A biochemical and immunological investigation into the physiological basis of the increased albumin filtration induced in hyperalbuminaemic female Wistar rats

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Summary

1. The level of proteinuria induced in female Wistar rats after bovine albumin injection intraperitoneally was highly dose dependent.

2. The proteinuria remained highly selective, with albumin constituting approximately 90% of the total protein excreted, even in the most severely affected rats.

3. Calculations relating the amount of bovine albumin available in the serum for filtration to the amount excreted in the urine indicated that complete saturation of the reticuloendothelial and tubular protein reabsorption systems may have occurred during the course of the 5 day injection period in rats given more than 3.5 mg of bovine albumin day⁻¹ g⁻¹ body wt.

4. When this situation was attained there appeared to be no further increase in glomerular permeability to either rat or bovine albumin and an equilibrium state seemed to exist where, when serum albumin levels were raised above the normal level, all the excess albumin passed across the glomerular filter to be excreted in the urine.

5. The passage of these large quantities of albumin across the glomerular filter may have resulted not from ultrastructural damage to the filter itself but rather from the generation of vastly increased concentration gradients across the glomerular basement membrane, which were sufficiently large to overcome the electrostatic repulsive forces which normally severely restrict albumin filtration.

Key words: albuminuria, kidney, proteinuria.

Introduction

Hyperalbuminaemia, produced by intraperitoneal albumin injection, is known to induce increased protein excretion in the rat [1–4], which is accompanied by loss of the normal complex glomerular epithelial cell foot process architecture and by the accumulation, within the epithelial cell cytoplasm, of electron dense protein droplets [5–9]. The sequential development of the proteinuria induced by hyperalbuminaemia has been monitored after the administration of human [3] and bovine [8, 9] albumins and both exogenous and endogenous proteins have been identified [2, 3] and measured [9–11] in serum and urine samples from rats with gross proteinuria. The published experimental data concerned with hyperalbuminaemic proteinuria are, however, sparse, ambiguous and difficult to interpret since no consistent experimental protocol has been used by the various workers in the field. Recent studies [12, 13] have shown that the response to hyperalbuminaemia depends on the sex and strain of rat injected and also, within a given sex or strain of rat, on baseline albumin excretion. There are also indications that the particular type of albumin administered ([8, 9, 14]; G. M. Lawrence & D. B. Brewer, unpublished observations) and the amount of protein injected [12] also affect the response to hyperalbuminaemia. The present investigation was undertaken therefore to study the response to hyperalbuminaemia within a well-defined experimental system in the hope that a clearer insight into the physiological
basis of the increased proteinuria associated with the condition could be attained.

**Materials and methods**

Proteinuria was induced in female Wistar rats by Cohn V bovine albumin (Sigma Chemical Co. A4503) solutions, with concentrations ranging from 50 to 200 g/l, injected intraperitoneally twice daily for 5 days [12]. Daily bovine albumin doses were calculated and divided into groups as previously described [12]. Blood samples were taken each day immediately after the first bovine albumin injection and 24 h urine collections made. Total urinary protein was measured by the biuret method and urinary and serum proteins were measured by single radial immunodiffusion [12]. Immuno-, cellulose acetate- and slab and tube sodium dodecyl sulphate/polyacrylamide gel-electrophoretic techniques were used to investigate urinary protein composition.

**Results**

In a previous report [12] the total amount of protein excreted by hyperalbuminaemic rats during a 5 day period of intraperitoneal injections of bovine albumin was shown to increase logarithmically with dose ($r = 0.86$, $n = 53$). Quantitative single radial immunodiffusion assays indicated that rat and bovine albumins made up the majority of this increased urinary protein excretion in all bovine albumin dose groups (Table 1). Furthermore, a good correlation existed between excretion and dose in both cases ($r = 0.85$ for rat albumin and $r = 0.88$ for bovine albumin, $n = 45$).

Daily rat and bovine albumin excretion patterns (Fig. 1) were similar to those previously reported for total protein excretion [12]. Maximum values were attained on day 3 in dose groups 2.0–3.0, 3.0–3.5 and 3.5–4.5 mg day$^{-1}$ g$^{-1}$ body wt. and on day 3 in the highest bovine albumin dose group. In the lowest group, total protein, rat albumin and bovine albumin excretion were still increasing on the fifth and final injection day. The relative amounts of rat and bovine albumin excreted each day were proportional to their serum concentrations, with rat albumin predominating only at low bovine albumin doses and during the first few injection days at higher doses when serum rat albumin concentrations were greater than the maximum levels attained by bovine albumin.

Control rats showed no increase in either total protein or albumin excretion during their 5 day injection period ($P > 0.40$ for total protein excretion and $P > 0.80$ for albumin excretion).

**Urinary protein composition**

All the techniques used to follow the development of proteinuria during the 5 day bovine albumin injection period indicated that bovine and rat albumins constituted the vast majority of the serum-derived protein excreted, irrespective of the bovine albumin dose given or the period of administration. Other serum proteins were, however, present in minor but increasing proportions as the levels of induced proteinuria rose. This was particularly well illustrated by chromoscan traces (Fig. 2) made from cellulose acetate strips run with daily urine samples collected from two hyperalbuminaemic rats which developed widely differing degrees of proteinuria. In these traces the area under each peak is proportional to the total amount of protein present.

The minor proteins present in hyperalbuminaemic rat urine were identified from their immunological and electrophoretic properties and their molecular weights as, in decreasing order of importance, transferrin, dimer albumin, immunglobulin G, α_1-anti-trypsin, Gc globulin and haemopexin.

**Table 1. Mean total protein, rat albumin (RSA) and bovine albumin (BSA) excretion over a 5 day period for female Wistar rats given intraperitoneal injections of phosphate-buffered saline containing BSA doses in the range 1.0–5.5 mg day$^{-1}$ g$^{-1}$ body wt. compared with control rats injected with the same volume of phosphate-buffered saline alone**

<table>
<thead>
<tr>
<th>Bovine albumin dose group (mg day$^{-1}$ g$^{-1}$ body wt.)</th>
<th>Mean total protein excretion (mg/5 days)</th>
<th>$n$</th>
<th>Mean RSA excretion (mg/5 days)</th>
<th>$n$</th>
<th>Mean BSA excretion (mg/5 days)</th>
<th>$n$</th>
<th>Mean RSA + BSA excretion (% of mean total protein excretion)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>30.0 ± 9.0</td>
<td>6</td>
<td>5.5 ± 2.0</td>
<td>6</td>
<td>15 ± 14</td>
<td>18</td>
<td>16</td>
<td>55</td>
</tr>
<tr>
<td>1.0–2.0</td>
<td>65 ± 47</td>
<td>18</td>
<td>21 ± 20</td>
<td>18</td>
<td>15 ± 14</td>
<td>18</td>
<td>55</td>
<td>18</td>
</tr>
<tr>
<td>2.0–3.0</td>
<td>467 ± 139</td>
<td>8</td>
<td>127 ± 32</td>
<td>4</td>
<td>201 ± 73</td>
<td>4</td>
<td>70</td>
<td>4</td>
</tr>
<tr>
<td>3.0–3.5</td>
<td>919 ± 607</td>
<td>4</td>
<td>305 ± 195</td>
<td>4</td>
<td>497 ± 331</td>
<td>4</td>
<td>87</td>
<td>4</td>
</tr>
<tr>
<td>3.5–4.5</td>
<td>1793 ± 629</td>
<td>12</td>
<td>575 ± 185</td>
<td>12</td>
<td>1019 ± 364</td>
<td>12</td>
<td>89</td>
<td>12</td>
</tr>
<tr>
<td>4.5–5.5</td>
<td>2334 ± 763</td>
<td>11</td>
<td>699 ± 237</td>
<td>7</td>
<td>1489 ± 625</td>
<td>7</td>
<td>94</td>
<td>7</td>
</tr>
</tbody>
</table>
Albumin filtration in hyperalbuminaemia

Fig. 1. Hyperalbuminaemic proteinuria induced by intraperitoneal injection of bovine albumin (BSA): variation in daily rat albumin (■) and bovine albumin (■) excretion patterns for each bovine albumin dose group over the 5 day injection period. The BSA dose groups studied were: (1) 1.0–2.0; (2) 2.0–3.0; (3) 3.0–3.5; (4) 3.5–4.5; (5) 4.5–5.5 mg of BSA day⁻¹ g⁻¹ body wt. (Error bars denote standard deviations.)

Fig. 2. Cellulose acetate electrophoresis: chromoscan traces of the development of proteinuria induced by intraperitoneal injection of bovine albumin in rats which excreted (a) 435 mg and (b) 1337 mg of total protein during the 5 day bovine albumin injection period (R, rat albumin; B, bovine albumin; T, transferrin; G, immunoglobulin G).

Rat albumin clearances

Daily rat albumin clearances were calculated by use of serum concentration values which were means of the two rat albumin concentrations determined at the start and end of each 24 h urinary collection period. The relationship between daily rat albumin clearance and bovine albumin dose for 13 rats in three different bovine albumin dose groups is illustrated in Fig. 3(a). Rat albumin clearance in 164 normal female Wistar rats had previously been found to be 16.5 nl/min, with an upper limit of normality of 99.5 nl/min (G. M. Lawrence & D. B. Brewer, unpublished observations). The maximum mean rat albumin clearances measured in the three groups of hyperalbuminaemic rats examined in the present study (162 ± 158, 1552 ± 574 and 6490 ± 858 nl/min) were, therefore, some 10-, 100- and 400-fold greater than normal values respectively. No further significant increases in rat albumin clearance were apparent after the third bovine albumin injection day in any of the three groups of rats.

Rat albumin clearances on the fifth and final bovine albumin injection day were determined in 41 rats and a good correlation was found between clearance and dose (r = 0.87). However,
FIG. 3. (a) Daily rat albumin (RSA) clearances in hyperalbuminaemic proteinuria induced by intraperitoneal injection of bovine albumin (BSA) as a function of BSA dose: ●, 1-0-2-0 mg day\(^{-1}\) g\(^{-1}\) body wt. \((n = 3)\); ▲, 2-0-3-0 mg day\(^{-1}\) g\(^{-1}\) body wt. \((n = 4)\); ■, 3-5-4-5 mg day\(^{-1}\) g\(^{-1}\) body wt. \((n = 6)\). (b) Relationship between rat albumin clearance on the fifth bovine albumin (BSA) injection day and BSA dose group (1-5) for hyperalbuminaemic proteinuric female Wistar rats (1, 1-0-2-0; 2, 2-0-3-0; 3, 3-0-3-5; 4, 3-5-4-5; 5, 4-5-5-5 mg of BSA day\(^{-1}\) g\(^{-1}\) body wt.). (Error bars denote standard deviations.)

when the data were examined after their normal division according to bovine albumin dose group (Fig. 3b) it was clear that there was no significant increase in rat albumin clearance above bovine albumin doses of 3-5 mg day\(^{-1}\) g\(^{-1}\) body wt.

Bovine albumin concentrations in the sera of hyperalbuminaemic rats

The serum bovine albumin concentrations determined in the present study at 24 h intervals could not be used to calculate meaningful values for daily bovine albumin clearances as, unlike rat albumin concentrations, serum bovine albumin levels changed in a complex manner throughout the 24 h urinary collection period as bovine albumin was introduced via the peritoneal cavity or removed by normal serum clearance mechanisms. However, if the assumption were made that the serum half life of bovine albumin was the same in normal and hyperalbuminaemic rats, the daily bovine albumin serum concentration data collected could be used in conjunction with information previously obtained from normal female Wistar rats [12] to explore the relationship between the amount of bovine albumin theoretically available in the serum for filtration and the quantity actually excreted in the urine.

With a value of 18-8 h for bovine albumin serum half life \((t_h)\) and experimentally determined values for the serum bovine albumin concentrations \((N_0)\) attained as a result of intraperitoneal injection of different bovine albumin doses [12], the serum bovine albumin concentrations \((N)\) expected any time \((t)\) as a result of each daily intraperitoneal injection were calculated for each bovine albumin dose group as previously described [12], with the equation \(\log(N_0/N) = \log(\text{predicted}/\text{measured})\). Predicted daily serum bovine albumin levels were then obtained by summing the serum bovine albumin concentrations contributed by each intraperitoneal injection given before each daily sampling time. The amount of bovine albumin available for filtration each day could then be calculated by multiplying the difference between predicted and measured serum bovine albumin levels by 6-3 ml, the serum volume of a 200 g rat [15].

In the lowest bovine albumin dose group (1-0-2-0 mg day\(^{-1}\) g\(^{-1}\) body wt.) the calculated amount of bovine albumin available for filtration each day was significantly greater than that excreted (Table 2). This was also true in the dose group 2-0-3-0 mg day\(^{-1}\) g\(^{-1}\) body wt. until day 2. On day 3, however, the amount of bovine albumin excreted was equivalent to calculated
Table 2. Daily comparison of the amount of bovine albumin (BSA) excreted and the quantity calculated to be available for filtration in female Wistar rats injected intraperitoneally with various doses of BSA.

<table>
<thead>
<tr>
<th>Dose group (mg day⁻¹ g⁻¹ body wt.)</th>
<th>Day no.</th>
<th>Measured urinary BSA excretion (mg/24 h)</th>
<th>BSA available for filtration (mg/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-0-3-0</td>
<td>n = 3</td>
<td>1.5 ± 1.2</td>
<td>0 ± 19</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.7 ± 3.4</td>
<td>31 ± 28</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.8 ± 7.2</td>
<td>31 ± 12</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.0 ± 8.0</td>
<td>21 ± 10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.9 ± 5.1</td>
<td>53 ± 15</td>
</tr>
<tr>
<td>2.0-3.0</td>
<td>n = 4</td>
<td>1.7 ± 1.6</td>
<td>0 ± 18</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.6 ± 3.8</td>
<td>13 ± 12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>38 ± 43</td>
<td>6 ± 30</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>96 ± 32</td>
<td>29 ± 27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>82 ± 18</td>
<td>65 ± 34</td>
</tr>
<tr>
<td>3.5-4.5</td>
<td>n = 5</td>
<td>48 ± 23</td>
<td>22 ± 21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>228 ± 68</td>
<td>152 ± 13</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>351 ± 58</td>
<td>201 ± 9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>329 ± 64</td>
<td>213 ± 16</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>258 ± 71</td>
<td>234 ± 35</td>
</tr>
</tbody>
</table>

Filtration values, and, at later times, exceeded these levels. At the highest bovine doses injected these discrepancies were apparent as early as day 1 (Table 2).

Discussion

The present study clearly demonstrates that, in hyperalbuminaemic rats, albumin constituted the vast majority of the increased protein excretion measured and that the main renal functional change induced must therefore have concerned the handling of albumin. Since the level of albuminuria is normally controlled by two processes, glomerular ultrafiltration and tubular reabsorption, alterations in the characteristics of either of these systems could have caused this increased albuminuria.

The presence of a semipermeable membrane (the glomerular basement membrane) between the glomerular capillary and Bowman’s space sets up a concentration gradient across the membrane which favours the passage of albumin from serum to the glomerular ultrafiltrate. Filtration of albumin is, however, normally severely restricted by electrostatic repulsion between the protein (pI = 4.5) and anionic sites within the membrane [16-18] and on the endothelial cell surface [19], in a process which relies on the maintenance of normal renal haemodynamics [20, 21]. Any small quantities of albumin that do cross the glomerular filter and escape uptake in the glomerular epithelium [22] are subject to tubular reabsorption, mainly in the proximal convoluted tubules [23-25]. Although microinjection and micropuncture experiments [26-31] have suggested that the albumin tubular reabsorption system operates at near saturation capacity in the normal rat, problems concerning the contamination of ultrafiltrate samples with extraneous albumin [31], and histochemical studies demonstrating that the amount of albumin present in proximal tubular epithelia rises markedly with increasing proteinuria ([25]; G. M. Lawrence, unpublished observations), suggest that it is probably a low affinity, high capacity system, similar to those involved with the reabsorption of other low-molecular-weight proteins such as ribonuclease, insulin and lysozyme, which normally operate well below their T_m values, removing a constant fraction of the filtered load [32].

In rats injected with bovine albumin larger concentration gradients would have automatically been generated across the glomerular filter at all doses because of the increased total serum albumin concentrations induced. At low doses this may have been the only factor causing the slightly raised albuminuria detected because, if tubular reabsorption does remove a constant fraction of the filtered albumin load, any increase in filtration would also have led to an increase in excretion. If the simple generation of increased concentration gradients were the only factor influencing albumin excretion in hyperalbuminaemic rats one might expect to see a linear relationship between serum albumin concentration (and hence bovine albumin dose) and albumin clearance. The 400-fold increases in rat albumin clearance measured when total serum albumin concentration was raised only twofold, and the logarithmic relationship between bovine albumin dose and induced proteinuria, suggest, therefore, that other changes also contributed to the increased albuminuria.

One possible explanation of this phenomenon would be that, under conditions of increased plasma viscosity caused by large rises in total serum albumin concentration in rats injected with bovine albumin (G. M. Lawrence, unpublished observations), associated decreases in renal plasma flow rate and erythrocyte stirring contributed [33, 34] to a thickening of the concentration–polarization layer of partially rejected serum proteins that is thought to exist on the luminal surface of the capillary endothelium in the normal state [35]. As albumin forms the greater part of the serum protein in hyperalbuminaemic rats, one might expect large amounts of albumin to be deposited in this layer...
thereby further increasing its concentration gradient across the glomerular filter. If this mechanism did operate, the proportional rise in albumin filtration could be many times greater than the relative rise in serum albumin concentration. Similar but smaller gradients created in the same way could also account for the small measured rises in excretion of transferrin and immunoglobulin G, proteins whose serum concentrations were not elevated during hyperalbuminaemia.

Another possible explanation is apparent from the discrepancies found in calculations relating the amount of bovine albumin theoretically available for filtration in hyperalbuminaemic rats to the quantity actually excreted. The discrepancies found can readily be explained if, as bovine albumin dose rose, firstly a relatively larger proportion of the injected protein was available for filtration than in normal rats and, secondly, if more of this filtered protein was actually excreted. These effects would occur if the cells of the reticuloendothelial system (which remove foreign proteins from the blood stream) and the cells of the renal tubular epithelium (which reabsorb excess filtered protein) were at first partially and then completely saturated in processes which depended both on the duration and size of the bovine albumin dose administered. The occurrence of the former effect finds support from the rise in aggregated albumin serum survival time seen in rats preinfused with colloidal carbon particles, which are avidly accumulated by the reticuloendothelial system [36], and the latter from studies indicating that prolonged intraperitoneal injection of bovine albumin prevents the accumulation (within the tubular epithelium) of haemoglobin-containing protein absorption droplets that normally occurs after a single intravenous injection of haemoglobin [37].

Assuming that, in hyperalbuminaemic rats, 42% of the bovine albumin injected into the peritoneal cavity was transferred to the blood stream just as it was in normal female Wistar rats [12], calculations show that complete saturation of the serum clearance and tubular reabsorption mechanisms must have occurred on day 3 in the dose group 3.5-4.5 mg day⁻¹ g⁻¹ body wt. since, from this time onwards, mean bovine albumin excretion (approximately 330 mg/24 h) was similar to the maximum amount calculated to be available for filtration (290-380 mg/24 h). [The latter quantity was computed by multiplying the dose of bovine albumin administered each day (3.5-4.5 mg day⁻¹ g⁻¹ body wt.) by the weight of the rat injected (200 g) and the percentage of the bovine albumin injected into the peritoneal cavity that was actually transferred to the blood stream (42%).] An identical situation existed in the dose group 4.5-5.5 mg day⁻¹ g⁻¹ body wt., where mean bovine albumin excretion on days 3, 4 and 5 was in the order of 400 mg/24 h (Fig. 1) and the calculated amount available for filtration approximately 380-460 mg. In the lowest bovine albumin dose group studied in detail (2.0-3.0 mg day⁻¹ g⁻¹ body wt.) serum clearance or tubular reabsorption or both could only have been partially saturated throughout the 5 day bovine albumin injection period as even the maximum mean bovine albumin excretion (96 mg/24 h) was less than 50% of the calculated amount available (170-250 mg).

If this interpretation of the bovine albumin excretion data is correct it indicates, firstly, that in the bovine albumin dose groups 3.5-4.5 and 4.5-5.5 mg day⁻¹ g⁻¹ body wt. there was no increase in glomerular filtration of bovine albumin after day 3 and, secondly, that glomerular permeability to bovine albumin in these two groups was similar, the increased excretion in the highest dose group being due simply to the increased amounts of bovine albumin available. These conclusions were supported by the rat albumin clearances reaching a plateau from day 3 onwards in the dose group 3.5-4.5 mg day⁻¹ g⁻¹ body wt. (Fig. 3a) and the fact that, although rat albumin clearance on the fifth bovine albumin injection day correlated well with dose, little further increase was apparent at bovine albumin doses above 3.5 mg day⁻¹ g⁻¹ body wt. When this stage was reached the amount of rat albumin excreted each day was equivalent to that normally synthesized (150-200 mg/24 h; [38]), indicating that an equilibrium state may then have existed, in which an amount of rat albumin equivalent to the quantity newly synthesized each day and all the injected bovine albumin that was available in the blood stream were filtered at the glomerulus and then excreted in the urine.

The results of the present study therefore suggest that increased concentration gradients generated during hyperalbuminaemia may have been sufficiently great to allow filtration of large amounts of albumin across a glomerular basement membrane which need not necessarily have suffered any ultrastructural alteration. This increased filtration may then in turn have been, together with the saturation of tubular reabsorption mechanisms at high bovine albumin doses, sufficient to account for even the greatest levels of proteinuria induced. Such an interpretation would be consistent with ultrastructural studies using the cationic probes alcian blue, ruthenium red and polyethyleneimine (T. P. Rollason & D. B.
Brewer, unpublished observations), which indicate that, during hyperalbuminaemic-induced proteinuria, there is no reduction in the number of fixed anionic sites present within the glomerular basement membrane and, with the highly selective proteinuria that was maintained in the present study, even by rats with very high levels of proteinuria. It also helps to explain the rapid recovery normally evident in most hyperalbuminaemic rats after the cessation of bovine albumin injections [9].

Acknowledgments
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References

