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Research Article

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Normal Glomerular Permeability and Its Modification by Minimal Change Nephrotic Syndrome

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ABSTRACT It has been suggested that the glomerular basement membrane restricts the passage of large molecules only, the barrier to filtration of smaller molecules being at the level of the epithelial slit pore. This hypothesis was investigated by measuring glomerular permeability to ^{125}I-labeled polydisperse polyvinyl pyrrolidone (PVP) in 16 children with idiopathic nephrotic syndrome (INS) and in 6 children of comparable age who had no evidence of renal disease. Studies were performed in the patients with INS before, during, and after treatment with steroids. PVP in blood and urine samples was separated according to molecular size by solumn chromatography, to permit the calculation of permeability to inert macromolecules of sizes ranging from 8,000 to 85,000 mol wt. In untreated INS, glomerular permeability to molecules > 40 Å was normal; permeability to smaller molecules was markedly reduced, frequently to 20% or less of normal. There was an average decrease in inulin clearance (C_{In}) of 24%. Glomerular permeability and C_{In} returned to normal in INS treated with steroids only when proteinuria disappeared. The results support the concept, derived from studies with ultrastructural tracers, that the final barrier to filtration may be at the level of the epithelial slit pore. Thus fusion of the epithelial foot processed with obliteration of the slit pores was associated with impaired passage of smaller molecules of PVP into the urine. Reversal of the pathologic abnormality resulted in return of permeability to normal. The decreased C_{In} seen in INS may not reflect true glomerular filtration rate, but may result from restricted passage of inulin molecules (mol wt 5,000) through the epithelial slit pore.

INTRODUCTION

Despite the recent and extensive studies on the dynamics of glomerular ultrafiltration (1–3), the mechanisms responsible for the permeability characteristics of the glomerulus are still incompletely understood. The endothelial cells lining the capillary surface of the glomerular basement membrane (GBM)[1] are unlikely to form an effective barrier to filtration, since their cytoplasm is penetrated completely by fenestrae of 400–1,000 Å in diameter (4). In contrast, the GBM itself appears to completely separate plasma from the glomerular filtrate. Traditionally this structure has been considered to behave as a semipermeable membrane containing cylindrical pores of 35–42 Å radius or rectilinear slits of half-width 36 Å (5). However, if the membrane does contain functional pores, it may be more heteroporous than originally suggested (6), and although these experiments suggest that the basement membrane contains pores, such pores have never been demonstrated. An alternate view conceives the GBM as a hydrated gel with glomerular filtration occurring by diffusion (7).

The function of the glomerular epithelial cells and their possible role as a barrier to filtration also remains to be delineated. These cells are characterized by trabecular foot processes that extend from the cell body to the external layer of the basement membrane in which they are embedded. With correct histologic prep-

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[1] *Abbreviations used in this paper:* C_{In}, inulin clearance; GBM, glomerular basement membrane; GFR, glomerular filtration rate; INS, idiopathic nephrotic syndrome of childhood; PAH, para-aminohippurate; PVP, polydisperse polyvinyl pyrrolidone.

aration, it has been shown that the foot processes are separated by narrow clefts approximately 200–300 Å wide (4) and are lined by a strongly anionic coat, termed the glomerular polyanion (8). It has been suggested from studies utilizing a variety of ultrastructural tracers that the epithelial slit pore or slit diaphragm may provide the final barrier to filtration and may contribute significantly to the characteristics of the glomerular filtrate (4).

The present study was undertaken to investigate human glomerular permeability in greater detail and to evaluate the role of the glomerular epithelial cell in determining permeability characteristics. Studies were performed in normal children and in those with untreated, minimal-change, steroid-responsive, idiopathic nephrotic syndrome of childhood (INS). This disease is characterized pathologically by swelling and fusion of the epithelial foot processes without any other consistently demonstrable glomerular abnormalities (9, 10), and provides a unique opportunity to study the role of the glomerular epithelial cell in glomerular filtration. Studies were repeated in the patients after the administration of steroids at a time when the epithelial foot processes had returned to normal and proteinuria had disappeared. Patterns of glomerular permeability were measured from the clearances of individual moieties of ^{125}I-labeled polydisperse polyvinyl-pyrrolidone (PVP) an inert macromolecule with molecules ranging in size from 8,000 to 85,000 mol wt.

In the present study, the swelling and fusion of the epithelial foot processes seen in untreated INS was associated with a decreased glomerular permeability to small molecules. However, reversal of this pathologic change was associated with a return to normal of glomerular permeability characteristics. Thus the results of the study suggest that modification of the epithelial foot processes by disease states alters the glomerular permeability characteristics and support the concept, derived from in vitro studies utilizing ultrastructural tracers, that the final filtration barrier for relatively smaller molecules may be at or about the level of the epithelial slit pore or slit diaphragm (4).

METHODS

28 measurements of glomerular permeability were performed in 11 patients with INS. Additional studies were undertaken in three children who had had INS but who had been in remission for a minimum of 2 yr at the time of study. Glomerular permeability was also measured in six healthy children who had no evidence of renal disease. The children with INS ranged in age from 1.4 to 14.8 yr (Table I), those in prolonged remission were aged 6, 8, and 11 yr, and the ages of the normal children ranged from 8 to 16 yr.

The diagnosis of INS was based on the clinical presentation with the classical features of the syndrome (11) and was subsequently supported by a typical response to steroid therapy. However, by the currently accepted criteria, tissue confirmation of the diagnosis was required in any patient who manifested any atypical features or who presented with nephrotic syndrome after the age of 5 yr; renal biopsy with the percutaneous needle technique being performed in 5 of the 11 patients before treatment (Table I). All tissue samples were processed for light, electron, and immunofluorescent microscopy by standard methodology. Each biopsy showed the typical minimal change lesion (9, 10) with no evidence of immune complex deposition on immunofluorescent or electron microscopy.

Of the 11 patients with INS studied before treatment was started, 2 were in relapse from previously treated INS and the remaining 9 were studied during the initial episode

TABLE I

Clinical Details of the Patients with INS at the Time of the Initial Study, before Therapy

Patient	Age	Biopsy	Relapse	Edema	Serum cholesterol	Urine protein		Serum protein		C_{in}	C_{PAH}	Filtration fraction
								Total	Albumin			
	yr			*% body wt*	*mg/100 ml*	*g/liter*	*g/day*	*g/100 ml*		*ml/min per 1.73 m²**		%
R. P.	1.4	−	−	18.5	285	7.9	2.9	3.6	1.2	75	292	24.4
D. R.	1.8	−	−	12.3	495	12.6	2.2	5.2	1.5	84	278	27.0
R. K.	1.9	−	−	16.1	322	17.3	2.8	4.2	1.2	99	484	22.0
E. B.	2.3	−	−	16.9	360	10.8	1.2	5.3	1.2	78	415	18.8
K. W.	2.6	+	−	22.0	567	8.6	1.7	6.0	1.9	88	515	17.1
D. T.	4.1	−	−	14.2	349	28.8	1.5	3.6	1.0	108	766	14.0
J. G.	5.4	−	+	18.7	505	28.8	4.3	4.6	1.6	73	526	21.6
J. P.	11.5	+	−	28.3	732	11.5	3.8	4.1	1.4	80	808	10.0
G. F.	12.3	+	−	40.1	293	15.8	4.8	3.9	1.4	82	660	11.2
D. W.	12.5	+	−	15.5	456	3.5	2.1	4.3	1.1	91	429	21.3
K. S.	14.8	+	+	11.5	315	21.6	11.4	4.9	1.4	117	627	18.7

Abbreviations: C_{in}, inulin clearance; C_{PAH}, PAH clearance.

* Patients' surface area calculated from standard tables and the patient's height and weight, when not edematous.

of the disease. In all instances, the patients were edematous and had proteinuria, hypoalbuminemia, and hypercholesterolemia at the time of study (Table I).

A second study was performed in each patient after therapy with steroids had been instituted. Six patients were subjected to a third study. The repeat studies in three patients were performed 7–10 days after starting prednisone, given daily in a single morning dose of 3.0–3.5 mg/kg body wt. At the time of these studies, none of the patients had shown any evidence of response to therapy. They had not undergone a diuresis, and proteinuria had not decreased; however, each patient subsequently responded to steroid therapy, undergoing complete remission. Seven repeat studies were performed in patients undergoing treatment with the prednisone regime described above, but at the time of study the patients had undergone a diuresis and had had protein-free urine for 7–10 days. At the time of these studies, steroids were being continued in full dosage; hypoalbuminemia and hypercholesterolemia were still present. Five repeat studies were performed on patients remaining in remission from nephrotic syndrome while steroid dosage was being progressively reduced. These studies were performed approximately 12 wk after steroid therapy had been started; the usual dose of prednisone at this time was 1–1.5 mg/kg body wt, taken as a single dose on alternate days. Two patients were studied on a third occasion, when in complete remission, having discontinued steroid therapy for 2 and 4 mo, respectively. Three additional patients who fulfilled all the criteria for INS described above, who had responded to prednisone and who had been in remission for at least 2 yr, were also studied.

The protocols used in the study were approved by the Washington University Committee for review of research involving human subjects. Informed consent was obtained from the parents of both the normal children and those with renal disease before any studies were undertaken.

Glomerular permeability was measured by methods similar to those described by Hulme and Hardwicke (12). All patients were given oral potassium iodide for 1 day before and 7 days after the study. After sampling venous blood and urine to measure inuloid and para-amino-hippurate (PAH) blank readings, priming doses of inulin and PAH calculated to raise plasma inulin and PAH levels to 20 mg/100 ml and 2 mg/100 ml, respectively, were injected intravenously. This was followed by a sustaining infusion of inulin and PAH dissolved in 0.45% saline, administered at a rate calculated to maintain plasma levels constant. After 60 min a pulse of [^{125}I]PVP (sp act 25 μCi/mg, Amersham/Searle Corp., Arlington Heights, Ill.) was injected intravenously in a dose of 20–40 μCi/m^2 surface area. After a further 15–20-min interval to allow the PVP to reach its volume of distribution, the patient's bladder was emptied completely; this urine was discarded. Two accurately timed urine collections were then obtained, each collection period lasting from 30 to 60 min, and a heparinized peripheral venous blood sample was obtained at the beginning and end of each urine collection.

Inulin concentrations in the four plasma and three urine samples were measured by the anthrone method (13) and PAH concentrations in the same samples were measured by the Smith modification of the Bratton-Marshall reaction (14). Inulin and PAH clearances were calculated by standard methodology, with allowances for inuloid and PAH blank readings.

Clearance of PVP molecules of different sizes were calculated after 2-ml portions of plasma and urine samples had been subjected to column chromatography with Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) in 2.5 × 100-cm columns with flow adapters. All samples were applied to the lower end of the column by constant speed syringe pump (model 341, Sage Instruments Div., Orion Research, Inc., Cambridge, Mass.) and were eluted against gravity by using an eluate containing 10 g/liter sodium chloride and 500 mg/liter sodium azide. The eluate was degassed before use and was applied at a rate of 20 ml/h from a Mariotte flask under a constant pressure of up to 15 cm water. All chromatography was performed at a constant ambient temperature of 70–72°F.

The urine and two plasma samples from any individual clearance period were always eluted on the same column, with a minimum of three bed volumes of eluate allowed to run through the column between samples. Effluent from the columns was separated into 5-ml fractions by a linear fraction collector (Fractomette 200, Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N. J.) in the drop-count mode. Each fraction was then assayed for [^{125}I]PVP in a gamma spectrometer (model 5219, Packard Instrument Co., Inc., Downers Grove, Ill.), with the appropriate window and gain settings for ^{125}I.

Human albumin (50 mg) was added to each 2-ml urine sample before it was subjected to column chromatography. Protein concentrations in the effluent fractions was measured by the method of Lowry, Rosebrough, Farr, and Randall (15), enabling plasma and urine samples to be aligned according to the effluent tube containing the protein peak. Clearance of each fraction of PVP was calculated from the formula UV/P, where U was the urine concentration of that moiety of PVP, V urine flow rate, and P the logarithmic mean concentration of PVP in the corresponding fractions from the two plasma samples obtained at the beginning and end of the clearance period.

The average molecular size of each fraction of PVP was calculated by a method based on the observation that the cross-linked dextran gels separate macromolecules on the basis of diffusion constant or molecular radius (16, 17). The elution constant (K_{av}) for each individual fraction of PVP was calculated from the formula of Laurent and Killander (17).

$$K_{av} = \frac{V_e - V_o}{V_t - V_o},$$

where V_e is the elution volume of that fraction, V_t is the bed volume of the column and V_o is the void volume determined from the elution volume of blue dextran (Pharmacia Fine Chemicals), a molecule of 2,000,000 mol wt, which is completely excluded from the gel. Molecular radius of the PVP fractions can then be calculated from the formula

$$\log R_s = 0.871 + 1.085\ (1 - K_{av}),$$

as described by Hardwicke, Hulme, Jones, and Ricketts (18), where R_s represents the radius of equivalent sphere. This formula was derived from the observation that $1 - K_{av}$ is directly proportional to log molecular radius over a considerable molecular size range (19). Calculations were performed on an appropriately programmed calculator (model 1775, Monroe Div., Litton Industries, Orange, N. J.).

In preliminary studies, it was found that recovery of [^{125}I]PVP from Sephadex columns was incomplete. This was rectified by running 500 ml of eluate containing 10 g/liter of unlabeled PVP (Sigma Chemical Co., St. Louis, Mo.) through each column before any sample containing

[^{125}I]PVP was chromatographed on the column. The concentration of sodium chloride in this eluate was appropriately reduced to maintain constant osmolality. With this procedure, the recovery of labeled PVP was consistently found to be complete. This procedure did not alter the distribution of molecular sizes of PVP recovered from the columns.

RESULTS

The results from a typical study of glomerular permeability in a normal subject are shown in Figs. 1 and 2. Fig. 1 depicts the elution patterns of PVP after column chromatography of the urine and the two plasma samples obtained from a single clearance period. The first plasma sample, collected at the beginning of the clearance period, was obtained 15 min after the intravenous injection of the PVP, and second plasma sample was obtained 30 min later, at the end of the clearance period. Concentration of PVP is plotted against tube number with the calculated theoretical radius of the PVP molecules (R_s) also being shown on the abscissa. The concentrations of the larger molecular-weight PVP molecules were virtually identical in the two plasma samples. The concentrations of the smaller molecules progressively declined in the second plasma sample and were considerably less than those seen in the initial blood sample. The distribution of PVP molecules in the urine was entirely different from that seen in either plasma sample. Larger PVP molecules were excluded from the urine. Smaller molecules had higher concentrations in the urine than in the blood, with the smallest molecules having the same urine-to-plasma concentration ratio as inulin.

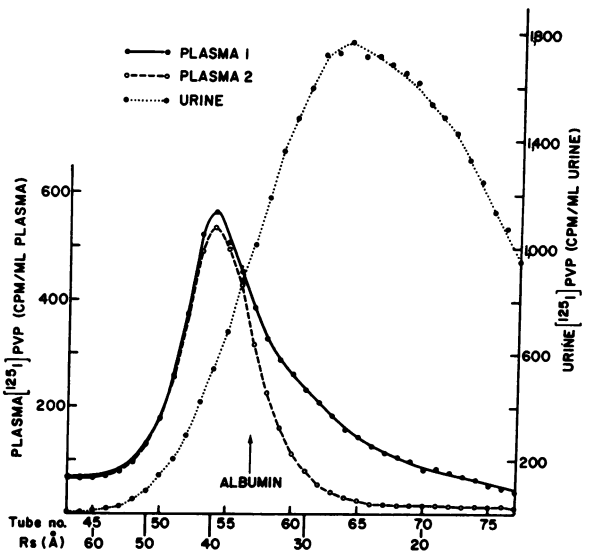

FIGURE 1 The elution patterns of [^{125}I]PVP from the urine and two plasma samples from a single study in a normal patient. The molecular radius of each fraction of the PVP (R_s) was calculated by the methods outlined in the text.

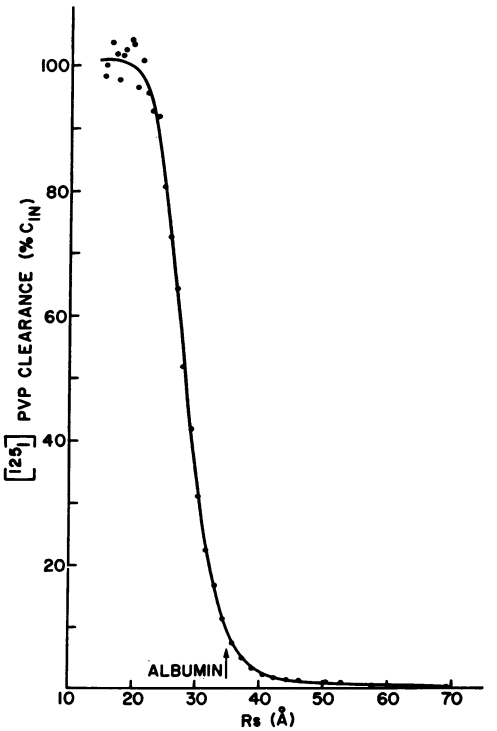

FIGURE 2 The pattern of glomerular permeability to PVP of different molecular sizes calculated from the data presented in Fig. 1.

The pattern of glomerular permeability derived from this data is shown in Fig. 2. The clearances of the individual moieties of PVP, expressed as a percentage of the clearance of inulin, are plotted against the calculated molecular radius of that fraction of PVP which permits the comparison of results from individuals of different age and size. The clearance of PVP molecules larger than 50 Å in size was virtually negligible. The clearances of smaller molecules progressively increased, being inversely related to molecular size, until a maximum value was reached, with molecules of approximately 15–20 Å in size having a clearance equal to that of inulin. The values for the clearances of molecules < 15 Å were unreliable, due to their rapid disappearance from the plasma and consequent negligible concentrations in the plasma sample obtained at the end of the clearance period.

Estimations of glomerular permeability were very reproducible. Virtually identical curves were obtained in successive clearance periods in any individual subject. Similarly, the results obtained when a single study was analyzed in duplicate on different Sephadex columns agreed closely with one another.

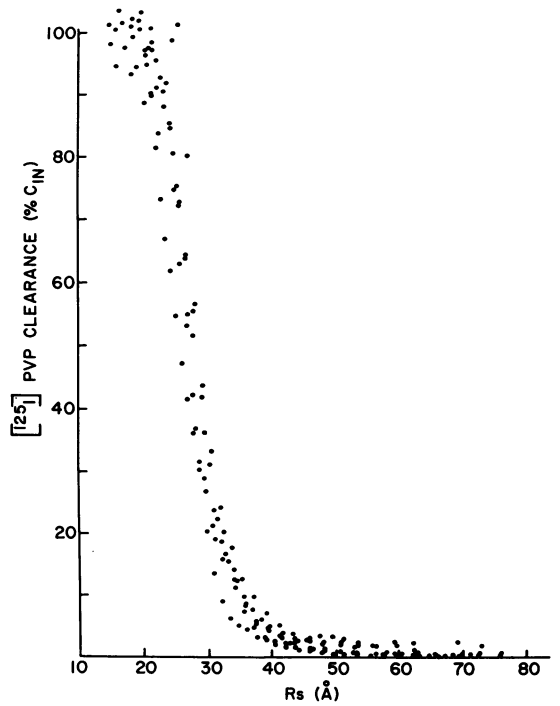

FIGURE 3 Values for glomerular permeability observed in the six normal subjects.

The values obtained from the six normal children are shown in Fig. 3. The ages of these subjects ranged from 8 to 16 yr; their absolute inulin clearances varied from 69.6 to 121.0 ml/min; the studies were carried out over an interval of more than 2 yr; and each of the studies was chromatographed on a different column: yet the patterns of glomerular permeability were remarkably consistent.

The clinical status of the 11 patients with INS at the time of their initial study before treatment was started is shown in Table I. Each had the typical features of idiopathic nephrotic syndrome, namely, marked proteinuria, hypoalbuminemia, hypercholesterolemia, and edema. Glomerular filtration was moderately reduced in 9 of the 11 patients, the mean value for the group being 88.6 ml/min per 1.73 m^2.

The values for glomerular permeability in these patients are depicted in Figs. 4 and 5, where they are compared to those obtained from the normal subjects. It is readily apparent that the results from the patients with INS demonstrated marked abnormalities. The clearances of larger mol wt PVP molecules (> 40 Å) were normal. Although there was considerable overlap between the clearances in the normal and nephrotic subjects of molecules of 25–40 Å in size, the mean values for the nephrotic subjects were significantly reduced from normal. However, even more marked differences were observed in the clearances of the smaller molecules. The clearance of PVP molecules 15–20 Å equaled inulin clearance in the normal subjects, but averaged only 67% of inulin clearance in the patients with INS. The reductions in the clearances of small PVP molecules were striking in individual studies. Thus in two patients, the clearance of the smaller molecular weight PVP never exceeded 20% of the clearance of inulin (Fig. 4).

Results obtained from patients with the first episode of nephrotic syndrome were similar to those obtained from patients in relapse from previously treated INS. There was no correlation between the severity of the decreased clearances of PVP and the degree of proteinuria, the serum albumin, the serum cholesterol, the degree of reduction from normal in GFR, or the interval between the initial clinical manifestation of nephrotic syndrome and the time of study.

The patterns of glomerular permeability in the three patients restudied 7–10 days after starting therapy with steroids remained unchanged. In each instance, abnormal curves with decreased clearances of the smaller mol wt PVP persisted. Since these studies were performed before there was any clinical evidence of

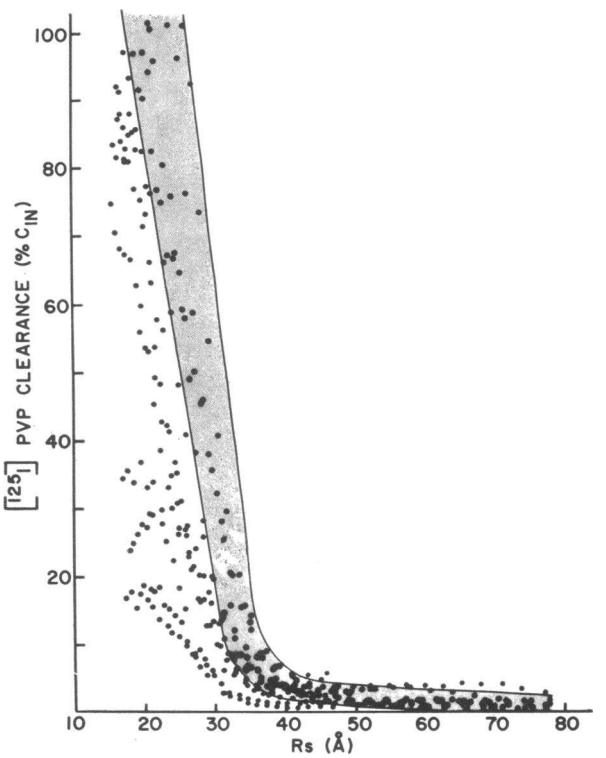

FIGURE 4 Glomerular permeability for the 11 patients with untreated idiopathic nephrotic syndrome. The values for glomerular permeability obtained from the normal subjects are depicted by the shaded area.

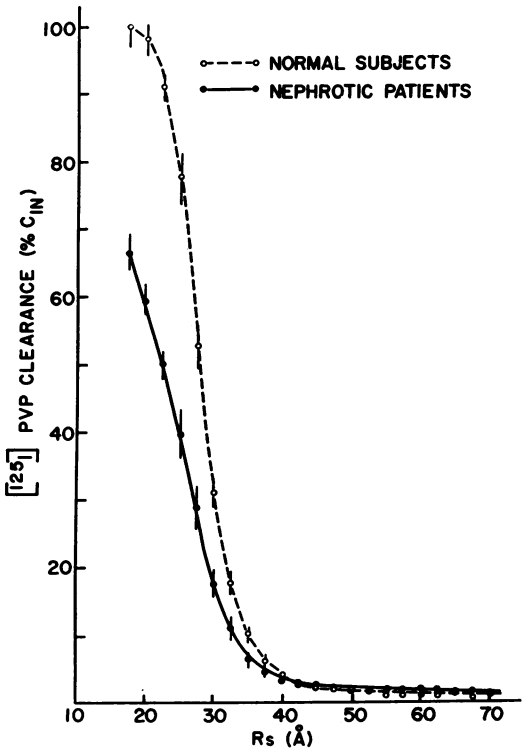

FIGURE 5 Mean values (±SEM) for glomerular permeability in 6 normal subjects and 11 patients with untreated INS. Values for the two groups were not significantly different for PVP molecules larger than 35 Å. Each of the values for PVP molecules less than 35 Å were significantly different ($P < 0.05$).

response to therapy, the patients, although taking steroids, remained edematous and were severely proteinuric at the time of study.

In contrast, the repeat studies, performed in the seven patients who had responded to steroid therapy and who had undergone diuresis and had had protein-free urine for at least 1 wk, indicated that glomerular permeability had reverted to normal in every instance (Fig. 6). Each of these patients was still receiving steroids in full dosage at the time of these studies. Although the urines were protein-free, serum albumin and cholesterol levels had not yet returned to normal and frequently were not different from pretreatment values. This dramatic alteration in glomerular permeability after response to therapy with steroids is further illustrated in Fig. 7 where the results obtained from a single patient before therapy was started are compared to those observed 3 wk later, subsequent to the patient's response to prednisone.

Four of the five patients restudied after a steroid-induced remission but during a later phase of the clinical course in which prednisone dosage was being progressively reduced showed normal permeability pat-

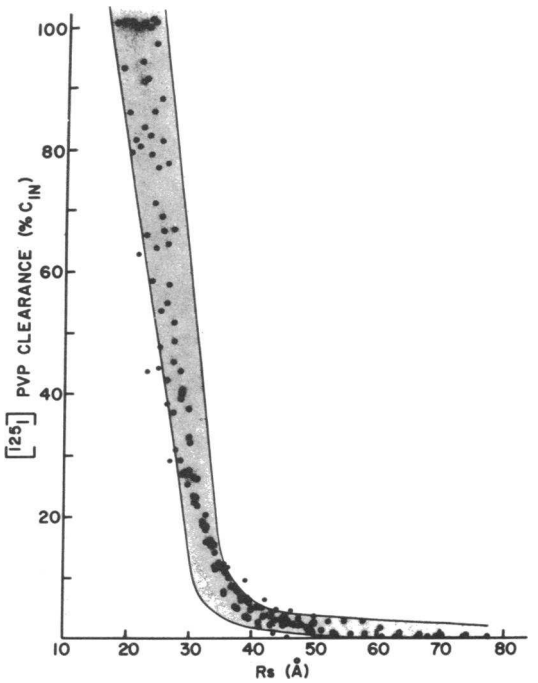

FIGURE 6 Results of glomerular permeability studies in seven patients with INS after response to steroid therapy. The shaded area represents the normal values.

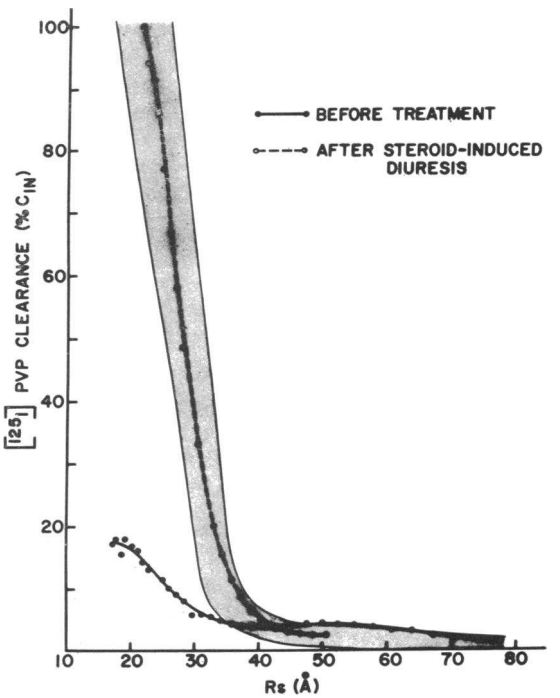

FIGURE 7 Two studies of glomerular permeability in patient R. P. The first study was performed before therapy was started, the second after proteinuria had disappeared after treatment with prednisone.

terns to PVP. The fifth patient showed a mildly decreased permeability to the smallest PVP molecules. Within 3 wk of this study, he redeveloped marked proteinuria and presented with a typical clinical relapse of his nephrotic syndrome. To date we have not had an opportunity to study additional patients just before relapse of their nephrotic syndrome to determine whether changes in glomerular permeability to PVP consistently precede the clinical manifestations of relapse of INS.

Glomerular permeability to PVP remained normal in patients in remission from INS and who were no longer taking steroids. Thus normal patterns were observed in the two patients studied after steroid therapy had been discontinued and in the three additional patients who had had a previous typical episode of INS, who had responded to therapy with prednisone and who at the time of study had been in remission without therapy for at least 2 yr.

To ensure that plasma or urine specimens did not modify the elution of PVP, aliquots of [^{125}I]PVP were added to isotonic saline and to plasma and urine samples obtained from normal subjects and from patients with untreated INS. These samples were then subjected to column chromatography. A typical result is shown in Fig. 8, which illustrates that the elution of PVP from nephrotic plasma or urine was identical to that observed for PVP dissolved in eluate. In addition, the elution patterns of albumin were identical from plasma and urine samples obtained from either normal or nephrotic subjects.

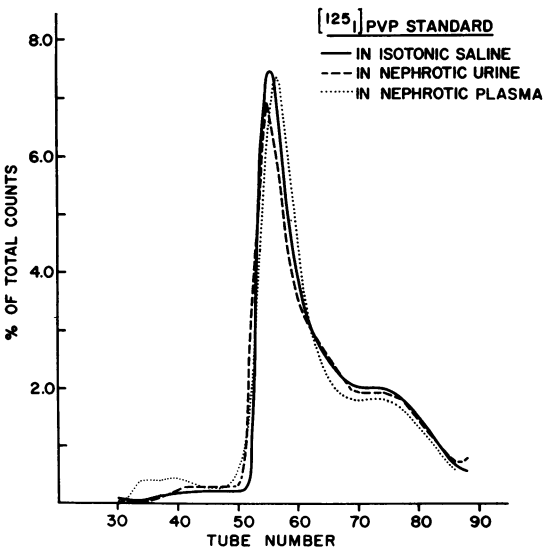

FIGURE 8 The elution patterns of [^{125}I]PVP added to isotonic saline and to urine and plasma obtained from an untreated nephrotic subject. Elution of each sample was performed on the same column.

TABLE II

Glomerular Function in 31 Normal Children and in 16 Children with INS before Treatment and after a Steroid-Induced Remission

		C_{in}	C_{PAH}	Filtration fraction
		ml/min per 1.73 m²		%
Normal subjects (n = 31)		120.0±16.3	589±112	21.1±5.2
INS (n = 16)	Before	81.6±32.6	506±195	16.7±7.9
	After	115.1±28.8	484±91	23.6±7.1

All values mean ±SD. Abbreviations used are those defined in Table I.

As shown in Table I, 9 of the 11 patients with untreated INS had moderate decreases in inulin clearances. Review of our previous experience indicated this to be a consistent feature in an additional 31 patients with INS. The significance of this observation was further explored by comparing the results of inulin, PAH clearances, and filtration fraction in patients before treatment with those obtained 2–4 wk after the patients had undergone a steroid-induced remission. Data was obtained from the 11 patients summarized in Table I and from an additional 5 patients with untreated INS. The results are shown in Table II, where values are compared to those obtained from 31 children of comparable age range who had no evidence of renal disease. In untreated INS, inulin clearance was significantly reduced from normal ($P < 0.001$). PAH clearance was reduced slightly from normal, but this difference was not statistically significant. In consequence, in untreated INS, filtration fraction was significantly reduced from normal ($P < 0.05$). After a steroid-induced remission, GFR increased in 13 of the 16 patients with INS and was virtually unchanged in the remaining three. These changes were highly significant when analyzed by Student's t test ($P < 0.01$) or by the paired t test ($P < 0.001$). The values for inulin clearance after treatment were not significantly different from normal. PAH clearance showed no consistent direction of change after treatment, although the mean value after treatment was slightly but insignficantly lower than before treatment. Filtration fraction increased in each of the patients after response to therapy, with the value after treatment being significantly increased above the pretreatment level ($P < 0.02$) and no longer significantly different from the normal value.

DISCUSSION

The proteinuria in patients with INS is highly selective (20), consisting principally of albumin. It is generally

considered to result from an increased glomerular permeability to serum proteins (21), with evidence suggesting that the primary defect is in the GBM. Thus, albumin is eluted before mannose from a column prepared from normal rat GBM, whereas the two compounds are eluted simultaneously from a column prepared from nephrotic rat GBM (22). In addition, the chemical composition of the GBM is altered in both human and experimental renal disease, and X-ray diffraction patterns suggest that there may be molecular rearrangement in this membrane with an increase in interstices between molecules (23–25). Similarly the clearances of macromolecules have been interpreted as indicating an increase in pore size in the GBM as the cause of proteinuria in nephrotic syndrome (26).

These abnormalities might be expected to increase glomerular permeability to PVP rather than to result in the selective decrease in the clearances of smaller PVP molecules, observed in our proteinuric patients with untreated INS. Such changes have been attributed to damage to part of the basement membrane with reduction of pore radius or to an increased thickness of the GBM (12). However, an alternate explanation appears more probable in INS. Recent studies utilizing ultrastructural tracers have suggested that the glomerular epithelial slit pores may contribute significantly to glomerular permeability characteristics (27–30). According to this view, there are two barriers to filtration in the glomerulus, the GBM acts only as a coarse filter for relatively large molecules, and the final filtration barrier for smaller molecules is located at or about the level of the epithelial slit pore or diaphragm (4). Although a functional role for the slit pores was proposed in 1957 (31), the pores seemed too wide to act as an effective filtration barrier. However, by using pathologic techniques designed to minimize shrinkage and separation of the foot processes, they are found to run a long, relatively straight course, with a width of only 200–300 Å (4).

The pathologic changes in the glomeruli in untreated INS are characterized by swelling and apparent fusion of the epithelial foot processes with obliteration of 80% or more of the epithelial slit pores. Thus the outer surface of the GBM, which appears normal itself, is covered by a rim of epithelial cytoplasm (9). If the interpretation of the ultrastructural tracer studies is correct, then these pathologic changes should be reflected by decreased permeability to the smaller PVP molecules, especially since the range of PVP molecular size utilized in the present study was similar to the size of tracers that appeared to be retarded by the epithelial slit pores. Thus, the finding of decreased glomerular permeability to the smaller PVP molecules in untreated INS supports the concept that the epithial slit pores or slit diaphragms act as a barrier to filtration, especially for smaller molecules.

Fusion of the epithelial foot processes may be a nonspecific reaction occurring whenever there is a markedly increased passage of protein across the GBM, such as in immune complex glomerulonephritis, especially when complicated by nephrotic syndrome (9), or after the infusion of albumin in doses sufficient to induce proteinuria (32, 33). Thus, this pathologic change could also explain the decreased glomerular permeability to low mol wt PVP seen by ourselves (unpublished observations) and by others (12) in some patients with nephrotic syndrome secondary to glomerulonephritis. These patients, in contrast to those with INS, also have an increased permeability to the larger PVP molecules, a change that may result from severe damage to the GBM secondary to the deposition of immune complexes in glomerulonephritis.

The return of glomerular permeability to normal after the use of steroids and loss of proteinuria in the patients with INS also suggests a role for the epithelial cells in determining permeability characteristics, since fusion of the foot processes reverts to normal in INS patients in remission from the disease (34). Again, this change in the epithelial cells could be secondary to changes in GBM permeability, since the administration of glucocorticoids modifies the chemical composition of the GBM in proteinuric rats (35).

If, as we suggest, the proteinuria in untreated INS results primarily from changes in the GBM, and the decreased permeability to PVP is secondary to changes in the epithelial cells, then the observed changes in permeability to PVP do not help to delineate the nature of the defect in the GBM in this disease. However, these arguments assume a purely mechanical barrier to filtration. The surface membranes of the epithelial foot processes are lined by glomerular polyanion (8). This negatively charged layer might contribute to glomerular permeability characteristics and repel any like-charged molecules, such as plasma proteins, that penetrate the GBM, preventing their entry into the urinary space. Such a mechanism could account for the markedly lower clearance of albumin than of equivalent-sized inert molecules seen in normal subjects in this and other studies (26). In untreated INS, albumin clearances were increased above normal, while PVP clearances, although still greater than those of albumin, were reduced. The loss of glomerular polyanion, which occurs in both human and experimental nephrotic syndrome (8), may account for the clearances of albumin and PVP altering in divergent directions in untreated INS. In this disease, an abnormal GBM may allow both albumin and equivalent-sized PVP to penetrate in proportionately increased amounts, the differences in the renal clearances of these molecules reflecting changes at the level of the epithelial cells. If albumin is normally excluded from the epithelial slit pore by the glomerular polyanion, any

that enters Bowman's space may have to pass through the epithelial podocytes rather than between them. In INS, loss of glomerular polyanion might permit increased passage of albumin through the slit pores, despite apparent fusion of the glomerular foot processes. In contrast, loss of glomerular polyanion would not modify the passage of the uncharged PVP. Instead, the physical changes in the slit pores would be the dominant factor, reducing permeability to PVP at this level and masking the increased permeability of the GBM to these molecules. Thus the discrepant clearances observed for molecules of similar size probably result from a combination of factors, including differences in the shape, internal bonds, and charge of individual molecules, similar discrepancies being observed with artificial membranes (26).

In summary, we suggest that in INS the GBM becomes increasingly permeable to albumin. Much of the albumin that penetrates the basement membrane may be reabsorbed or metabolized by the glomerular epithelial and proximal tubule cells (36), and there is loss of glomerular polyanion. Swelling of the epithelial foot processes with obliteration of the epithelial slit pores results. This change modifies glomerular permeability to uncharged molecules and reduces the ability of smaller PVP molecules to enter the urine. These glomerular changes may occur before proteinuria develops, since abnormal glomerular permeability to PVP redeveloped in a patient with INS before a clinically apparent relapse. Presumably at this time, the GBM protein leak did not exceed the renal ability to reabsorb protein.

Alternate explanations for the altered patterns of PVP permeability appear unlikely. An artifactual elevation of inulin clearance in untreated INS could have resulted in apparent depression of the PVP clearances, since these values were expressed as a percentage of inulin clearance. Although creatinine clearances may not measure GFR in proteinuric patients (37), inulin clearance is still regarded as a true reflection of GFR, even in the presence of renal disease. Moreover, in untreated INS, inulin clearances were decreased rather than elevated. Thus, factoring PVP clearances by inulin clearance reduced the apparent magnitude of the changes, the absolute clearances of the smaller PVP molecules being depressed even more markedly than is apparent in Figs. 4 and 5. Similarly, the abnormalities in the permeability curves do not appear to result from selective tubular reabsorption of PVP. The identical values for the clearances of inulin and of smaller PVP molecules in our normal subjects indicate, as do other studies (18, 38), that PVP is not reabsorbed by the normal renal tubule. Equivalent data is not available in renal disease, but inulin, an inert molecule of similar size, does not penetrate the renal tubule even under conditions resulting in proteinuria (39). Moreover, if renal disease was associated with generalized tubular reabsorption of inert molceules, this would not necessarily result in the decreased ratios of PVP to inulin clearances observed in untreated INS. Finally, our studies indicated that the nephrotic plasma and urine samples did not modify the elution of PVP from the columns to account for the abnormal permeability patterns.

Decreases in glomerular permeability like those demonstrated for PVP may occur with other inert molecules of equivalent size. The clearance of the polysaccharide fructosan (approximate mol wt 8,000) equals that of inulin in normal man, but averages only 60% of GFR in patients with glomerular disease, and similar observations have been made for a second larger polysaccharide (40). In addition, the ratios of hemoglobin (mol wt 68,000) to inulin clearances are reduced below normal in proteinuric patients with the nephrotic syndrome (41). If inulin is affected similarly, the decreased clearances of inulin seen in untreated INS may result from impaired movement of inulin into the glomerular filtrate secondary to fusion of the epithelial foot processes. This would explain the decreased filtration fraction seen in untreated INS and the return of both inulin clearance and filtration fraction to normal at a time when proteinuria had subsided but when other features of the nephrotic syndrome, especially hypoalbuminemia, persisted. It is apparent from the data presented in Figs. 4 and 5 that if there is decreased glomerular permeability to inulin in INS, it is of a less marked degree than that shown for PVP. If permeability through epithelial slit pores is related to molecular size and/or to charge, then fusion of the foot processes may decrease inulin clearance but may not modify permeability to very small molecules, such as water or electrolytes. Although this possibility was not explored in the present study, such a phenomenon would result in the clearance of inulin not reflecting true glomerular filtration rate in renal diseases associated with fusion of the glomerular epithelial foot processes.

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