Cytological basis of protein leakage into the eye following paracentesis

An electron microscopic study

George K. Smelser and Yen Fen Pei

If the blood aqueous barrier is destroyed and a plasmoid aqueous formed, marked structural changes occur which permit the leakage of protein from the ciliary capillary to the aqueous humor. This is largely due to disjunction of the endothelial cells and enlargement of intercellular spaces of both epithelial layers of the ciliary process. The electron dense particles used in these experiments were seen to pass from the ciliary capillaries between the endothelial cells, although some passage via cytoplasmic vesicles and pores may have occurred. Diffusion across the connective tissue spaces appears to be unimpeded, but the basement membranes of the capillaries and of the epithelium restrict, but do not prevent, passage of such particles. The pathway through both the pigment and surface epithelium was almost exclusively intercellular. The large spaces were formed by disengagement of the interdigitations of the epithelial cells, and disruption of their desmosomal linkages. The basement membrane covering the surface cells and lining the posterior chamber appeared also to offer some, but by no means great, restriction to the passage of particles of the size used.

In the ciliary processes of the normal eye, there is a barrier between the blood and the aqueous humor which is largely a physiologic concept, based on the difference in concentration of various substances in the intraocular and vascular fluids. When the anterior chamber is emptied by paracentesis, or the eye is treated with drugs, such as disisopropyl fluorophosphate or nitrogen mustards, the anterior chamber becomes filled with a protein rich or plasmoid aqueous humor. In this condition, the blood aqueous barrier is said to have been broken. The anatomical aspects of this condition have been studied repeatedly and very well reviewed by Davson. It has been held that larger molecules, such as protein, normally enter the aqueous humor very slowly, and are restrained first by the capillary wall, and finally by the epithelium of the ciliary processes. They pass the latter, presumably via intercellular spaces. It is known, from studies of the fine structure, that these spaces are narrow and tortuous, and that desmosomes firmly join the pigment and surface cell layers.

It is the purpose of the experiments reported here to discover the route taken by substances, normally retained by the capillary wall, from the blood to the aqueous humor when the blood aqueous barrier is broken. This is an effort to extend in greater

From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, N.Y.

This investigation was supported by Research Grant NB 01202-08 and Training Grant 5 TI NB 5324-01 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, United States Public Health Service.
detail the older light microscopic stud-
ies\(^1\)-\(^8\) by the application of electron
microscopy to this problem, and to add to
the report of Pappas and Tennyson,\(^9\) whose
observations were restricted to the mor-
phology of the capillaries.

Methods

The anterior chambers of five-pound albino rab-
bits, anesthetized with sodium pentobarbital,
were opened by a corneal keratome incision. The
iris was not touched. Immediately thereafter, 10
ml. of Thorotrast (a 25 per cent stable suspension
of thorium dioxide in an aqueous dextrin solution;
Testagar & Co., Inc., Detroit, Michigan) was in-
jected via the carotid artery at the rate of 1.0
ml. per minute. Single particles of the thorium
dioxide are about 100 \(\text{Å}\) in diameter, but clumps
of particles are frequently seen which are much
larger. Five minutes after cessation of the intra-
carotid injection, the eye fixed in vivo with 1 per
cent osmium tetroxi.de in Caulfield or Millonig
buffer at pH 7.5-7.6, and the animal killed. The
ciliary processes were embedded in Epon or
Maraglas, sections were cut with glass knives on a
Porter-Bloom microtome, and studied with a Sie-
mens microscope, Elmiskop I. Inflammation of
the ciliary processes was also produced by an in-
travenous injection of 10 meg. per kilogram body
weight of endotoxin (lipopolysaccharide S. flex-
neri, Difco). Two hours following the injection
the aqueous humor was turbid, indicating that it
contained an abnormal amount of protein. Thoro-
trast was then injected via the carotid artery and
the tissue processed as before.

Observations

Fifteen minutes after a plasmoid aqueous
had been produced by paracentesis, and the
Thorotrast suspension injected as de-
scribed, thorium-dioxide particles were
found in most of the ciliary processes. The
particles were present in the lumen of the
capillaries and the surrounding connective
tissue stroma and also, although less fre-
quently, in the epithelial layers and the
posterior chamber.

The capillaries of the ciliary processes
of normal adult rabbits consist of a single
layer of endothelial cells surrounded by a
basement membrane in which occasional
pericytes are embedded. The endothelial
cells are variable in thickness, possessing
areas which are very thin, containing, at
irregular intervals, fenestrations. These
"openings" are approximately 350 \(\text{Å}\) in di-
ameter and are bridged by a thin mem-
brane, or diaphragm. Where the endothelial
cells join one another, they often overlap
and appear to be firmly attached. At such
junctions a terminal bar is found on the
luminal surface. External to the endothelial
cell the basement membrane covers the en-
tire outer surface of the capillary, and is
continuous over the fenestrations. In the
experimental animals, the most obvious
change in the capillaries was a separation
between the endothelial cells at their junc-
tion. At this point, a large amount of
thorium-dioxide particles could often be
found leaving the capillary through the
opening made by the separation (Fig. 1).
There was regularly an accumulation of
these electron dense particles between the
endothelial cell and the basement mem-
brane which covered the capillary. Where
a pericyte was located, the thorium-dioxide
particles were found in the basement mem-
brane between the two cells. Elsewhere, it
was noted that the Thorotrast had found
its way through the basement membrane
around pericytes, when they were present,
and into the connective tissue spaces of the
stroma (Figs. 2 and 3). In addition to the
leakage of Thorotrast particles out of the
capillaries at the loosened endothelial cell
junctures, blood platelets or erythrocytes
(Fig. 2) were also found leaving the capil-
lar lumen. Many areas were seen in which
the basement membrane, even between
endothelial cell and pericyte, appeared to
offer some resistance to the passage of the
particles. They were found in greater con-
centration between the basement mem-
brane and the endothelial cell, or above
the basement membrane, next to the peri-
cyte cytoplasm, than in the stroma. This
distribution indicated strongly that the base-
ment membrane did restrict their outward
movement (Figs. 2 and 3). Thorotrast
particles also left the capillary lumen by
means of membrane-bounded vesicles which
were found within the endothelial cell cyto-
plasm (Fig. 1). Occasionally, particles were
found within the endothelial cell fenestra-
Protein leakage into eye following paracentesis

Tions immediately against the diaphragm which closed the fenestration. Single particles or clusters of thorium dioxide particles were found both immediately inside and outside the capillary attached to, or resting on, the diaphragm. None was found actually within the thin diaphragm. The inflammation produced by endotoxin injections affected the vessels in the same manner as described.

External to the capillaries, the Thorotrast particles appeared to diffuse through the connective tissue space freely. Often a somewhat higher concentration of the particles was found near or on collagen fibers. Phagocytosis of the Thorotrast particles by histiocytes was observed (Fig. 4). Thorotrast particles diffused freely through the connective tissue stroma, and came to lie against the basement membrane underlying the pigment epithelium which, for the most part, remained intact (Fig. 5). It was reported earlier that damage to the blood aqueous barrier by paracentesis or intravenous injection of endotoxin resulted in the formation of large intercellular spaces.

This observation, which confirmed the

Text continued on p. 258.
Fig. 2. A separation of the endothelial cells with the opening closed by the basement membrane. Part of an erythrocyte (Er) and clumps of Thorotrast particles can be seen in the opening (arrow). Some particles are on the outside of endothelial cell, possibly restricted by the basement membrane (Bm). Numerous particles are scattered in the stroma (St). P, process of a pericyte. (Lead stain, Maraglas.)

Fig. 3. The endothelial junction in this micrograph appears to be closed (arrow); however, note the accumulation of Thorotrast particles just outside. Particles are scattered in the stroma (St), but others are retained by the basement membrane (Bm) and pericyte (P). F, fibroblast. (Lead stain, Epon.)

Fig. 4. A phagocyte in the stroma of a ciliary process. It has already engulfed large quantities of Thorotrast which is contained in vacuoles (arrow). Formation of a new vacuole has just been completed (arrow on right). (Lead stain, Epon.)
Fig. 4. For legend see opposite page.
Figs. 5 and 6. For legend see opposite page.
Fig. 7. In this section the basement membrane (Bm) is interrupted by a "passage" (arrow) which leads between two pigment epithelial cells. V, intercellular vesicle between pigment epithelial cells. Note the numerous Thorotrast particles in these spaces. (Lead stain, Maraglas.)

Fig. 5. A large intercellular vesicle (V) free of Thorotrast between widely spaced processes of pigment epithelial (P.E.) cells and the basement membrane (Bm). Note the large amount of Thorotrast particles in the stroma (St) which has not passed the basement membrane of the pigment epithelium.

Fig. 6. This figure is similar to Fig. 5 except that the basement membrane (Bm) appears less compact, and the Thorotrast particles have entered the intercellular vesicle in high concentration. (Lead stain, Epon.)
Fig. 8. An enlarged intercellular space containing "villus projections" at 1, but with normal "tight junctions" and desmosomes shown at 2 and 3. Note that Thorotrast particles are present in the enlarged intercellular space. P.E., pigment epithelium; S.E., surface cell; P.C., posterior chamber. (Lead stain, Epon.)

Fig. 9. In this micrograph, the pigment epithelial cells appear quite normal, except for an enlargement of the intercellular space which contains a few Thorotrast particles (arrows). A few particles are in a nearby small vesicle (arrow). (Lead stain, Epon.)
Fig. 9. For legend see opposite page.
Fig. 10. A moderate enlargement of the intercellular space between two surface cells which contains clusters of Thorotrast particles (arrow). The apparent intracellular vacuole (lower left arrow) is thought to be a diverticulum of intercellular space. (Lead stain, Epon.)

earlier findings of Greef¹ and Poos,² was also made in the present experiments. The intercellular vesicles formed extremely large, apparently empty, spaces between pigment epithelial cells and the basement membrane on which they rested. In many of these areas, the thorium-dioxide particles were completely prevented from entering such open spaces by the basement membrane (Fig. 5). In contrast, however, other areas, apparently similar in nature, did contain the Thorotrast particles which had penetrated the basement membrane (Fig. 6). In some instances, what may be interpreted as gross breaks in the membrane were observed (Fig. 7), in which cases the concentration of Thorotrast within the intercellular space was high.

The surface cells lining the posterior chamber are, in the normal animal, joined together by many complicated interdigitations, but attachment devices such as desmosomes are either very rare or absent. The pigment epithelial cells also fit together with intercellular interdigitations which are far less complicated and numerous than those of the surface cell layer and here, also, no desmosomes or similar structure is found. The cells of the surface and pigment layers themselves are, however, firmly attached to each other by numerous desmosome, "tight junctions," and occasional simple interdigitations or villus-like projections between the cells of the two layers illustrated in Fig. 8.

This firm juncture was disrupted in many places as a result of the experimental treatment. In such regions large intercellular spaces or vesicles were formed, into the lumen of which tongues of surface epi-
Protein leakage into eye following paracentesis

Fig. 11. Enlarged intercellular space between surface epithelial cell containing large clusters of Thorotrast particles (arrow) and large dark masses of fibrin (f). Note the absence of particles in the posterior chamber (P.C.). (Lead stain, Epon.)

Epithelial cells protruded as if, in fact, the two cell layers had just separated by withdrawing from each other (Fig. 8). Such spaces contained Thorotrast particles distributed more or less evenly throughout. All regions of a given ciliary process, or, indeed, all ciliary processes in one eye, were not equally affected by the experimental treatment. The thorium-dioxide particles were found in spaces where the disruption of cellular unions was greatest. Other areas of essentially normal appearing cells without enlarged intercellular areas contained no Thorotrast particles. The amount of particles present in these layers was in direct proportion to the severity of the morphologic change. When the intercellular space was only moderately enlarged, very few particles of thorium were found either there or in nearby intracytoplasmic vesicles (Fig. 9). In the areas in which severe morphologic changes had occurred, with extremely large intercellular vesicles, or spaces, the cytoplasm of the cells was often compressed into thin processes, and much of the regular cytoplasmic detail was lost.

The surface epithelial cells lining the posterior chamber normally possess numerous and very complicated intercellular interdigitations. The intercellular pathway, therefore, from the pigment epithelium to the posterior chamber is normally extremely narrow, long, and tortuous. This pathway, in the abnormal eyes, becomes much sim-
Fig. 12. This portion of the ciliary epithelium contains nearly normal membranous and cytoplasmic structures in one cell. The cytoplasm of the second cell is abnormally lucent. Thionin contrast particles (arrow) are present in the posterior chamber (P.C.). S.E., surface epithelium.

(Lead stain, Maraglas.)
pler, shorter, and wider. The complicated interdigitations disappear to a very large extent, and dilatations or intercellular vesicles appear. In addition, the more markedly affected cells appear to be lower in height than normal. In other instances the general cytoplasm appeared to be more lucent than normal (Figs. 10, 11, and 12). In other cells the cytoplasmic components did not show any significant change and the mitochondria were normal in appearance. Where the intercellular spaces were dilated, Thorotrast particles were found (Figs. 9 and 10). Occasionally, particle-filled vesicles were also seen in the cytoplasm, but in many instances these were clearly diverticula from the intercellular space rather than pinocytotic vesicles in transit through the cytoplasm. Fig. 10 demonstrates the Thorotrast particles between two surface cells. In many instances the intercellular spaces between the surface cells were grossly dilated, containing clusters of Thorotrast particles. In these severely changed areas, the large spaces contained, in addition to the Thorotrast, masses of fibrous material believed to be fibrin (Fig. 11). This was identified on the basis of its similarity to intravascular fibrous material found in the same specimens and to published figures of this material. The fibers exhibited a periodicity which could be seen with higher magnification. The surface cells bordering the posterior chamber were covered by a basement membrane that also appeared in many areas to restrict the passage of the Thorotrast particles, which were seen to accumulate behind it in greater concentration than was found in the posterior chamber proper (Fig. 11). Fig. 12 is a tangential section near the surface of the ciliary epithelium in which the posterior chamber appears as a narrow cleft. Most of the cells on one side of this figure have retained essentially their normal architecture. In other areas the cytoplasm of the cells is more lucent than normally. The posterior chamber can be seen clearly delimited by the basement membrane which covers the ciliary epithelial cells.

Within the lumen of the posterior chamber, Thorotrast particles are readily demonstrable, indicating that they have passed from the capillaries through the connective tissue, epithelial layers, and entered the posterior chamber.

Discussion

With the advent of methods of morphologic investigation permitting greater resolution, attempts have been made to determine the nature of capillary changes in inflammation. Many of the investigations used electron dense tracers and electron microscopy to determine how the increase in capillary permeability takes place. Farquhar and Palade, studying the capillary permeability of normal and nephrotic rat kidney, concluded that the tracer material (ferritin and colloidal gold) left the capillary lumen via pores, or fenestrations. These structures, however, differ from those of the ciliary process in that no diaphragm, or membrane, closing the pore, is visible. In addition to the trans-pore route, their markers were transported out of the capillary via intracytoplasmic vesicles. The endothelial cell junctures were not the site of increased capillary permeability in this structure. Pappas and Tennyson, in contrast, found, as we have reported here, that the electron dense markers left the capillary lumen through openings formed by detachments of the endothelial cells. Both Farquhar and Palade's results, and the ones reported here, emphasize the basement membrane as an important structure in restriction of the outward movement of material from the capillary lumen. Studies of capillary permeability of inflamed tissue were conducted by Majno and Palade in which the capillary bed of the cremaster muscle was treated with histamine or serotonin. These amines caused an immediate increase in vascular permeability, and electron microscopy showed that the leakiness of the vessels was due to opening of the interendothelial junctures, through which their electron dense markers were seen to pass. Therefore, the break in the blood
aqueous barrier caused by paracentesis is due in part to a capillary change similar to that which occurs in inflammation induced in muscle tissues, as described by Majno and Palade, or by Movat and Fernando Neil, Cotran and Majno, or Florey. Formed elements of the blood also exit the capillaries by this route, as observed by Marchesi. Platelets, fibrin, and possibly an erythrocyte were seen to be leaving the capillaries in the present experiments through endothelial cell disjunction, accompanied by masses of Thorotrast. There was no evidence of restriction of the passage of thorium-dioxide particles in the connective tissue spaces between the capillaries and epithelium, although often particles were seen lying along the collagenous fibers, sometimes arranged in a pattern suggesting that they were adsorbed in an orderly manner with respect to the periodicity of the collagen fiber. This was not sufficiently frequent to suggest that diffusion of the particles was inhibited. The basement membrane underlying the epithelium, however, did clearly impede movement of the electron dense markers, as seen in Fig. 5, indicating that both the vascular and epithelial basement membranes play an important role in the complex called the blood aqueous barrier. In general, the particles appeared to have diffused through both the vascular and epithelial basement membranes. Some gross interruptions or very thin areas in the basement membranes underlying the pigment epithelium (Fig. 7) or covering the vascular endothelium were found. In the latter, blood platelets and/or fibrin were seen leaving the capillaries. Such conditions in both locations allowed unrestricted passage of Thorotrast. As long as the desmosomes, "tight junctions," and interdigitations remained intact between the pigment and surface cell layers, no Thorotrast particles were seen between them, indicating that if they enter this intercellular space they do so rarely. The width of the intercellular spaces is sufficient to permit their passage, if the spaces are free of material, not visible with the electron microscope, which would impede such passage. In our material there is no evidence of desmosomes or other intercellular attachments or of a zonula occludens in the ciliary epithelium which would restrict such movement, as in Descemet's endothelium.

The pathway of materials, on the order of the size of proteins, from the vascular channels to the posterior chamber, when the blood aqueous barrier is "broken," appears to be intercellular through large open passageways created by separation of the epithelial cells, which may form intercellular vesicles. That the thorium particles used were accompanied by proteins is indicated by the occasional presence of fibrin with them (Fig. 11). These findings, therefore, support the conclusion of physiologists that larger molecules entered the aqueous humor via pores, or intercellular channels. They also verify the nature of the vacuoles, described in 1894 by Greef and later by Poos. The conclusions of von Sallmann, based on light microscopy, that the blue dye, T1824, attached to protein, passed from the capillaries to the aqueous humor via intercellular channels, is also supported.

The changes seen in the ciliary epithelium and the underlying capillaries after paracentesis, or endotoxin injections, were drastic. Presumably, these cells recover speedily and re-form their complicated intercellular relationships and cytoplasmic structure, thereby re-establishing the normal blood aqueous barrier. Although such changes may not be as extreme in the primate as in the rabbit eye, the recovery of normal ocular function following surgical procedures, intraocular inflammation, or treatment with diisopropyl fluorophosphate re-emphasizes the enormous adaptability of cells.

We wish to express our appreciation to Professor J. Rhodin for his advice and particularly for the many hours' instruction he has given one of us (Y. F. Pei).

REFERENCES

Protein leakage into eye following paracentesis


