The soluble proteins of the bovine cornea

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The soluble proteins of calf cornea, both epithelial and stromal, were fractionated by Sephadex G-200 gel filtration followed by DEAE-cellulose ion-exchange chromatography. Each fraction was further analyzed using a variety of techniques, including disc electrophoresis in polyacrylamide gels, Ouchterlony immunoprecipitation, and immunoelectrophoresis. From the corneal epithelium some 20 or more distinct protein zones are demonstrated by electrophoresis. A single protein appears to comprise about 40 per cent of the epithelial proteins and a significant part of the stroma as well. The epithelium is devoid of serum proteins. The stroma, however, contains at least five serum proteins, albumin, 7S-gamma globulin, transferrin, a lipoprotein, and an unidentified protein of beta electrophoretic mobility. These serum proteins constitute over half of the soluble stromal proteins. Of the remainder, there is one stromal protein identical with the principal epithelial protein, a few are glycoproteins, and none are soluble collagen.

Although the insoluble components of the cornea, such as collagen and the sulfated mucopolysaccharides, have been investigated in detail, very few reports exist describing the soluble protein components. A few report the relative amounts of corneal protein which are soluble; one suggests the presence of certain serum proteins, two utilize the fluorescent antibody technique to demonstrate the presence of serum albumin and immunoglobulins, and one older paper studied soluble glycoproteins. Maurice and Watson used serum albumin as a model for calculating the diffusional properties of the cornea and demonstrated that a protein with the electrophoretic mobility of albumin was present in rabbit corneas. Certain studies suggested that soluble proteins were involved in the immunologic sensitization of the recipients of corneal grafts. The present study was undertaken to gain knowledge of the soluble corneal proteins as a basis for possible further study of corneal transplantation antigens.

Methods

The corneal epithelium of calf eyes was scraped off with a scalpel blade, homogenized in 0.01 M phosphate buffer, pH 7.8 usually was used. Following centrifugation at 15,000 x g for 30 minutes, the supernatant fluid was dialyzed overnight against the same buffer. The resulting solution contained about 15 mg per milliliter of soluble epithelial proteins. The stroma was then excised, minced with scissors, and thoroughly homogenized in a small Waring blender. The resulting fine slurry was stirred overnight in the cold, centrifuged 30 minutes at 15,000 g, and the supernatant solution decanted and saved. The pellet resuspended

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in buffer, stirred overnight, and recentrifuged. The two supernatants were combined, dialyzed for 24 hours against a large excess of buffer, and concentrated to 10 to 15 mg. per milliliter by placing the solution, contained in dialysis tubing, in dry Sephadex G-200 power overnight. Protein concentrations were estimated by the optical density and Lowry methods.

Sephadex G-200 gel filtration was performed in 1.7 by 50 cm. columns, approximately 75 mg. of stromal or epithelial soluble proteins being applied. DEAE-cellulose chromatography was performed on each of the main Sephadex fractions in 1.7 by 50 cm. columns previously equilibrated with 0.01 M phosphate, pH 7.6. The first DEAE fraction was eluted with a 0.01 M phosphate wash, the remaining fractions being eluted at various points along a concentration gradient achieved with a Marriott flask arrangement in which 175 ml. of 0.15 M phosphate, pH 7.6, was added drop by drop with mixing to 120 ml. of 0.01 M phosphate. This gradually raised the eluting molarity for 0.01 to 0.12 M over 175 ml. Next, 250 ml. of 0.2 M phosphate was similarly added, gradually raising the eluting molarity from 0.12 to 0.19. All the proteins were eluted well before this point.

Disc electrophoresis was performed on each Sephadex and DEAE fraction by the method of Davis, except that the separating gel was 6.5 per cent acrylamide. Densitometry of the stained gels was done with a disc electrophoresis densitometer attachment to the Gilford recording spectrophotometer (Gilford Institute, Oberlin, Ohio). The mobility of a protein zone relative to the protein standards was determined from direct measurements of the gels. In certain cases, the gels were stained for glycoproteins by the modified periodic acid-Schiff (PAS) method of Zacharius and co-workers. Staining of the gels for lipoproteins and serum transferrin was done by the methods of Beaton, Selby, and Wright, and for haptoglobin by the method of Smithies. Nucleic acid content of the fractions was estimated by the orcinol method. Hydroxyproline was estimated by the method of Neuman and Logan, with protein hydrolysis and removal of chromogens by the methods of Prockop and Udenfriend.

Rabbit antiserum to soluble calf corneal proteins was produced in 5 to 8 pound albino rabbits by a series of 8 immunizations, the first 5 at weekly intervals, the last 3 at two week intervals. At each immunization, a total of 10 mg. soluble corneal proteins from homogenates of the whole cornea mixed 1:1 in Freund’s complete adjuvant was given intradermally in four separated sites. The animals were bled by marginal ear vein initially and 3 days after each immunization. The sera were assayed for antibody activity by the Ouchterlony method. No improvement in titer was noted after the sixth immunization. Commercial antisera used were rabbit antiovine serum proteins (GIBCO, Grand Island, N. Y.), rabbit antiovine gamma globulin, and rabbit antiovine serum albumin (Behringwerke AG, obtained through Certified Blood Donor Service, Inc., Woodbury, N. Y.).

Double-diffusion immunoprecipitation was performed by a micromodification of the basic Ouchterlony technique, using 1.5 per cent agar in 0.05 M phosphate, pH 7.6. Immunoelectrophoresis was basically by the micromethod of Scheidegger, using 2 per cent agar in Veronal buffer, pH 8.2, ionic strength 0.05.

Results

The average wet weights and yields of proteins extracted from calf corneas are presented in Table I. Assuming the dry weight of the stroma to be 25 per cent of the wet weight, soluble protein represents 11.5 per cent of the stromal dry weight. This and other figures in Table I are in reasonable agreement with data of previous studies. The soluble proteins were fractionated according to the general scheme outlined in Fig. 1. In designating the protein fractions, “E” denotes that the fraction is of epithelial origin, “S” denotes stromal, “G” denotes a Sephadex G-200 fraction, and “D” one from DEAE.

Gel filtration on Sephadex G-200 of either epithelial or stromal soluble proteins yielded two fractions, as shown in Fig. 2. Blue dextran used as a marker eluted from the column just ahead of the first stromal fraction, S-G1, suggesting it is composed of proteins retarded slightly by the gel. Peak S-G1 exhibited a “knee” on its descending arm. Disc electrophoresis (Fig. 5, gels 1 and 2) revealed that this knee represents the addition of new protein not present in the earlier part of peak S-G1. The relative amounts of protein eluting in each fraction are given in Tables II and III. Epithelial fraction E-G1 exhibited greater absorption at 260 than at 280 m., suggesting material containing nucleic acid. The ratio of nucleic acid to protein, each in milligrams per milliliter, was calculated for each Sephadex fraction. For E-G1 it was 1.2, whereas for E-G2
Table I. Extraction of corneal soluble proteins

<table>
<thead>
<tr>
<th></th>
<th>Wet weight</th>
<th>Soluble protein</th>
<th>Per cent soluble protein (mg. sol. prot.) (mg. wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>Per cent of total</td>
<td>mg.</td>
</tr>
<tr>
<td>Epithelium</td>
<td>14</td>
<td>6.2</td>
<td>1.98</td>
</tr>
<tr>
<td>Stroma</td>
<td>211</td>
<td>93.8</td>
<td>5.89</td>
</tr>
<tr>
<td>Entire cornea</td>
<td>225</td>
<td>100</td>
<td>7.87</td>
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</tbody>
</table>

Table II. Approximate yields of soluble epithelial proteins in the Sephadex and DEAE fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein per cornea (mg.)</th>
<th>Per cent of soluble epithelial protein</th>
<th>Per cent of fraction E-G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-G1</td>
<td>0.5</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>E-G2</td>
<td>1.5</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>E-G2-D1</td>
<td>0.26</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>E-G2-D2</td>
<td>0.78</td>
<td>39</td>
<td>52</td>
</tr>
<tr>
<td>E-G2-D3</td>
<td>0.10</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>E-G2-D4</td>
<td>0.36</td>
<td>18</td>
<td>24</td>
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</table>

Table III. Approximate yields of soluble stromal proteins in the Sephadex and DEAE fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein per cornea (mg.)</th>
<th>Per cent of soluble stromal protein</th>
<th>Per cent of fraction S-G1 or S-G2</th>
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</thead>
<tbody>
<tr>
<td>S-G1</td>
<td>3.2</td>
<td>55</td>
<td>100</td>
</tr>
<tr>
<td>S-G2</td>
<td>2.7</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>S-G2-D1</td>
<td>0.29</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>S-G2-D2</td>
<td>0.83</td>
<td>14</td>
<td>31</td>
</tr>
<tr>
<td>S-G2-D3</td>
<td>0.53</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>S-G2-D4 &amp; 5</td>
<td>1.0</td>
<td>17</td>
<td>38</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.7</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Rf. 58</td>
<td>0.15</td>
<td>(2.5)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.15</td>
<td>(2.5)</td>
<td></td>
</tr>
</tbody>
</table>

it was only 0.11, for S-G1, 0.17, and for S-G2, 0.09, thus confirming this impression. Disc electrophoresis of E-G1 is shown in Fig. 4.

Further purification of each epithelial and stromal Sephadex fraction was attempted on DEAE-cellulose columns. Fraction E-G1 did not yield discrete peaks and no further analysis was pursued. Stromal Sephadex fraction S-G1 was separated by DEAE into several very small peaks and a single large one which eluted at a phosphate buffer concentration of about 0.08 M. Disc electrophoresis (Fig. 5, gel 3) revealed that the large 0.08 M peak contained the two principal S-G1 protein zones in large amounts and relatively free of contaminants. Accordingly, tubes eluting from 0.075 to 0.035 M phosphate were pooled, labeled as fraction S-G1-D3, and used in further studies.

The Sephadex fractions E-G2 and S-G2 were also subjected to DEAE chromatography, with the results illustrated in Fig. 3. The DEAE fractions are labeled D1 through D5 with a prefix E-G2 or S-G2 denoting the Sephadex fraction of origin. Peaks E-G2-D1 and S-G2-D1 were eluted with the 0.01 M phosphate wash. Subsequent E-G2 peaks were eluted at 0.045, 0.075, 0.10, and 0.17 M phosphate. The four subsequent S-G2 peaks were eluted at approximately 0.050, 0.080, 0.11, and 0.18 M phosphate. The relative amounts of protein in each fraction are given in Tables II and III. Each of these fractions was collected, concentrated, and further analyzed by disc electrophoresis.

Disc electrophoresis was performed on each of the Sephadex and DEAE fractions, as illustrated in Figs. 4 through 7. The protein zones resolved by electrophoresis can be categorized in terms of their Rf values, an approximate scale of which...
appears beside the gels in these figures. In Fig. 4, the first epithelial Sephadex fraction, E-G1, reveals only a few faint zones. The second Sephadex fraction, E-G2, is more complex, showing at least 11 bands clearly. Of the five DEAE fractions into which E-G2 is resolved most contain several protein zones. The fraction E-G2-D2, however, which makes up 52 per cent of E-G2, appears to contain a single protein, labeled "e," with Rf 0.32. Fractions E-G2-D4 and -D5 had similar electrophoretic appearances, so only E-G2-D4 is illustrated. Disc electrophoresis of stromal Sephadex fraction S-G1 is shown in gels 1 and 2 of Fig. 5. Gel 3 in the figure is the result of electrophoresis of the DEAE fraction S-G1-D3, described earlier as coming from that portion of S-G1 eluting off DEAE at 0.075 to 0.085 M phosphate. It contains a single sharp zone, labeled "s," and a broader heterogeneous zone "g," the identities of which will be discussed later.

The second stromal Sephadex fraction, S-G2, resolved into a number of protein zones on disc electrophoresis, as seen in gels 4 and 5 of Fig. 5. The more concentrated, tube 5, shows certain minor components to better advantage, while the
Fig. 2. Elution of soluble stromal and epithelial proteins from Sephadex G200. Two major fractions are eluted from both stroma and epithelium. These are labeled as indicated by the bars under each peak and as outlined in Fig. 1 and the text. In the curves depicted, 100 mg. of stromal and 60 mg. of epithelial proteins were applied to the respective columns.

less concentrated, tube 4, shows the major zones better. DEAE chromatography of S-G2 gave the 5 fractions described earlier, the electrophoretic analysis of which is presented in Fig. 6. Of the many protein zones revealed in each fraction certain ones were studied further. Fraction S-G2-D2 contains 2 proteins, labeled "e" and "t," migrating closely together. These are also shown in the gel D2(a), which is taken from an intermediate fraction midway between S-G2-D2 and S-G2-D3. The "e" zone will be shown later by immunoprecipitation to be the same protein as the principal epithelial protein described earlier in fraction E-G2-D2. Fraction S-G2-D3 appears to have traces of the "e" and "t" zones as well as a heavily staining, more rapid component, Rf about 0.65. DEAE factions S-G2-D4 and S-G2-D5 had similar electrophoretic appearances, and so only -D4 is illustrated. The heaviest -D4 zone, Rf 0.76, labeled "a" in Fig. 6, will later be shown to be serum albumin. Migrating behind albumin is a second heavily stained zone (labeled "c") and a series of regularly spaced zones of progressively decreasing concentration and mobility, labeled by dots. The appearance of these suggests a series of aggregates of a basic structural unit such as is seen in the human serum haptoglobins.21

Each of these electrophoresis gels was stained by the PAS method for glycoproteins,18 and those with positive results are shown in Fig. 7. Although no glycoproteins were demonstrated by this method in any epithelial fractions the stroma contained several. Stromal fraction S-G1 exhibited 2, the sharp "s" zone and the diffuse "g" protein. The "t" zones of fractions S-G2-D2 and S-G2-D2(a) stained clearly, as did a diffuse zone just above. The most prominent stromal glycoprotein is in fraction S-G2-D3, labeled "p."

The production of a potent anticerneal protein antiserum proved difficult, even after repeated immunizations. Ours reacted with an unfractionated homogenate of soluble corneal proteins to give 4 precipitin
Soluble proteins of bovine cornea

Fig. 3. DEAE-cellulose chromatography of the two Sephadex fractions, epithelial fraction E-G2 (squares), and stromal fraction S-G2 (circles). The peaks are labeled as described in the flow diagrams of Fig. 1 and in the text. The dashed line represents eluting buffer molarity. Each of these peaks was further studied by disc electrophoresis and immunoprecipitation.

lines. Two of these were removed after absorption against calf serum, leaving two corneal specific antigens. One of these corneal specific antigens proved to be present only in stromal fraction S-G2-D4, as shown in Fig. 8, A. The other was present in the epithelial fraction E-G2-D2, and must therefore correspond to the Rf 0.32 ("e") electrophoretic zone (see Fig. 4). This same antigen is also present in the stromal fraction S-G2-D2 and in trace amounts in S-G2-D1 and -D3, as is illustrated in Fig. 8, B. This antigen probably corresponds to the "e" zone in Fig. 6. This "e" zone represents about 39 per cent of the soluble epithelial protein and a significant part of the stromal fraction, S-G2.

The antiserum to calf serum proteins delineated no serum proteins in any epithelial fraction but revealed several in the stroma, as is illustrated by immunoelectrophoresis in Figs. 9, A and B. The most cathodic precipitin arc in S-G1, labeled "g," is shown in Fig. 9, B to be gamma globulin. Although the antiserum used is polyspecific for all gamma globulins, the antigen delineated here is most likely a 7S globulin, as 19S globulins are unable to diffuse into the cornea. This S-G1 antigen probably corresponds to the electrophoretic zone "g" of Fig. 5 as this resembles the known disc electrophoretic appearance and mobility of 7S gamma globulin. The positive PAS staining of this electrophoretic zone (Fig. 7) is supportive evidence, as gamma globulin is a glycoprotein. Densitometry showed this zone to make up 56 per cent of fraction S-G1 (Table III), which means that about 31 per cent of the soluble stromal protein is gamma globulin, this being about 1.8 mg. per calf cornea.

A second serum antigen in fraction S-G1 is represented by the arc labeled "s" in
Fig. 4. Disc electrophoresis of soluble epithelial proteins. Gels, left to right, are: unfractionated epithelial proteins (E), Sephadex fractions E-G\(_1\) and E-G\(_2\), and DEAE fractions E-G\(_2\)-D\(_1\), -D\(_2\), -D\(_3\), -D\(_4\) (see Fig. 1). Migration is from top to bottom. The Rf scale is approximate and meant only as a general guide. Labels: e-the "principal" epithelial protein, also found in stromal fraction S-G\(_2\)-D\(_2\) (see Fig. 6).

Fig. 5. Disc electrophoresis of stromal proteins. Gel 1: fraction S-G\(_1\) from ascending limb of elution curve S-G\(_1\); gel 2: S-G\(_1\) from descending limb showing addition of a new protein Rf 0.59; gel 3: that portion of S-G\(_1\) eluting at approximately 0.08 M phosphate from DEAE and labeled S-G\(_1\)-D\(_3\); gel 4: S-G\(_2\), 65 \(\mu\)g sample applied, gel 5: S-G\(_2\), 100 \(\mu\)g applied. Labels: g-7S gamma globulin, s-the Rf 0.08 serum lipoprotein, and a-albumin.
Fig. 6. Disc electrophoresis of stromal S-G2-DEAE fractions. Tubes are designated, from left to right, fractions S-G2, S-G2-D1, S-G2-D2, S-G2-D2 (a), S-G2-D3, and S-G2-D4. Labels: e-the Rf 0.32 principal epithelial protein, also present in stroma, t-transferrin, a-albumin, c-the Rf 0.57 stromal protein, and dots-the repeating stromal protein, as described in the text.

Fig. 7. Glycoprotein staining of selected stromal fractions. Gels 1a, 2a, and 3a are stained with Coomassie blue, a protein stain, while 1b, 2b, and 3b are stained by the PAS method for glycoproteins. Gels 1a and 1b: fraction S-G1, gels 2a and 2b: S-G2-D2, and gels 3a and 3b: S-G2-D3. Labels: g-gamma globulin, s-the Rf 0.08 serum lipoprotein, t-transferrin, and p-the principal stromal glycoprotein.

Figs. 9, A and B, and corresponds to the other major electrophoretic zone in S-G1, labeled “s” in Fig. 5. This disc electrophoretic zone gives a positive glycoprotein stain (Fig. 7) and was the only zone to stain positively with oil Red-O, a lipid stain. By densitometry, this zone makes up 14 per cent of S-G1, or about 7.7 per cent of the soluble stromal proteins. The third serum antigen in S-G1, labeled “u” in Fig. 9, A, was not seen when smaller concentrations of sample were applied, as in Fig. 9, B, and therefore probably represents a serum protein present in small amounts. Benzidine staining for heptoglobulin was negative and the identity of this protein was not established.

The S-G2 antigen with alpha mobility, labeled “a” in Fig. 9, A, appears to be serum albumin. In Fig. 8, A, fraction S-G2-D4
was shown to contain one serum and one corneal specific antigen, and Fig. 8, C illustrates that the serum antigen is albumin. This figure also shows the lack of albumin in the other S-G2 DEAE fractions. The "a" electrophoretic zone of fraction S-G2-D4 in Fig. 6 is thus almost certainly albumin, exhibiting the expected rapid mobility and characteristic ability to bind the bromphenol blue tracking dye during electrophoresis. By densitometry, this "a" zone makes up 70 per cent of S-G2-D4 and -D5 (Table III) and albumin thus represents about 12 per cent of the soluble stromal protein. The cornea-specific antigen demonstrated in Fig. 8A must correspond to one of the other electrophoretic zones of S-G2-D4, but it is uncertain which one.

The precipitin arc with beta mobility in Fig. 9, A, labeled "t," was found to be localized to stromal fraction S-G2-D2 by Ouchterlony analysis. This fraction contains only two proteins by electrophoresis (Fig. 6) and two antigens by Ouchterlony analysis (Fig. 8, B). Of these two, the corneal specific antigen has already been related to the Rf 0.32 "e" protein electrophoretic zone. Thus, the serum antigen must correspond to the series of two to three closely spaced electrophoretic zones labeled "t" in Figs. 6 and 7, the mobility and appearance of which closely resemble that of bovine transferrin, an iron-binding serum beta glycoprotein. Specific staining of...
this fraction for transferrin by the iron-Nitroso R salt method gave positive results with these Rf 0.36 to 0.40 zones. Transferrin is a glycoprotein; thus the positive PAS staining of these zones in Fig. 7 is confirmatory. The final precipitin arc in fraction S-G2, labeled "g" in Fig. 9, A, bottom, was shown by Ouchterlony studies not illustrated to represent traces of gamma globulin in fraction S-G2-D3.

None of the protein fractions contained collagen in amounts that could be detected by the assays used. The negative results are consistent with those of Polatnick, Tessa, and Katz on who could extract no soluble collagen at neutral pH.

Discussion

This study was undertaken to survey the soluble corneal proteins of the calf using standard methods. Even though complete purification of each of the main corneal proteins was not achieved, the existence of a large number of discrete proteins has been demonstrated, and the use of various methods has permitted several deductions and conclusions to be made about them. The quantitative considerations presented in Tables II and III must be considered approximate, especially those based on densitometry of the electrophoretic gels, as this method clearly distinguished the major zones but not the minor and closely spaced ones. The data are presented because few other quantitative estimates of the various soluble corneal proteins are available.

The corneal epithelium is shown to contain a host of discrete proteins, mostly in small quantities, and presumably, mostly of intracellular origin. In the light of this, it is interesting that one protein fraction, with Rf 0.32 on disc electrophoresis, makes up about 40 per cent of epithelial soluble proteins. A possible importance of this protein is that it is also found in the stroma, particularly in S-G2-D2. In addition to forming such a large part of the corneal soluble protein, it was one of only two intrinsic (i.e., nonserum) corneal proteins which were sufficiently antigenic to stimulate antibody production in rabbits immunized against unfractionated corneal proteins. At present all that is known about this protein fraction is that it has a "beta" mobility on polyacrylamide gel electrophoresis, elutes from DEAE-cellulose at about 0.045 M, and is not a serum protein, glycoprotein, lipoprotein, or a soluble collagen. Other epithelial proteins are present in smaller amounts. Of these, the four or five in E-G2-D1 constitute 17 per cent of E-G2, whereas the other fractions are mainly in trace amounts. The lack of detectable serum proteins in the epithelium was consistent with the findings of other studies.

The present study documents the existence of serum albumin and 7S-gamma globulin in the stroma by the use of specific antiserum against these two proteins and relates these antigens to certain disc electrophoretic zones. The identity of the S-G2-D2 serum protein as serum transferrin rests on the specific staining of the transferrin-iron complex by Nitrosos-R salt plus the characteristic disc electrophoretic patterns. The presence of a serum lipoprotein is suggested by the correlation of a serum antigen of beta mobility with the disc electrophoretic zone Rf 0.08 in S-G1 which stains with oil Red-O. There is also present in S-G1 a fifth serum antigen of slow beta mobility, the identity of which was not demonstrated. The suspicion that it was haptoglobin could not be documented by benzidine staining.

The four serum proteins demonstrated make up 3.3 mg. of the 5.9 mg. (56 per cent) of soluble protein in the stroma, the remaining 44 per cent being made up of proteins intrinsic to the stroma and the unidentified serum protein. Expressed differently, of the 2.8 per cent of the stromal wet weight which is soluble protein, 1.2 per cent is stromal-specific protein and 1.6 per cent is of serum origin. The serum proteins present in the stroma are those with the highest concentrations in blood except for the 19S and lipidic macroglobul-
lins,22 which are too large to enter the cornea by diffusion. If serum proteins enter the cornea by diffusion from the limbal capillaries, then their corneal concentrations should be proportional to those in serum. A ratio of the absolute values of the serum concentrations of each of these four proteins in grams per 100 ml. as given in a standard text23 to the stromal concentrations in milligrams per stroma as found in the present study, gives the following values: albumin 4 to 6, 7S-gamma globulin 0.5 to 1.0, transferrin 1.0, and lipoprotein 0.75 to 1.0. A ratio of about 1.0 appears to hold except for albumin, which is present in the cornea in much smaller proportion than the other three. This can be explained by assuming a diffusional loss of albumin into the aqueous as suggested by the calculations of Maurice and Watson.7 The value of 0.7 mg. albumin per stroma converts to roughly 0.3 Gm. per 100 ml. stroma, about 1/10 the average serum value, a figure which is consistent with Maurice and Watson's predictions. The estimate of 1.8 mg. gamma globulin per calf cornea is consistent with Stock and Aronson's4 figure of 0.5 mg. per human cornea.

The nonserum proteins found in the stroma comprise 44 per cent of the soluble stromal proteins. None are collagen. Certain proteins are present in fairly large amounts. Of these, the Rf 0.6 to 0.7 zone of S-G2-D3 stains strongly with PAS and must have a high carbohydrate content. Also in this fraction are a number of more faintly staining glycoprotein zones. Perhaps some of these correspond to the soluble neutral and acid mucopolysaccharides described by Dohlman and Balazs6 in 1957. The series of repeating electrophoretic zones in S-G2-D4 may represent a stromal protein or proteins made up of numbers of basic units in varying degrees of aggregation, perhaps analogous to the human haptoglobin types 2-1 and 2-2 seen in disc and starch gel electrophoresis.15, 21

The possible relation of the several serum, stromal, and epithelial soluble proteins to the problem of host sensitization to donor material in keratoplasty is an area which is in need of exploration. If, in fact, soluble corneal components are important in graft rejection, then such techniques as prior soaking of the graft and transplantation antigen typing would assume more importance.

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