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Glomerular charge alterations in human minimal change nephropathy

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Glomerular charge alterations in human minimal change nephropathy. A theoretical model of charge and size selectivity for the glomerulus has been applied to human data. Using previously published values for GFR, renal plasma flow, systemic oncotic pressure, and fractional clearances of neutral dextrans, albumin, salivary amylase, and transferrin, membrane parameters describing the glomerular barrier were determined for normal individuals under control conditions and during lysine infusion (which retards tubule protein reabsorption), and for patients with minimal change nephropathy (MCN). To permit the estimation of membrane charge from fractional clearances, molecular charge values for human transferrin (−9.4 Eq/mole) and human salivary amylase (−4.1) were determined by measuring electrophoretic mobilities of these proteins in polyacrylamide gels. Assuming no large changes in the transmural hydraulic pressure difference (ΔP), the glomerular ultrafiltration coefficient (K_f, the product of hydraulic permeability and capillary surface area) was calculated to be reduced by >50% in MCN. The effective pore radius (~55 Å) is virtually unaltered in MCN, suggesting that the decline in K_f is due to a reduced number of pores. The degree of albuminuria observed in MCN is attributable to an approximately 50% reduction in the concentration of fixed negative charges in the glomerular capillary wall. The concentrations of fixed charges calculated from albumin data in normal individuals (140 to 160 mEq/liter) and in patients with MCN (60 to 90 mEq/liter) are insensitive to the assumed values of ΔP.

Altérations de la charge glomérulaire dans la néphropathie humaine à lésions minimes. Un modèle théorique pour la sélectivité par la charge et la taille pour le glomérule a été appliqué aux données humaines. En utilisant des valeurs déjà publiées pour le GFR, le débit plasmatique rénal, la pression oncotique systémique, et les clearances fractionnelles de dextrans neutres, d'albumine, d'amylase salivaire et de transferrine, les paramètres membranaires décrivant la barrière glomérulaire ont été déterminés pour des individus normaux, dans des conditions contrôles, et pendant une perfusion de lysine (qui retarde la réabsorption tubulaire des protéines), et chez des malades ayant une néphropathie à lésions glomérulaires minimes (MCN). Afin de permettre l'estimation de la charge membranaire à partir des clearances fractionnelles, les valeurs de la charge moléculaire de la transferrine humaine (−9,4 Eq/mole) et de l'amylase salivaire humaine (−4,1) ont été déterminées en mesurant la mobilité électrophorétique de ces protéines sur des gels de polyacrylamide. En postulant qu'il n'y a pas de grandes modifications dans la différence de pression hydraulique transmurale (ΔP), le coefficient d'ultrafiltration glomérulaire (K_f, produit de la perméabilité hydraulique et de la surface capillaire) apparaît par le calcul être réduit de <50% dans la MCN. Le rayon effectif des pores (~55 Å) est virtuellement inchangé dans la MCN, suggérant que la diminution de K_f est due à une diminution du nombre des pores. Le degré d'albuminurie observé dans la MCN est attribuable à une réduction d'environ 50% de la concentration des charges négatives fixées sur la paroi capillaire glomérulaire. Les concentrations de charges fixées calculées à partir des données obtenues avec l'albumine chez des individus normaux (140 à 160 mEq/litre), et chez des patients avec une MCN (60 à 90 mEq/litre) sont insensibles aux valeurs supposées de ΔP.

A number of studies in rats have demonstrated that molecular charge, in addition to molecular size, is an important determinant of the glomerular filtration of macromolecules. It has been shown by using various derivatives of dextran [1–3], ferritin [4, 5], and horseradish peroxidase [6], ranging in charge from cationic to anionic, that the filtration of polyanions is reduced, and that of polycations enhanced, relative to that of neutral macromolecules of comparable size and structure. The need to account for effects of both molecular size and charge has motivated development of a theoretical model which treats the glomerular capillary wall as an isoporous membrane containing a certain density of fixed negative charges [7]. In this model the filtration barrier is characterized completely by three parameters: the ultrafiltration coefficient (K_f, product of hydraulic permeability and capillary surface area), the effective pore radius (r_o), and the apparent concentration of fixed negative charges (C_m). These quantities have been estimated for normal rats and for rats with certain forms of experimental glomerular injury leading to proteinuria [7, 8].

In rats with nephrotoxic serum nephritis (NSN) and puromycin aminonucleoside nephrosis (PAN), it has been found that the filtration of neutral dextran is decreased while the transport of anionic dextran sulfate is increased [9–11]. This suggests that the increased filtration of albumin and other anionic proteins in these experimental disorders is due to the depletion of membrane charge, rather than increases in the number or size of pores. Calculated values of C_m declined from a normal range of 120 to 170 mEq/liter to 25 mEq/liter in NSN and 100 mEq/liter in PAN [7]. Ultrastructural studies employing various cationic stains qualitatively confirm a reduction in fixed charge content in these and other proteinuric disorders, including minimal change nephropathy in man [10, 12–14].

Previous studies in humans, to our knowledge, have not attempted to model the effect of membrane charge on macromolecule filtration in health or disease. Quantification of membrane charge alterations should allow a clearer comparison of

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various disease entities and may well have implications for therapy and pathogenesis. Accordingly, in this study we estimate membrane charge in humans in health and minimal change nephropathy (MCN), a condition of particular interest since recent data suggest that proteinuria in this disorder results entirely from a reduction in the fixed charge density of the glomerular capillary wall [14]. Furthermore, the selective proteinuria characteristic of MCN [14, 15] and the failure to find an increased clearance of large neutral molecules in MCN [16] suggest that it can be realistically represented using the isoporous model that has been applied to the rat. The analysis of membrane charge presented here is based necessarily on data for certain endogenous plasma proteins, since no other charged test macromolecule (such as dextran sulfate) has been available for human use. We use previously published clearance data for albumin, transferrin, and salivary amylase obtained under normal conditions [17, 18], during the inhibition of tubule protein reabsorption with lysine [17] and in MCN [15]. Clearances of neutral dextran in health and MCN are also used in the calculations [15]. Molecular charge estimates for two of the proteins, transferrin and amylase, have not been available previously and were obtained from electrophoretic mobility measurements reported here. We show that despite uncertainties stemming from protein reabsorption and the inaccessibility of the human glomerulus to direct measurement of pressures and flows, probable ranges of C_m in health and MCN can be estimated with reasonable confidence. These values of C_m for humans prove to be quite similar to those reported previously for the rat [7, 8].

Methods

Calculation of membrane parameters. The fixed-charge model of Deen, Satvat, and Jamieson [7] was used to estimate values for the membrane parameters, K_f, r_o, and C_m. Since the model has been described in detail elsewhere, only a brief summary of the governing equations and assumptions is presented here. The model assumes that the glomerular capillary can be described as a tube of constant circumference, the wall of which is perforated by pores of radius r_o and length δ. S refers to total capillary surface area and y denotes normalized axial position along a capillary (y = 0 at afferent end, and y = 1 at efferent end)[1]. The membrane is assumed to contain a concentration C_m of fixed negative charges. The primary effect of these fixed charges is to impose Donnan potentials at the plasma and Bowman's space membrane interfaces, the intramembrane potential being negative relative to either of the external solutions. Potential differences within the membrane are negligible [7].

Mass balance equations. Under the assumptions given above, the following differential equations relate the changes in plasma flow rate (Q) and concentration of solute i (C_i) with position along a glomerular capillary to the fluxes of volume (J_v) and solute i (J_i) from plasma to Bowman's space:

$$dQ/dy = -J_vS \quad (1)$$

$$d(QC_i)/dy = -J_iS \quad (2)$$

The initial conditions required are the afferent values of plasma flow rate (Q_A) and solute concentration (C_{iA}). Equation 2 is applied to three solutes: total protein, sodium, and the test macromolecule (dextran or specific protein). For total protein, the right side of equation 2 is set equal to zero, based on the assumption that the total amount of protein filtered is a very small fraction of that presented to the kidney in afferent plasma. This assumption is supported by the data of Carrie, Salyer, and Myers [14] and Carrie and Myers [15] in MCN, which indicate this fraction to be less than $\sim 10^{-3}$ even at the nephrotic levels of proteinuria studied.

Flux equations. The small fractions of protein filtered justify continued use of the Starling relation to describe the transcapillary volume flux:

$$J_vS = K_f(\Delta P - \pi_G) \quad (3)$$

ΔP is the transcapillary hydraulic pressure difference[2] and π_G the intracapillary colloid osmotic pressure (calculated from total protein concentration, C_p, using equation 8). Note from equation 1 that only the product of J_v and S is required. For a solute of molecular charge z_i and diffusion coefficient (in bulk solution) D_i, the flux is given by:

$$J_i = \frac{J_vC_iK_C\phi \exp[z_i\Delta\psi(0)]}{1 - \exp(-\alpha)\{1 - K_C\phi \exp[z_i\Delta\psi(\delta)]\}} \quad (4)$$

where

$$\alpha = \frac{K_C(\Delta P - \pi_G)r_0^2}{8\eta K_D D_i} \quad (5)$$

In equation 4, the potential differences across the membrane surfaces adjacent to the capillary lumen and to Bowman's space are given by $\Delta\psi(0)$ and $\Delta\psi(\delta)$, respectively. These are the magnitudes of the Donnan potentials at the respective membrane interfaces and are positive numbers. Here ψ is a dimensionless potential, referenced to RT/F, where R is the universal gas constant, T is the absolute temperature, and F is Faraday's constant. In equation 5, η is the viscosity of water at 37°C. The remaining quantities in equations 4 and 5 (K_C, K_D, ϕ) all involve hindrances to molecular transport based on the ratio of molecular radius to pore radius. These functions describe the size-selectivity of the capillary wall and are calculated from a hydrodynamic theory of transport in pores [19]. For small solutes such as sodium, K_C, K_D, and ϕ are approximately equal to unity, whereas for large solutes they approach zero.

Evaluation of membrane parameters. For a given set of membrane parameters (K_f, r_o, C_m) and other input quantities the mass balance equations (equations 1 and 2) are integrated numerically to determine all fluxes and concentrations as

[1]This model is based on the assumption of identical capillaries in parallel, so that the mass balance and flux equations apply equally well to a single capillary, a single glomerulus, or the sum of all glomeruli, depending on the interpretation of S and plasma flow rate (Q). The single glomerulus is the most appropriate basis in the rat, since micropuncture data are available at that level. In humans the natural basis is the sum of all glomeruli in both kidneys.

[2]In this study ΔP is assumed to be essentially independent of position along a glomerular capillary, as suggested by findings in the rat [31].

functions of y. This allows calculation of the ratio (θ) of the macromolecule concentration in Bowman's space to that in afferent plasma, which is given by:

$$\theta = \frac{\int_0^1 J_i \, dy}{C_{iA} \int_0^1 J_v \, dy} \qquad (6)$$

It should be noted that for a solute that is not reabsorbed or secreted, θ equals the fractional clearance, the ratio of the urinary clearance of i to that of inulin. Reabsorption of filtered protein requires that a distinction be made between θ and urinary fractional clearance, denoted by θ_U.

The ultrafiltration coefficient (K_f) is evaluated using the combined solutions of equations 1 and 2 (the latter applied to total protein), by matching the calculated efferent value of C_p to that measured. Since ΔP is not directly measurable in humans, values of K_f will be reported for various assumed values of ΔP. For a neutral macromolecule ($z_i = 0$) of specified size, θ depends only on K_f and pore radius (r_o). With K_f already determined as just described, r_o is estimated by adjusting its value until the calculated value of θ matches that observed. For a charged macromolecule, θ depends on one additional parameter, the fixed charge concentration (C_m). Given an assumed value of C_m, the Donnan potentials in equation 4 are calculated by applying equation 2 to sodium and using conditions of overall electroneutrality in each compartment [7]. An iterative procedure is then used to find the value of C_m that provides the best fit to the value of θ observed for the charged macromolecule.

Estimation of molecular charge. Application of the fixed charge model requires evaluation of the effective molecular charge (z) of the macromolecule under consideration. We used electrophoretic mobility measurements in polyacrylamide gels to estimate z for human transferrin (Sigma Chemical Co., St. Louis, Missouri) and for human salivary amylase (Sigma Chemical Co.). A gel electrophoresis cell was fabricated to allow for large and equal volumes (~3L) in the upper and lower buffer chambers, to minimize pH variation due to the electrochemical reactions. The power supply (model 500) and electrophoresis reagents were obtained from Bio-Rad Laboratories, Richmond, California. Buffer chambers were filled with sodium phosphate buffer at pH 7.4 and 0.15 M ionic strength. The cell was immersed in a large water bath and maintained at 37°C. Polyacrylamide gels of approximately 70 mm length were formed in glass tubes (5 mm I.D. × 125 mm) by polymerization of acrylamide in the presence of the cross linking agent N,N'-methylene-bis-acrylamide (BIS) as described previously [7].

At the beginning of an electrophoresis run 100 μl of a 20% sucrose solution containing about 0.04 mg of protein was layered carefully on top of each gel. Generally, 16 tubes were used, four at each gel concentration. In one or two tubes at each concentration, purified human serum albumin (Sigma Chemical Co.) was used as a reference standard. The remaining tubes were loaded with the protein being studied. Two sets of 16 tubes were run for both transferrin and amylase. Gels with indistinct bands and gels distorted during removal from the tubes were discarded. The electrophoresis was interrupted at 40-min intervals; the pH in each buffer chamber was determined and readjusted toward 7.4. This procedure resulted in a maximum range of pH from 7.35 to 7.45 in both chambers throughout each experiment. Electrophoresis was carried out for 180 min for transferrin and 240 min for amylase at a power supply setting delivering a constant current of 8.1 mA/tube. Standard protein staining and destaining procedures with Coomassie blue [8] allowed the determination of the migration distances. The monomeric albumin band (highest mobility) was used as the reference. The relative mobility was determined at each gel concentration by dividing the average migration distance of the test protein by that of the albumin band.

Results at various gel concentrations[3], G, were extrapolated to zero gel concentration using the equation [20]:

$$ln\,(M_{alb}/M_{test}) = ln\,(M_{alb}/M_{test})_o - KG \qquad (7)$$

where (M_{alb}/M_{test}) is the ratio of albumin mobility to that of the test protein at gel concentration G, and $(M_{alb}/M_{test})_o$ is the mobility ratio in the free solution. Here K is a constant that represents the relative retarding influence of the gel and may be positive or negative, according to whether the gel retards albumin more or less than the test protein. The constants $ln\,(M_{alb}/M_{test})_o$, K, and the correlation coefficient r were calculated using linear regression, and the 95% confidence intervals for the relative mobilities determined in standard fashion [21]. The molecular charge for albumin at pH 7.4 ($z = -19$) was calculated from potentiometric titration results corrected for chloride binding [22, 23]. Given their free solution mobilities, relative to albumin, the molecular charges of transferrin and amylase were determined through application of the Henry-Gorin hard sphere model for the migrating colloid [24, 25], an appropriate choice for globular proteins.

Results

Electrophoretic mobility and molecular charge

Transferrin. Each gel contained a distinct band corresponding to the major component. Faint bands of lower mobility, present in some tubes, were not reliably detected and were ignored. The results are summarized in Figure 1 and Table 1. The mobility of albumin relative to transferrin at $G = 0$ is 2.12, with a 95% confidence interval of (1.68, 2.70). The molecular radius of transferrin used in this study (38 Å), is the mean of two values (36 Å, 40 Å) reported by Laurent and Killander [26]. These data allow estimation of the molecular charge of human transferrin at physiologic pH as $z = -9.4$. The 95% confidence interval for z shown in Table 1 is calculated from the confidence interval for the relative free solution mobility.

Salivary amylase. All gels contained one band of major intensity. Additional bands of lower intensity were variably present and were ignored. Since all electrophoretic variants of salivary amylase are reported to have similar molecular weights [27], and since serum amylase activity displays only one peak in gel filtration chromatography [28], the major band is assumed to have a molecular radius of 29 Å, corresponding to that peak. The results are summarized in Figure 2 and Table 1. The free solution mobility of albumin relative to salivary amylase is 3.71 with a 95% confidence interval of (2.31, 5.98). The estimated

[3]Gel concentration (expressed as percent) is given by the total weight of BIS and acrylamide per 100 ml solvent.

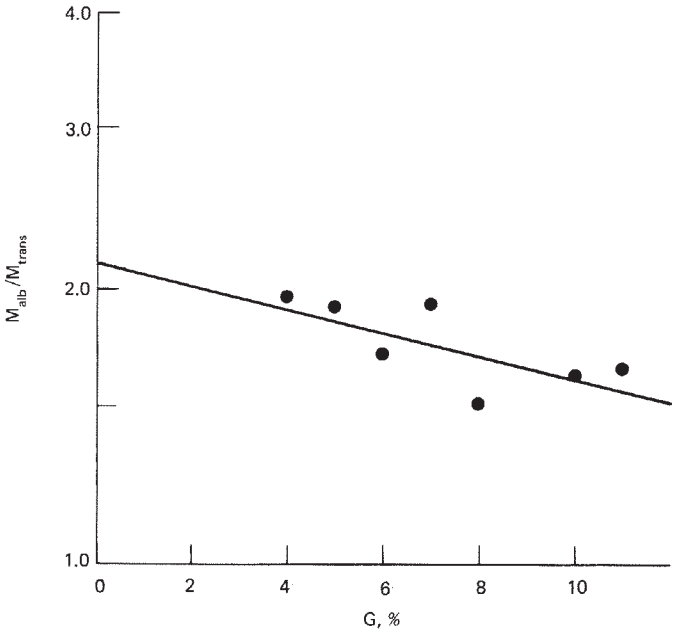

Fig. 1. *Relative mobility of albumin to transferrin (M_{alb}/M_{trans}) as a function of gel concentration G, plotted on semilogarithmic coordinates.* The *linear regression line* shown has correlation coefficient r = −0.667. At G = 0, the relative free solution mobility is 2.12.

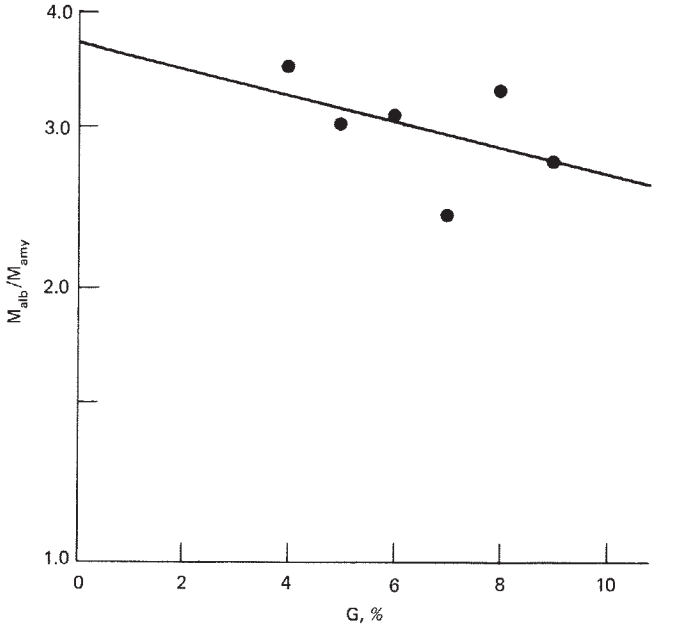

Fig. 2. *Relative mobility of albumin to amylase (M_{alb}/M_{amy}) as a function of gel concentration G, plotted on semilogarithmic coordinates.* The *linear regression line* shown has correlation coefficient r = −0.473. At G = 0, the relative free solution mobility is 3.71.

molecular charge of salivary amylase at pH 7.4 is z = −4.1. The finding here of only one major salivary amylase band on electrophoresis is consistent with the results of Mayo and Carlson [28], who found that the four salivary isoamylases

Table 1. Physico-chemical data for selected proteins

Protein	$(M_{alb}/M_{test})_o$	z	95% Confidence interval for z	Effective molecular radius, Å
Albumin	1.00	−19	—	36
Transferrin	2.12	−9.4	(−7.4, −12)	38
Amylase	3.71	−4.1	(−2.6, −6.6)	29

Abbreviations: z, effective molecular charge; $(M_{alb}/M_{test})_o$, mobility ratio in the free solution.

consist of two isoamylases with isoelectric point (IEP) = 5.9, and two isoamylases with IEP = 6.4. All four isoamylases, however, formed a single composite band on disc gel electrophoresis, implying that the molecular charge of all salivary isoamylases may be similar despite disparate isoelectric points. This belief is consistent with the finding that all isoamylases, both salivary and pancreatic, are cleared by the kidney to a similar extent [18].

Analysis of human filtration data

Ultrafiltration coefficients, K_f. The pore radius as calculated here is a function of both K_f and ΔP. Under conditions of filtration pressure disequilibrium ($\pi_E < \Delta P$, where π_E is the efferent oncotic pressure), a unique value of K_f can be calculated for each assumed value of ΔP. As filtration pressure equilibrium is approached ($\pi_E \simeq \Delta P$), the calculated value of K_f tends toward infinity and only a minimum estimate can be obtained reliably. Glomerular capillary oncotic pressure (π_G) and total plasma protein concentration (C_P) are related as follows[4]:

$$\pi_G = a_1 C_P + a_2 C_P^2 \qquad (8)$$

where a_1 = 1.629 mm Hg/(g/dl) and a_2 = 0.2935 mm Hg/(g/dl)^2. C_P in the afferent arteriole (C_{PA}) was calculated from values of systemic oncotic pressure reported by Carrie and Myers [15] in normal individuals and in MCN. These values of C_{PA}, together with the other needed inputs reported in the same study, GFR (inulin clearance) and renal plasma flow rate (RPF, estimated from PAH clearance) are shown in Table 2. Mogenson and Solling [17] reported that GFR and RPF did not change significantly during lysine infusion in normal volunteers. Accordingly, we have used the same values of C_{PA}, GFR, and RPF in our calculations for lysine infusion as for normal individuals. C_P in the efferent arteriole (C_{PE}) was obtained in each case from C_{PA} and filtration fraction (FF = GFR/RPF), using the relation:

$$C_{PE} = \frac{C_{PA}}{1 - FF} \qquad (9)$$

π_E (from C_{PE} and equation 8) averaged 25.5 mm Hg in MCN and 39.0 mm Hg in healthy individuals.

The ultrafiltration coefficient, K_f, calculated for both MCN and normal humans as a function of assumed ΔP is illustrated in Figure 3. These calculations demonstrate a marked reduction in K_f in MCN at any assumed constant value of ΔP. For ΔP = 40

[4]Equation 8, with the same values of a_1 and a_2, has been shown to be applicable both in normal individuals and in MCN [37].

Table 2. Model inputs for man

Quantity U	Normal	Lysine infusion	Minimal change nephropathy
GFR, *ml/min*	99	99	80
RPF, *ml/min*	513	513	371
C_{PA}, *g/dl*	7.33	7.33	5.45
ΔP, *mm Hg*	40	40	40
K_f, *ml/min · mm Hg*	19.7	19.7	4.29
r_o, *Å*[a]	54.5	54.5	54.5
z_pC_p, *mEq/liter*[b]	16.0	16.0	11.9
C_{Na}, *mEq/liter*[c]	152	152	152
θ_U albumin[d]	1.7×10^{-6}	5.7×10^{-5}	1.75×10^{-3}
θ_U transferrin[d]	1.3×10^{-6}	1.1×10^{-4}	—
θ_U amylase[d]	1.8×10^{-2}	—	—

[a] Glomerular pore radius calculated from neutral dextran clearances.
[b] Total protein charge concentration in systemic plasma; z_p is assumed to be constant.
[c] Total cation concentration in systemic plasma.
[d] Urinary fractional clearance.

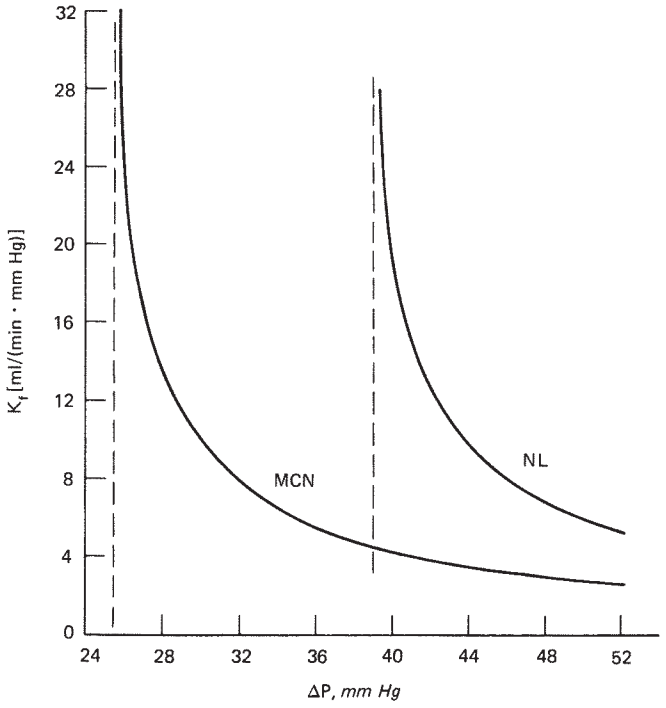

Fig. 3. *Whole kidney ultrafiltration coefficient* K_f, *as a function of the assumed value of* ΔP *in normal man* (NL) *and in minimal change nephropathy* (MCN), *on the basis of the parameters in Table 2.*

mm Hg, K_f is reduced by >75% in MCN, and for ΔP = 50 mm Hg, K_f is reduced by >50% relative to control. That ΔP in MCN varies little from normal values is suggested by micropuncture measurements of glomerular and tubule hydraulic pressure in normal and PAN rats [11]. At ΔP = 40 mm Hg, on a two-kidney basis, K_f = 19.7 ml/(min · mm Hg) in normal man (Table 2). The corresponding single nephron K_f = 0.16 nl/(sec · mm Hg), assuming 2×10^6 nephrons in the two kidneys. This is roughly twice the typical K_f value in the rat [31]. Note that for a single nephron, K_f = kS, the product of the apparent hydraulic permeability, k, and the total glomerular capillary surface area, S. The average surface area of the human glomerulus has been estimated at S = 0.0038 cm^2 [29], twice the value for the rat glomerulus [30]. Thus, the hydraulic permeabilities of human and rat glomeruli appear to be virtually identical.

Pore radius. Values of r_o were calculated as a function of ΔP using the clearance data for neutral dextrans of Carrie and Myers [15] for both MCN and controls, with the results shown in Figure 4. In both cases r_o is in the approximate range of 50 to 60 Å and changes by only about 10% over the range examined. For the particular value of ΔP = 40 mm Hg, which we have chosen as a base case, identical values of r_o (54.5 Å) are obtained for normal individuals and MCN, as indicated in Table 2 and Figure 4. Values of r_o during lysine infusion were taken to be the same as for normal individuals, at any assumed value of ΔP.

Estimates of the membrane charge density, C_m. Values of the glomerular membrane charge density in normal humans and patients with MCN are estimated in Table 3. Values of the Bowman's space-to-plasma concentration ratio (θ) for transferrin, albumin, and amylase are estimated from the urinary fractional clearance (θ_U) in normal subjects [17, 18], during lysine infusion [17], and in MCN [15], and are given in Table 2. Due to tubule protein reabsorption, these values underestimate θ and result in the calculation of upper bounds on C_m. As shown in Table 3, the upper bound on the normal membrane charge density, C_m, ΔP = 40 mm Hg, ranges from 220 to 480 mEq/liter for the three macromolecules. These upper bounds are higher than the estimated values for normal Munich-Wistar rats, which range from 100 to 170 mEq/liter, based on both micropuncture data for albumin and fractional clearances of anionic, neutral, and cationic dextrans [7, 8].

Mogenson and Solling [17] interpreted their results from lysine infusion in normal volunteers as showing near complete inhibition of tubule reabsorption of albumin and a variety of other proteins. As already mentioned, the model input values used for the lysine infusion group are assumed to be identical to the normal group with the exception of the values for θ (Table 2). The upper bounds on C_m during lysine infusion (160 to 260 mEq/liter, Table 3) are much lower than the upper bounds computed without the inhibition of protein reabsorption (220 to 480 mEq/liter), and presumably are a much closer approximation to actual charge values for normal individuals. In MCN, the upper bound on C_m (90 mEq/liter) computed from the urinary clearance of albumin is substantially lower than the value of 160 mEq/liter obtained for albumin during the inhibition of tubule albumin reabsorption by lysine infusion in normal volunteers.

Lower bounds on C_m must be based on estimated upper bounds on θ. Recall that for a molecule which is neither secreted nor reabsorbed, the urinary fractional clearance (θ_U) is equal to the Bowman's space-to-plasma concentration ratio (θ) for that molecule. Since plasma proteins are reabsorbed (to varying degrees), the urinary fractional clearance underestimates θ. For both MCN and control groups, reasonable assumptions about the extent of albumin reabsorption allow estimation of an upper bound on θ, denoted by θ_{max}. Numerous micropuncture experiments in normal and nephrotic rats allow calculation of the fractional reabsorption of albumin. In a group of such studies reviewed by Galaske, Baldamus, and Stolte [32], the fraction of filtered albumin reabsorbed varied from 57

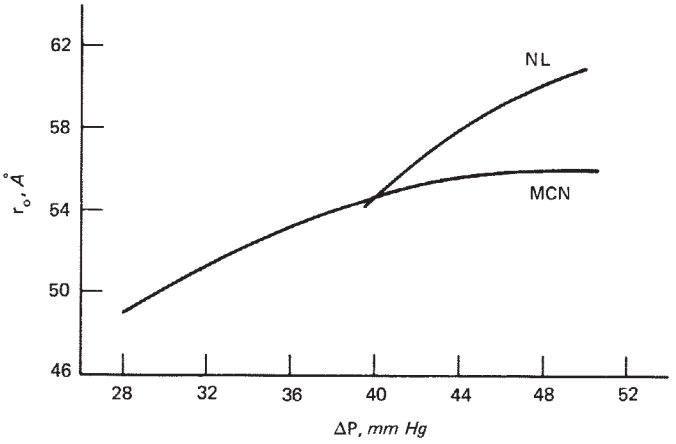

Fig. 4. *Pore radius, r_o, as a function of the assumed value of ΔP, calculated from neutral dextran clearances.* In *MCN*, pore radius is not increased relative to normal *(NL)*, assuming no large changes in *ΔP*.

Table 3. Estimates of glomerular wall fixed negative charge density C_m(mEq/liter)[a]

Protein	Upper bound C_m	Lower bound[b] C_m
Normal		
Albumin	220	140
Transferrin	480	—
Amylase	305	—
Lysine infusion		
Albumin	160	—
Transferrin	260	—
Minimal change nephropathy		
Albumin	90	60

[a] Values were calculated to the nearest 5 mEq/liter.
[b] Assumes < 99% reabsorption of albumin normally and < 80% reabsorption of albumin in minimal change nephropathy.

to 96% in normal rats and from 16 to 20% in rats with glomerulopathy induced by antiglomerular basement membrane antibody. If we denote the urinary fractional clearance (without lysine) as θ_U then conservative assumptions are that $\theta_{max} = 100\ \theta_U$ normally, and $\theta_{max} = 5\ \theta_U$ in MCN. Thus, based on the values in Table 2 for man, $\theta_{max} = 1.7 \times 10^{-4}$ normally, and $\theta_{max} = 8.75 \times 10^{-3}$ in MCN. This is equivalent to the assumption that less than 99% of filtered albumin is reabsorbed normally, and less than 80% of filtered albumin is reabsorbed with nephrotic levels of proteinuria in MCN. The corresponding lower bounds on C_m are 140 mEq/liter in normal individuals and 60 mEq/liter in MCN, as shown in Table 3. Thus, the best estimates of θ for albumin give charge values ranging from 140 to 160 mEq/liter in normal individuals and 60 to 90 mEq/liter in MCN.

The estimates of C_m in Table 3 are based on the assumption that $\Delta P = 40$ mm Hg in each experimental condition. The effect on these calculations of the assumed value of ΔP is shown in Table 4. Measured values of π_E discussed above indicate that $\Delta P > 39$ mm Hg in normal individuals and $\Delta P > 26$ mm Hg in MCN. As indicated in Table 4, little or no effect on the calculated C_m is observed when ΔP is varied throughout the probable physiological range for normal subjects (40 to 50 mm Hg) or patients with MCN (30 to 50 mm Hg). The variations in C_m due to the uncertainty in ΔP are only ± 0 to 8%. Thus, the unavailability of micropuncture data on ΔP for humans does not seriously hinder the quantitative estimation of membrane charge.

Discussion

The calculated upper bounds on C_m in humans based on normal urinary fractional clearances (220 to 480 mEq/liter) are well above the actual range of values for C_m (100 to 170 mEq/liter) in normal Munich-Wistar rats [7, 8]. This discrepancy is undoubtedly due largely to tubule reabsorption of albumin, salivary amylase, and transferrin. From the data of Mogenson and Solling [17], one can estimate that normal reabsorption rates of albumin and transferrin exceed 97 and 99%, respectively, of the filtered loads. Since the ratio of amylase to creatinine clearance is constant over a wide range of GFR and serum amylase concentrations, past authors concluded that amylase is not reabsorbed significantly [35]. However, amylase reabsorption was demonstrated in rats in a later study by Noda using stop-flow analysis [36]. More recently, direct measurements of the clearance of salivary isoamylase showed four to fivefold increases during acute pancreatitis, suggesting that at least 75% of filtered amylase is reabsorbed normally [18]. Thus, the precise degrees of albumin, transferrin, and amylase reabsorption are not known, but it is fairly certain that reabsorption of these three proteins is normally not negligible. For this reason, estimates of C_m based on albumin and transferrin clearances during inhibition of protein reabsorption by lysine infusion (160 to 260 mEq/liter) should be much more accurate than those obtained from normal urinary clearances. These estimates are dependent of course on the assumption that lysine infusion selectively inhibits tubule protein reabsorption while not altering the glomerular barrier, as argued by Mogenson and Solling [17]. Fractional clearance measurements during lysine infusion for nonreabsorbed macromolecules (such as dextran) would provide a good test of this assumption, but such data are not currently available.

The molecular charge of transferrin at physiologic pH, −9.4 Eq/mole, was found to be one-half that of albumin. Since the molecular radius of transferrin (38 Å) is similar to that of albumin (36 Å), electrostatic interactions with the fixed negative charges on the glomerular capillary wall should cause albumin fractional clearance to be less than that of transferrin. In accord with this expectation, the urinary fractional clearance (θ_U) of transferrin is approximately twice that of albumin, when tubule protein reabsorption has been reduced substantially by lysine infusion (Table 2). In contrast, one would expect clearances of these two proteins to be more similar during any form of glomerular injury in which charge selectivity is impaired. Almost precise equivalence between albumin and transferrin clearances has indeed been observed frequently in a variety of human glomerulopathies, including MCN [33, 34]. On a more quantitative level, the higher "upper bound" value of C_m obtained for transferrin than for albumin (260 versus 160 mEq/liter) indicates that, according to the theoretical model, the normal ratio of transferrin to albumin clearance (neglecting reabsorption) should be even larger than the observed ratio of

Table 4. Sensitivity of estimates of C_m to uncertainty in ΔP

Protein	Upper bound C_m	Lower bound C_m
Normal, $\Delta P = 40 \rightarrow 50$ mm Hg		
Albumin	(220 → 220)	(140 → 140)
Transferrin	(480 → 520)	—
Amylase	(305 → 325)	—
Lysine infusion, $\Delta P = 40 \rightarrow 50$ mm Hg		
Albumin	(160 → 160)	—
Transferrin	(260 → 290)	—
Minimal change nephropathy, $\Delta P = 30 \rightarrow 50$ mm Hg		
Albumin	(85 → 90)	(60 → 65)

1.9, approximately 60. Whether this discrepancy in C_m values is due to greater residual reabsorption of transferrin than that of albumin during lysine infusion, or to some effect (like differences in molecular configuration) not included in the model, cannot be determined at this time. Until a better understanding is available of both protein reabsorption kinetics and effects of molecular configuration on filtration, inferences regarding differences in C_m between normal and pathological states should be based on data for the same macromolecule.

The changes in membrane properties (K_f, r_o, C_m) of the human glomerulus due to MCN are remarkably similar to those reported previously for a morphologically similar disorder in rats, puromycin aminonucleoside nephrosis (PAN) [7, 11]. In PAN rats, K_f determined from direct micropuncture measurements of the required input quantities (including ΔP) was reduced by approximately 70% compared to normal controls [11]; depending on the assumed values of ΔP in humans, we infer K_f in MCN to be reduced by approximately 50 to 75% (Fig. 3). Effective pore radius (r_o) was virtually unchanged in PAN and MCN, perhaps decreasing slightly. This implies a large reduction in the number of filtering pores, perhaps accompanied by an increase in their length. Similar conclusions for MCN were reached by Winetz et al [37]. The reduction in C_m inferred here from the albumin data (140 to 160 mEq/liter in normal individuals to 60 to 90 mEq/liter in MCN) again is quite similar to that calculated for PAN rats [7], based on fractional clearances of dextran derivatives (120 to 170 mEq/liter in controls to 100 mEq/liter in PAN). Thus, the proteinuria both in PAN rats and in humans with MCN is most readily explained by a 40 to 50% loss of fixed negative charges from the glomerular capillary wall.[5] As shown by the results in Table 4, the unavailability of ΔP measurements in humans has little effect on the charge estimates.

[5] Albumin filtration in PAN rats is at least roughly approximated by the measured total rate of protein excretion [11], since proteinuria in this disorder is highly selective and fractional reabsorption of albumin is probably quite small. Assuming this to be true, we calculate that C_m = 70 mEq/liter in PAN is sufficient to account for the observed level of proteinuria. Although this charge concentration in PAN rats is somewhat lower than that obtained from the dextran data (100 mEq/liter), it still represents a 40 to 50% reduction below the corresponding C_m values calculated from albumin data in normal animals, 100 to 130 mEq/liter [8].

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