GLOMERULAR PERMEABILITY TO HIGH MOLECULAR WEIGHT DEXTRANS IN ACUTE ISCHAEMIC RENAL FAILURE AND POSTURAL PROTEINURIA

PAMELA R. MACLEAN, J. J. B. PETRIE AND J. S. ROBSON

Renal Unit, Department of Medicine, and Department of Clinical Chemistry, Edinburgh Royal Infirmary

(Received 9 June 1969)

SUMMARY

1. Renal permeability to dextran of a molecular weight range approximating to that of the plasma proteins has been studied in six patients with acute ischaemic renal failure, four patients with postural proteinuria and six healthy subjects.

2. Results are expressed in terms of dextran selectivity indices which relate the clearance of dextran to its molecular weight. Indices of dextran selectivity were found to be high in acute ischaemic renal failure, postural proteinuria and in normal subjects. Comparable indices of plasma protein selectivity in these groups were low.

3. It is suggested that in postural proteinuria and acute ischaemic renal failure the proteinuria is not glomerular in origin, and that in these conditions macromolecules are filtered quite normally and urinary protein arises from a post glomerular source characterized by a lack of selectivity.

The renal clearance of macromolecules has been studied by several workers in order to assess glomerular permeability in health and disease. Plasma proteins have been widely used for this purpose, results being expressed as indices of selectivity (Blainey et al., 1960; Joachim et al., 1964; Robson, 1968). Proteins are not ideal macromolecules with which to study glomerular permeability since they are reabsorbed by the renal tubules. This is particularly relevant in the investigation of normal glomerular function and in minor degrees of proteinuria, when almost all the protein filtered by the glomeruli is reabsorbed and only small amounts escape into the urine (Dirks, Clapp & Berliner, 1964).

Dextran is a more suitable macromolecule for this purpose, since virtually all the dextran filtered at the glomerulus appears in the urine and no demonstrable amount is reabsorbed by the tubules (Brewer, 1951; Wallenius, 1954). Normal glomerular permeability to dextran has been studied by Arturson & Wallenius (1964a, b) and the relationship between renal clearance and molecular weight over the range 16 000–60 000 has been defined. Petrie, MacLean & Robson (1968) also studied the glomerular permeability to higher molecular weight

Correspondence: Dr Pamela R. MacLean, Department of Clinical Chemistry, The Royal Infirmary, Edinburgh EH3 9YW.
Pamela R. MacLean, J. J. B. Petrie and J. S. Robson
dextran in patients with different forms of glomerulonephritis and compared the results with protein studies carried out simultaneously. In proliferative glomerulonephritis, dextran selectivity studies gave results in marked disagreement with those of protein; on the other hand, in minimal lesion and membranous glomerulonephritis the selectivity results using dextran and protein were substantially the same. These findings are attributed to differences in the degree of uniformity of the glomerular lesions in the two groups of patients.

The protein clearance patterns in patients with acute ischaemic renal failure and in postural proteinuria are unselective (MacLean & Robson, 1966). The lack of selectivity is surprising in view of the almost normal glomerular appearance reported by most authors (Dalgaard & Pederson, 1961; Ruckley et al., 1966). In view of this, glomerular permeability to dextran and plasma proteins of comparable molecular size has been compared in patients with acute renal failure and postural proteinuria. The glomerular permeability to dextran was also studied in healthy subjects.

METHODS

Total urinary protein was measured by the biuret method of Hiller, Grief & Beckman (1948) and by a modification of the microbiuret method of Itzhaki & Gill (1964). An AutoAnalyzer (Technicon) was used to estimate creatinine in serum and urine (Stevens et al., 1962).

Protein selectivities were determined by the immunodiffusion technique of Soothill (1962) as modified by MacLean & Robson (1967). The index of protein selectivity by this method is denoted by $-k$.

Dextran selectivities were determined as described by Petrie et al. (1968). The method is similar in principle to that used to determine protein selectivity by gel filtration (MacLean & Petrie, 1966), but the coefficient of variation for the dextran method is higher (9%) and most of the estimations were therefore performed in duplicate and the average taken. The index of dextran selectivity ($D$) represents the change in renal clearance of dextran, calculated over a fixed range of molecular size, and is arithmetically and methodologically comparable to the index of protein selectivity ($\Delta$) when determined by gel filtration. Since $\Delta$ is related to $-k$ by the equation $\Delta = 0.76 (-k) - 0.08$ (MacLean & Petrie, 1966) then the indices of $D$ and $-k$ should be compared according to the equation $D = 0.76 (-k) - 0.08$. High values for indices of selectivity indicate that the glomerulus is filtering macromolecules in a selective fashion and allowing very few large molecules to escape into the urine. Conversely low values for indices of selectivity indicate that the glomerulus is allowing a higher proportion of large molecules to escape into the urine.

The diagnosis of acute ischaemic renal failure and of postural proteinuria was based on appropriate clinical findings and light and ultrastructural appearances of renal biopsies (Ruckley et al., 1966). Patients with postural proteinuria were shown to have proteinuria which was intermittent and related to posture.

RESULTS

Table 1 shows indices of dextran ($D$) and protein ($-k$) selectivity in six patients with acute ischaemic renal failure, at the beginning of the diuretic phase, in four patients with postural proteinuria, and in six healthy subjects. Fig. 1 shows these results and, for comparison, dextran
Glomerular permeability to dextrans

and protein indices of selectivity in minimal lesion and membranous glomerulonephritis (Petrie et al., 1968). Table 1 also includes values for total 24 hr urine protein, creatinine clearance, and serum:urine albumin concentration ratio. Values for $D$ were high, ranging from 1·99 to 3·28 (mean 2·41) for patients with acute ischaemic renal failure, from 2·38 to 3·47 (mean 3·08) for patients with postural proteinuria and from 2·38 to 3·65 (mean 2·91) for normal subjects. In contrast, the values for protein selectivity in patients with acute ischaemic renal failure were low, ranging from 0·25 to 1·40, mean 0·84. These patients all had low creatinine clearances, and the total protein excretion was over 200 mg/24 hr. The albumin serum:urine concentration ratio was also abnormal, the upper limit in normal subjects being 1600 (MacLean & Robson, 1966). The values for protein selectivity in postural proteinuria were also low, ranging from 1·05 to 1·41 (mean 1·22) and the serum:urine albumin concentration ratio was abnormal.

**DISCUSSION**

The results show that the glomerular permeability to dextran in acute ischaemic renal failure, postural proteinuria and normal subjects is highly selective. The normal glomerulus has

### Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>$D$</th>
<th>$-k$</th>
<th>Alb S:U</th>
<th>Creatinine clearance (ml/min)</th>
<th>Total urine protein (mg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute ischaemic renal failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1·99</td>
<td>0·77</td>
<td>96</td>
<td>8</td>
<td>600</td>
</tr>
<tr>
<td>2</td>
<td>2·02</td>
<td>—</td>
<td>—</td>
<td>41</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>2·10</td>
<td>—</td>
<td>—</td>
<td>22</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>2·59</td>
<td>0·93</td>
<td>256</td>
<td>13</td>
<td>600</td>
</tr>
<tr>
<td>5</td>
<td>2·65</td>
<td>0·25</td>
<td>448</td>
<td>10</td>
<td>800</td>
</tr>
<tr>
<td>6</td>
<td>3·28</td>
<td>1·40</td>
<td>224</td>
<td>4</td>
<td>200</td>
</tr>
<tr>
<td>Postural proteinuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2·38</td>
<td>1·05</td>
<td>180</td>
<td>62</td>
<td>600</td>
</tr>
<tr>
<td>8</td>
<td>3·47</td>
<td>1·41</td>
<td>330</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>3·26</td>
<td>1·22</td>
<td>370</td>
<td>134</td>
<td>900</td>
</tr>
<tr>
<td>10</td>
<td>3·22</td>
<td>1·18</td>
<td>220</td>
<td>95</td>
<td>500</td>
</tr>
<tr>
<td>Normal subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2·38</td>
<td>—</td>
<td>—</td>
<td>93</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>2·50</td>
<td>—</td>
<td>—</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>13</td>
<td>2·76</td>
<td>—</td>
<td>—</td>
<td>37*</td>
<td>87</td>
</tr>
<tr>
<td>14</td>
<td>2·98</td>
<td>—</td>
<td>—</td>
<td>104</td>
<td>21</td>
</tr>
<tr>
<td>15</td>
<td>3·19</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>45</td>
</tr>
<tr>
<td>16</td>
<td>3·65</td>
<td>—</td>
<td>—</td>
<td>92</td>
<td>15</td>
</tr>
</tbody>
</table>

$D =$ index of dextran selectivity; $-k =$ index of protein selectivity by immunodiffusion; Alb S:U = albumin serum:urine concentration ratio. Values for $-k$ in the four patients with acute ischaemic renal failure are similar to those previously obtained (MacLean & Robson, 1966).

* Subject 13 had one kidney.
previously been suggested to be highly selective (Hulme & Hardwicke, 1966), in spite of the unselective pattern of protein excretion (Rowe & Soothill, 1961; MacLean & Robson, 1966). Similarly this study shows that in acute ischaemic renal failure and postural proteinuria a highly selective dextranuria accompanies an unselective proteinuria.

The methods used to determine protein and dextran selectivity are different in principle.

Fig. 1. Dextran (○) and protein (●) selectivity, denoted by $-k$ (left ordinate) and $D$ (right ordinate) respectively. The scales for $-k$ and $D$ differ according to the relationship $D = 0.76(-k) - 0.08$. In acute ischaemic renal failure and postural proteinuria (two left-hand columns) there is wide disparity between the dextran and protein values for selectivity. By contrast in minimal lesion and membranous glomerulonephritis (extreme right-hand column) values for dextran and protein selectivity are comparable (Petrie et al., 1968). Values obtained in healthy subjects for dextran (from Table 1) and for protein (from MacLean & Robson, 1966) are also shown.

In proteinuria greater than 1 g/day, results of gel filtration and immunodiffusion correlate well (MacLean & Petrie, 1966). In proteinuria of less than 1 g/day the gel filtration method is a doubtful measure of glomerular selectivity because urinary tract protein is present in the urine in a relatively greater amount. However, the immunodiffusion method appears to be reliable at lower protein concentrations of 0.2–1 g/day. In postural proteinuria
and acute ischaemic renal failure values of selectivity are constant in the face of wide variations in protein excretion (Ruckley et al., 1966; MacLean & Robson, 1966). Although it is theoretically possible that degraded protein in the urine will react antigenically and influence the value of selectivity, studies of urinary proteins show in general that the molecular weights are similar to those of the serum proteins (Gitlin & Janeway, 1952; Neale, 1955; Hardwicke & St. Cyr, 1961). Although small molecular weight immunoglobulins can be detected in the urine there is evidence that they are derived by plasma clearance rather than by degradation (Fagelman, McGhee & Chaplin, 1966).

Protein and dextran differ considerably in molecular configuration. Dextran has the more expanded structure, and the effective molecular radius for dextran of molecular weight 70,000 is substantially larger than that for a globular protein of the same molecular weight (Laurent & Granath, 1967; Anderson & Stoddart, 1967). This fact results in dextrans being eluted earlier from a gel filtration column than proteins of the same molecular weight, and, compared to protein, leads to a difference between the lines relating clearance to molecular size. This shift makes only a small difference in the range of molecular size used for calculating selectivity and does not influence the slope of the line. Since indices of selectivity are a function of this slope, and do not depend on precise measurements of molecular weights, comparisons between dextran and protein selectivities are valid.

In discussing the mechanism of dextran and protein excretion in glomerulonephritis, Petrie et al. (1968) concluded that the differences in dextran and protein selectivity in proliferative glomerulonephritis resulted from different tubular treatment of these macromolecules by different nephrons. Since dextran is not reabsorbed by the renal tubules the urinary dextran reflects the behaviour of every functioning nephron, whereas estimates of selectivity based on protein can reflect only the behaviour of glomeruli which are associated with tubules where the reabsorptive capacity is saturated. It follows that when the glomerular lesions are uniform throughout all nephrons, protein and dextran selectivities would be expected to agree as, e.g., in minimal lesion and membranous glomerulonephritis (Fig. 1), whereas when the glomerular lesion is patchy, values for dextran selectivity will be higher than those of protein, assuming of course that the normal glomerulus is highly selective.

However, in acute ischaemic renal failure this mechanism is unlikely to account for the association of a highly selective dextranuria and a highly unselective proteinuria. In this condition, there is no histological evidence of a patchy glomerular lesion and the glomeruli appear uniformly almost normal. The high dextran selectivity, which is similar to that found in normal subjects, is in accord with the virtually normal glomerular appearances. The source of the urinary proteins in this condition is uncertain, but it has been suggested that fragmented tubules may allow the passage of plasma protein into the tubular lumen from the peritubular fluid (MacLean & Robson, 1966). The finding that the range of dextran selectivity in acute ischaemic renal failure is slightly lower than in normal subjects is compatible with the view that the glomerular dextran of high selectivity may be admixed with dextran from a more unselective route, which could be the source of the urinary protein.

In postural proteinuria the dextran values are also highly selective and have a range similar to that of healthy subjects, indicating that the glomeruli are functioning normally. The unselective protein pathway appears to make no significant contribution to the dextran selectivity, possibly because in these patients there is a good renal function and the amount of glomerular dextran is large in relation to the small amount of dextran from any other source. Thus al-
though the origin of the urinary protein is obscure it is most unlikely to be glomerular. Renal haemodynamic changes have been postulated as a cause of postural proteinuria (Bull, 1948; King & Baldwin, 1954; Robinson et al., 1963). Because of the renal portal system, back pressure from the renal veins could conceivably result in a tubular leak of protein. However, there is no histological or electron microscopic evidence of rupture of the tubular basement membrane in postural proteinuria. Lowgren (1955) suggested a post-glomerular mechanism derived from the renal lymphatic system and, although there is little information in support of this, studies comparing lymph and serum protein patterns suggest that lymph is produced by a relatively unselective process (Schultze & Heremans, 1966). Although our results do not resolve the question of the origin of the protein in postural proteinuria, the normal glomerular permeability, indicated by the high dextran selectivity, coupled with the low protein selectivity provides indirect support for a renal lymphatic origin.

While the source of urinary proteins in normal subjects is uncertain, the dextran results confirm that protein selectivity at normal levels of protein excretion does not reflect glomerular permeability and that the normal glomerulus is highly selective.

ACKNOWLEDGMENT

This research was supported by a grant from the Scottish Hospital Endowments Research Trust as a research fellowship for P.R.M.

REFERENCES


Glomerular permeability to dextrans


