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An Electron Microscopic Study of the Permeability of Iris Capillaries to Horseradish Peroxidase in the Vervet Monkey (*Cercopithecus aethiops*)

TORGEIR VEGGE
Anatomical Institute, University of Oslo, Oslo, Norway

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Summary. Anesthetized vervets were given intravenous injections of horseradish peroxidase. Subsequent studies of iris capillaries with the electron microscope showed peroxidase reaction product within the lumen of the vessels and in endothelial vesicles, but no peroxidase had penetrated the vascular endothelium. The normal ultrastructure of the vascular wall was retained.

Key Words: Iris — Capillaries — Horseradish peroxidase — Protein barrier — *Cercopithecus aethiops.*

Introduction

The composition of aqueous humour is to some extent modified by the blood vessels of the iris (Kinsey and Palm, 1955; Davson, 1963). In separate papers we have reported on the normal ultrastructure of vervet iris capillaries (Vegge, in press), and on the permeability to horseradish peroxidase in the ciliary processes (Vegge, 1971). Studies by Bill (1964, 1968) have indicated different permeability to serum proteins in the capillaries of the ciliary body as compared to those of the iris.

The present study is an attempt to clarify further vascular phenomena with a bearing on aqueous humour problems, and deals primarily with the permeability of iris capillaries to horseradish peroxidase in the vervet monkey.

Material and Methods

Both eyes from 6 vervets (*Cercopithecus aethiops*), weighing from 2 to 3 kg, were used. After an intramuscular injection of either 20 mg phencyclidine hydrochloride (Sernylan "Parke-Davis") or 15 to 30 mg methohexital sodium (Brietal "Lilly"), 10 to 15 mg mebumal sodium per kg body weight was given intravenously. When anesthesia was satisfactory, each of 4 animals was given an intravenous injection of 170 to 400 mg horseradish peroxidase (Sigma Type II, Sigma Chemical Company, St. Louis, Mo., U.S.A.) dissolved in 5–10 ml 0.9% NaCl. The eyes were enucleated 2 to 9 minutes after the peroxidase injection. In all cases respiration and pulse were regular until after the enucleations were completed. The eyes from these 4 animals are identical with those used in a previous study of the ciliary processes (Vegge, 1971), and details of the further treatment (fixation, incubation etc.) will be found there.

Parts of both eyes from 2 animals that did not receive peroxidase, were used as control specimens. After dissection, tissue sectors of the anterior segments of one of these animals were fixed for 2–3 hours in icebath-cooled 1.5% glutaraldehyde in 0.09 M sodium cacodylate with 0.025% $CaCl_2$ at pH 7.3–7.4. The other animal was fixed by perfusion of fixative through

the aorta. Details of this fixation is found under procedure 4 in a paper on the normal ultrastructure of the vervet iris vessels (Vegge, in press). After aldehyde fixation, the iris tissue from both these animals was treated identical to the peroxidase preparations (incubation etc.).

Ultrathin sectioning was done on LKB Ultrotomes. Sections were stained with lead citrate or with an aqueous solution of uranyl acetate, or both, and examined in Siemens Elmiskop 1 b or 1 A.

Observations

The normal ultrastructure of vervet iris capillaries have been described in a separate article (Vegge, in press). The only change observed in the present study from this normal structure, was the appearance of the black peroxidase reaction product.

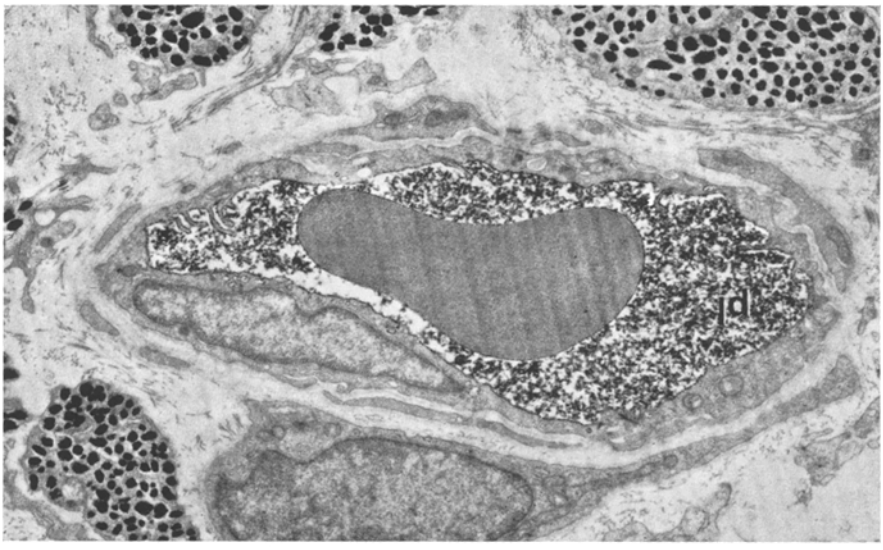


Fig. 1. Black, granular reaction product in the lumen of the capillary (*pl*). No reaction product outside the endothelium. ×7500

The black, granular, reaction product was found intravasally in varying amounts (Fig. 1). As a rule, all vessels contained reaction product. However, in some sections only some of the vessels had a distinct blackening of their lumen, whilst other lumens were quite pale. In many cases, even though the lumen showed little or no reaction product, there was evidence of ingestion of peroxidase by the endothelial cells (Fig. 2). The findings were similar in all peroxidase preparations, and the differences mentioned above could not be ascribed to different survival times. Fig. 3 shows a capillary from the same animal as that in Fig. 2. The preparation in Fig. 3 is from the right eye, enucleated 7 minutes after the injection of peroxidase, whereas Fig. 2 is from the left eye, enucleated 4 minutes after the injection. It will be clearly seen, that the amount of reaction product is greater in the vessel in Fig. 3 than in the one in Fig. 2. The preparation in Fig. 1, with a capillary rich in reaction product, is from the same eye as that in Fig. 2.

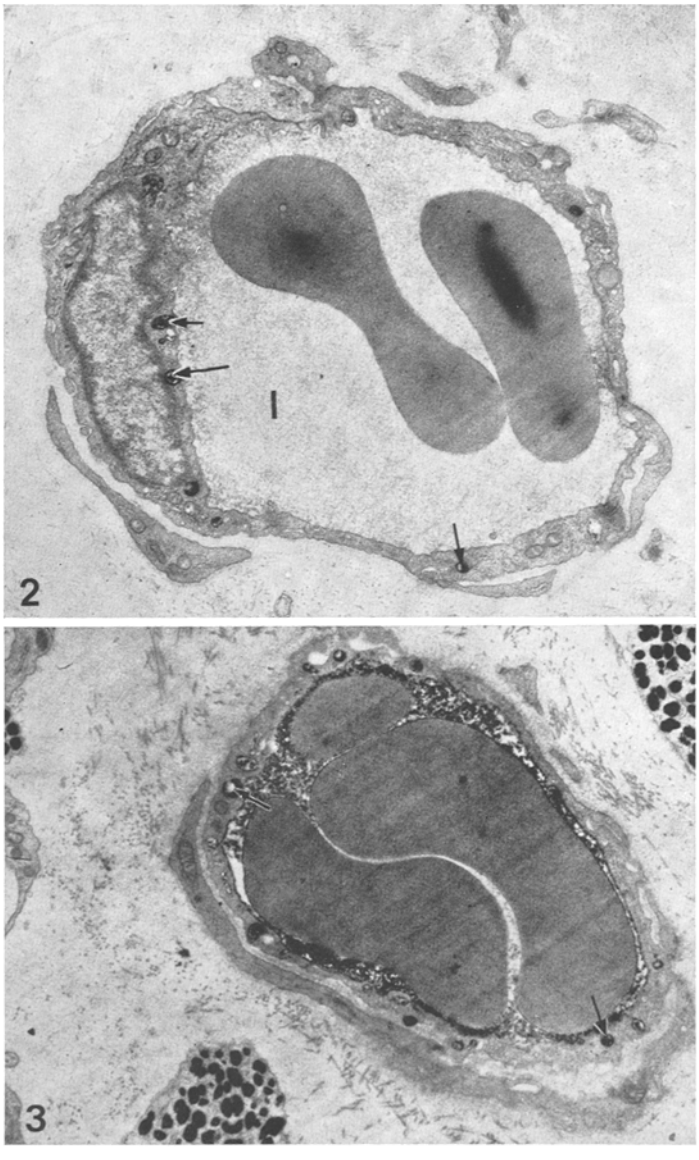
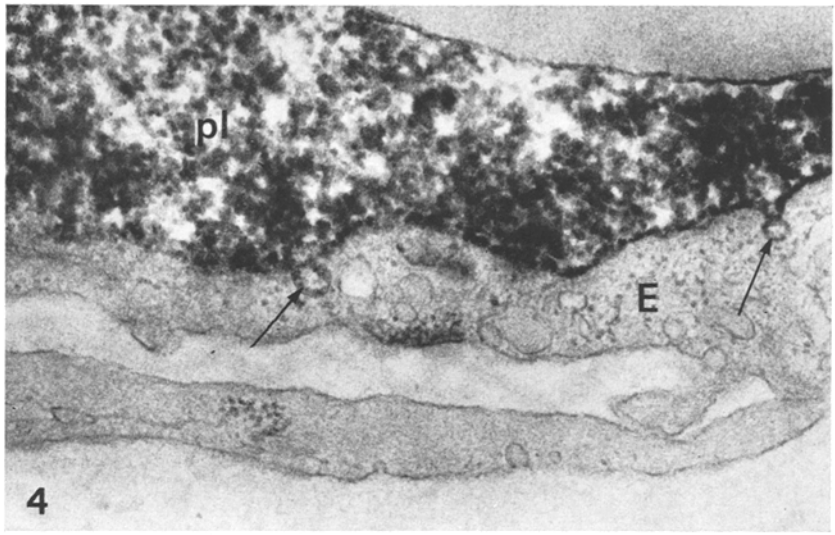


Fig. 2. Very little reaction product is seen in the lumen (*l*) of the capillary. Vesicles with reaction product are seen in the endothelium (*arrows*). Same eye as Fig. 1. ×7500

Fig. 3. Capillary lumen rich in reaction product. The product is also present in endothelial vesicles (*arrows*). Same animal as in Figs. 1 and 2, other eye. ×7500

Micropinocytic invaginations on the luminal surface of the endothelium were filled with reaction product (Fig. 4). Within the cytoplasm of the endothelial cells, vesicles partly filled with the product were also found. A few of these were of a size corresponding to the micropinocytic invaginations (Fig. 5), but often their diameter was much greater, up to 0.3 μ (Figs. 2, 6). Invaginations



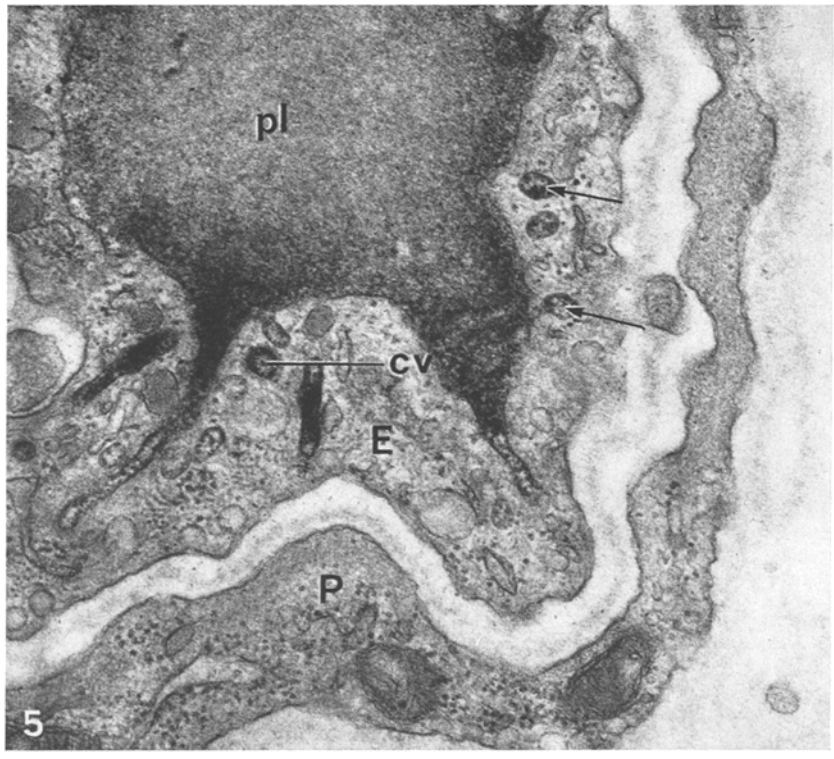


Fig. 4. Reaction product (*pl*) in capillary lumen and in micropinocytic invaginations (*arrows*) of luminal surface of the endothelial cell (*E*). ×45000

Fig. 5. Reaction product in the lumen (*pl*) is more dense peripherally. Several vesicles of micropinocytic size in the endothelium (*arrows*) contain reaction product. One coated vesicle (*cv*) is also seen to contain the product. Note that none of the micropinocytic invaginations on the basement membrane side show reaction product. Endothelial cell (*E*), pericyte (*P*). ×45000

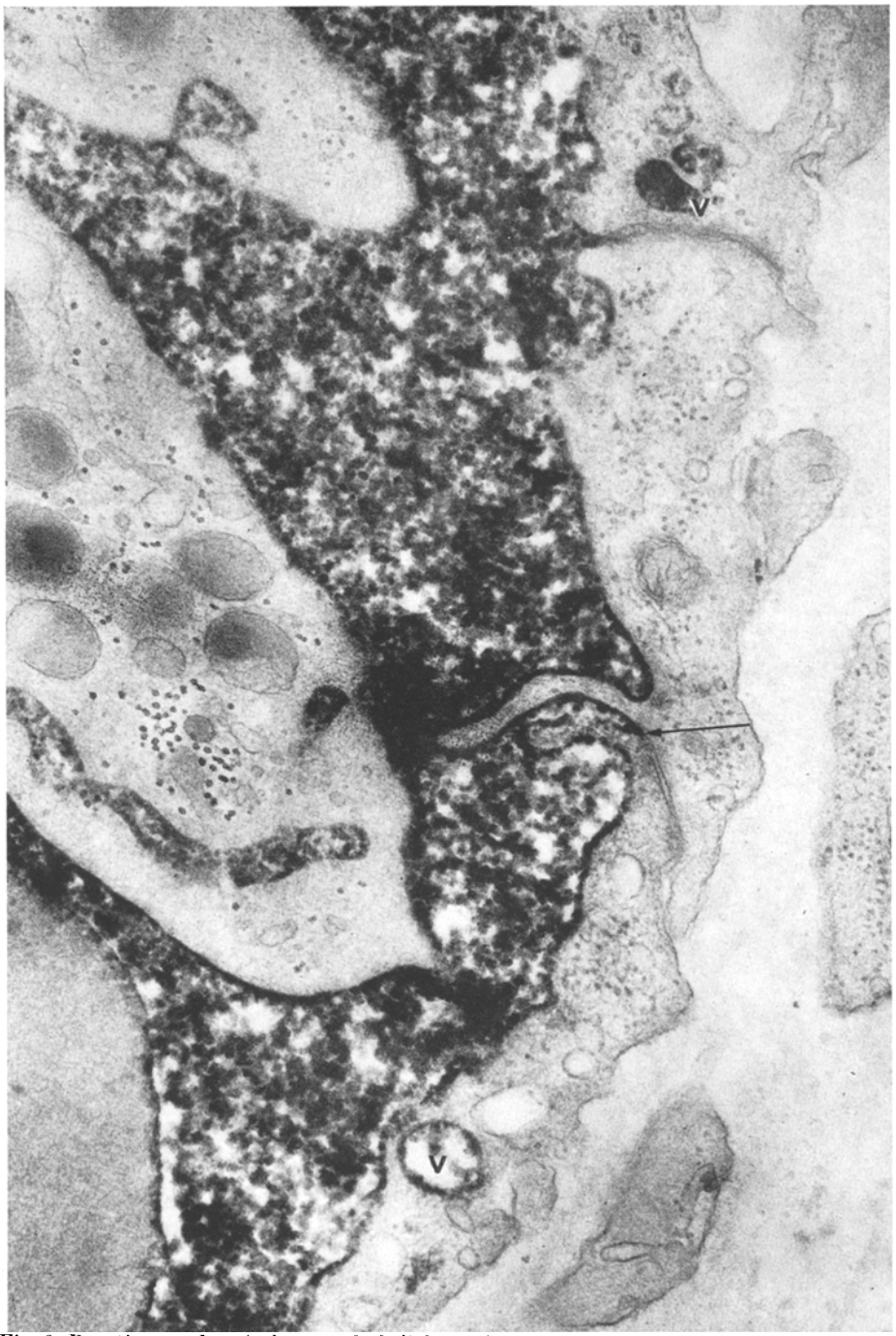


Fig. 6. Reaction product in large endothelial vesicles (*v*), and in the apical end of the intercellular cleft (*arrow*) between marginal folds. Note absence of product in the cleft peripheral to the luminal junction. Reaction product is also seen in two platelets in the capillary lumen. ×45000

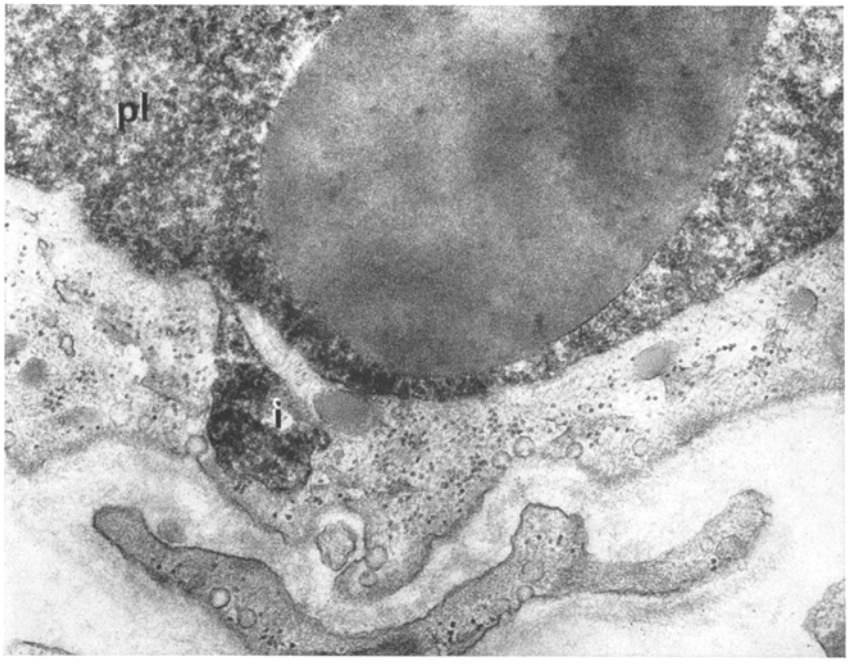


Fig. 7. Reaction product in lumen of capillary (*pl*). Note also reaction product in large invagination (*i*). ×40000

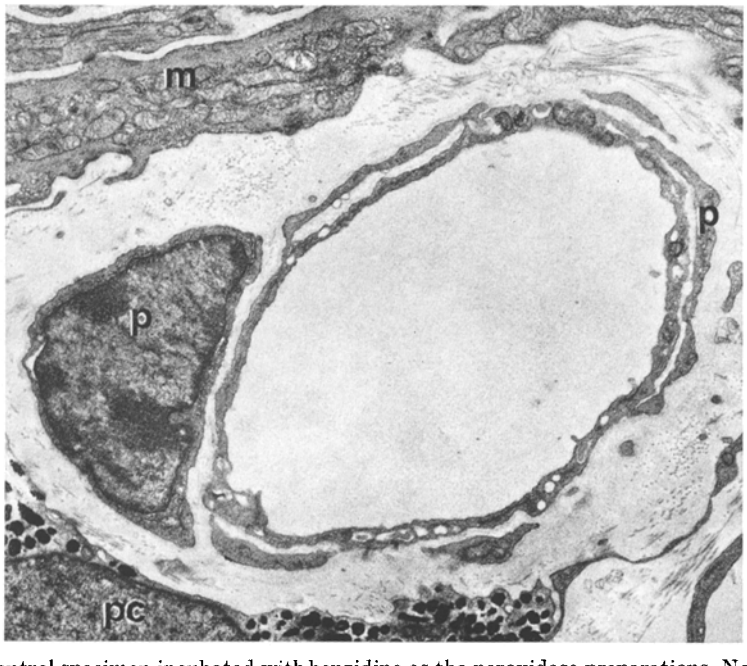


Fig. 8. Control specimen incubated with benzidine as the peroxidase preparations. No reaction product. Sphincter muscle cell (*m*), pigmented stroma cell (*pc*), pericytes (*p*). ×7500

of the cell surface corresponding in size to these larger vesicles, were also found (Fig. 7), although single sections do not show whether such invaginations are shaped like vesicles or grooves. Such invaginations were often associated with the marginal folds of the endothelial cells.

The reaction product was also found in the luminal part of intercellular clefts in the endothelium, but not peripheral to the intercellular junction (Fig. 6). The product was never found outside the endothelium, nor in micropinocytic invaginations on the basal aspect of the endothelial cells.

Tissue from the control specimens (Fig. 8) showed no reaction product.

Discussion

In a previous study, it was shown that the capillaries of the ciliary processes in the vervet are readily permeable to horseradish peroxidase (Vegge, 1971). However, a protein barrier was found to exist in the epithelial covering of the processes. This was in agreement with the findings of Bill (1964), that serum proteins penetrate into the stroma of the ciliary body, but do not enter the aqueous humour. Bill's studies also indicated that the permeability of the iris vessels differed from that of the ciliary body vessels (Bill, 1968). In the present study his findings are verified, insofar as it is shown that horseradish peroxidase, which has lower molecular weight than that of all the serum proteins (Vegge *et al.*, in press), with a molecular diameter of 44–47 Å, does not penetrate the wall of iris capillaries at time intervals up to 9 minutes. Compared with studies on the very same animals (Vegge, 1971), this shows a definite difference in capillary permeability in the two areas. It seems thus established that in the iris, a blood-aqueous barrier to protein exists in the vessel wall itself.

Cotran and Karnovsky (1967) believed that in some species, capillary permeability is artificially increased by the injection of horseradish peroxidase, perhaps due to histamine liberation. Winther (1971) showed that no such liberation took place in the guinea pig. The possibility that the permeability to horseradish peroxidase found in the ciliary processes of the vervet monkey (Vegge, 1971), should be induced by histamine liberation, seems to be ruled out by the present study. The animals in this study are identical with those used in the ciliary process study, and it seems hardly conceivable that such an artificial effect should be limited to the ciliary body, with no effect on the iris.

We should like at this point to emphasize that permeability studies of this type with macromolecules or heavy metal colloids as tracers, primarily give evidence about the presence or absence of "functional pores" in relation to the particular tracer used. Conclusions about the permeability to other particles, even of the same size or smaller, should be made with caution. Furthermore, when an enzyme molecule such as peroxidase is the tracer, one is hampered also by the fact that the amount of blackening seen in micrographs has no known ratio to the amount of tracer molecules present. Such studies, therefore, do not give quantitative information.

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Dr. Torgeir Vegge
Anatomical Institute
University of Oslo
Karl Johansgt. 47
Oslo 1, Norway