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An Electron Microscopic Study on the Blood-Optic Nerve and Fluid-Optic Nerve Barrier *

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Summary. Utilizing horseradish peroxidase as a tracer, electron microscopic studies were done on the blood-optic nerve and fluid-optic nerve barrier to the peroxidase diffusion. Following intravenous injection the peroxidase was observed to fill the lumen of the capillaries of the laminar, prelaminar and orbital portions of the optic nerve but there was no penetratation of the capillary walls. The obstruction of the tracer diffusion out of capillary walls was attributed to the tight junctions between the endothelial cells. Peroxidase penetration was also absent in the capillaries of the pia and dura mater, however, was observed in pinocytotic vesicles of the endothelial cells.

Lateral diffusion from the surrounding choroid into the optic nerve was detected but diffusion from the prelaminar optic nerve into the juxta-optic nerve retina was prevented by the Kuhnt intermediary tissue. Tight junctions which prevented peroxidase diffusion were found between the glial cells of the Kuhnt tissue, and this tissue was the barrier between the prelaminar optic nerve and the juxta-optic nerve retina.

Peroxidase which was given into the lateral ventricle of the brain appeared in the subarachnoidal space around the optic nerve and penetrated freely into the optic nerve. The pial surface of the optic nerve possess no barrier activity. Peroxidase could be traced along the intercellular space between glial cells and optic nerve fibers. The basal lamina of the optic nerve capillaries was filled with peroxidase but diffusion into the capillary lumen was obstructured by the tight junctions between the endothelial cells.

Zusammenfassung. Mit Hilfe von Meerrettich-Peroxidase wurden elektronenmikroskopische Tracer-Studien an der Blutsehnerven- und Liquorsehnervenbarriere unternommen. Nach intravenöser Injektion von Peroxidase füllte diese das Lumen der Capillaren des laminären, prälaminären und orbitalen Anteiles des Nervus opticus aus, ohne daß eine Penetration der Capillarwände zu beobachten war. Die Verhinderung einer Tracerdiffusion durch die Capillarwand wurde den "tight junctions" zwischen den Endothelzellen zugeschrieben. Auch bei den Capillaren der Pia und Dura fand sich keine Penetration von Peroxidase. Es wurden jedoch mit Peroxidase gefüllte pinocytotische Vesikel im Endothel beobachtet. Während eine laterale Diffusion von Peroxidase von der umgebenden Chorioidea in den Sehnerven festgestellt wurde, wurde eine Diffusion des Tracers vom prälaminären Anteil des Sehnerven in das umgebende Retinagewebe durch das Kuhnt'sche Zwischengewebe blockiert. "Tight junctions", die eine Diffusion von Peroxidase verhinderten, fanden sich zwischen den Gliederzellen des Kuhnt'schen Gewebes, so daß dieses Gewebe als Barriere zwischen dem prälaminären Anteil des Sehnerven und der juxtapapillären Netzhaut wirkte.

Peroxidase, die in einen Seitenventrikel des Gehirns appliziert wurde, erschien in dem den Sehnerven umgebenden Subarachnoidalraum und diffundierte ungehindert in den Sehnerven. Die Pia Mater des Sehnerven hatte somit keine Barrierenfunktion. Peroxidase konnte entlang den Intercellularspalten zwischen Gliazellen und Sehnervenfasern verfolgt werden. Die Basalmembran der Capillaren des Sehnerven war angefüllt mit Peroxidase. Eine Diffusion in das Capillarlumen wurde jedoch durch die "tight junctions" zwischen den Endothelzellen verhindert.

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A number of studies have covered the blood-ocular barriers but there are only a few reports on the existance of the blood-optic nerve barrier as demonstrated using electron microscope. Peyman and Apple (1972), in squirrel monkeys, utilizing electron microscope and horseradish peroxidase as tracer demonstrated that following intravenous injection, peroxidase was not detected external to the capillary walls of the optic nerve. In a preveous study (Yamashita *et al.*, 1973), utilizing light microscope, the existance of the blood-optic nerve barrier of the albino mice eyes was studied by tracing the horseradish peroxidase diffusion which was given into the vein or lateral ventricle of the brain. The present paper reports a result of our study on the same subject with electron microscope.

Material and Methods

Adult albino mice weighing 25–30 g were used. In a group of animals, under pentobarbital (Nembutal) anesthesia, 3 mg of horseradish peroxidase (HRPO) (Type II, Sigma Chemical Company) dissolved in 0.5 ml of isotonic saline was injected into the tail vein. At intervals of 15 and 30 seconds, 3, 5, 10, 30 and 60 minutes the animals were sacrificed and eyes were enucleated together with the long optic nerves and then immediately fixed in the fixative of 4% formaldehyde-5% glutaraldehyde (pH 7.4) for 3–5 hours at a temperature of 4° C. The eyes were then washed overnight in 0.1 M phosphate buffer at pH 7.4 at a temperature 4° C. Frozen sections of 10–20 μ thickness were made and each was incubated for 15 minutes in a solution consisting of 5 mg of 3,3-diaminobenzidine tetrahydrochloride and 0.1 ml of 1% H_2O_2 in 10 ml of tris-HCL buffer at pH 7.4 (Graham and Karnovsky) at room temperature. After incubation, specimens were washed in distilled water and postfixed for 60 minutes in 2% osmium tetroxide. Subsequent steps in preparation for electron microscopy were routine. They were dehydrated in alcohols and embedded in Epon 812. Ultrathin sections, cut on a Porter-Blum MT-2 Ultramicrotome, were examined in a HU-12 or JEM 7 electron microscope.

On the other group of animals, 0.12–0.15 mg of horseradish peroxidase dissolved in 0.02 to 0.03 ml of isotonic saline was injected into the lateral ventricle of the brain. Some animals were administered 0.01 ml of horseradish peroxidase solution into the lateral ventricle 3 times at intervals of 15 minutes. Fifteen minutes after injection the tissue was fixed by perfusion via either the left ventricle of the heart or aortic arch with 2% formaldehyde-2.5% glutaraldehyde mixture (pH 7.2) for 60 minutes at 4° C. The eyes together with the long optic nerves were removed and immersed in the same mixture for 60 minutes. Subsequent steps in preparation for electron microscopy were the same as used for the animals given an intravenous injection. Some sections were stained with lead citrate and uranyl acetate or uranyl acetate. Some of the specimens were stained en bloc with uranyl acetate.

In addition, the eyes not involved with any administration were used as controls.

Results

In the animals injected with horseradish peroxidase, (HRPO) intravenously HRPO was seen to fill the lumen of the capillaries of the orbital, laminar and prelaminar portion of the optic nerve, but the tracer diffusion out of the capillary walls was obstructed by tight junctions between endothelial cells (Fig. 1). HRPO penetration was likewise not detected in the capillaries of the pia and dura mater. However, HRPO was detected in pinocytotic vesicles of endothelial cells. At 8–10 minutes, HRPO which is derived from the surrounding choroid and sclera was evident in the intercellular space of glial cells and optic nerve fibers of the laminar and prelaminar portion. The basal lamina of the capillaries of this region was filled with HRPO but diffusion into the capillary lumen was obstructed. Diffusion of HRPO from the laminar and prelaminar part of the optic nerve into

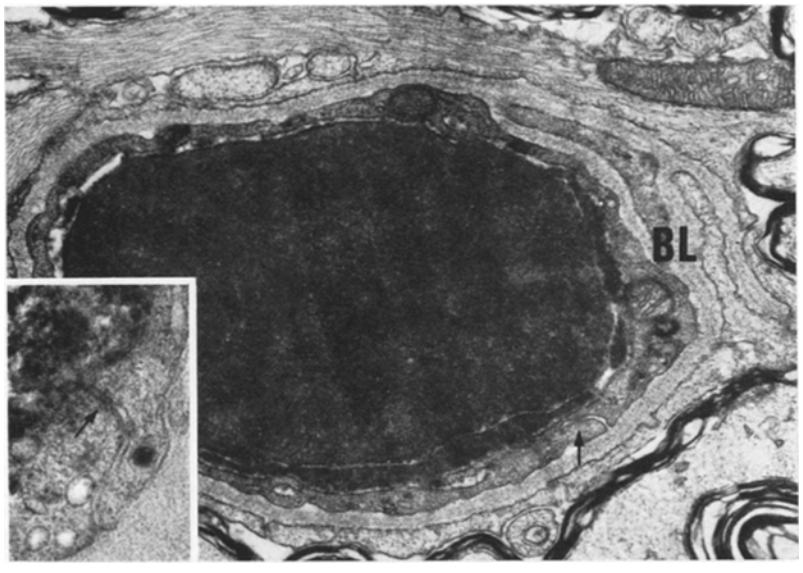


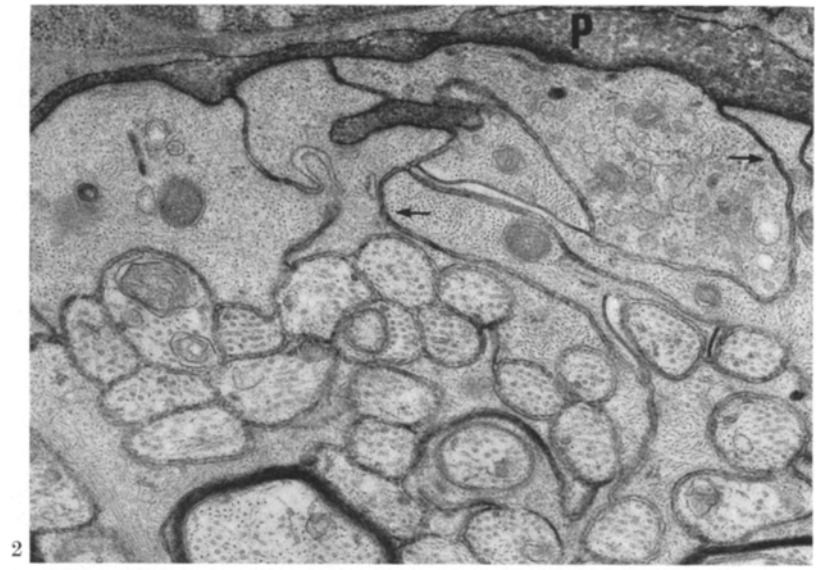
Fig. 1. A capillary of the orbital portion of the optic nerve 10 min after intravenous injection of horseradish peroxidase (HRPO). HRPO fills the capillary lumen, but outward diffusion of HRPO is obstructed by the tight junction (arrow) between endothelial cells. *BL* Basal lamina. Uranyl and lead stained. × 29000. Inset shows the tight junction at higher magnification. × 42000

the juxta-optic nerve retina was obstructed by the tight junctions between glial cells of the Kuhnt intermediary tissue.

Following intraventricular injection, HRPO appeared in the subarachnoidal space around the optic nerve within 15 minutes, diffused freely and could be traced along the intercellular space between glial cells and optic nerve fibers (Figs. 2–4) and connective tissue septa. This fact corresponds to the concept of transglia. The basal lamina of the optic nerve capillaries was filled with HRPO, but diffusion into the capillary lumen was obstructed by the tight junction of endothelial cells. However, HRPO was evident in pinocytotic vesicles of endothelial cells. HRPO could also be observed along the intercellular space between glial cells and optic nerve fibers of prelaminar (Fig. 5) and laminar (Fig. 6) optic nerve, intercellular space of glial cells of the Kuhnt intermediary tissue (Fig. 7) and in the connective tissue of the border between the optic nerve and sclera (Fig. 6). Diffusion of HRPO from laminar and prelaminar portion of the optic nerve into the juxta-optic nerve retina was prevented by the tight junctions between glial processes of the Kuhnt intermediary tissue and HRPO was not evident in the retina (Fig. 7). Glial processes of the Kuhnt intermediary tissue showed numerous microvillous projections on the retinal side (Fig. 7).

Discussion

According to Tansley (1956) the lamina cribrosa is very poorly developed in mice. In most mice, transverse fibers are not observed to cross the optic nerve (Tansley, 1956). In this paper, however, we used the terms of "prelaminar" and



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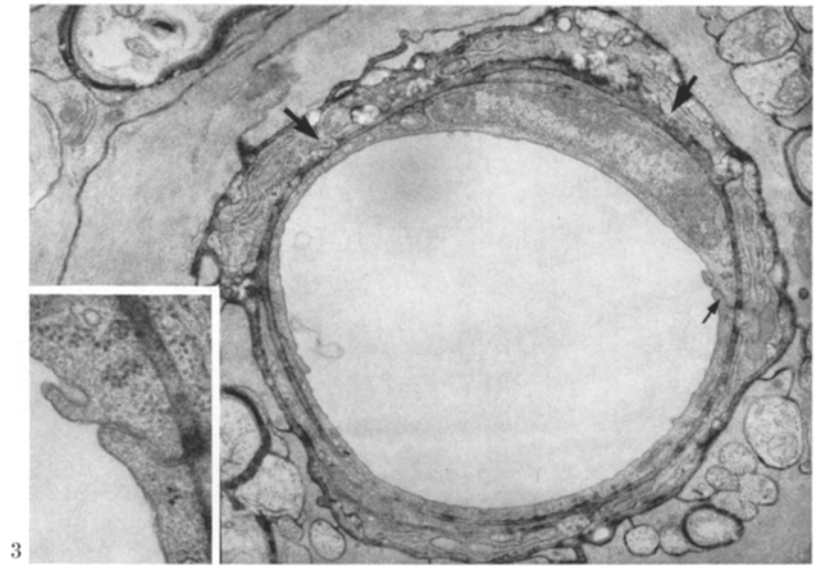

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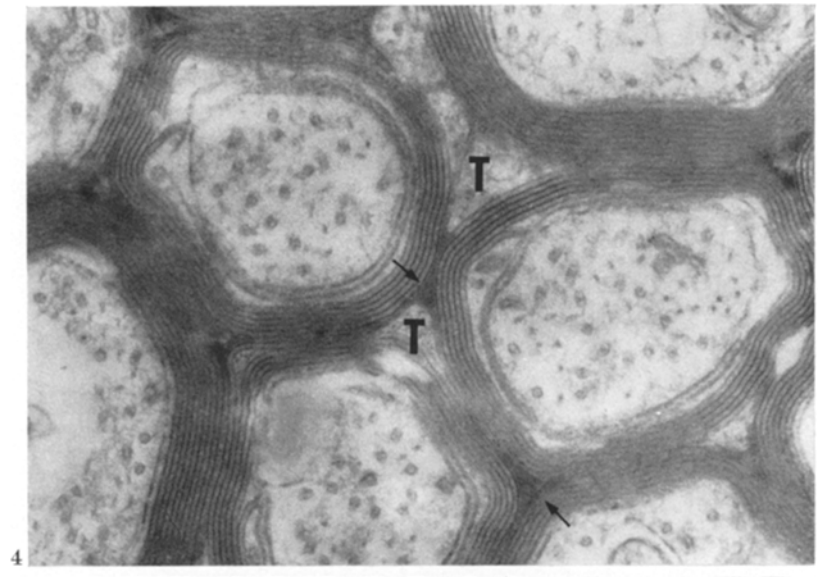
Fig. 2. HRPO from the subarachnoidal space can be traced along intercellular space of glial cells and optic nerve fibers (arrow). Uranyl en bloc stained. × 20000

Fig. 3. A capillary in the orbital portion of the optic nerve 15 min after intraventricular injection of HRPO. HRPO can be observed along intercellular space of glial cells and optic nerve fibers. The basal lamina of the capillary is filled with HRPO (thick arrow) but diffusion into the capillary lumen is obstructed by the tight junction (thin arrow) of endothelial cells. Uranyl en bloc stained. × 10000. Inset shows the tight junction at higher magnification. × 36000



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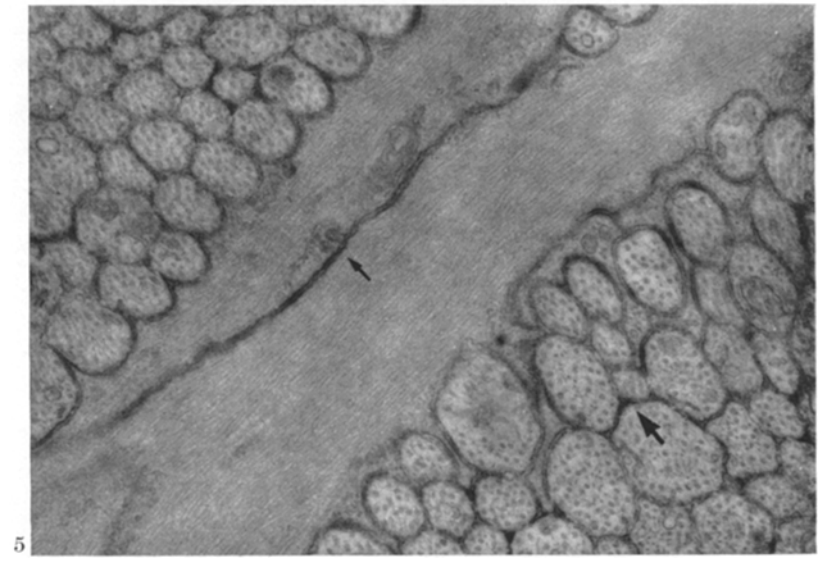

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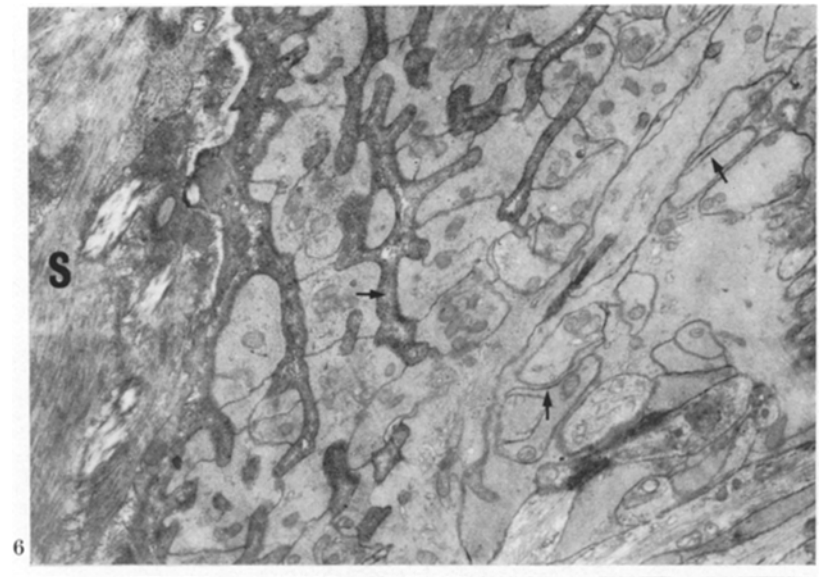
Fig. 4. An electron micrograph illustrating HRPO in intercellular space between glial cells and nerve fibers (arrow). Fifteen min after injection of HRPO into the lateral ventricle of the brain. *T* tangue-like processes of glial cells. Uranyl stained. × 59000

Fig. 5. An electron micrograph illustrating nerve fibers and glial processes of prelaminar region. Fifteen min after intraventricular injection. HRPO can be traced along intercellular space between glial processes (thin arrow) and nerve fibers (thick arrow). Uranyl stained. × 25000



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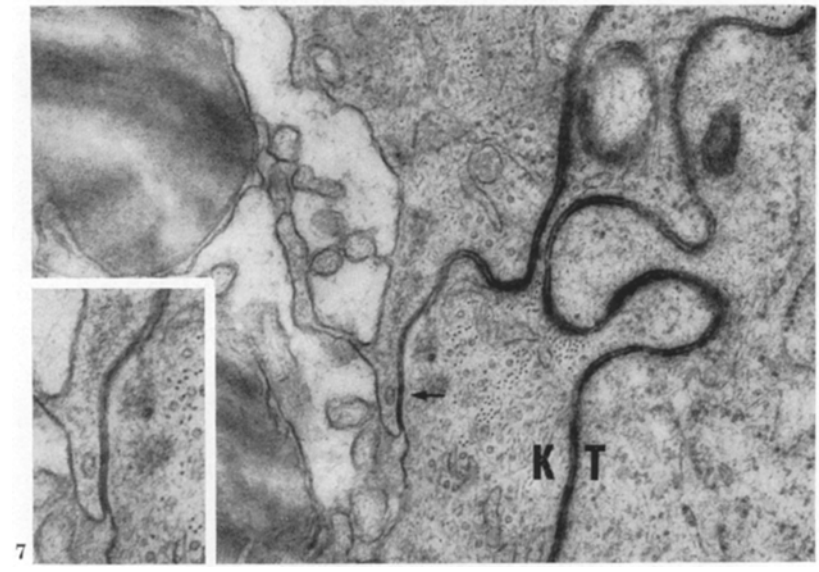

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Fig. 6. Border area between sclera and optic nerve at the posterior scleral foramen. Fifteen min after injection of HRPO into the lateral ventricle of the brain. HRPO appears in intercellular space between glial cells and nerve fibers (arrow) and connective tissue is stained by HRPO. (arrow). *S* sclera. Uranyl en bloc stained. × 7000

Fig. 7. An electron micrograph illustrating a tight junction (arrow) between glial processes of the Kuhnt intermediary tissue (*KT*). HRPO can traced along intercellular space of glial processes but diffusion into the retina is obstructed by the tight junction and HRPO is not detected in the retina. OS outer segment of photorecepter. Microvillous projections can be observed on the retinal side of the Kuhnt tissue. Fifteen min after intraventricular injection. Uranyl en bloc stained. × 42000. Inset whows the tight junction between glial cells at higher magnification. × 67000

"laminar" to show the corresponding area of the optic nerve. In our preveous study (Yamashita *et al.*, 1973) using horseradish peroxidase HRPO as tracer and a light microscope, we observed that HRPO had arrived at blood vessels of the optic nerve, choroid and retina 15 seconds after intravenous injection. At 30 seconds HRPO was evedent in the laminar and retrolaminar part of the optic nerve and at 30 seconds HRPO was found along the intercellular space of glial cells of the Kuhnt intermediary tissue. The most intensively stained tissue by HRPO's reactive products in the areas of prelaminar and laminar optic nerve was observed 8 minutes after injection. Peyman and Apple (1972), utilizing an electron microscope and HRPO as tracer, demonstrated that following intravenous injection no peroxidase was detected external to the capillary walls of the optic nerve. The same results were obtained in the present study. HRPO diffusion barrier was demonstrated in the capillaries of prelaminar, laminar and orbital portion of the optic nerve of the mice. HRPO penetration was not detected in the capillaries of the dura mater, arachnoid and pia mater. HRPO diffusion out of the capillary wall was obstructed by the tight junctions between endothelial cells.

Grayson and Laties (1971) and we (Tsukahara and Ota, 1974) indicated that in the monkey, sodium fluorescein could leak from the sourrounding choroid into the optic nerve at the laminar and prelaminar part of the optic nerve. Recently Cohen (1973) suggested that potential anatomical defects exsisted in the blood-optic nerve barrier in this region. The data obtained in the present study also suggest the existence of a lateral diffusion of the tracer from the nearby choroid or from the nearby choroid and sclera into the laminar and prelaminar optic nerve. However, diffusion of HRPO from the prelaminar and laminar area into the adjacent retina (juxta-optic nerve retina) was obstructed by the tight junctions between glial cells of the Kuhnt intermediary tissue and HRPO was not evident in the retina. It seems probable that the Kuhnt intermediary tissue is responsible for the barrier among the prelaminar optic nerve and adjacent retina. Rodiguez-Peralta (1966) utilizing diaminoacridine as tracer and light microscope pointed out the barrier activity of the Kuhnt intermediary tissue but he thought that this tissue prevented diffusion of the tracer from the choroid into the optic nerve. However, in our opinion, the tracer penetrates from the choroid into the optic nerve but the Kuhnt tissue prevents the diffusion from the optic nerve into the juxta-optic nerve retina.

Following intraventricular injection HRPO in the subarachnoidal space around the optic nerve travels freely into the optic nerve. Pial surface of the optic nerve posses no barrier activity as in the brain.

Anderson (1969) demonstrated the tight junctions between mesothelial cells on the surface of the optic nerve but he did not determine whether or not there was a continuous closure of the intercellular junction among mesothelial cells. The data of the present study suggest that the mesothelium of the pia mater of the optic nerve does not act as a barrier to the diffusion of substances and there is no continuous closure of the intercellular junction.

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