GLOMERULAR PERMEABILITY DURING PROTEINURIA INDUCED BY PLASMA INFUSION

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SUMMARY

1. Glomerular permeability to protein and dextran was studied in four patients with bleeding disorders who had normal renal function and in whom proteinuria occurred during the large infusions of plasma needed for treatment of the primary disorder.

2. Results are expressed in terms of selectivity indices which relate clearance to molecular weight.

3. Dextran selectivity was high both in the presence and absence of proteinuria and protein selectivity during proteinuria was also high.

4. Proteinuria induced by plasma infusion is likely to be glomerular in origin and the results indicate that the normal glomerulus is highly selective to plasma protein as well as to dextran.

In recent years, clearances of plasma proteins have been used in the assessment of patients with proteinuria, and the results have been interpreted in terms of glomerular permeability. This function is usually expressed in terms of the degree of selectivity of the glomerulus in permitting the passage of macromolecules of different sizes (Blainey, Brewer, Hardwicke & Soothill, 1960; Joachim, Cameron, Schwartz & Becker, 1964; Cameron & White, 1965; Hardwicke, 1965; Hitzig, Auricchio & Benninger, 1965; Ruckley, MacDonald, MacLean & Robson, 1966; Robson, 1968). In selective proteinuria the glomerulus restricts the passage of larger protein molecules to a very marked degree, and the clearance of albumin is about 1000 times that of $\alpha_2$ macroglobulin. In unselective proteinuria the passage of large molecules is much less restricted and the clearance of albumin is about five to 100 times that of $\alpha_2$ macroglobulin.

The normal glomerulus is highly selective for dextran and polyvinyl pyrrolidone (Hulme & Hardwicke, 1966; MacLean, Petrie & Robson, 1970), but the excretion pattern of the small amount of protein which appears in normal urine is highly unselective (Rowe & Soothill, 1961; MacLean & Robson, 1966; Poortmans, 1968). It has been suggested that this disparity may be

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due to the fact that a high proportion of normal urinary protein arises from the renal tract (Grant, 1957) and differs immunologically from serum protein, and/or that a relatively high proportion of the plasma protein in normal urine is derived from tubular or more distal sources rather than from glomerular filtration.

In health, filtered protein is largely reabsorbed by the renal tubules (Rather, 1952; Dirks, Clapp & Berliner, 1964) and only very small amounts escape into the urine. In glomerulonephritis on the other hand, it is believed that the tubular reabsorptive capacity for protein is saturated and that the bulk of the urinary protein is derived from glomerular filtration (Hardwicke & Squire, 1955). Only under such circumstances can studies of protein clearance be expected to be a valid reflection of glomerular permeability to protein and these conditions clearly do not prevail in health.

Proteinuria, however, can be induced in normal dogs (Brull, 1934; Terry, Hawkins, Church & Whipple, 1948; Vernier, 1961) and in human subjects not previously showing proteinuria (Waterhouse, Bassett & Holler, 1949) by the intravenous infusion of large amounts of albumin or plasma. This urinary protein is almost certainly of glomerular origin, the protein infusion leading to an increase in the protein content of the glomerular filtrate which is sufficient to saturate tubular reabsorption. Although electron microscopy studies in the dog indicate that this type of proteinuria is associated with loss of structure of the epithelial foot processes of Bowman's capsule, its disappearance following cessation of protein infusion suggests that the renal damage is of minor degree (Vernier, 1961).

Patients with bleeding disorders frequently require large infusions of plasma or plasma products to achieve haemostasis, and a proportion of such patients develop transient, heavy proteinuria with this treatment. The estimation of protein clearances in these circumstances thus provides the opportunity to estimate glomerular permeability to protein in what are believed to be normal human kidneys. In the present study, proteinuria was induced by plasma infusion in four patients with bleeding disorders, but with no evidence of renal disease, and protein selectivity was estimated. Since plasma expansion per se may cause haemodynamic changes resulting in altered glomerular permeability (Chinard, Lauson, Eder, Greif & Hiller, 1954; Malmendier, de Koster & Lambert, 1960), glomerular permeability to dextran was also measured both in the absence of proteinuria and after it had been induced.

METHODS

The patients studied suffered from haemophilia or Christmas disease and were admitted to hospital for treatment of haemorrhagic complications or for haemostatic therapy prior to elective surgery. Haemostatic therapy was controlled by the staff of the Department of Haematology and no alterations in therapy were made for the purpose of this study. Of ten patients infused with more than 2.5 l of plasma at a rate of over 800 ml/day, six developed proteinuria of over 1.0 g/day. Four of these patients have been studied in detail. In addition to fresh frozen platelet-poor plasma (prepared by the Blood Products Unit, Edinburgh), four received purified antihaemophilic fraction (Cohn fraction I) and case 3 received Prothrombin Complex (concentrate of factors II, VII, IX, X). The protein concentrations and volumes of the fresh frozen plasma and protein fractions are shown in Table 1.

Protein selectivities and urine to serum ratios of albumin were determined by a modification
Proteinuria induced by plasma infusion

Table 1. Details of protein containing solutions given to patients prior to onset of persistent proteinuria.
The infusion period is the time taken for proteinuria to develop.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Platelet-poor plasma (ml)</th>
<th>Antihaemophilic fraction (g)</th>
<th>Prothrombin complex (ml)</th>
<th>Total protein infused (ml)</th>
<th>Infusion period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Haemophilia</td>
<td>2400</td>
<td>132</td>
<td>-</td>
<td>3000</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>Christmas disease</td>
<td>7400</td>
<td>407</td>
<td>-</td>
<td>7440</td>
<td>407</td>
</tr>
<tr>
<td>3</td>
<td>Haemophilia</td>
<td>8400</td>
<td>462</td>
<td>2000</td>
<td>10400</td>
<td>522</td>
</tr>
<tr>
<td>4</td>
<td>Haemophilia</td>
<td>7200</td>
<td>396</td>
<td>2400</td>
<td>9600</td>
<td>468</td>
</tr>
</tbody>
</table>

Fig. 1. Calculation of $-k$, the index of selectivity for protein in patient 1. The renal clearance, expressed as a percentage of the clearance of albumin, is plotted against molecular weight for each of four proteins, using a double logarithmic scale. $-k$ is the slope of the regression line obtained, using the method of least squares. In this case the proteinuria is selective and $-k$ is $3.13$. 
of the immunodiffusion technique of Soothill (MacLean & Robson, 1966; MacLean & Robson, 1967). The index of selectivity obtained is denoted by the symbol $-k$. (Fig. 1).

Dextran selectivities were determined by a method which involves the separation of serum and urinary macromolecules by Sephadex G 200 on the basis of molecular size (Petrie, MacLean & Robson, 1968). The dextran selectivity index is denoted by the symbol $D$.

Creatinine in the serum and urine, total serum protein and serum albumin were estimated employing the AutoAnalyzer (Technicon Methodology, Files N11b and N19b, Northam & Widdowson, 1967). Total urinary protein was measured by the biuret method (Hiller, Grief & Beckman, 1948), and by a modification of a microbiuret method (Itzhaki & Gill, 1964).

RESULTS

The four patients studied (Table 2) all had less than 30 mg/100 ml of protein in their urine, or a urine to serum albumin ratio of less than 1:1600 both before the protein infusions, and 5 days after the cessation of treatment. The values for creatinine clearance ranged from 76 to 150 ml/min. Plasma protein levels prior to the protein infusions ranged from 6-9 to 7-8 g/100 ml, with albumin concentrations of between 3-8 and 4-2 g/100 ml. The serum protein concentration in these patients at the time of proteinuria ranged from 8-3 to 9-4 g/100 ml with albumin levels ranging from 4-4 to 5-0 g/100 ml.

Protein selectivities measured during the period of proteinuria ranged from $-k$ values of 2-49 to 3-13. These values indicate a highly selective pattern of protein excretion, comparable for example to that seen in glomerulonephritis with minimal lesions (Robson, 1968). The technique for determining dextran selectivities differs somewhat from the immunological technique used in assessing protein selectivities, but gel filtration selectivity values have been shown to be related to $-k$ values for protein selectivity by the formula $D = 0.76 (-k) - 0.08$ (MacLean & Petrie, 1966). D values measured during proteinuria ranged from 2-47 to 3-15 and are therefore comparable to the simultaneously measured $-k$ values for protein. These D values show the highly selective excretion pattern which is characteristic of the normal kidney (MacLean et al., 1970). Essentially similar values for dextran selectivity were obtained in the absence of proteinuria. These ranged from 2-44 to 3-03.

DISCUSSION

The proteinuria induced by plasma infusion is highly selective and simultaneously measured dextran clearances show a similar pattern. There are three possible explanations of the results.

Firstly, the glomeruli may be structurally normal, but the increase in plasma volume produced by protein infusion leads to stretching of 'glomerular pores'. In this event the glomerular clearance of protein would be slightly raised and this, along with the increased serum protein concentration, would result in an increased amount of protein being delivered to the tubules, with consequent saturation of tubular protein reabsorption. This mechanism has been shown to occur in patients with the nephrotic syndrome following albumin infusion (Chinard et al., 1954; Malmendier et al., 1960). However, since no alteration in dextran selectivity could be demonstrated following plasma infusion in our patients 'pore stretching' due to plasma expansion appears unlikely.

Secondly, the glomeruli may be structurally and functionally abnormal. The abnormality, if
Table 2. \(-k\) = index of protein selectivity, \(D\) = index of dextran selectivity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Creatinine clearance (ml/min)</th>
<th>Serum protein (g/100 ml)</th>
<th>Serum protein (during proteinuria) (g/100 ml)</th>
<th>Urine protein (no proteinuria) (mg/24 h)</th>
<th>Urine protein (during proteinuria) (g/14 h)</th>
<th>Albumin serum: urine ratio (no proteinuria)</th>
<th>(-k)</th>
<th>(D) (during proteinuria)</th>
<th>(D) (no proteinuria)</th>
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<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>7.7</td>
<td>8.3</td>
<td>19</td>
<td>12.6</td>
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<td>3.13</td>
<td>3.15</td>
<td>2.89</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>7.3</td>
<td>8.8</td>
<td>7</td>
<td>4.2</td>
<td>5430</td>
<td>2.49</td>
<td>2.47</td>
<td>2.44</td>
</tr>
<tr>
<td>3</td>
<td>129</td>
<td>6.9</td>
<td>9.2</td>
<td>20</td>
<td>4.5</td>
<td>7920</td>
<td>2.66</td>
<td>2.48</td>
<td>2.99</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
<td>7.8</td>
<td>9.4</td>
<td>23</td>
<td>11.8</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
it exists, coincides in time with the plasma infusion and is therefore presumably caused by it. All the patients studied had had repeated previous infusions of plasma, and may well have possessed antibodies to various components of the infused material. It is known that antigen-antibody complexes can be deposited in the basement membrane with resultant glomerular damage and proteinuria (Dixon, 1968; Cochrane, 1968). While the present results do not exclude glomerular damage of this type, this mechanism is unlikely. In the first place the proteinuria is highly selective. Secondly, it occurs only after the serum protein concentration has been raised significantly. It also disappears promptly following cessation of protein infusion when the plasma protein concentration falls towards normal.

Finally, the glomeruli may be structurally and functionally normal, the proteinuria being due entirely to saturation of tubular reabsorption. This saturation can be attributed to more protein than usual being filtered through normal glomeruli as a result of the raised concentration of plasma proteins. In this event the selectivity is a valid reflection of glomerular permeability.

The haemostatic disorder in these patients naturally prevented renal biopsies being undertaken during or after the proteinuria induced by the protein infusion. In view of the values for dextran selectivity however and the transient nature of the proteinuria it is believed that glomerular function and structure in these patients is not significantly abnormal. The results therefore indicate that the normal glomerulus filters plasma protein in a highly selective manner.

ACKNOWLEDGMENT

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REFERENCES


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