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UNDESCRIBED ENDOTHELIAL PROCESSES OF THE CHORIOCAPILLARIS EXTENDING TO THE RETINAL PIGMENT EPITHELIUM OF THE CHICK*†‡

BY

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DURING a fine structural analysis of the chick retina, unusual endothelial processes of the choriocapillaris were occasionally found in the matrix of Bruch's membrane. Such processes have not previously been observed in this area. The functional significance of these structures remains as yet unknown. Wolter (1955) observed delicate argentaffin fibre bundles passing through the choroidal matrix and dividing like roots of a tree in the outer layer of Bruch's membrane. It was believed these fibre bundles served as anchors. In this present study, processes of endothelial cells of the choriocapillaris vessels are shown to traverse Bruch's membrane and to come into very close relationship with the retinal pigment epithelium.

The purpose of this paper is to describe such endothelial processes of the choriocapillaris and to suggest their possible function.

Material and Methods

Chicks 5 to 10 days old were anaesthetized with chloroform and the eyes fixed by vascular perfusion through the aorta either with 6·25 per cent. glutaraldehyde (pH 7·2) buffered with 0·067 M phosphate buffer or with 1 per cent. osmium tetroxide (pH 7·4) buffered with 0·067 M phosphate buffer. Following the perfusion, the eyes were enucleated and further fixed in unbuffered 2 per cent. osmium tetroxide at 4°C. for 2 hours. The fixation and subsequent histological procedures have been described previously (Matsusaka, 1967). Ultrathin sections of these eyes were observed with a JEM-6C electron microscope (Japan Electron Optics Laboratory Co., Tokyo, Japan).

Observations

The endothelium of the choriocapillaris was characterized by a markedly attenuated cytoplasm, about 40 mμ thick, on the side of the capillary adjacent to Bruch's membrane (Fig. 1). The several layers of homogeneous and fibrous elements which compose Bruch's membrane (Fig. 2) were less distinct in the current chick material than usually described. The main dense layer (see Fig. 2*c*, arrow) was discontinuous, but could be recognized near the capillary endothelium. The basal lamina of the choriocapillaris vessels was not clearly demarcated, but that of the retinal pigment epithelium was regular and continuous.

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Bruch's membrane was about 1·5 μ thick. Though previously thought to be without cellular elements, endothelial processes were observed traversing it (Fig. 1).

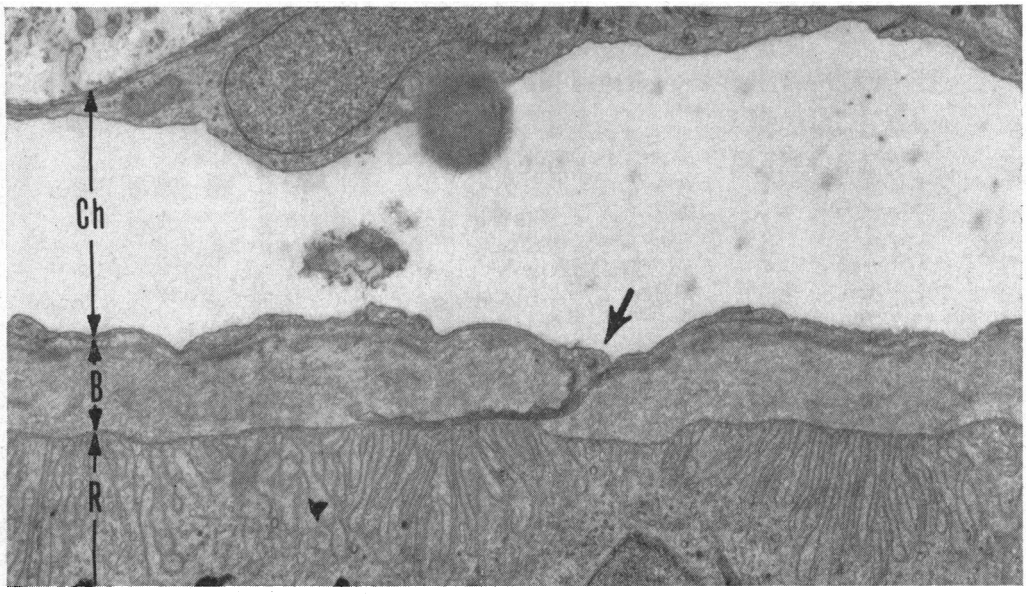


FIG. 1.—Electron micrograph of the choriocapillaris (Ch), Bruch's membrane (B), and retinal pigment epithelium (R), showing a cytoplasmic process arising from the endothelial cell of the choriocapillaris and approaching closely the retinal pigment epithelium (arrow). Glutaraldehyde fixation. Uranyl acetate-lead hydroxide stain. ×8,400.

These arose from local thickenings of endothelial cells of choriocapillaris vessels close to Bruch's membrane. When sectioned parallel to and including their course, the processes were seen to be approximately 4μ long. Serial sections showed them to be slender finger-like processes (Fig. 2*a–c*). After emerging from the endothelial cell, they pierced the intervening fibrous dense layer and passed through the main substance of Bruch's membrane in direct contact with it. The processes extended along the basal surface of the pigment epithelium, which here showed regular infoldings. However, an ill-defined dense layer about 120 Å thick was constantly present between the processes and the pigment epithelium; thus the processes were not in direct contact with the pigment epithelial cells. In occasional areas there were small profiles of membrane-limited cytoplasm in Bruch's membrane which seemed to be cross-sections of these endothelial processes (Fig. 6, arrows).

The luminal surfaces of choriocapillaris endothelial cells showed a few mitochondria, dense bodies, caveolae, and vesicles at sites where these processes originated (Figs 2, 4, and 5). However, these organelles were not found in the processes themselves. The processes contained a large number of parallel fibrils approximately 80 Å in diameter (Fig. 3) which seemed to fuse with the plasma membrane at the cytoplasmic thickenings, characterizing the origins of the processes (Fig. 2*b*).

Discussion

In as much as retinal vasculature of the chick is limited to the pecten, it seems evident that not only the retinal pigment epithelium but the entire retina must derive its nutrition from

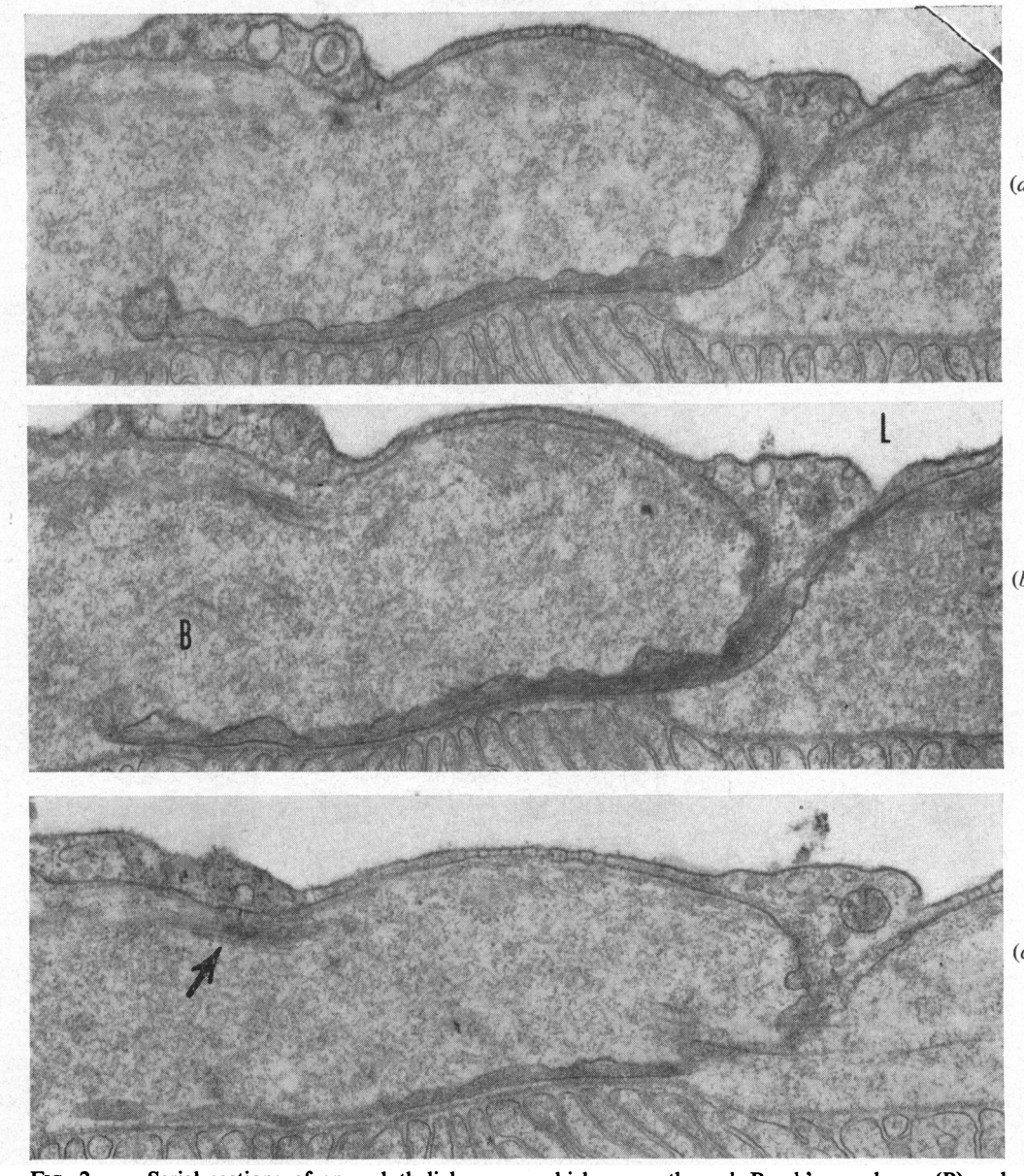


FIG. 2*a–c*.—Serial sections of an endothelial process which passes through Bruch's membrane (B) and comes to lie very close to the surface of the retinal pigment epithelium. Many fine fibrils can be seen in the cytoplasm of the process. L. capillary lumen. Arrow indicates intervening dense layer. Glutaraldehyde fixation. Uranyl acetate-lead hydroxide stain. ×26,400.

the choroid, presumably the choriocapillaris. However, direct evidence for the morphological relationship has not been previously apparent. The electron microscope showed the endothelial cytoplasm of the choriocapillaris to be extremely attenuated and to display capillary pores. Previous studies (Bernstein, 1961; Hogan and Feeney, 1961; Taniguchi, Ueno, Sumita, and Nakamizo, 1961; Missotten, 1962; Okuda, 1962; Bairati and Orzalesi,

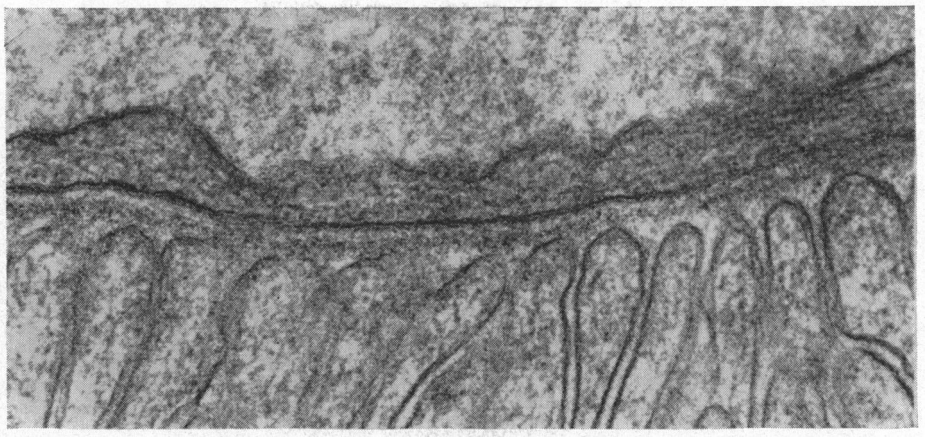
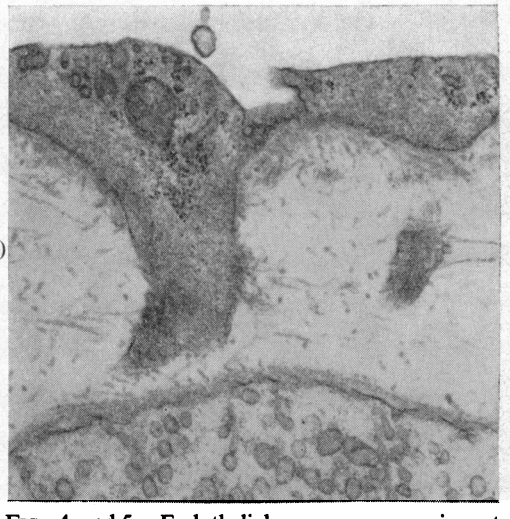
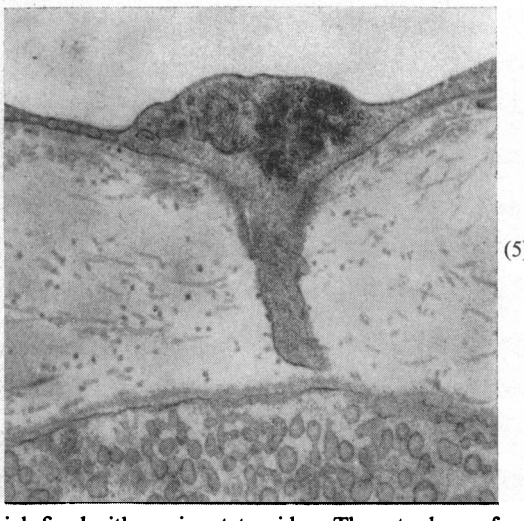


FIG. 3.—Higher magnification electron micrograph of Fig. 2*b*, showing an intimate relationship between the plasma membrane of an endothelial process and that of an adjacent retinal pigment epithelial cell. An ill-defined dense extracellular layer intervenes. ×84,500.

(4)





(5)

FIGS 4 and 5.—Endothelial processes as seen in materials fixed with osmium tetroxide. The cytoplasm of endothelial cells contains vesicles, ribosome particles, mitochondria, and dense bodies. Lead hydroxide stain. ×28,000.

1963; Lerche, 1963; Nakaizumi, 1964*a* and *b*; Nakaizumi, Hogan, and Feeney, 1964; Karli, Stoeckel, and Porte, 1965; Hogan and Alvarado, 1967) indicate Bruch's membrane to consist of homogeneous and fibrous materials, arranged in five layers:

(1) the basal lamina of the pigment epithelium;
(2) the inner collagenous zone;
(3) the interrupted elastic tissue zone;
(4) the outer collagenous zone;
(5) the basal lamina of the choriocapillary endothelium.

Leeson and Leeson (1967) reported cytoplasmic "slips" of fibroblasts and fibrocytes extending into Bruch's membrane in the rat. However, the extremities of these processes were not defined. In the present study, processes of endothelial cytoplasm not previously

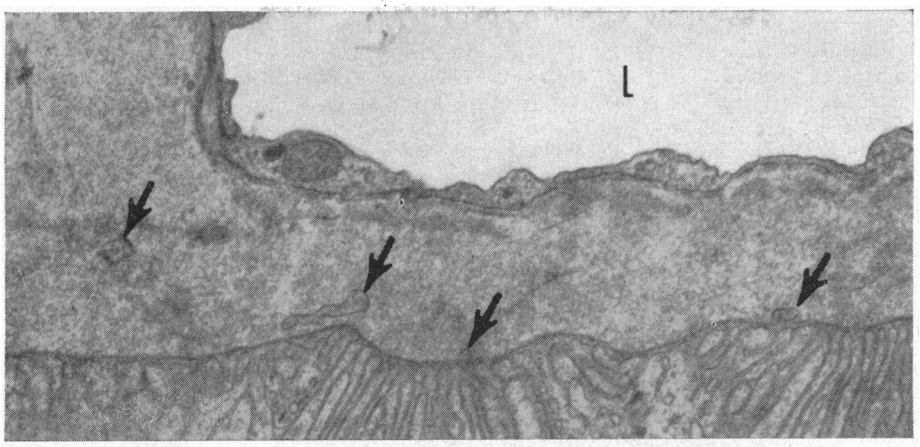


FIG. 6.—Small profiles of membrane-limited cytoplasm (arrows) in Bruch's membrane, representing cross-sections of endothelial processes. L. capillary lumen. Glutaraldehyde fixation. Uranyl acetate-lead hydroxide stain. ×14,000.

described were seen to pass through Bruch's membrane and terminate close to the retinal pigment epithelium. These processes seemed to connect the choriocapillaris with the retinal pigment epithelium. It could not be determined from morphological evidence how strong the strength of the connexions is, nor whether the fine fibrils in the processes serve a contractile function. Several investigators have suggested that fine fibrils in other capillary endothelial cells may mediate contraction (Fawcett, 1959; Hama, 1961; Zwillenberg and Zwillenberg, 1963; Bensch, Gordon, and Miller, 1964; Rhodin, 1967).

Processes arising from the outer surface of capillary endothelial cells have been observed by Suter and Majno (1965) in the skin and muscle of the newborn rat and by Shakib and Ashton (1966) in the retinal arterioles of cats with experimentally-induced ischaemia. Additionally, Rhodin (1967) demonstrated similar processes in arterioles and precapillaries in rabbit lung tissue. It is interesting to note that in these examples, as in the choriocapillaris endothelium described here, endothelial cells send out processes which make contact with another kind of cell. These associations suggest an important functional relationship. The fine fibrils in the processes resemble those of the intestinal microvilli (Ito, 1965; Rostgaard and Barrnett, 1965; Cardell, Badenhausen, and Porter, 1967). In the present study, the presence of caveolae and vesicles associated with the luminal surface of the choriocapillaris vessels may indicate that an active pinocytotic process takes place here. The cytoplasm at the origin of the processes also contains mitochondria, dense bodies, and dense particles, suggesting special oxidative and other physiological transactions at these sites. Since the endothelial cytoplasm of the processes is in close relation with the retinal pigment epithelium, the processes are considered to serve as pathways which facilitate transfer of metabolites between the vascular endothelium and the retinal pigment epithelium.

Summary

Eyeballs of 5 to 10-day-old chicks were fixed by vascular perfusion, either with 6·25 per cent. glutaraldehyde or with 1 per cent. osmium tetroxide, and prepared for electron microscopy. Fine structural analysis of the chick retina revealed hitherto undescribed endothelial processes of the choriocapillaris extending to the retinal pigment epithelium. A possible function of the processes is suggested.

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