The authors have obtained evidence that destruction of the retinal pigment epithelium (RPE) causes choriocapillaris (CC) atrophy. The observations led us to hypothesize that the RPE modulates CC structure and function. Rabbits received injections of sodium iodate, which selectively destroyed the RPE. The authors killed the rabbits at various times after iodate and examined the RPE and CC by fluorescein angiography, fundus photography, and light and electron microscopy. Fluorescein angiography and fundus photography revealed a pattern of retinopathy similar to that described by other investigators, e.g., blood-retinal barrier breakdown and the patchy nature of the RPE/CC degeneration. One week after injection of iodate, the RPE transformed into a mixture of flattened, depigmented cells and plump, highly pigmented ones lying along Bruch's membrane. The CC appeared normal by light microscopy, but electron microscopy revealed changes indicating CC atrophy: degenerating endothelial cells (EC), EC that appeared normal but had reduced numbers of fenestrae, and pericapillary basal laminae that looped away from the endothelium, as if the latter had shrunk. One month after iodate, patches of Bruch's membrane were devoid of RPE, which was replaced by scar tissue. The CC was markedly atrophic over these patches, having reduced numbers of profiles and smaller lumina in those which remained. The CC appeared normal over areas where RPE remained. Eleven weeks after iodate, the light microscopic picture paralleled that seen 1 month after injection, but the patchy RPE degeneration was more extensive. By electron microscopy, the CC profiles over areas devoid of RPE showed severe atrophy. Degenerating EC were more numerous. EC adjacent to areas of RPE loss had few or no fenestrae. Here, capillaries were encased in dense, collagenous, connective tissue, unlike the CC of normal rabbits. These changes were not seen where the RPE still covered Bruch's membrane. These observations suggest that RPE modulates CC structure and function. The authors propose that a diffusible vascular modulating factor produced by RPE cells does this. Invest Ophthalmol Vis Sci 25:1135-1145, 1984

The causes of chorioretinal degenerations remain obscure. Initial choriocapillaris (CC) dysfunction, with secondary ischemic degeneration and atrophy of adjacent retinal pigment epithelium (RPE) is an oftencited mechanism. Rarely, however, has there been any consideration of the effect of RPE and/or outer neural retinal damage on the overlying CC. Sarks is one of the few proponents supporting the view that, in human disorders such as senile macular degeneration and retinal drusen formation associated with aging, the CC degenerates consequent to RPE absence. Despite extensive structural and functional investigations, iodate-induced retinopathy has never been used to explore the relationship between RPE and CC. For example, Ringvold et al did note reduced CC lumina and fenestrae in rabbits with iodate retinopathy, but they did not link these changes to the status of the RPE. In this report, we present experimental evidence that RPE destruction by sodium iodate precedes and initiates CC changes. We hypothesize that the RPE affects CC morphology and, in turn, its function.

Materials and Methods

Seven adult New Zealand (pigmented) rabbits were divided into three groups of two each (A, B, C) plus one control. All animals underwent baseline color fundus photography and fluorescein angiography. The six experimental animals received sodium iodate (0.08 M at pH 7.4) via an ear vein, in two divided doses (25 mg/kg) 4–6 hr apart. The control animal received injections of normal saline. Fundus photography and fluorescein angiography were repeated as follows: group A at 24 hours, 3 days, and 7 days; group B at 7 days, 14 days, and 28 days; group C at 21 days, 35 days, and 77 days; and control as in group B.
Fig. 1. Angiograms and fundus photos, control rabbit (A) and iodate rabbits (B-D). C and D are from color originals. A, Fluorescein angiogram (early phase) of a control rabbit. No evidence of leakage across the RPE. B, Fluorescein angiogram (early phase) from a rabbit that received sodium iodate 24 hr previously. Diffuse fluorescence indicates leakage across the RPE. C, Fundus photo of a rabbit, 3 weeks after iodate. Patchy RPE degeneration is seen in the lower right of the field. The area is seen better 11 weeks after iodate (Fig. 1D). D, Same fundus, 11 weeks after iodate. Part of area of RPE degeneration seen in C has advanced, presumably becoming scar tissue (arrowheads denote boundary). Light and electron microscopy of such boundary regions are seen in Figures 7 and 8.

Animals received 25 mg/kg of nembutal and 300 mg/kg of urethane intravenously as anesthesia prior to photography. Two percent phenylephrine and 0.2% atropine drops were instilled topically in the conjunctival sac to dilate the pupils. Photography was performed using a Zeiss Fundus Flash camera and Ektachrome 64 film. Fluorescein angiography then was performed using standard fluorescein filters and Tri-X film. One ml of 0.5% sodium fluorescein was injected via an ear vein. Only the right eye of each animal underwent angiography.

The animals were perfused with 200 ml of 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer via the ascending aorta after ligation of the descending aorta for light and electron microscopy. The eyes were enucleated and immersed overnight in fixative at 4°C after removal of the anterior segment. The specimens then were dissected into smaller pieces, postfixed for 2 hr in 2% osmium tetroxide in phosphate buffer, alcohol dehydrated, and embedded in epoxy resin. Two-micron thick sections stained with toluidine blue were used for light microscopy. Thin sections were stained with lead citrate and uranyl acetate and examined in a Zeiss EM9S electron microscope. Rabbits were examined 1 week (1 rabbit), 4 weeks (1 rabbit), and 11 weeks (2 rabbits) after iodate. The control rabbit also was processed for electron microscopy.
The use of animals in this investigation conformed to the ARVO Resolution on the Use of Animals in Research.

Results

Angiography and Fundus Photography

Angiography revealed changes similar to those described by others. After 1 week, we saw no fluorescein leakage.

Fundus photography showed the development of scar tissue subsequent to RPE loss and the patchy nature of the RPE/CC degeneration (Figs. 1C, D).

Light and Electron Microscopy

The control rabbit RPE and CC appeared normal (Figs. 2A, D).
One week after iodate: The RPE was replaced by a layer of flattened, depigmented cells (probably modified RPE cells) along Bruch's membrane (Figs. 2B, 3A). The cells were connected by junctional complexes and still bore adherent junctions along Bruch's membrane, as described by Miki et al. But
other signs of their RPE nature were reduced or absent, such as basal folds, apical villi, and pigment granules (Fig. 3B).

Large, round, pigmented cells were scattered among the flattened cells (Figs. 2B, 4). While clearly RPE in nature (they contained many pigment granules), they, too, lacked the surface specializations of normal RPE cells, such as basal folds, apical folds, and junctional complexes.

The CC appeared normal by light microscopy, or had only slightly reduced lumina (cf, Fig. 2A, B). However, electron microscopy revealed several changes: (1) The pericapillary basal lamina no longer tightly invested many of the CC profiles; it looped away, as if left behind while the CC atrophied (Figs. 4, 5). (2) Endothelial cells (EC) had reduced numbers of fenestrae on their side facing Bruch’s membrane (Fig. 5). (3) EC degenerated (Fig. 6). (4) Some fibroblasts and granulocytes infiltrated the CC pericapillary space. (5) Some cell processes were inserted between the CC and the RPE (Fig. 3B). We presumed these were pericyte processes because at high magnification they had filaments and submembranous densities characteristic of these cells, and they were connected to pericyte cell bodies in some fortuitous sections.

Four weeks after iodate: The RPE showed patchy degeneration (Figs. 7A–C). The CC over apparently normal RPE also appeared normal, whereas that over stretches of degenerated RPE was reduced or absent.

Eleven weeks after iodate: The major change evident by light microscopy was the occurrence of stretches of Bruch’s membrane with no apparent RPE cover (Fig. 2C, 8A). In these areas, there were multilayered cellular laminae composed of Müller cell processes and some flattened cells containing filaments and a few pigment granules—presumably transformed RPE.

The CC over these scars had reduced lumina and EC with few or no fenestrae (Figs. 8D, E) as compared with nearby areas where the RPE appeared normal (Figs. 8B, C). The CC over areas devoid of RPE was encased in dense collagenous connective tissue (Fig. 8E, 9)—presumably the result of fibroblasts invading the pericapillary space. By comparison, the capillaries over areas with RPE present retained loosely organized pericapillary connective tissue like that seen in the control rabbit (cf, Figs. 8B, D).

Degenerated EC were seen frequently in the CC over areas devoid of RPE (Fig. 9). They were more evident than in the 1-week model.
Fig. 5. One week after iodate, electron micrograph of CC. The endothelium has shrunk away from the pericapillary basal lamina (arrowheads). The pericapillary connective tissue (ct) appears denser than in control rabbits (cf, Fig. 2D)—a process that culminates in the even denser connective tissue encasing some degenerating CC at later stages (see Figs. 8, 9). The dense inclusions in the RPE are pigment granules (see Fig. 3B) (X22,100).

In both the 4-week and 11-week animals, we examined over a dozen sections spanning areas where the RPE stopped and was replaced by scar tissue. In every one, we made the correlation illustrated in Figures 7 and 8. No instances of the reverse, ie, CC loss where RPE was still present, were observed.

Fig. 6. Electron micrograph, 1 week after iodate. An atrophic capillary overlies affected RPE cells. All that remains of the capillary is its pericyte (p); the endothelium has degenerated, leaving some debris and the pericapillary basal lamina (arrowheads). A wandering blood cell is in the lumen (X5,700).
Discussion

Observations in human eyes suggest that RPE influences, or modulates the CC. Sarks\(^5\) most directly addresses this idea, stating explicitly that the CC degenerates or atrophies after RPE loss. Her opinion is based on histologic examination of over 500 cases of human senile macular degeneration and drusen formation. For example, only after RPE is lost over the drusen does the CC drop out. Gartner and Henkind\(^13\) describe CC loss after adjacent RPE loss in cases of retinitis pigmentosa, and Miller et al\(^14\)
Fig. 8. Correlative light and electron microscopy, 11 weeks after iodate. A, Light micrograph, retina with continuous RPE (left of dashed line) bordering retina with discontinuous RPE and focal scar tissue (right of dashed line). The CC is more abundant to the left than to the right (×340).

Fig. 8. B–E, Electron micrographs are from adjacent thin sections. Electron micrographs of CC over continuous RPE (Figs. 8B, C; to left of dashed line in Fig. 8A) and discontinuous RPE (Figs. 8D, E; to right of dashed line in Fig. 8A). Note presence of fenestrae (f) in Figs. 8B, C, over intact RPE (their basal folds and lipid droplets are at the bottom of the pictures). Fenestrae are absent in CC overlying retinal scar tissue in Figures 8D, E. Note these capillaries are encased in denser connective tissue than those over intact RPE (cf. Fig. 8B) or in normal retina (cf, Fig. 2D) (×12,800 [B, C], ×13,500 [D], and ×10,300 [E]).
describe CC loss where RPE degenerates in their ultrastructural study of a case of presumed thioridazine retinopathy. These observations point to some influence by RPE on CC structure (eg, the presence of fenestrae) and probably function—Federman suggests that number of fenestrae are a marker, or "barometer" of fluid and small molecule transport across the CC.

Our observations are the first attempt to prove the idea that RPE modulates CC structure and function.

**Fig. 8 (continued)**

**Fig. 9.** Electron micrograph, 11 weeks after iodate. A degenerated endothelial cell (arrow) occurs in the CC overlying retinal scar tissue (s). The pericapillary connective tissue is denser than normal (cf, Fig. 2D) (**×**12,500).
in an animal model. The sodium iodate retinopathy is suited for this. The chemical appears to selectively destroy the RPE in a patchy manner. This is seen by elimination of the retinal C-wave, which is due to RPE electrical activity, and RPE degeneration shortly after injection. 

If iodate affected the CC directly, we would expect to see evidence of CC degeneration over patches of normal RPE. We did not, nor have other investigators. 3-11

Neither was inflammation the primary cause of the CC changes. Although lymphocytes and macrophages occurred, they were not abundant at the times we sampled. We did not see lymphocytes, platelets or fibrin occluding CC lumena. This led us to believe that vascular occlusion due to inflammation did not cause the observed CC degeneration.

We expected little or no response in the CC after damaging the RPE and outer neural retina; when the neural retina is destroyed or compromised after optic nerve section, there is no apparent effect on the retinal vasculature. 16 To our surprise, however, we noted a clear-cut correlation between RPE loss and CC atrophy. This relationship was most evident in our 11-week model, where sections bridging patches of apparently normal RPE and neighboring scar tissue, outlined nicely in fundus photos (eg, Fig. 1D), revealed CC atrophy only over the latter region. We failed to find CC atrophy over areas of normal RPE.

This led us to hypothesize that the CC depends on the RPE, for example. In animals with a tapetum, the tapetal CC is fenestrated only where it bulges into tissue, outlined nicely in fundus photos (eg, Fig. 1D), revealed CC atrophy only over the latter region. We failed to find CC atrophy over areas of normal RPE. This led us to hypothesize that the CC depends on the intact, functioning RPE for its own survival.

Observations in other animal models support our hypothesis. In rats and monkeys, Kuwabara et al 17 mimicked human gyrate atrophy by giving intravitreal injections of ornithine hydrochloride. Choriocapillaris atrophy occurred only where the RPE degenerated. In monkeys with photic and thermal RPE damage, Kuwabara 18 also showed reduced CC fenestrae over areas devoid of RPE. Also in monkeys, Okisaka et al 19 described loss of fenestrae in ciliary body capillaries after destroying the pigmented ciliumary epithelium by perfusing hypertonic solutions through the internal carotid artery.

Even in normal animals, evidence of the RPE's control over CC structure has been seen. Choriocapillaris fenestrae are concentrated on the side facing the RPE, for example. In animals with a tapetum, the tapetal CC is fenestrated only where it bulges into RPE cells, 20 eg, in cats (Fig. 1 of reference 12).

Bellhorn and co-workers have explored the relationships between RPE and endothelia in rats with phototoxic and urethane retinopathies. 21,22 When photoreceptors are destroyed by light or subcutaneous injections of urethane, retinal capillaries become embedded in the RPE. The segments of capillary so enclosed develop fenestrae—but not the portion of the capillary remaining in the neural retina, ie, removed from intimate contact with RPE. Korte et al 23 have shown that the development of fenestrae in these intraepithelial capillary segments is associated with changes in their permeability. Indeed, the segments within the RPE assume the same permeability characteristics as the CC: intravenously injected horseradish peroxidase readily penetrates them, but larger molecules, like catalase, do not. The RPE exerts a profound effect on intraretinal, and probably choriocapillary, endothelial structure and function.

How does it do this? We propose that RPE releases a diffusible factor, a "vascular modulating factor" (VMF), which affects EC. This factor probably diffuses locally, rather than circulating, as the RPE's influence on endothelia always requires their approximation. This would explain why normally nonfenestrated retinal capillaries became fenestrated when embedded in the RPE of rats with phototoxic or urethane retinopathy, 22,23 or why the CC lost its fenestrae when the RPE was destroyed. In the case of the CC, the dependence of the VMF goes beyond merely maintaining structural specializations, like fenestrae, and may influence EC survival.

We admit that VMF is speculative. But it does explain observations (discussed above) drawn from human pathology, animal models and normal histology. It also provides a conceptual framework for experiments to determine if VMF exists, and if it is a product of RPE cells.

Key words: choriocapillaris, pathology, retinal pigment epithelium, sodium iodate, vascular modulating factor

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References