Effects of paracentesis on the blood-aqueous barrier: a light and electron microscopic study on cynomolgus monkey

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The effects of paracentesis on the blood-aqueous barrier of cynomolgus monkeys were studied by light and electron microscopy. The following regions of the eye were examined: ciliary body, iris, Schlemm's canal, juxtacanalicular connective tissue, and trabecular meshwork. Prominent structural alterations were seen in the ciliary epithelium at the anterior portion of the pars plicata, but the epithelium at the posterior portion of the pars plicata and at the pars plana appeared less disrupted. Plasma proteins which crossed the capillary endothelium accumulated in the stroma, and appeared in the posterior chamber through the enlarged extracellular spaces of the ciliary epithelium. The plasma proteins in the ciliary stroma of the anterior portion of the pars plicata moved to the stroma of the iris root; whereas in the iris the capillary endothelium provide an impermeable barrier to proteins. Following paracentesis, the lumen of Schlemm's canal was invaded by blood, and through newly formed gaps in the endothelial lining of the inner wall of the canal, the plasma proteins and red blood cells rapidly diffused into the juxtacanalicular connective tissue, trabecular meshwork, and anterior chamber. On the basis of the present experiments, the protein of the aqueous humor can be considered as originating from both the anterior portion of the pars plicata and the inner wall of Schlemm's canal.

Key words: paracentesis, blood-aqueous barrier, intraocular pressure, light and electron microscope, ciliary body, iris, drainage angle, cynomolgus monkey.

There is a barrier between the blood and the aqueous humor in the ciliary processes of the normal eye. When aqueous humor is drained away, the anterior chamber becomes filled with a protein-rich or plasmoid aqueous humor. The anatomic aspects of this condition have been studied repeatedly and very well reviewed by Davson. These studies suggest that the protein-containing exudate entering the eye after paracentesis is derived from the ciliary body rather than the iris and appears in the anterior chamber by flow through the pupil. In a recent paper Raviola pointed out that paracentesis does not affect the permeability properties of either the blood-aqueous barrier.
ciliary epithelium or the iris vessels, and (2) following paracentesis the plasma proteins gain access to the lumen of Schlemm's canal, permeate the tissue of the sclerocorneal angle, and appear in the anterior chamber.

The purpose of the experiments presented here was to discover the route of the plasma proteins, normally retained by the capillary wall, once the blood-aqueous barrier is broken.

Material and methods

Fourteen cynomolgus monkeys weighing 2.0 to 2.5 kilograms were anesthetized by intravenous injection of sodium pentobarbital, with corneal transparency maintained by frequent irrigation with physiologic saline.

Paracentesis was achieved by penetrating the anterior chamber with a 25-gauge needle; 0.10 ml. of aqueous humor was withdrawn, with great care taken that the tip of the needle did not touch the anterior surface of the iris. The intraocular pressure (IOP) was measured with a Schiötz and Perkins' tonometer at various times following paracentesis.

Eyes were enucleated at various intervals after paracentesis (5, 15, and 45 min., 2 and 24 hr., and 7 days), and fixed immediately in 4 per cent glutaraldehyde in 0.15M phosphate buffer solution (pH 7.2) for 20 min. at room temperature. During the initial fixation, the tissue to be studied was carefully dissected out and trimmed into small pieces. The tissue was postfixed in 1 per cent osmium tetroxide in the same buffer solution for 90 min. at 4° C. The small pieces were dehydrated in a series of ethyl alcohol, treated with propylene oxide, and embedded in an epoxy resin following Luft's method. Sections 1 /* thick were stained with toluidine blue for general histologic study, and ultrathin sections stained with uranyl acetate and lead citrate were examined on a Siemens Elmiskop-1 electron microscope.

A series of 50 consecutive 1 μm thick sections were cut from each of four positions of the angle (3, 6, 9, and 12 o'clock), with the angle aligned vertical to the axis of Schlemm's canal. The number of endothelial nuclei and vacuoles per length of Schlemm's canal were counted, using 100× objective lens.

Results

The effects of paracentesis on IOP, ciliary body, iris, and drainage angle are described.

Intraocular pressure. The time course of

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Vacuoles per 1,000 μ</th>
<th>Nuclei per 1,000 μ</th>
<th>Vacuoles per nuclei</th>
<th>Intraocular pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.2 ± 8.5</td>
<td>67.9 ± 6.3</td>
<td>0.96 ± 0.23</td>
<td>14.0 ± 0.3</td>
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<tr>
<td>5 min.</td>
<td>63.5 ± 6.3</td>
<td>53.4 ± 3.1</td>
<td>0.84 ± 0.10</td>
<td>0</td>
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<tr>
<td>15 min.</td>
<td>39.1 ± 4.1</td>
<td>72.8 ± 7.2</td>
<td>0.54 ± 0.09</td>
<td>0.5 ± 0.3</td>
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<tr>
<td>45 min.</td>
<td>11.3 ± 1.3</td>
<td>62.3 ± 6.3</td>
<td>0.18 ± 0.03</td>
<td>1.5 ± 0.3</td>
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<tr>
<td>2 hr.</td>
<td>49.2 ± 8.2</td>
<td>58.9 ± 7.5</td>
<td>1.19 ± 0.21</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td>24 hr.</td>
<td>68.1 ± 8.5</td>
<td>72.2 ± 9.2</td>
<td>0.94 ± 0.08</td>
<td>14.0 ± 0.3</td>
</tr>
<tr>
<td>7 days</td>
<td>63.1 ± 7.9</td>
<td>72.8 ± 8.8</td>
<td>0.87 ± 0.20</td>
<td>14.0 ± 0.2</td>
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IOP recovery after paracentesis is shown in Fig. 1 and Table I. IOP increased rapidly and returned to normal value about 2 hr. after paracentesis. IOP reached its highest value (about 20 mm. Hg) at about 3 hr., and returned to normal 6 to 9 hr. after paracentesis.

Ciliary body. Very severe changes were observed in the anterior portion of the pars plicata, rather than in the posterior portion of the pars plicata and the pars plana.

Fifteen minutes after paracentesis, at the posterior portion of the pars plicata and at the pars plana, the capillaries showed dilatation and congestion (Fig. 2, a*). Poorly defined fenestrae were detected at some capillary endothelium (Fig. 2, b). Dense amorphous material was seen in the dilated ciliary channel between the pigmented and nonpigmented epithelium (Fig. 2, c). These changes remained similar up to 2 hr. after paracentesis. No remarkable changes were seen in the junctional complexes.

On the other hand, at the anterior portion of the pars plicata, the capillary endothelium revealed swelling, increase of membrane-bound vesicles, slight enlargement of intercellular spaces, and poorly defined fenestrae (Fig. 3). The endothelial
basement membrane was detected with difficulty. Scattered aggregate of fibrin was seen at the expanded space between the capillary wall and the pigmented epithelium (Fig. 4, a). The pigmented epithelium showed complete disappearance of basement membrane and basal infoldings. Enlargement of the intercellular space was seen between the adjacent cells of the pigmented epithelium, and between the pigmented and nonpigmented epithelium. These spaces contained plasma proteins (Fig. 4, b).

The changes at the anterior portion of the pars plicata progressed until 2 hr. after paracentesis. At 45 min., enlargement of the intercellular space progressed between the adjacent cells of the nonpigmented epithelium and these spaces contained fibrin (Fig. 5). The cell organelles of both pigmented and nonpigmented epithelium did not show any abnormal changes. The posterior chamber contained a mass of fibrin.

Twenty-four hours after paracentesis, the capillaries returned to normal. The spaces between the pigmented and nonpigmented epithelium and the adjacent nonpigmented epithelium were still enlarged. Both pigmented and nonpigmented epithelium revealed newly formed basement membrane and basal infoldings, and their mitochondria were slightly swollen. The posterior portion of the pars plicata and the pars plana showed no remarkable changes.

At 7 days, morphologic differences between the anterior and posterior portion of the pars plicata were still noted. At the anterior portion of the pars plicata both pigmented and nonpigmented epithelium showed marked dilatation of the cisternae of rough endoplasmic reticulum and many lysosomes containing melanin granules. At the posterior portion of the pars plicata the ciliary epithelium was of normal structure except for an accumulation of basement membrane-like material in the widely opened basal infoldings of the nonpigmented epithelium.

Iris. The earliest change was detected 45 min. after paracentesis. The stroma at the iris root was slightly edematous and its ground substance was composed of very loose connective tissue containing plasma proteins (Fig. 6, a). The general morphology of the endothelial cells of the capil-

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**Fig. 1.** Effect of paracentesis on IOP of monkey. The arrow indicates paracentesis at time zero.
Fig. 2. Ciliary processes at the posterior portion of the pars plicata 15 min. after paracentesis. 

a, Dilatation of the capillary is pronounced. (x350.) b, Capillary endothelium shows intact fenestrae (thin arrow) and poorly defined fenestrae (thick arrows). (x25,000.) c, Dense amorphous material is seen in the dilated ciliary channel (asterisks). (x19,000.)

Fig. 3. The capillary at the anterior portion of the pars plicata 15 min. after paracentesis. Capillary endothelium shows swelling, increase of membrane-bound vesicles, and poorly defined fenestrae (arrow). Basement membrane is not detectable. (x30,000.)
Fig. 4. Ciliary process at the anterior portion of the pars plicata 15 min. after paracentesis. 
a. Scattered aggregate of fibrin (asterisk) is seen at the space between the dilated capillary 
and the pigmented epithelium. (×450.) b. The pigmented epithelium shows complete disappearance of basement membrane and basal infoldings (arrows). Enlargement of the intercellular space is seen between the pigmented and nonpigmented epithelium (asterisks). (×10,000.)

Fig. 5. Ciliary epithelium at the anterior portion of the pars plicata 2 hr. after paracentesis. Enlargement of the intercellular space is seen between the adjacent nonpigmented epithelial cells, and these spaces contain fibrin (asterisks). (×6,000.)
laries was not different from that of the control animals. At the anterior border layer the intercellular spaces between fibroblasts and melanocytes were enlarged (Fig. 7). The epithelium did not show any abnormal changes except the enlargement of the spaces between adjacent myoepithelium (Fig. 8). No abnormal change was seen in the pupillary sphinctor muscle region (Fig. 6, b).

At 2 hr., plasma proteins were barely detectable in the stromal ground substance at the iris root. Twenty-four hours after paracentesis, the iris structure was normal.

**Drainage angle.** The earliest changes were seen 5 min. after paracentesis. A moderate amount of red blood cells and plasma proteins are seen at the external collector channel and Schlemm's canal. Plasma proteins were seen also in the intercellular spaces of the juxtacanalicular connective tissue. Discontinuity of the endothelium and enlargement of the intercellular clefts between the endothelial cells of the inner wall of the canal were seen, although the endothelial pinocytotic vesicles did not show any changes (Fig. 9).

At 15 min., red blood cells and plasma proteins were trapped in the juxtacanalicular connective tissue and the lumen of the corneoscleral meshwork. The number of endothelial vacuoles of the inner wall of Schlemm's canal decreased moderately, as compared with control (Fig. 10). These changes in the drainage angle continued until 45 min. after paracentesis, except for a very marked decrease in the number of the endothelial vacuoles in the inner wall of Schlemm's canal (Fig. 11).

Two hours after paracentesis, the lumen of the canal was free from red blood cells. Small numbers of red blood cells and a little plasma protein were trapped in the juxtacanalicular connective tissue. Macrophages and red blood cells were seen between strands of corneoscleral meshwork. The number of endothelial vacuoles returned to normal.

At 24 hr. and at 7 days after paracentesis, the drainage angle was normal except for the presence of macrophages in the corneoscleral meshwork.

Fig. 12 summarizes the path of leakage and movement of plasma protein at the anterior portion of the pars plicata, iris root, and drainage angle.

**Discussion**

The present study indicates the existence of a different reaction between the anterior and posterior portions of the pars plicata of the ciliary epithelium after paracentesis.

At the anterior portion of the pars plicata, the plasma proteins have reached the stroma across the capillary endothelium as demonstrated by poorly defined fenestrae, slight opening of the intercellular spaces, and an increase of membrane-bound vesicles. The accumulation of plasma proteins between the capillary wall and the pigmented epithelium forms a cystlike space containing a mass of fibrin. This edematous change may cause the disappearance of the basement membrane of capillary endothelium. The direct leak of fibrin through the enlarged spaces between adjacent cells of the nonpigmented epithelium undoubtedly contributes significantly to the protein-rich aqueous fluid. On the other hand, at the posterior portion of the pars plicata and at the pars plana, the dilated ciliary channels between the pigmented and nonpigmented epithelium contain plasma proteins. They may be transported to the posterior chamber through the intracytoplasmic vesicles. Thus, one of the sites of protein leakage following paracentesis has to be sought in the ciliary body.

The present experiment, using monkeys, reveals the extreme sensitivity of the anterior portion of the pars plicata after paracentesis. The same finding is shown in monkeys after topical administration of prostaglandin E1. In rabbits, both the iridial processes and the tips of the ciliary processes have manifested extreme sensitivity not only in response to paracentesis, but also to prostaglandins, to systemic endotoxin, and to vasodilator...
Fig. 6. a, The stroma at the iris root 45 min. after paracentesis. The endothelial cells of the capillary are normal, though the surrounding loose connective tissue contains plasma proteins (asterisks). (×10,000.) b, The stroma at the pupillary sphincter muscle region 45 min. after paracentesis. The endothelial cells of the capillary and the surrounding loose connective tissue are normal. (×10,000.)

Fig. 7. Anterior border layer at the iris root 45 min. after paracentesis. The stroma contains plasma proteins (asterisks). Enlarged intercellular spaces contain plasma proteins (arrow), and these spaces communicate with the anterior chamber which contains fibrin. (×3,000.)
Fig. 8. Iris epithelium at the root 45 min. after paracentesis. The stroma beneath the myoepithelium contains plasma proteins (asterisks). (x4,000.)

Fig. 9. Schlemm’s canal, juxtacanalicular connective tissue 5 min. after paracentesis. Schlemm’s canal contains red blood cells and plasma proteins. Plasma proteins (asterisks) and red blood cell (rbc) are seen in intercellular spaces of the juxtacanalicular connective tissue. (x4,000.)
Fig. 10. The drainage angle before paracentesis. The endothelial cells of the inner wall of Schlemm's canal have giant vacuoles (arrows). ($\times$1,000.)

Fig. 11. The drainage angle 45 min. after paracentesis. Red blood cells are seen in Schlemm's canal, juxtanuclear connective tissue, and trabecular meshwork. The endothelial cells lining Schlemm's canal show a marked decrease in the number of giant vacuoles, and their nuclei are flat (arrow). ($\times$1,000.)

Fig. 12. Schematic drawing of the movement of plasma protein from uveal vessels after paracentesis. Arrows indicate the path of leakage of plasma protein.
drugs such as bradykinin, acetylcholine, and histamine.\textsuperscript{1}

It has been also observed that paracentesis is accompanied by a higher content of prostaglandins in the aqueous humor.\textsuperscript{10, 11} Prostaglandins are thought to be synthesized and released in response to a wide variety of inflammatory or irritative stimuli.\textsuperscript{18} The morphologic alteration of the ciliary body after paracentesis may be due mainly to the action of prostaglandins.

When aqueous humor is drained away, ciliary capillaries are permeable to plasma proteins, and the covering epithelium is also permeable, mainly at the anterior portion of the pars plicata; whereas in the iris the capillary endothelium is impermeable.

The plasma proteins in the stroma of the anterior portion of the pars plicata moved towards the stroma of the iris root 45 min. after paracentesis in this study. Raviola\textsuperscript{15} used perfusion fixation and found no change in the permeability properties of either the ciliary epithelium or the iris vessels after paracentesis. She does not state in her article which portions of the ciliary body and the iris were studied. The discrepancy among them might be due to fixation method. Scheie and associates\textsuperscript{17} observed that, in the completely iridectomized cat's eye, the re-formed aqueous humor after paracentesis contains only 10 to 15 per cent of protein found in the re-formed fluid of the contralateral normal eye. If there was the possibility that the anterior portion of the pars plicata were damaged during the total iridectomy, paracentesis could bring little change of the aqueous protein content. If such were the case, the idea that the iris could be such a significant source of plasma proteins in plasmoid aqueous humor\textsuperscript{5, 17} might need to be changed.

The results of the present experiments clearly demonstrate that as a result of the sudden fall of IOP, blood flows back from the episcleral veins into Schlemm's canal, and gaps appear in its endothelial lining large enough to permit the passage of plasma proteins and red blood cells into the juxtacanicular connective tissue, trabecular meshwork, and anterior chamber. This interpretation is consistent with the observation of Feeney and Wissig\textsuperscript{1} that red blood cells appear in the juxtacanicular connective tissue following removal of small amount of aqueous humor. Following topical administration of prostaglandin E\textsubscript{1} to monkey eyes, gaps do not appear in the endothelium of Schlemm's canal, and plasma proteins and red blood cells do not escape into the juxtacanicular connective tissue.\textsuperscript{13} Thus, the morphologic alteration of the drainage angle after paracentesis may be due to a mechanical factor such as the sudden fall in IOP, or to the release of additional substances as well as prostaglandins.

The endothelial cells of the inner wall of Schlemm's canal have giant vacuoles. In the present experiments, the number of endothelial vacuoles decreased gradually after paracentesis, and reached a minimum at 45 min. Two hours after paracentesis the endothelial cells returned to normal. Many aspects of the factors involved in the endothelial vacuolation cycle have been discussed.\textsuperscript{22} The incidence of vacuoles varies significantly in individual eyes and even in the same eye at different levels of sectioning.\textsuperscript{22} Thus, the tissue samples were taken from each of four different regions as described above. Shabo and co-workers\textsuperscript{19} suggest that giant vacuoles are the result of rapidly occurring postmortem change. The immersion method of fixation adopted in the present experiment may not be entirely free from this criticism. But the present observations do represent a real change, since all material was fixed by the same method and consistent differences were observed between control and experimental eyes.

Recent investigations have demonstrated that graded increase in IOP can induce corresponding alteration in outer meshwork.\textsuperscript{5, 6} Our morphologic evidence suggests that a decrease in the number of endothelial vacuoles in the inner wall of Schlemm's canal occurs when episcleral venous pressure is higher than IOP. Johnston and Grant\textsuperscript{8} have suggested that these changes may be due to a mechanism for
bulk outflow of fluid and particles, with a one-way valve action preventing reflux of blood from Schlemm's canal into the anterior chamber. But our experiments indicate that the one-way valve action of the endothelial cells does not hold when there are rapid changes in IOP.

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