of $\overline{\Delta P}$, Q_A, C_{PA}, K_f, and $[\theta_{SB}]_{exp}$ in the two experimental states, assuming that the membrane parameters are unchanged by plasma volume expansion. Eqs. 14 and 21 in ref. 19 are solved iteratively, values of ωS and σ for a given solute radius being adjusted simultaneously using a method similar to that of Stusnick (31) until $|[\theta_{SB}]_{calc} - [\theta_{SB}]_{exp}| < 0.001$ for each dextran in both experimental conditions. The dependence of ωS on effective dextran radius is illustrated in Fig. 6 A. Vertical bars again denote the sensitivity of the calculated values to ± 1 SE variations in the measured inputs. As can be seen, ωS decreases markedly with increasing molecular size, falling by some two orders of magnitude over the range of dextran radii studied. These findings suggest that for the largest dextrans, diffusion is no longer an important mechanism for transport across the glomerular capillary wall. Fig. 6 B illustrates the relationship between

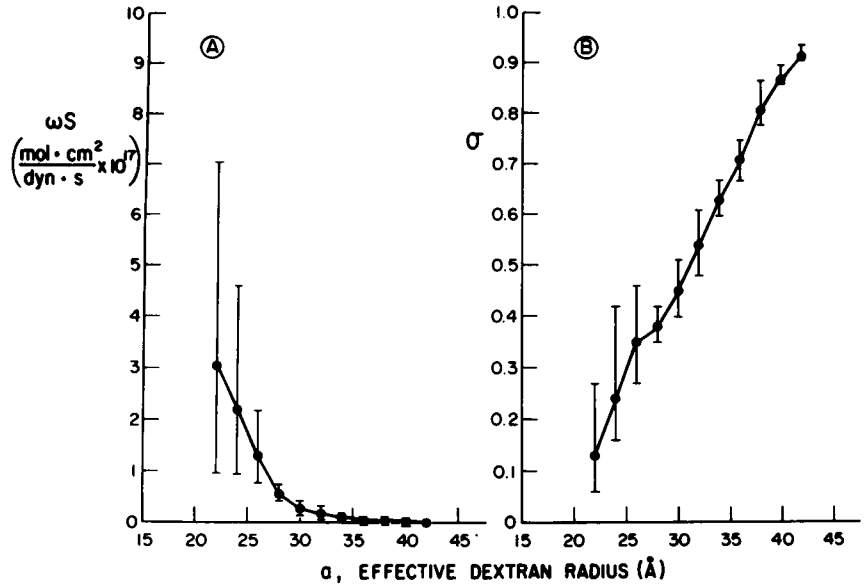

FIGURE 6 (*A*) The relationship between ωS, the product of solute permeability and total glomerular capillary surface area, and effective dextran radius, a, assuming that the membrane parameters were unchanged by plasma volume expansion. Vertical bars in panels *A* and *B* denote ± 1 SE variations in the measured inputs. (*B*) The relationship between σ, the solute reflection coefficient, and effective dextran radius, a.

dextran size and the reflection coefficient, σ. σ increases in essentially a linear manner with increasing dextran radius. There is no selective restriction by the capillary wall to transport of the smallest dextrans for which σ approaches zero. In contrast, for the largest dextrans σ approaches one, denoting the progressive reduction in dextran transport by bulk flow. Glomerular filtrate is normally almost totally devoid of these large molecules since, as shown in Fig. 6 A, effective diffusivities of these large molecules are also essentially zero. It is for the intermediate-sized dextrans, with radii of roughly 25–35 Å, that bulk flow and diffusive transport are both quantitatively significant. Nevertheless, since $\sigma > 0$ for these intermediate molecules and ω is not infinite, their transport is restricted relative to that of inulin or water.

DISCUSSION

Fractional solute clearance profiles similar to that shown in Fig. 4 for the normal hydropenic rat have been reported previously in man and experimental animals (12–15). The finding that fractional dextran clearances were influenced appreciably by alterations in glomerular pressures and flows induced by plasma volume expansion (Fig. 4) is in keeping with the theoretical results of the companion study (19), where it was shown that if some macromolecule is transported across the glomerular capillary wall to a significant extent by diffusion, its fractional clearance would be altered by

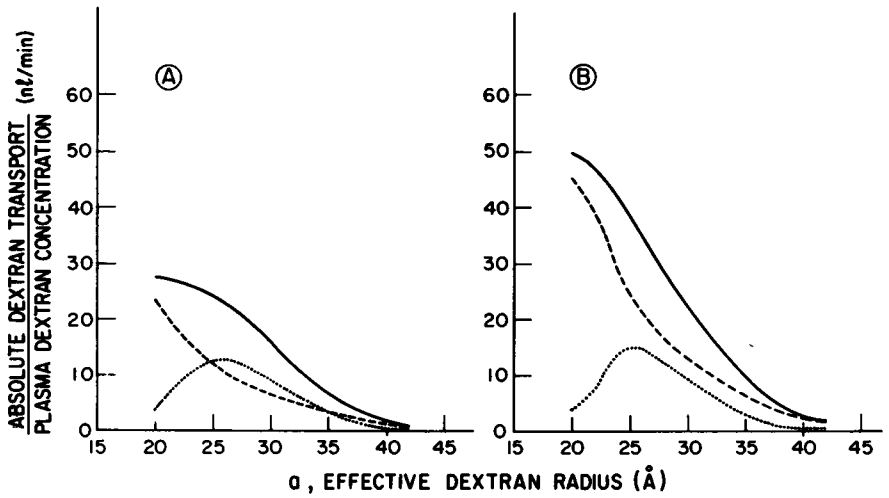

FIGURE 7 Relationship between dextran size and single nephron dextran clearance based on pore theory and measurements during normal hydropenia (*A*) and plasma volume expansion (*B*). Solid, dashed, and dotted lines represent total dextran transport, convective transport alone, and diffusive transport alone, respectively.

changes in the determinants of SNGFR, namely, Q_A, $\overline{\Delta P}$, C_{PA}, and K_f. This result is also in accord with theoretical expressions derived by previous workers (12, 15–17), which predict an inverse relationship between fractional clearance of a macromolecule and SNGFR when diffusion contributes appreciably to macromolecular transport. Of interest is the experimental demonstration by Lambert and Grégoire (18) of an inverse relationship between whole kidney glomerular filtration rate and fractional clearance of hemoglobin, which provides additional evidence of the significant contribution of diffusion to the glomerular transport of macromolecules. More recent results from this same laboratory (15) obtained using PVP molecules have also emphasized the role of diffusion.

The relative importance of convection and diffusion to dextran transport is examined quantitatively in Fig. 7 for pore theory and Fig. 8 for the Kedem-Katchalsky flux equations (19). In these figures, total dextran transport is represented by solid lines, convective transport alone by dashed lines, and diffusive transport alone by dotted lines. Since the ordinates in Figs. 7 and 8 represent single nephron dextran clearances, the solid lines approach the mean values of SNGFR (27.6 nl/min in normal hydropenia and 51.2 nl/min in plasma volume expansion) as dextran size diminishes and dextran clearance approaches inulin clearance. In general, the contribution of diffusion to dextran transport, while appreciable, is less than that of convection, a conclusion also reached by Verniory et al. (15). Diffusion exceeds convection only for dextrans of 25–35 Å radius in normal hydropenia (Fig. 7 A) for pore theory. Based on the Kedem-Katchalsky equations, diffusion accounts for less than 25% of the total

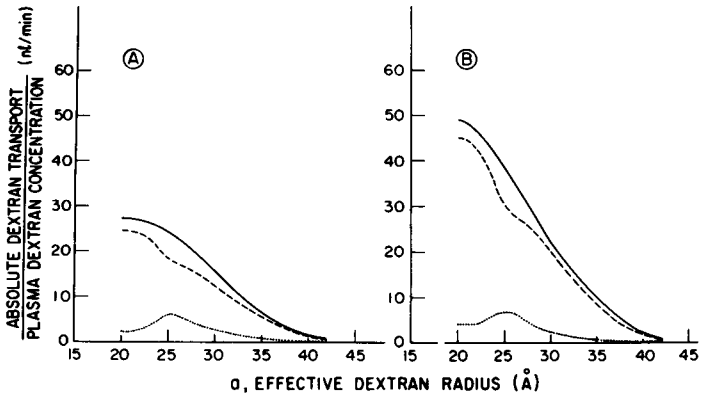

FIGURE 8 Relationship between dextran size and single nephron dextran clearance based on the Kedem-Katchalsky equations and measurements during normal hydropenia (*A*) and plasma volume expansion (*B*). Solid, dashed, and dotted lines are as in Fig. 7.

dextran transport (Fig. 8). It is of interest that for both experimental conditions, using either theory, diffusive transport is maximal for dextrans of ~25 Å radius. The relative importance of diffusion has also been found to be maximal for intermediate-sized PVP molecules (15). This can be explained qualitatively by noting that diffusive transport for a given dextran is the product of its effective diffusivity and mean transcapillary concentration difference. Although dextrans smaller than 25 Å have larger effective diffusivities, their mean concentration difference is much less because $\theta_{SB} = (U/P)_{\text{dextran}}/(U/P)_{\text{inulin}}$ is relatively close to one. The reverse is true for larger dextrans, where the concentration driving force is greater (θ_{SB} lower) but the effective diffusivity is much less. This reasoning also explains why plasma volume expansion, which has the effect primarily of decreasing θ_{SB} for any given dextran (Fig. 4), results in slight increases in the absolute rates of diffusive transport (Figs. 7 and 8). Plasma volume expansion diminishes the relative importance of diffusion, however, since solute transport by convection is augmented to a much greater extent than is transport by diffusion.

From consideration of the measured changes in the determinants of glomerular filtration rate (see Table IV), it is possible using the theoretical results in ref. 19 to estimate the relative contribution of each determinant to the observed change in the fractional dextran clearance profile. It is unlikely that K_f contributed to the observed changes in fractional dextran clearances, since we have shown previously that K_f remains unaffected by alterations in Q_A and $\overline{\Delta P}$ brought about by a similar degree of plasma volume expansion (4). Moreover, since both $\overline{\Delta P}$ and C_{P_A} increased slightly following plasma volume expansion, their net effect would have been to offset one another. The measured increase in $\overline{\Delta P}$ would tend to decrease fractional dextran clearance (Figs. 6 and 11 in ref. 19), whereas the increase in C_{P_A} would have a nearly equal and opposite effect (Figs. 7 and 12 in ref. 19). Thus, the change in the fractional

dextran clearance profile shown in Fig. 4 must have resulted solely from the measured large increase in Q_A (Figs. 3 and 9 in ref. 19).

A measure of the utility of isoporous theory in describing transport of macromolecules across the glomerular capillary wall is the extent to which the parameters r_o and S'/l are found to be independent of molecular size. If the glomerular capillary wall indeed behaves as if it were perforated by cylindrical pores of uniform radius, then r_o should be independent of dextran size. That is, the same fixed set of pores presumably should be available for each size of dextran. As shown in Fig. 5 A, constancy of r_o is found when the effective dextran radius exceeds 28 Å, but r_o increases progressively as dextran size is reduced below this level. A possible explanation for these increases in apparent pore radius for smaller molecules is that pore theory systematically overestimates the hindrance to transport of small molecules. As a result, fractional clearances for the smaller dextrans shown in Fig. 5 A are larger than can be accommodated by the pore radius (~52 Å) that correlates the data for the larger dextrans. Verniory et al. (15), in the most sophisticated application of pore theory to the glomerular capillary wall available to date, also found r_o and S'/l to depend on solute radius. Their results are extremely difficult to interpret, however, in that their reported values of S'/l do not vary as r_o^{-2}, a relationship which must hold if Eq. 7 is to be satisfied. Using the Kedem-Katchalsky flux equations, similar tests of consistency cannot be made between the values of σ and ωS obtained in the present study (Fig. 6), since this approach does not predict a relationship between σ and ωS. Determination of values for σ and ωS for a given molecular size is more difficult experimentally and computationally than is application of pore theory, because calculation of the two unknown membrane parameters (in addition to K_f) requires data from two experimental periods (e.g., measurements before and after plasma volume expansion). Although, as just discussed, isoporous theory does not yield internally consistent results in the smaller molecular size range using the present data, it nonetheless possesses the advantages of intuitive and computational simplicity.

Several recent studies (13–15) have reported estimates of the mean driving pressure for glomerular ultrafiltration, $\overline{\Delta P} - \overline{\Delta \pi}$, based on whole kidney clearance data for PVP or dextran. Using isoporous theory (14, 15), such an approach involves calculation of r_o and S'/l, from which an effective filtration coefficient (analogous to K_f) can be derived. Dividing the measured glomerular filtration rate by this filtration coefficient yields an estimate of $\overline{\Delta P} - \overline{\Delta \pi}$. The present study affords the first opportunity to test this approach, since, in addition to dextran clearance data, measurements of all of the glomerular pressures and flows needed for a direct calculation of K_f and $\overline{\Delta P} - \overline{\Delta \pi}$ (30) were obtained. This direct method, justified in detail elsewhere (30), does not depend on the dextran clearance data or on the specific assumptions inherent either in pore theory or in the Kedem-Katchalsky flux equations, and thus affords an independent and highly reliable means for calculating K_f and $\overline{\Delta P} - \overline{\Delta \pi}$. Table V compares results from the two approaches. Using equations derived by Verniory et al. (15) and employed also by Gassée (14), values of r_o and S'/l were

TABLE V

COMPARISON OF VALUES OF THE ULTRAFILTRATION COEFFICIENT (K_f) AND MEAN ULTRAFILTRATION PRESSURE ($\overline{\Delta P} - \overline{\Delta \pi}$) COMPUTED FROM THE PRESENT DATA USING THE METHOD OF VERNIORY ET AL. (15) AND THOSE EMPLOYED IN THE PRESENT STUDY (19, 30)

Condition	SNGFR	Method of calculation	r_o	S'/l	K_f	$\overline{\Delta P} - \overline{\Delta \pi}$
	nl/min		*Å*	*cm*	*nl/(s·mm Hg)*	*mm Hg*
Normal hydropenia	27.6	Ref. 15	50.8	24.1	0.148	3.1
		Refs. 19, 30	51.3*	11.4*	0.071‡	6.5
Plasma volume expansion	51.2	Ref. 15	50.8	24.3	0.149	5.7
		Refs. 19, 30	54.9*	9.9*	0.071	12.0

*Values of r_o and S'/l are given for a dextran radius of 30 Å. See Fig. 5 for values of these quantities at other dextran radii.

‡K_f cannot be computed directly in normal hydropenia because of the existence of filtration pressure equilibrium (30). Since K_f has been shown to be insensitive to changes in glomerular plasma flow rate induced during plasma volume expansion (4), the value of K_f for normal hydropenia is assumed to be the same as that determined for plasma volume expansion.

found which best fit the dextran clearance curves in Fig. 4. The values of r_o and S'/l shown for ref. 15 in Table V, obtained by minimizing the total error (14, 15)

$$\sum \left[\frac{[\theta_{SB}]_{calc} - [\theta_{SB}]_{exp}}{([\theta_{SB}]_{exp})^{1/2}} \right]^2$$

using data for dextran radii of 18–42 Å, were essentially identical to those obtained using a more restricted range of dextran sizes, 28–42 Å. As indicated in Table V, these values of r_o and S'/l, calculated from our dextran data using the approach given in ref. 15, are remarkably similar for normal hydropenia and plasma volume expansion, but yield values of K_f (from Eq. 7) slightly more than twice that obtained directly (30) using the glomerular pressures and flows measured in the present study during plasma volume expansion (Table IV). Since $\overline{\Delta P} - \overline{\Delta \pi}$ = SNGFR/K_f, the method of Verniory et al. (15) underestimates the mean driving pressure in both periods in the present study by a factor of two. Also shown are values of r_o and S'/l calculated at one representative dextran radius (30 Å) using the present method (19), in which S'/l is found from the directly determined value of K_f and the value of r_o needed to fit the dextran data. Comparison of the two sets of results in Table V (also see Fig. 5) indicates that the overestimates of K_f and underestimates of $\overline{\Delta P} - \overline{\Delta \pi}$ obtained using ref. 15 result primarily from overestimates of S'/l. It is noteworthy that Arturson et al. (13), assuming two discrete pore sizes, obtained an estimate of $\overline{\Delta P} - \overline{\Delta \pi}$ of less than ~0.7 mmHg from dextran clearances in normal human subjects; Verniory et al. (15) and Gassée (14) obtained much higher values for hydropenic dogs using PVP clearances (15–20 mm Hg). Based on the comparisons in Table V, it seems doubtful that clearance data alone can be used to provide reliable estimates of

K_f and $\overline{\Delta P} - \overline{\Delta \pi}$, and it is thus unclear whether these different estimates for man and dog reflect true species differences or are artifacts of the methods of calculation. Unfortunately, because these methods (13–15) use curve fitting procedures, it is difficult to predict in a given case whether the values of $\overline{\Delta P} - \overline{\Delta \pi}$ calculated from clearances would be expected to overestimate, underestimate, or agree with values obtained more directly. Of the mammals thus far studied, only the Munich Wistar rat and squirrel monkey, which have glomeruli accessible to micropuncture, offer the possibility of measuring all of the glomerular pressures and flows needed for a direct determination of $\overline{\Delta P} - \overline{\Delta \pi}$.

The authors are indebted to Dr. James Whitehead for his advice concerning gel chromatographic and dextran labeling procedures.

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This work was presented in part at the 66th Annual Meeting of the American Institute of Chemical Engineers, Philadelphia, Pa., 12 November 1973 (paper no. 57B), and at the 31st Annual Meeting of the American Federation for Clinical Research, Atlantic City, N.J., 4 May 1974 (*Clin. Res.* **22**:519A, 1974).

This study was supported in part by grants from the National Institutes of Health (HE14945 and AM13888). Computer time was made available by the School of Engineering, Stanford University.

Received for publication 6 November 1974 and in revised form 14 March 1975.

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