Molecular sieving in suprachoroidal fluid formation in man

Leo T. Chylack, Jr., and A. Robert Bellows

Suprachoroidal fluid (SCF) obtained at the time of the surgical evacuation of a clinically significant choroidal detachment (CD) was analyzed for its chemical and cellular components in four distinct subgroups: (1) CD following cataract surgery, (2) CD (nonhemorrhagic) following glaucoma surgery, (3) CD (hemorrhagic) following glaucoma surgery, and (4) intraoperative CD during glaucoma surgery in patients with elevated episcleral venous pressure. The fluid obtained in groups 1, 2, and 4 was clear and slightly xanthochromic and contained low-molecular-weight substances in concentrations essentially equal to serum. Proteins and other high-molecular-weight substances were present in lesser amounts than in serum. Albumin, α2-antitrypsin, and transferrin were present in amounts approximately equal to those in serum, whereas α2-macroglobulin, IgM and IgG were decreased. β-Lipoprotein and β-complement were absent. It is postulated that this distribution of serum proteins is a manifestation of molecular sieving and is consistent with the existence of an isoporous membrane between the intravascular and suprachoroidal space with a pore diameter of 144 Å. In the intraoperative choroidal effusions, there was evidence for exclusion of more of the lower, as well as all of the higher, molecular weight proteins. This suggested that the degree of molecular sieving increased with increasing filtration rate. In the hemorrhagic SCF, the distinctive character of the fluid and the protein concentrations indicated that the integrity of the capillary membrane was markedly disrupted, thereby allowing higher-molecular-weight proteins and cellular elements to enter the space.

Key words: suprachoroidal fluid, human, molecular sieving

Choroidal detachment (CD) is a clinical term describing the formation of large, discrete or confluent, smooth, hemispherical mounds in the posterior segment of the human eye as fluid collects between the choroid and sclera in the suprachoroidal space. Classically, it has occurred after cataract or glaucoma surgery in patients with flattened (or collapsed) anterior chambers. It occurs 2 to 3 weeks postoperatively in the former condition and only 3 to 10 days in the latter. Estimates of incidence vary, but some studies have shown small, low CD's to be present in 93% of eyes after cataract extraction. Large CD's occur much less frequently. Small CD's frequently clear spontaneously without treatment; large CD's usually do not, and surgical evacuation of the suprachoroidal fluid (SCF) is indicated. Little is known about the composition of SCF, and even less about the mechanism of SCF formation.

We have attempted to characterize SCF removed at surgery and compare it to serum removed simultaneously to answer the following specific questions. (1) Is SCF composition similar among patients with CD's of

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different origin? If not, is the degree of difference sufficient to provide clues as to the mechanism of SCF formation? (2) Is SCF a transudate of serum alone, or is it, in part, derived from aqueous humor or vitreous? Is there an inflammatory component in SCF formation? (3) Is the fluid similar to extracellular fluid in the normal choroid, or are there significant differences which might indicate altered capillary membrane permeability in patients with choroidal detachment? (4) Are our conclusions about capillary pore size in the choriocapillaris consistent with anatomical studies of the same tissue?

Methods and materials

Samples of SCF and serum were collected simultaneously from patients undergoing surgical evacuation of SCF at the Massachusetts Eye & Ear Infirmary. Extreme care was taken to avoid contamination of SCF with blood. Specimens were collected at the lips of the sclerostomy, with an olive-tipped needle attached to a 1 ml tuberculin syringe. Samples of SCF and serum were immersed in ice, and within 2 hr, centrifuged in Corex tubes to remove cellular components and occasional fibrin clots. The cellular components were placed on microscopic slides and stained with Wright’s stain for microscopic identification. All samples not immediately assayed were frozen at —76° C until analysis was accomplished.

Ascorbate assay. Aliquots (50 µl) of serum and SCF were placed in 6 by 50 mm glass tubes; trichloroacetic acid (TCA) was added, and the samples were kept at 0 to 4° C until assayed according to the method of Lowry et al., modified to use TCA without Norit.

Electrolytes. Aliquots of 30 to 100 µl of serum and SCF were sufficient for electrolyte assay; Na⁺ and K⁺ levels were determined by flame photometry with a microtechnique employing a Model 343 flame photometer (Instrumentation Laboratories, Inc., Lexington, Mass.) in the clinical laboratory at the Massachusetts Eye & Ear Infirmary.

Protein assay. Serum and SCF (20 µl) were diluted to 1.0 ml with deionized water and kept at —20° C until assayed by the method of Lowry et al.⁶

Agarose electrophoresis and single radial immunodiffusion. Aliquots of serum and SCF (30 µl) were kept in 12 by 75 mm glass tubes at —76° C until assayed by agarose electrophoresis according to the method of Johansson⁷ or by single radial immunodiffusion with Behring Diagnostic Assay Kits (Behring Diagnostics, Somerville, N. J.).

Episceral venous pressure measurement. In three selected patients, one with advanced open-angle glaucoma and increased episcleral venous
pressure and two with Sturge-Weber syndrome, episcleral venous pressure was measured by a modification of a pressure chamber device⁸ attached to a Haag-Streit biomicroscope; it provided direct visualization of the vessel through a synthetic membrane. Episcleral venous pressure was recorded from a water manometer, with the lowest pressure at which the vessel wall collapsed used as end point.

Results

A total of 25 specimens were analyzed and, in most cases, compared with simultaneously obtained serum samples. The following four definite categories of CD were encountered:

1. After cataract surgery
2. After glaucoma surgery (nonhemorrhagic)
3. After glaucoma filtering surgery (hemorrhagic)
4. Intraoperative

In addition to the previously well-recognized forms of serous CD following cataract and glaucoma surgery, two distinct additional forms were recognized. The first was a hemorrhagic CD which developed suddenly and was characterized by severe pain and associated with acute shallowing of the anterior chamber. At the time of evacuation of the suprachoroidal contents, a dark, maroon, nonclotting, viscous fluid was discovered. This fluid is quite different from the clear, slightly xanthochromic, less viscous fluid usually recovered at the time of choroidal tap following cataract and glaucoma surgery. The cellular elements of these two distinct varieties are quite different. The more characteristic serous choroidal effusion has an occasional red cell and a very few white blood cells or other cells indicative of inflammation; in the hemorrhagic variety, a large number of red blood cell ghosts⁹ were found, with a small proportion of fresh red blood cells and rare white cells.

The second previously unrecognized form of CD occurred during the intraoperative period of glaucoma filtering surgery in patients with prominent episcleral vessels and documented elevated episcleral venous pressures.¹⁰ This entity presented as a rapid accumulation of SCF that caused shallowing of the anterior chamber and anterior rotation of the ciliary body immediately after the intraocular pressure had been lowered to zero. Posterior sclerostomy permitted evacuation of a clear straw-colored fluid from the suprachoroidal space that continued to accumulate during the course of the operation.

When all samples were analyzed, insignificant differences existed between the sodium and potassium levels of SCF and serum in almost all of these subgroups (Table I).

Ascorbic acid level was used as a marker to evaluate the possible contribution of aqueous humor and vitreous in the collection of suprachoroidal effusion. The ascorbic acid level

<table>
<thead>
<tr>
<th>CD associated with</th>
<th>No. of specimens</th>
<th>Na⁺ (mEq/L) SCF</th>
<th>Na⁺ (mEq/L) SER</th>
<th>K⁺ (mEq/L) SCF</th>
<th>K⁺ (mEq/L) SER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract</td>
<td>(10)</td>
<td>145 ± 3</td>
<td>142 ± 3</td>
<td>4.3 ± 1.1</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.001 &lt; p &lt; 0.01</td>
<td></td>
<td>p = 0.6</td>
<td></td>
</tr>
<tr>
<td>Filter (NH)</td>
<td>(7)</td>
<td>142 ± 3</td>
<td>135 ± 4</td>
<td>4.2 ± 0.5</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02 &lt; p &lt; 0.05</td>
<td></td>
<td>0.4 &lt; p &lt; 0.5</td>
<td></td>
</tr>
<tr>
<td>Filter (H)</td>
<td>(4)</td>
<td>126 ± 20</td>
<td>135 ± 4</td>
<td>4.0 ± 0.6</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 &lt; p &lt; 0.4</td>
<td></td>
<td>0.3 &lt; p &lt; 0.4</td>
<td></td>
</tr>
<tr>
<td>Intraoperative*</td>
<td>(4)</td>
<td>124</td>
<td>136 ± 2</td>
<td>3.7</td>
<td>4.3 ± 0.3</td>
</tr>
</tbody>
</table>

*Only one of the intraoperative SCF samples was large enough to do all assays; therefore S.D. and P values could not be calculated for this group.

¹Numbers in parentheses represent percent of serum total protein in the SCF.
### Table

<table>
<thead>
<tr>
<th>Ascorbic acid (mg/dl)</th>
<th>TP (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF</td>
<td>SER</td>
</tr>
<tr>
<td>1.11 ± 0.51</td>
<td>1.36 ± 0.85</td>
</tr>
<tr>
<td>0.3 &lt; p &lt; 0.4</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>1.10 ± 0.72</td>
<td>1.21 ± 0.39</td>
</tr>
<tr>
<td>0.6 &lt; p &lt; 0.7</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>0.79 ± 0.57</td>
<td>1.18 ± 0.23</td>
</tr>
<tr>
<td>0.1 &lt; p &lt; 0.2</td>
<td>0.01 &lt; p &lt; 0.02</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

in the SCF was consistently lower than it was in serum in all of the samples that were tested.

There was consistently less total protein in the SCF than in the serum. The SCF from cataract and both filtering surgery groups was found to have approximately 65% of the protein found in serum. The protein level in the intraoperative choroidal effusion cases was even lower than the other three varieties. Agarose gel electrophoresis revealed a selective loss of certain serum proteins from all groups of the SCF (Fig. 1). Bands of β-lipoprotein and β-complement were absent. Alpha-2 macroglobulin was markedly diminished, and IgM and IgG were decreased. Bands for low-molecular-weight proteins (albumin, antitrypsin, and transferrin) were present and stained with an intensity approximately equal to that of serum.

To quantitate the changes in these proteins, single radial immunodiffusion measurements of the amounts of nine serum proteins and immunoglobulins were made on 14 matched pairs of SCF and serum (Table II). This technique confirmed the impression derived from the agarose electrophoresis study that certain high-molecular-weight proteins were being excluded from the SCF: in 10/13 samples, there was no detectable α2-macroglobulin; in 12/13, no IgM; and in 9/9 samples, there was no β-lipoprotein. The molecular weights of these excluded proteins ranged from 820,000 to 2.4 million daltons. However, there were certain other samples in which these high-molecular-weight species were present. In all the hemorrhagic samples tested, there were easily detectable levels of α2-macroglobulin and IgM; β-lipoprotein was not measured. These findings may signify a significant increase in chorio-capillaris permeability in the hemorrhagic group.

If the amount of each protein is normalized to the total amount of protein present and this ratio plotted against the logio molecular weight, the data suggest that in the cataract and nonhemorrhagic filter group there was a relative barrier to molecules with molecular weights greater than 900,000 daltons (Fig. 2).

### Discussion

CD that required surgical intervention has been documented to occur in four distinct, identifiable clinical entities: (1) delayed (days or weeks) in patients with prolonged hypotony following cataract or (2) glaucoma surgery; (3) delayed (days or weeks) hemorrhagic following glaucoma filtering surgery; (4) immediate (minutes) intraoperative in patients with elevated episcleral venous pressure undergoing glaucoma filtering surgery.

Analyses of small molecules in SCF suggest that there is no restriction to the exchange of Na+, K+, and probably other small molecules between the intravascular and suprachoroidal space.

It is known that ascorbic acid is present in low levels in serum and high levels in primary aqueous humor and vitreous. The level in secondary aqueous is less than in primary aqueous but is still higher than serum. That the ascorbic acid level in SCF was consistently lower than in serum suggests that most of the SCF is derived from the intravascular compartment and not aqueous humor.

That there is a restriction to the passage of serum proteins which increases with increasing molecular size suggests that molecular sieving is occurring. This process is affected by the filtration rate across a capillary membrane; equations 16, 17, and 18 in reference...
Table II. Results of single radial immunodiffusion assays of proteins in serum (SER) and SCF

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CD type*</th>
<th>SCF</th>
<th>SER</th>
<th>SCF</th>
<th>SER</th>
<th>SCF</th>
<th>SER</th>
<th>SCF</th>
<th>SER</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB-1</td>
<td>C</td>
<td>105</td>
<td>180</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>308</td>
<td>875</td>
</tr>
<tr>
<td>MB-2</td>
<td>C</td>
<td>120</td>
<td>230</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>307</td>
<td>1200</td>
</tr>
<tr>
<td>MB-3</td>
<td>C</td>
<td>158</td>
<td>320</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>312</td>
<td>1050</td>
</tr>
<tr>
<td>HP-1</td>
<td>C</td>
<td>230</td>
<td>480</td>
<td>—</td>
<td>74</td>
<td>80</td>
<td>616</td>
<td>1880</td>
<td></td>
</tr>
<tr>
<td>HP-2</td>
<td>C</td>
<td>216</td>
<td>340</td>
<td>—</td>
<td>210</td>
<td>290</td>
<td>596</td>
<td>1410</td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>F-NH</td>
<td>115</td>
<td>480</td>
<td>—</td>
<td>—</td>
<td>360</td>
<td>345</td>
<td>1330</td>
<td></td>
</tr>
<tr>
<td>FI</td>
<td>F/NH</td>
<td>136</td>
<td>210</td>
<td>—</td>
<td>296</td>
<td>330</td>
<td>770</td>
<td>1600</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>F-NH</td>
<td>—</td>
<td>—</td>
<td>2.84</td>
<td>3.90</td>
<td>120</td>
<td>220</td>
<td>350</td>
<td>970</td>
</tr>
<tr>
<td>RW</td>
<td>F-H</td>
<td>125</td>
<td>125</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>470</td>
<td>1040</td>
</tr>
<tr>
<td>NI</td>
<td>F-H</td>
<td>152</td>
<td>90</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>770</td>
<td>1000</td>
</tr>
<tr>
<td>MG</td>
<td>Io</td>
<td>—</td>
<td>—</td>
<td>4.19</td>
<td>5.96</td>
<td>187</td>
<td>—</td>
<td>900</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>Io</td>
<td>—</td>
<td>—</td>
<td>2.45</td>
<td>3.00</td>
<td>160</td>
<td>—</td>
<td>900</td>
<td></td>
</tr>
<tr>
<td>MM-1</td>
<td>Io</td>
<td>80</td>
<td>142</td>
<td>3.14</td>
<td>3.25</td>
<td>54</td>
<td>187</td>
<td>248</td>
<td>1160</td>
</tr>
<tr>
<td>MM-2</td>
<td>Io</td>
<td>—</td>
<td>—</td>
<td>0.96</td>
<td>3.77</td>
<td>15</td>
<td>147</td>
<td>60</td>
<td>545</td>
</tr>
</tbody>
</table>

* C = cataract, F = filtering surgery, H = hemorrhagic, NH = nonhemorrhagic, Io = intraoperative.

15 suggest that at low rates of bulk flow across a capillary membrane, diffusional exchange, even of large molecules, is significant. At high rates of bulk flow across a membrane, diffusional exchange of large molecules is of lesser significance and molecular sieving is increased.16 In the delayed (cataract and no hemorrhagic glaucoma) SCF data, molecules with weights greater than 900,000 are excluded. However, in the intraoperative SCF data, molecules with weights greater than 339,000 daltons are excluded and the total amount of protein is reduced. Presumably, the filtration rate is increased in the latter group as a result of the elevated episcleral venous pressure. In the hemorrhagic group there is passage of large molecules and cellular elements into the SCF, indicating considerable disruption of the capillary membrane.

A fundamental question remains unanswered by these data. Is this selective permeability in the human choroid (presumably in the choriocapillaris) present in the normal eye? Allansmith17 has published a qualitative and quantitative description of the location, type, and amount of immunoglobulin in the human eye. Although not conclusive, her data suggest that (1) there are significant amounts of the lower-molecular-weight immunoglobulins in the extracellular space of the normal choroid and (2) there is apparent exclusion from the extracellular compartment of measurable amounts of IgM, a protein with a molecular weight of 900,000 daltons. It was possible to review the immunofluorescent stained sections of choroid with Dr. Allansmith (personal communication) to confirm that lower-molecular-weight proteins were present in both the intravascular and extravascular compartments whereas IgM was excluded from the extracellular compartment. Allansmith saw no evidence of plasma cells in the uveal tract and assumed that the albumin and immunoglobulins were derived from blood.

It is possible to derive from our results an estimate of the pore size which excludes \( \alpha_2 \)-macroglobulin and larger proteins from the suprachoroidal space. Pore diameter calculated thus is 144 Å. (See Appendix.)

In a recent study by Spitznas and Reale18 the fenestrations (or pores) in human choriocapillaris were studied by the freeze-fracture technique. After parallel cleaving through the pores, it was apparent that the over-all pore diameter was 600 Å, with a central thickening of 300 Å. If one assumes that permeability is greatest through the thinnest part of the pore diaphragm, then the peripheral zone, concentric to the central thicken-
ing, has an effective pore diameter of 150 Å. This is almost exactly the value predicted by our data.

Our data strongly suggest that the formation of SCF in the cataract group, many of the filtering surgery patients, and those patients with intraoperative choroidal effusions associated with elevated episcleral venous pressure occurs through a normal choriocapillary membrane. Only in the hemorrhagic group does there appear to be significant alteration of this membrane. Molecular sieving appears to occur in the formation of normal interstitial fluid in the choriocapillaris and in the formation of SCF after surgery. The markedly lower protein concentrations in the intraoperative group with elevated episcleral venous pressure is consistent with increased molecular sieving of large molecules under conditions of an accelerated filtration rate due to the pressure differential created by elevated extraocular venous pressure.

In view of the probable absence of any significant change in membrane permeability preceding or associated with CD, we must try to postulate a mechanism for the formation of SCF. A profound drop in intraocular pressure occurs almost universally in humans after glaucoma filtering surgery and in some patients following cataract surgery complicated by wound leak. Hypotony is usually prolonged in patients who develop CD. If Starling’s equation is applied to the eye, intraocular pressure is equivalent to tissue turgor. It is entirely possible that the drop in intraocular pressure leads to a net flow of fluid from the intravascular to the extravascular space. Capper and Leopold demonstrated that both hypotony and trauma were necessary to produce CD’s in rabbit eyes. Even uncomplicated surgery may be sufficient trauma to contribute to CD in humans. Experimental vortex vein occlusion has been shown to produce localized congestion in the corresponding segment of iris, ciliary body, and choroid. Accordingly, an increased intravenous pressure as manifested in patients with increased episcleral venous pressure could lead to accelerated net flow into the extravascular space, explaining the development of intraoperative choroidal effusions.

When CD occurs later in the postoperative period, there appears to be slower bulk flow across capillary membranes, and the contribution of diffusion to the net movement of large molecules from the intravascular to the suprachoroidal space is increased. Under these conditions, even large molecules may approach a diffusion equilibrium between the two compartments. Our data suggest that the change in filtration rate is responsible for the
apparent shift in effective pore size from that in the intraoperative CD in which molecules of 339,000 daltons are excluded to that in the cataract-filter group in which molecules up to 900,000 daltons pass through the membrane.

Since there are no anastomoses between different anteriovenous segments in the choriocapillaris,21 embarrassment of arterial inflow could lead to regional ischemia. Depending on the age of the patient and the condition of the vessels, such a vascular embarrassment could lead to transient edema (young patient) or a hemorrhagic infarction (older patient). This speculation has also been raised by Hayreh.19 The occurrence of non-hemorrhagic and hemorrhagic SCF is consistent with this speculation.

We express our sincere appreciation to the surgeons of the Massachusetts Eye & Ear Infirmary who collected serum and suprachoroidal fluid samples for us to assay. Without their cooperation, this study could not have been done. The technical assistance of Mr. Fred Schaefer, Ms. Julie Chien, Ms. Barbara Hodichek, and the staff of the Clinical Laboratory at the Massachusetts Eye & Ear Infirmary is also gratefully acknowledged. We are indebted to Dr. Stephen Flicker for the use of his computer.

Appendix

Using our data, one can calculate the diameter of the α2-macroglobulin (α2M) molecule as follows:

\[ V_{20} = 0.776 \text{ cc/gm (ref. 22), where } V_{20} = \text{partial specific volume at 20° C.} \]

\[ M.W. (\alpha_2M) = 820,000 \text{ gm/mol} \]

\[ (V_{20}) \cdot (M.W.) = 636,320 \text{ cc/mol \ Avogadro's \ #} = 6.061 \times 10^{23} \text{ molecule/mol} \]

\[ V_{\text{sphere}} = 4/3\pi R^3 = 1.050 \times 10^{-18} \text{ cc/molecule, where } V = \text{volume, } R = \text{radius of the sphere, } D = \text{diameter.} \]

\[ R = 63 \text{ Å} \]

\[ D = 126 \text{ Å} \]

From the following equation (Ferry23 and Pappenheimer19) for the diffusion of spherical particles through circular openings, the pore size can be calculated:

\[ D' = \frac{1 - \frac{a^2}{r^2}}{1 + 2.4\frac{a^2}{r^2}} \]

where \( D' \) = restricted diffusion coefficient, \( D \) = diffusion coefficient, \( a \) = radius of the spherical particle, \( r \) = radius of pore.

If one assumes that the diffusion of molecules the size of \( \alpha_2M \) is reduced to 0.5%, then solution of this equation for \( r = 72 \) Å is as follows:

\[ 0.005 = \frac{\left(1 - \frac{63^2}{r^2}\right)}{1 + 2.4\left(\frac{63}{r}\right)} \]

The pore diameter would, of course, be 144 Å.

REFERENCES


