Peroxidase diffusion in the normal and laser-coagulated primate retina

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Diffusion processes in the normal and laser-coagulated squirrel monkey retina were studied by electron microscopy and peroxidase tracer techniques. In a group of monkeys, the right eye was coagulated with an American Optical Ruby laser. Two to three weeks later, peroxidase was injected through the pars plana, close to the surface of the retina, in both eyes of each animal. The animals were then put to death at time intervals ranging from 20 minutes to four hours after injection. In a second series of animals, the chorioretinal diffusion process was studied following laser treatment. In this group, peroxidase was injected into the cubital vein. The animals were then put to death at intervals ranging from five minutes to two hours after injection. Following intravascular injection, peroxidase diffused rapidly through the intercellular spaces in the noncoagulated retina but stopped at the tight junctions (zonulae occludentes) of the pigment epithelium. After intravenous injection, peroxidase diffused through the fenestrated endothelium of the choriocapillaries and through Bruch's membrane but, again, was unable to pass through the tight junctions of the pigment epithelium. No diffusion of peroxidase from the retinal capillaries into the tissue was observed. Laser coagulation produced a retinal scar through which intercellular peroxidase diffusion was allowed to take place in both directions across the site of the original junctional barrier. Retina-to-choroid diffusion was far more extensive than chorioretinal diffusion. Laser treatment also destroyed some choriocapillaries and altered the fenestrated endothelium of those that remained. This study complements previous findings on the avascular rabbit retina in which xenon arc photocoagulation was employed. These studies suggest that, following laser coagulation, the breakdown of pigment epithelial junctional complexes and alteration in the number and permeability of choriocapillaries are responsible for the disappearance of subretinal and intraretinal fluid in various retinal diseases.
Figs. 1 and 2. For legend see opposite page.
It was shown that intercellular lanthanum diffusion from the scleral side was stopped by the tight junctions between pigment epithelial cells. That these tight junctions formed an efficient diffusion barrier was subsequently confirmed by others with the use of Graham and Karnovsky's peroxidase tracer technique.

Lasansky and Wald showed that ferrocyanide, placed in the vitreous chamber, could readily gain access to retinal intercellular spaces. This was also demonstrated by Smelser and colleagues with the use of ferritin. Lasansky and Shakib have confirmed and expanded the details of this mechanism with the use of peroxidase. Peyman and associates showed that, when injected intravitreally, peroxidase can diffuse rapidly through retinal intercellular spaces until it, like lanthanum, is stopped by the tight junctions between pigment epithelial cells.

In previous experiments, changes in diffusion brought about as a result of xenon arc photoocoagulation were demonstrated in the nonvascularized rabbit retina. Lasansky and Wald showed that ferritin has diffused through the retinal intercellular spaces from the vitreal surface. It is observed between apical processes of the pigment epithelium and, for a small distance, between adjacent cell bodies of this layer (arrows). Peroxidase diffusion has stopped at the tight junction (zonula occludens) between the pigment epithelial cells.

The intercellular space (IS) extending from the tight junction to Bruch's membrane (BM) is free of peroxidase. A rod outer segment (OS) is also visible. (× 20,000.) 2. Normal squirrel monkey pigment epithelium 30 minutes after an intravitreal peroxidase injection. Bruch's membrane (BM) is filled with the protein, as are the basal infoldings (BI) of the pigment epithelium. Peroxidase has diffused through the intercellular space (arrows) and has stopped at the tight junction (ZO). A rod outer segment (OS) is also seen. (×20,000.)
Figs. 3, 4, and 5. Normal retinal capillary 30 minutes after an intravenous injection. Peroxidase (P) is retained within the capillary lumen. An erythrocyte (E) and an endothelial cell nucleus (N) are also visible. The area outlined by the rectangle is magnified in Fig. 4. (x13,000.) 4. Magnified view of area outlined in Fig. 3. Diffusion of peroxidase (P) from the capillary is stopped by the pentalaminar tight junction (arrow) between contiguous elements of the endothelial cell. (x42,250.) 5. Normal choriocapillary endothelium (CE), Bruch's membrane (BM), and basal area of normal pigment epithelium (PE) 30 minutes after an intravenous injection. Peroxidase (P) is present in the capillary lumen, Bruch's membrane, and basal infoldings (BI) of the pigment epithelium. Its relationship to fenestrations (arrows) in the capillary endothelium is also seen. (x45,200.)

stained with uranyl acetate and lead citrate, and examined with a Siemens IA electron microscope.

Results

Normal retina. When peroxidase was injected intravitreally, it diffused rapidly through the inner limiting membrane and intercellular spaces of the monkey retina and accumulated between the outer segments and on the vitreal side of the pigment epithelium. Closer examination showed that it came to an abrupt stop on the vitreal side of the tight junctions between pigment epithelial cells (Fig. 1). Diffusion was complete 20 minutes after injection and did not progress further in animals which were put to death thereafter. When peroxidase was injected intravenously, it diffused through the fenestrated endothelium of the choriocapillaries (Fig. 5). Having done this, it penetrated Bruch's membrane and filled the basal infoldings of
the pigment epithelium. Further diffusion was halted on the scleral side of the tight junctions between adjacent pigment epithelial cells (Fig. 2). Peroxidase did not pass beyond these junctions at any subsequent time during the experiment, although it was present in pinocytic vesicles within the pigment epithelial cytoplasm after both routes of injection.

In contrast to choriocapillaries, retinal capillaries did not allow peroxidase diffusion through their walls (Fig. 3). Instead, its progress was stopped by tight junctions between contiguous surfaces of individual endothelial cells (Fig. 4). Peroxidase was seen occasionally in pinocytic vesicles within the cytoplasm of endothelial cells.

Laser-coagulated retina. Two to three weeks after laser coagulation, a portion of the normal retina was replaced by a scar which usually extended beyond the outer nuclear layer. Depending on the amount of energy absorbed, the treatment produced varying effects on part of the choroid, pigment epithelium, and photoreceptor layer, including the inner nuclear layer. The retinal layers close to the inner limiting membrane were less affected. After photocoagulation, choriocapillaries were destroyed in some areas and replaced by scar tissue. Vessels which were damaged rather than destroyed lacked the fenestrations...
typical of normal capillaries (Fig. 6). The regenerated pigment epithelium was smooth on its scleral surface and was without its usual basal infoldings (Figs. 8 and 9). Most significantly, tight junctions normally present between pigment epithelial cells of the untreated retina were lacking in the laser-treated retina.

Following intravitreous injection of the laser-treated eye, peroxidase diffusion from the vitreous to the choroid was unobstructed (Figs. 7 to 10). Peroxidase readily passed through the scar tissue and entered Bruch’s membrane (Figs. 8 to 10). From Bruch’s membrane it passed into intercellular spaces of the choroidal scar and...
Figs. 9 and 10. Retinal scar, Bruch's membrane (BM), and choriocapillary of laser-coagulated specimen four hours after an intravitreal injection. Peroxidase has passed through intercellular spaces (arrows) of the retinal scar and through Bruch's membrane. In addition, it has passed through intercellular spaces (arrows) of choroidal cells and entered the lumen (CL) of a choriocapillary. Basement infoldings of the scar cells lining Bruch's membrane are very small or absent. The area inside the rectangle is magnified in Fig. 10. (x11,450.) 10, Magnified view of area indicated in Fig. 9. Peroxidase (P) is shown entering Bruch's membrane. (x31,200.)
Fig. 11. Laser-treated retina two hours after an intravenous injection of peroxidase. Inner retina shows absence of peroxidase in the intercellular spaces (arrows). However, peroxidase (P) is present in the lumen of a retinal vessel, from which it cannot escape. (x8,900.)

Discussion

The results presented here for the vascularized primate retina complement previous results for the nonvascular rabbit retina.\textsuperscript{9,15,16} The access to retinal intercellular spaces for the bulk transport of metabolites is evident from the rapid diffusion of material into these spaces from the vitreous. The pigment epithelium, on the other hand, with its tight intercellular junctions represents a diffusion barrier to materials which leave the choriocapillaries. Therefore, under normal conditions, chorioretinal transport must occur across the pigment epithelium via active transport or by pinocytic mechanisms. No bulk transport occurs from choriocapillaries to the retina or vice versa. The tight junctions of the pigment epithelium represent the only known physical barrier to this transport.

The ophthalmoscopic threshold at which tight junction disruption occurs cannot be measured accurately. A finite amount of energy will cause visible changes in one area but will have somewhat different effects if applied elsewhere. Likewise, the disruption of junctional complexes is incomplete in the extreme periphery of coagulation even though ultrastructural...
changes are evident in photoreceptor outer segments. As a rule of thumb, however, the disruption of pigment epithelial junctions is complete when a gray discoloration with some gas formation is evident at the time of coagulation. Once disruption occurs, a junctional complex is not built again (although this is true for a retinal scar, it may not be the case in other tissues). The coagulation site in the pigment epithelium can be refilled by macrophages, pigment epithelial cells, and retinal glial cells, but the new occupants of this region lack complete tight junctions (zonulae occludentes).

Differences in the morphology and permeability of the two capillary systems that supply the retina have already been pointed out by several investigators, and this study complements those findings. In addition, it demonstrates the site of unobstructed diffusion from the choriocapillaries (endothelial fenestrations) and also shows the site of the diffusion barrier in retinal capillaries (tight junctions). It is only under certain pathologic conditions (neovascularization) that this retinal vascular barrier does not exist. Absence of the barrier in retinal capillaries results in abnormal transudation into the tissue, as is evident by fluorescein angiographic examination. Of these two capillary systems, the choriocapillaries are affected more by laser coagulation, since most of the energy is absorbed by the pigment epithelium. The loss of fenestrations from the endothelium facing Bruch's membrane probably results in reduced permeability for these vessels and is probably responsible for the reduced leakage implicated in fluorescein angiographic studies.
The finding that peroxidase has diffused from choriocapillaries into the intercellular spaces of external scar cells appears to contradict the results of fluorescein studies. In lesions of the type discussed here, fluorescein angiography quite often shows a white border around the chorioretinal scar, which probably represents only a slight diffusion of dye into these areas. Our results suggest a more marked diffusion into the scar, perhaps for two reasons. First, the technique employed in this study is far more sensitive than fluorescein angiography. A small amount of peroxidase can catalyze a large amount of reaction product from 3,3'-diaminobenzidine; as a result, very small amounts of peroxidase can be detected in the intercellular space. Second, sudden decompression of the eyeball during removal of the anterior segment for fixation (see Methods and materials) probably causes an increased flux of tracer from the choroid into the retina.

In addition to the alteration of choriocapillaries, photocoagulation, by destruction of the bulk diffusion barrier, connects the retinal intercellular spaces with those of the choroid. This, we believe, carries significant implications for the treatment of central serous retinopathy and retinoschisis. Interruption of the barrier between the retina and choroid could explain the rapid disappearance of abnormal subretinal or intraretinal fluid accumulation such as is observed after photocoagulation in central serous retinopathy and retinoschisis. Removal of the diffusion barrier probably produces the conditions for enhanced transport of fluid from the retina. We should emphasize that the effect of photocoagulation in central serous retinopathy is not a sealing off of the pigment epithelium, as is classically taught. On the contrary, photocoagulation disrupts the pigment epithelial barrier. As stated earlier, the remaining choriocapillaries are sealed off partially as well. In our experience, the destruction of choriocapillaries by ruby laser coagulation is not as pronounced as with xenon arc photocoagulation. This may explain why xenon arc photocoagulation is the more effective method in the treatment of central serous retinopathy.

Studies with the squirrel monkey retina as a model and earlier studies of a similar type on the rabbit retina suggest the following beneficial effects of photocoagulation on a variety of retinal disorders: (1) destruction of the site of abnormal transudation, (2) formation of a chorioretinal adhesion, and (3) increased reabsorption of abnormally accumulated retinal fluid by destruction of junctional complexes between pigment epithelium.

We would like to extend our sincere thanks to Monica Guthrie and Lucia Vedeges for their tissue sectioning skills, to Marcia Lloyd for tissue sectioning and some of the electron microscopy, and to Roger Witucki for preparation of the figures.

REFERENCES

8. Shakib, M., and McDonald, E. D.: Studies of the permeability of the blood-retinal barrier. VI. Junctional complexes of the pigment epithelium and their role in the permeability of the chorio-retinal barrier. Presented at the
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Spring Meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Fla., May 1 to 5, 1970.


