Electron microscopic study of pseudo-exfoliation of the lens capsule

I. Lens capsule and zonular fibers

Norman Ashton, Manoucher Shakib, Robert Collyer, and Rolf Blach

Considerable controversy has arisen as to the nature and origin of the blue-gray flecks seen in the anterior segment of the eye in so-called pseudo-exfoliation of the lens capsule. An electron microscopic study has been carried out in an attempt to clarify this problem and Part 1 of this work deals with the findings in the lens and lens capsule. The filamentous nature of the deposit is confirmed and its ultrastructure further defined, but its exact chemical composition could not be identified. Exfoliative material was demonstrated directly upon the lens capsule and also within its substance, particularly at the anterior equatorial region. In this region also a new discovery is reported, namely, the presence within the inner half of the lens capsule proper of a degenerative band, apparently containing exfoliative material. Reasons are given for believing this band to be a characteristic feature of pseudo-exfoliation. The lens epithelium was normal and there is insufficient evidence to incriminate the zonular lamella or the zonular fibers as a source of the exfoliative material. It is concluded, however, that the lens capsule is undoubtedly involved in pseudo-exfoliation.

Since the original observations by Lindberg on the condition now known as pseudo-exfoliation of the lens capsule, there has been much speculation on the origin of the blue-gray flecks seen in the anterior segment of the eye in this disease. Vogt made a detailed study of the condition, and in 1925 named it "exfoliation of the anterior lens capsule," although he originally thought the flecks were remnants of the pupillary membrane and later that they were exudates. The first histologic study was made by Busacca, who suggested that the flecks arose from the aqueous humor and not from the lens capsule, while Trantas thought of the condition as a syndrome affecting all the glass membranes of the eye. Theobald, while not committing herself as to the origin of the flecks, agreed that they did not arise from the lens capsule and she, therefore, called the condition "pseudo-exfoliation of the lens capsule"—a name now in general use. Sunde maintained that this material originated locally in some unknown way and that its deposition was related to the close proximity of the affected surfaces. Gifford held the view that the deposits were reticulin-like and derived from the zonular lamella and the zonular fibers, while Ashton speculated that the material originated in the...
ciliary epithelium or its basement membrane. These are some of the examples of the confusion which prevails, but the literature has been fully reviewed by both Sunde and Tarkkanen to whose papers the reader is referred. It was to be hoped that electron microscopy might finally solve this problem, but, although it has added considerably to our knowledge of the disease, the exact source of the material remains an enigma. In the following work describing our electron microscopic findings we have therefore retained for the present Theobald’s name of “pseudo-exfoliation of the lens capsule.”

Material and methods

Table I gives details of the nature and source of the material used in this study. The lenses from eight patients were studied; all were cataractous, and five showed pseudo-exfoliation; the remaining three served as controls. In every case the lens was extracted intracapsularly without the use of chymotrypsin. Only those parts of the zonule adherent to the lens and ciliary body were available for examination. The average age of the patients was 72. Glaucoma was present in only four of the cases of pseudo-exfoliation.

Details of the methods employed in the electron microscopic study are shown in Table II. All the material was obtained at the time of operation, and fixed and embedded by the methods indicated in Table II. Fixation was carried out for 1 to 2 hours at room temperature. Specimens were then dehydrated in graded alcohol. Ultrathin sections were cut with a Huxley microtome, with glass knives, and transferred either to carbon or to colloidized 150 mesh copper grids, and examined with an AEI EM 6 electron microscope. Other sections, 2 μ in thickness, were cut for phase contrast microscopy.

For the purpose of this examination the lens capsule was divided into regions as shown in Fig. 1:

Region A, anterior central disc.
Region B, intermediate zone.
Region C, peripheral ring.
Region D, area between peripheral zone and equator.
Region E, anterior equator.
Region F, posterior equator.
Region G, posterior central.

In addition to electron microscopy, light microscopy was undertaken on Case 2 and on a control eye. Specimens were fixed with osmic acid and embedded in paraffin wax. The sections were bleached with 5 per cent hydrogen peroxide for 30 minutes, stained with Alcian blue, and counterstained with periodic acid-Schiff (P.A.S.). Other sections were stained with P.A.S. alone or with hematoxylin and eosin.

Table I. Source and nature of material studied

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Cataract present</th>
<th>Glaucoma present</th>
<th>Type of operation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With pseudo-exfoliation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. EM 50</td>
<td>72</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td>2. EM 53</td>
<td>72</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td>3. EM 16</td>
<td>67</td>
<td>F</td>
<td>Yes</td>
<td>No</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td>4. EM 9</td>
<td>72</td>
<td>F</td>
<td>Intumescent lens</td>
<td>Acute attacks</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td>5. EM 8</td>
<td>?</td>
<td>?</td>
<td>Yes</td>
<td>Absolute</td>
<td>Enucleation</td>
</tr>
<tr>
<td>6. EM 10</td>
<td>85</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td>7. EM 64</td>
<td>78</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td><strong>Without pseudo-exfoliation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. EM 39</td>
<td>70?</td>
<td>?</td>
<td>Yes</td>
<td>No</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td>9. EM 60</td>
<td>72</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
<tr>
<td>10. EM 63</td>
<td>66</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>Intracapsular cataract extraction and iridectomy</td>
</tr>
</tbody>
</table>
### Table II. Material and methods employed

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Specimen</th>
<th>Fixative</th>
<th>Stain</th>
<th>Embedding media</th>
<th>Areas studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lens</td>
<td>Millonig's isotonic phosphate-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Sections on grid stained with 1% PTA in 50% alcohol or lead citrate</td>
<td>Prepolymerized methacrylate</td>
<td>A, B, C, D, E, F, G</td>
</tr>
<tr>
<td></td>
<td>Iris</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Sections on grid stained with 1% PTA in 50% alcohol or lead citrate</td>
<td>Prepolymerized methacrylate</td>
<td>Pupillary margin</td>
</tr>
<tr>
<td>2</td>
<td>Lens</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with ( \text{KMnO}_4 )</td>
<td>Prepolymerized methacrylate</td>
<td>A, B, C, D, E, F, G</td>
</tr>
<tr>
<td></td>
<td>Iris</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Epon, Araldite</td>
<td>C, D, E, F</td>
</tr>
<tr>
<td>3</td>
<td>Lens</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Araldite</td>
<td>A, C, E, F, G</td>
</tr>
<tr>
<td>4</td>
<td>Lens</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Araldite</td>
<td>C, E</td>
</tr>
<tr>
<td>5</td>
<td>Lens</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Araldite</td>
<td>C, E</td>
</tr>
<tr>
<td>6</td>
<td>Ciliary body</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Araldite</td>
<td>Ciliary processes</td>
</tr>
<tr>
<td>7</td>
<td>Iris</td>
<td>Palade's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Araldite</td>
<td>Pupillary margin</td>
</tr>
<tr>
<td>8</td>
<td>Lens</td>
<td>Caulfield's Veronal-buffered ( \text{OsO}_4, \text{pH} 7.3 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Araldite</td>
<td>A, E, F</td>
</tr>
<tr>
<td>9</td>
<td>Lens</td>
<td>Palade's ( \text{OsO}_4 )</td>
<td>Sections on grid stained with 1% PTA in 50% alcohol</td>
<td>Prepolymerized methacrylate</td>
<td>A, B, C, D, E, F, G</td>
</tr>
<tr>
<td>10</td>
<td>Lens</td>
<td>Caulfield's ( \text{OsO}_4 )</td>
<td>Tissue stained with 1% PTA in absolute alcohol</td>
<td>Araldite</td>
<td>E, F</td>
</tr>
</tbody>
</table>

### Observations

The morphology of the exfoliative material (ExM). Our ultrathin sections have in general confirmed the morphologic descriptions of Blackstad, Sunde, and Traetteberg. The material consists of interlacing filaments with a characteristic and remarkably constant appearance (Fig. 2). Each individual filament measures about 200 Å in width and, in any particular section, up to 1 μ in length. Throughout its length the filament is accompanied by irregular granules of varying electron density which impart a fluffy indefinite outline, and which seem to aggregate at fairly regular intervals to form dense uneven bands giving an impression of segmentation. The filaments tend to aggregate...
into felty masses and are occasionally associated with finer, more nondescript filaments and with free granules; they stain rather better with phosphotungstic acid (PTA) than with lead citrate. The material seen in the ciliary body and iris was identical in appearance with that on the zonule and lens capsule, but in one specimen of iris the whole texture of the exfoliative material was rather thinner and finer (see Part II).

Description of the lens capsule. All our specimens of pseudo-exfoliation showed essentially the same appearances, which will be described according to the regions previously indicated.

Region A (anterior central disc). The lens capsule appeared to be normal in all cases except for isolated filaments of exfoliative material on its anterior surface. Clinically these appear as the central disc seen in Fig. 1.

Region B (intermediate zone). This region was entirely normal in all cases and there was no exfoliative material.

Region C (peripheral ring). Exfoliative material was seen on the surface of the lens capsule in typical bushlike aggregations. Electron opaque granules, possibly of a similar nature, were seen in the superficial layer of the lens capsule (Fig. 3).

Region D (area between peripheral zone and equator). In this area the changes were similar to those in Region C, but there was less exfoliative material on the surface of the lens, while the granularity of the capsule itself was somewhat more marked.

Region E (anterior equator). This region consistently showed the most marked changes in comparison with the normal (Figs. 4A and 4B). The accumulation of exfoliative material on the surface of the lens capsule was especially evident at the insertion of the zonular fibers, and was also seen within the superficial layers of the lens capsule (Figs. 5 and 6). Confined to this area the lens capsule itself showed a grossly abnormal appearance, which we believe to be characteristic of pseudo-exfoliation. In its inner half it had undergone a degenerative change, presenting as a coarsely fibrogranular spongy band (FGB, Text continued on p. 151.)
Fig. 2. Case 2, Region C. Electron micrograph (EM) of exfoliative material (Ex M) on the surface of the capsule proper (CP) showing fluffy outline of the filaments and electron-dense areas. (KMnO$_4$-stained, methacrylate-embedded. Original magnification ×50,000; inset ×200,000.)
Fig. 3. Case 2, Region C. EM of lens capsule showing exfoliative material (Ex M) on capsule proper (CP). Electron-dense granules (G) can be seen in the superficial layers of the capsule. Epithelium (Ep). Nucleus (N). (KMnO₄-stained, methacrylate-embedded. Original magnification ×7,000. Upper right inset, higher magnification, ×30,000.)
Fig. 4A. Case 8, Region E. Survey EM of normal lens capsule showing normal epithelium (Ep), capsule proper (CP), and zonular lamella (ZL). (Methacrylate section stained with PTA. Approximately ×3,000.)

Fig. 4B. Case 2, Region E. Survey EM of lens capsule showing lens epithelium (Ep) and a deep fibrogranular band (FGB) in the capsule proper (CP). Zonular lamella (ZL), zonule (Z), exfoliated material (Ex M). Arrows point to pigment granules. (Permanganate-stained, methacrylate-embedded. Approximately ×4,000.)
Fig. 5. Case 2. Region E. EM of lens capsule showing exfoliative material (Ex M) inside the capsule proper (CP). (KMnO₄-stained, methacrylate-embedded. Approximately ×6,500.)
Fig. 6. Case 4, Region E. EM of lens capsule showing exfoliative material in the superficial layer of the lens capsule proper (CP). (PTA-stained, Araldite-embedded. Original magnification ×38,000.)

Fig. 7. Case 4, Region E. EM of lens capsule showing deep fibrogranular band of the capsule proper (CP). Note the granular filaments often appearing in streams perpendicular to the surface of the lens. (PTA-stained, Araldite-embedded. Original magnification ×38,000.)
Fig. 8. Case 3, Region E. Light microscope picture of the lens capsule showing epithelial cells and degenerative fibrogranular band in the inner aspect of the capsule proper. (OsO$_4$-fixed, paraffin waxen-embedded. Original magnification ×700.)

Fig. 9. Case 2, Region F. EM of lens capsule showing normal capsule proper (CP) and zonule (Z) and exfoliative material (Ex M) on the surface. Lens fibers (LF). (KMnO$_4$-stained, methacrylate-embedded. Original magnification ×9,000.)
Figs. 4B and 5), which on high-power examination showed granular filaments in every way resembling those of exfoliative material, streaming through a vacuolated lens substance, often perpendicular to the surface (Fig. 7). The lens epithelium appeared normal and no deposits could be seen within the cells, between the cell membranes, or between their membranes and the adjacent degenerate capsule, but the line of junction appeared markedly serrated. No exfoliative material was found within the adjacent lens fibers.

After the demonstration of this "fibrogranular" band in the inner capsule by the electron microscope the lens was re-examined with the light microscope. In paraffin sections of osmium-fixed material the band could be seen quite clearly, especially when stained with Alcian blue and P.A.S. (Fig. 8). When older sections of this condition were re-examined, fixed in formalin, and embedded either in celloidin or paraffin and stained by conventional methods, this layer was again readily recognized. It should be emphasized that in light microscopy this layer appears to be subcapsular (Fig. 8), but electron microscopy shows it to be within the deep aspect of the capsule itself (Fig. 4B).

Region F (posterior equator). In this region the capsule itself was quite normal, but exfoliative material was present on its surface in close association with the zonular fibers (Fig. 9).

Region G (posterior central). In this region the lens capsule showed no abnormality and no exfoliative material was seen. These findings are summarized in Fig. 10.

Discussion

Our findings in the "control" cataractous lenses agree with those of other workers who have studied the normal lens epithelium and the normal lens capsule. We would emphasize, however, that in electron microscopy the zonule and zonular lamella are readily differentiated from the lens capsule, and it can be seen that the zonular lamella exists only at the equator and in the regions immediately anterior and posterior to it; that is, the capsule, both anteriorly and posteriorly, is homogeneous and shows no separate strata. The electron microscope does not, therefore, show a "pericapsular membrane" in the pupillary area as described in histologic preparations by Theobald; or a "reticulin-like membrane" covering the anterior and posterior capsule as described by Gifford.

We have shown that the exfoliative material, whether found in relation to the zonular fibers, or upon or within the lens capsule, has a highly characteristic filamentous structure, but we have not been able to identify its nature and are not aware of any normal or abnormal substance with an exactly comparable electron microscopical appearance. Histochemical studies have been more informative in this respect and, although there is some confusion, most authors agree that the material shows the staining reactions of acid mucopolysaccharide, contains tyrosine, gives negative reactions for hyalin, amyloid, colloid, and fat, is resistant to collagenase and hyaluronidase, and is digested by...
Ashton et al. Investigative Ophthalmology April 1965

trypsin and pepsin only in strong concentrations or after long periods. It would seem that a continuation of these investigations in histochemistry or microchemistry is more likely to lead to the identification of the material than is further electron microscopy.

Although Theobald demonstrated a clear unstained zone between the exfoliated material and the capsule proper, this must be an artifact of light microscopy for electron microscopy has shown that there is no such zone. Moreover, exfoliative material may be seen inside the capsule, making it impossible to exclude this structure as a possible site of the material's origin.

A most interesting finding and perhaps the most important structural abnormality disclosed in this study was the degenerative change in the deep layer of the lens capsule in the region of the anterior equator (Region E). This band was clearly demonstrated in all specimens with pseudo-exfoliation and appears to be specific for this condition, having been found both by electron microscopy and conventional histology in pseudo-exfoliation, but not demonstrated in other pathologic conditions examined. The filaments and granules seen in this band closely resembled those of the exfoliative material, so that this region must also be considered as a possible point of origin of pseudo-exfoliation.

We have been able to study the zonular fibers only at their attachments to the ciliary body and lens capsule. At the attachment of the fibers to the lens there is always a great accumulation of exfoliative material and changes in the lens capsule are most marked in the region of this insertion. On the other hand, exfoliative material is only occasionally seen within the substance of the zonular fibers and many are unaffected; moreover, it will be recalled from histologic examinations that the zonular fibers in the region of the pars plana are almost always free from exfoliative material. We have, therefore, insufficient evidence to incriminate the zonule as a possible source of this material.

No further comments on the pathogenesis of pseudo-exfoliation would be justified without a knowledge of the changes to be found in the iris and ciliary body and this will be the subject of Part II of this work.

REFERENCES
1. Lindberg, J. C.: Kliniska undersökningar över depigmentering av pupillarranden och genomlysbarhet av iris vid fall av alderstarr samt i normala ögon hos gamla personer, D.s.s., Helsingfors, 1917.
16. Blackstad, T. W., Sunde, O. A., and Trassee,


