An Analysis of Post Transplantation Proteinuria

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It has been recently demonstrated that the pattern of proteinuria changes after transplantation both as a function of time and as a function of the graft tolerance (Braun & Merrill, 1970; Debray-Sachs, 1970; Laterre et al, 1970; Revillard et al, 1970; Stihlke & Hermann, 1971). It has been suggested that the changing electrophoretic pattern of proteinuria reflects a changing selectivity of the glomerular barrier (Revillard et al, 1970). That this might occur in acute rejection was demonstrated by Braun and Merrill (1970) who studied glomerular selectivity by measuring the relative clearance of individual proteins. The present study was undertaken in order to evaluate the changes in proteinuria noted after transplantation and to correlate them with modifications of the selectivity of the glomerular membrane as evaluated by the relative clearance of individual molecules (Joachim et al, 1964).

MATERIAL AND METHOD

One hundred and two 24 hour urine collections were obtained in 43 patients between one day and 68 months after transplantation. Details of the immuno-suppressive therapy have been published elsewhere (Laterre et al, 1970). No instance of acute rejection was encountered. Chronic rejection was diagnosed when renal function decreased and proteinuria increased. These findings were often accompanied by an elevation of arterial blood pressure.

Collection methods have already been described (Laterre et al, 1970). Total protein excretion was measured by trichloracetic precipitation and an electrophoresis in acrylamide agarose gel was obtained (Laterre et al, 1970). Classification of the electrophoretic patterns into physiologic, tubular, glomerulotubular and glomerular has been previously described (Manuel & Laterre, 1970). Seven proteins: albumin, transferrin, caeruloplasmin, IgG, IgA, α₂ macroglobulin and IgM were measured in plasma and in concentrated urine by the radial immunodiffusion method of Mancini et al (1965). Individual protein clearances were calculated. Results for five of these proteins
(albumin, transferrin, IgG, $\alpha_2$ macroglobulin and IgM) were plotted on a log-log graph with clearance on the ordinate and the molecular weight on the abscissa. Whenever a statistically significant ($p < 0.05$) correlation was found between clearance and molecular weight for these five proteins, a straight line was calculated by the method of least squares. The line was characterised by its angle with the horizontal axis (angle $\theta$), a value taken to express selectivity (Joachim et al., 1964; Braun & Merrill, 1970).

RESULTS

EVOLUTION OF PROTEINURIA AS A FUNCTION OF TIME

In the absence of rejection, proteinuria decreased progressively over the first six weeks after transplantation and stabilised subsequently around a mean value of 330mg/day (extreme 13 and 1958mg/day).

Table I. Percentage of samples with a given degree of proteinuria demonstrating a specific electrophoretic pattern

<table>
<thead>
<tr>
<th>Proteinuria g/day</th>
<th>&gt; 1</th>
<th>0.5 - 1</th>
<th>0.15 - 0.5</th>
<th>&lt; 0.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular</td>
<td>70</td>
<td>13</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Glomerulotubular</td>
<td>30</td>
<td>39</td>
<td>39</td>
<td>10</td>
</tr>
<tr>
<td>Tubular</td>
<td>0</td>
<td>48</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Physiologic</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>Number of samples</td>
<td>23</td>
<td>23</td>
<td>36</td>
<td>20</td>
</tr>
</tbody>
</table>

The electrophoretic pattern was predominantly glomerular or glomerulotubular initially (90% of 20 samples during the first two weeks). Six weeks after transplantation it became predominantly physiologic or tubular (80% of 24 samples after 6 weeks). Chronic rejection resulted usually in a significant increment in proteinuria and in the reappearance of glomerular or glomerulotubular electrophoretic patterns (60% of 39 samples after 6 weeks).

As shown in Table I, there is a good correlation between proteinuria and electrophoretic pattern ($X^2$ analysis: column 1 versus column 2, 3 and 4; column 2 versus column 4; column 3 versus column 4: $p < 0.001$).

INDIVIDUAL PROTEIN CLEARANCES
Relationship between individual protein clearances and proteinuria

In the present study 24 hour proteinuria ranged from 13 to 10,000 mg. Individual clearances ranged as follows:
range of clearances $\times 10^{-4}$ ml/min

<table>
<thead>
<tr>
<th>Protein</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>albumin</td>
<td>0.5 to 2772</td>
</tr>
<tr>
<td>transferrin</td>
<td>0.4 to 2046</td>
</tr>
<tr>
<td>caeruloplasmin</td>
<td>0.9 to 1816</td>
</tr>
<tr>
<td>IgG</td>
<td>0.3 to 823</td>
</tr>
<tr>
<td>IgA</td>
<td>0.2 to 12,450</td>
</tr>
<tr>
<td>$\alpha_2$ macroglobulin</td>
<td>0.1 to 205</td>
</tr>
<tr>
<td>IgM</td>
<td>0 to 457</td>
</tr>
</tbody>
</table>

Significant correlation ($p<0.001$) between individual clearances and proteinuria was found for albumin ($r = 0.82$), transferrin ($r = 0.73$), IgG ($r = 0.93$), $\alpha_2$ macroglobulin ($r = 0.74$) and IgM ($r = 0.80$). It is of interest to note that the slope of these relationships was markedly influenced by molecular weight. The light molecules, albumin and transferrin, have a slope of 196 and 201 respectively as compared with a slope of 90 for IgG and 13 and 24 for $\alpha_2$ macroglobulin and IgM respectively (Figure 1). The lack of correlation between proteinuria and caeruloplasmin or IgA clearances results probably from the known instability of these molecules (Trip et al, 1970; Bienenstock & Tomasi, 1968) and perhaps also from the fact that IgA may be secreted by the urinary tract. For these reasons, further correlation between individual protein clearances and molecular weight will not include these two proteins.

![Figure 1. Relationship observed between the clearances of individual proteins and 24 hour proteinuria. The lines were calculated by the least square method. In each instance, the correlation coefficient was highly significant.](image-url)
Relationship among the different protein clearances.
The determination of selectivity

A significant correlation (p< 0.05) between the logarithm of individual protein clearances and the logarithm of the molecular weight was absent in 25 of the 102 urine collections. The frequency of this phenomenon tended to decrease as a function of time: it was found in 10 out of 20 specimens collected within 2 weeks after transplantation, in 6 out of 19 samples obtained during the following 4 weeks and in 9 out of 63 samples collected subsequently. No relationship was found between the magnitude of proteinuria and the absence of correlation between clearance and molecular weight. In the 77 collections in which a correlation was found, a straight line was calculated by the method of the least squares and its angle ($\theta$) with the horizontal was determined. Previous studies have suggested that $\theta$ might be used as an index of selectivity (Joachim et al, 1964; Braun & Merrill, 1970).

The reproducibility of this value was evaluated by comparing $\theta$ in two urine collections obtained within ten days in the same patient and in which the amount and the electrophoretic pattern of urinary proteins was stable. In 11 out of 13 pairs $\theta$ varied by less than 5°. In the other two it varied by less than 7°.

Factors influencing selectivity

Results of the samples collected more than a week after transplantation were grouped according to increasing $\theta$ values, each group encompassing a 10° interval. No relationship was found between $\theta$ and electrophoretic patterns of urinary proteins: glomerular and glomerulotubular patterns were observed in 63% of the samples in the 25 - 35° group versus 43% in the 35 - 45° group, 46% in the 45 - 55° group and 66% in the 56 - 65° group.

Similarly, $\theta$ did not show any correlation with the degree of 24 hour proteinuria. $\theta$ averaged 44° in the group excreting more than 1 g of protein, 42° in the group excreting between 0.5 and 1 g, 49° in the group excreting between 0.50 and 0.15 g and 43° in the group excreting less than 0.15 g daily.

Angle $\theta$, however, increased significantly as a function of time. From a mean value (+ SEM) of 29 ± 4° during the first week after transplantation it rose to 35 ± 2° during the next week, 40 ± 2° during the following two weeks. It stabilised subsequently around that level: 44 ± 1° during the second month, 47 ± 3° during the third month, 43 ± 3° during the subsequent three months, 45 ± 1° during the next six months, 43 ± 3° and 49 ± 2° during the second and third year respectively and 48 ± 4° during the fourth and fifth year after transplantation. Mean $\theta$ during the first two weeks is significantly different (p<0.02) from the mean value reached by the sixth week. The latter value is significantly lower (p<0.01) than that attained during the third month.

The effect of chronic rejection on $\theta$ was analysed in the 39 samples
collected beyond 120 days after transplantation, when θ does not change as a function of time.

θ averaged (± SEM) 46 ± 2° in 13 samples obtained from patients showing no sign of rejection and 44 ± 2° in 26 samples obtained from patients rejecting progressively their kidney. This difference is not significant (p>0.5).

DISCUSSION

The present study confirms and extends previous observations (Laterre et al., 1970) on the evolution of proteinuria after transplantation. Proteinuria is usually massive immediately after transplantation but decreased progressively over the subsequent six weeks and remains stable thereafter. Simultaneously, the electrophoretic pattern which was mainly glomerular or glomerulotubular becomes tubular or physiologic. In the presence of rejection, however, proteinuria increases and the electrophoretic pattern becomes mainly glomerular. The relationship between changes in protein excretion and alteration of the selectivity of the glomerular membrane remains largely unexplored. It has been suggested by Revillard et al (1970) that modifications of the electrophoretic patterns of urinary proteins reflect changes in selectivity. The present data cast some doubt on this hypothesis. Indeed, in the 77 urine collections in which a correlation existed between the clearances of proteins and their molecular weight, no relationship could be found between selectivity and electrophoretic patterns of the urine. Similarly, no relationship was found between total proteinuria and selectivity: neither total proteinuria nor the electrophoretic pattern of the urine provide a basis for predicting the selectivity of the glomerular filter.

It is of interest to note that selectivity appeared to change as a function of time: immediately after transplantation, proteinuria is poorly selective. Subsequently selectivity increases progressively and reaches a plateau within 8 to 12 weeks. A similar observation has been reported by Braun and Merrill (1970). This observation is not fortuitous: it does not result from the fact that only good kidneys were observed long after transplantation, whereas the group followed immediately after transplantation was less homogeneous. Indeed, 10 out of the 14 grafts studied during the first 6 weeks are still functioning 2 to 2 1/2 years later. Two other kidneys worked satisfactorily for more than a year but the patients died from neurological disease. Furthermore, we were able to collect multiple samples on a few patients. In some of them, it was possible to show by covariance analysis that the slope of the protein clearance — molecular weight relationship increased significantly as a function of time.

Chronic rejection does not appear to modify selectivity. Indeed, in 29 urine samples collected more than four months after transplantation, when selectivity appears to be stable, mean selectivity was virtually identical in
the group of non rejecting kidneys and in the group of well tolerated grafts. This finding stands in contrast with the observation of Braun and Merrill (1970) who observed drastic changes in selectivity during acute rejection. This difference probably reflects the difference in histological lesions of the kidney noted in acute and chronic rejection.

The significance of the relationship between the clearances of proteins and their molecular weight has been recently challenged by Lambert et al (1970) for theoretical reasons and by Pesce et al (1970). These latter authors pointed out that the urinary to serum ratio of proteins with virtually identical molecular weight was not identical. We have noted similar discrepancies between the clearances of IgA, IgG and caeruloplasmin. However, these findings might result from technical difficulties insofar that the radial immunodiffusion method does not discriminate between the whole protein and its pieces. The well documented fragility of caeruloplasmin and IgA might account for the lack of correlation observed in the present studies between clearances of these proteins and total proteinuria.

Nevertheless, it is of interest to note that a significant correlation was found between individual clearances and molecular weight in 77 out of 102 samples, a finding similar to that of Pesce et al (1970). Furthermore, whatever the meaning of the relationship, it is also noteworthy that the frequency of statistical correlation was the same whatever the quantity of excreted proteins, a finding which suggests that in transplanted kidneys tubular protein reabsorption does not affect the relative protein clearances.

SUMMARY

1. Protein excretion decreases over the first six weeks and stabilises subsequently.
2. Initially, glomerular electrophoretic patterns predominate. After six weeks, tubular or physiologic patterns predominate.
3. Rejection is accompanied by increased proteinuria and a reversal of the electrophoretic pattern.
4. There is no correlation between selectivity and electrophoretic patterns.
5. Chronic rejection does not modify selectivity.
6. Selectivity increases progressively over the first three months and stabilises subsequently.

ACKNOWLEDGMENT

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REFERENCES

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OPEN DISCUSSION

J S CAMERON (London): I don't think, Dr van Ypersele, you need be surprised at the lack of correlation between your electrophoretic patterns and the selectivity of the differential protein clearances. I think there is a very large body of evidence to suggest that differential protein clearances are a much better method of assessing proteinuria in primary glomerular disease. Finding this in the transplant situation is simply confirmation of this. We, also, have some data in transplanted patients on protein clearances which in general are in agreement with yours.

E KEMP (Denmark): I would like to ask you if you have any experience with primary glomerular diseases. Compared with this series it would be very interesting. The second question I would like to ask is whether you have investigated the importance of steric configuration of the proteins?

van YPERSELE: First, we have no data in situations other than renal transplantation. One of our problems when we first looked at the results was that we really found no correlation between molecular weight and each of the seven proteins which we studied. At first we were surprised that we found
there was no correlation between IgA clearances and total proteinuria. However, this is easily understood because IgA may polymerise and really what is filtered in the glomerulus is not always the IgA which has the specific molecular weight which we know. There is a difference in the slope between transferrin and albumin and I think that you are probably right and that the steric configuration of the molecules may explain how transferrin, a slightly heavier protein than albumin, clears at a higher rate.

W O'DONOOGHUE (London): Do you have any information on the effect of indomethacin or steroids on proteinuria after transplantation?

van YPERSELE: No, it's very difficult to have any data on this since all the patients received steroids. However, I think that in patients with the nephrotic syndrome receiving steroids it has been shown by several workers that treatment with steroids does not change selectivity.