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AN EVALUATION OF NORMAL STRIAL CAPILLARY TRANSPORT USING THE ELECTRON-OPAQUE TRACERS FERRITIN AND IRON DEXTRAN

J. Santos-Sacchi[1] and William F. Marovitz[2]

From the [1]Center for Biomedical Education and [2]Dept. of Otolaryngology, Mount Sinai School of Medicine, City University of New York, New York

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Abstract. Enzymatic tracer techniques to study normal and pathologic strial capillary transport pose various problems. The use of electron opaque tracers can circumvent many of these problems. Iron dextran (mol. diam 20–70 Å) and ferritin (mol. diam 110 Å) were injected intravenously and the mice sacrificed at intervals of ½, 1, 2, 5, and 24 h. The iron dextran results were unusual in that from ½ to 5 h after administration the tracer was present within the cytoplasmic matrix of endothelia, but by 24 h it had been cleared out. No transendothelial exchange was noted. The ferritin results were in conflict with previous results using horse-radish peroxidase. Transport of ferritin was minimal regardless of time sacrificed. No more than a few molecules were scattered about the capillary basal lamina. Those molecules transported across capillaries were apparently delivered by means of the micropinocytotic system. The results suggest a blood-strial barrier similar to the blood-thymic and blood-myenteric barriers. Experimental as well as control animals exhibited strial light cells which contained ferritin-like particles within their cytoplasmic matrices. These light cells are probably reticuloendothelial type cells. Ferritin may be useful to gauge strial capillary transport alterations associated with auditory pathologies.

The functional role of the stria vascularis, which includes the production of the endocochlear DC potential and ionic composition of the endolymph, is important for normal inner ear function (Honrubia & Ward, 1969; Konishi et al., 1966). The stria vascularis lines the lateral wall of the cochlear duct and is composed of three cell layers, termed marginal, intermediate and basal (Smith, 1957). The tissue is effectively a separate compartment sealed off from the endolymphatic space and spiral ligament by zonulae occludens which occur between the cell membranes of abutting marginal cells and abutting basal cells (Jahnke, 1976). This tight junctional seal around the stria limits the nutrient inflow from neighboring sources; therefore, the high strial metabolic requirements (Chou & Rodgers, 1962) must be met essentially by intrinsic capillary networks (Reale et al., 1975). Thus the permeability functions of the strial capillaries are paramount when considering the survival and function of the strial cells; accordingly, strial capillary permeability is important for normal cochlear function.

Tracer molecules have been used to map the transport of materials through strial capillaries (Yamamoto & Nakai, 1964; Duvall et al., 1971; Winther, 1971; Gorgas & Jahnke, 1974). Tight junctions have been found to exist between strial endothelial cells as have been found to exist between capillary endothelia of most CNS tissue (Duvall et al., 1971; Gorgas & Jahnke, 1974; Reese & Karnovsky, 1967). Tight junctional seals are known to prevent the passage of lipid insoluble materials through intercellular clefts. Therefore, passage of lipid insoluble materials through the capillaries of the stria vascularis appears to be limited to the micropinocytotic vesicular system (Duvall et al., 1971; Gorgas & Jahnke, 1974). By means of the vesicular system, transport of the tracer molecule horseradish peroxidase (HRP, mol. wt. 43 000, mol. diam 55 Å) is very rapid. As early as 1–2 min following HRP injection, Duvall et al. (1971) observed the HRP reaction product distributed through strial capillary basal lamina. In muscle, however, more than

5 min following HRP injection were required before Karnovsky (1967) found capillary basal lamina densely stained. Although transport of HRP in muscle capillaries was primarily by means of pinocytosis, intercellular cleft transport also occurred.

In contrast with other permeability studies, Osako & Hilding (1971) have reported that HRP is transported across strial capillaries by a route between endothelial cells and that micropinocytotic vesicles are absent within strial endothelia of both Ames Waltzer mice and normal Swiss strain mice.

Yamamoto & Nakai (1964) studied strial capillary transport in the Guinea pig using iron dextran. At no time point after injection (10 min to 72 h) was the tracer observed in capillary basal lamina, although membrane bound accumulations were frequently found within endothelia. While these authors determined the size of iron dextran to be 100 Å in diameter, others have shown that the diameter of this tracer ranges from 20–70 Å (Muir & Goldberg, 1961; Richter, 1959). Molecules this size should be transported across strial endothelia if indeed the HRP results are sound. In fact, if one considers that the transport rate of HRP (mol. diam 55 Å) is more rapid through strial than muscle capillaries and that transport in both cases primarily involves pinocytosis, then it is difficult to account for the iron dextran results in light of the successful transport of molecules as large as ferritin (mol. diam 110 Å) across muscle endothelia (Bruns & Palade, 1968). Clearly, the literature on strial capillary permeability is in conflict.

It should be noted that the use of HRP as a tracer to study strial capillary permeability has certain drawbacks. For example, the enzyme HRP was noted by Duvall et al. (1971) to cause strial capillary alterations, consisting of intraluminal protrusions of endothelial cell membranes and pores within capillary walls. Cell protrusions have been known to occur in injured cells (Trump & Arstila, 1974). The artifactual production of pores in strial capillaries could have resulted in the release of HRP to the basal lamina. Such changes do not occur in adjacent spiral ligament vessels, where no HRP exists. Whether strial capillary alteration is a manifestation of HRP toxicity to select portions of the inner ear is not clear; however, it has been found that perilymphatic perfusion of HRP in vivo is acutely ototoxic (Ross et al., 1977).

Other problems of HRP use include: (1) the enzyme must retain its catalytic activity until and throughout incubation; (2) quantitative analysis of transported tracer substance cannot be performed since a) the HRP itself is not directly visualized and b) the quantity and quality of the reaction product is dependent upon the quality of both the enzyme preparation and incubation medium, the penetration of the medium into the tissue and the duration of the incubation; (3) histamine, which affects vessel permeability and strial capillary blood flow, is released in some species in response to HRP injection; (4) an endogenous peroxidase reaction exists which may interfere with the interpretation of experimental findings.

These problems are especially troublesome when evaluating capillary permeability changes in experimentally induced pathologies. For example, Duvall et al. (1974) used the tracer substance HRP to evaluate strial capillary permeability after intense noise exposure. They suggested that some of their unusual experimental results may have been due to the degradation of the protein HRP by lysosomal enzymes. Of course, if HRP is inactivated by lysosomal hydrolases, then the electron dense HRP reaction product, which indicates the presence of HRP, will not be produced. Therefore, the transport of the tracer cannot be determined.

Certainly, then, HRP and similar enzymatic tracers pose serious problems when used as a means to gage strial capillary transport during pathologic as well as normal conditions. A reliable method of evaluating the function of strial capillaries without the limitations imposed by the use of an enzymatic tracer would be beneficial.

Table I. *Number of mice per experimental condition*

	Post injection sacrifice time (h)				
Tracer	½	1	2	5	24
Ferritin	6	4	3	4	7
Iron dextran	2	2	2	2	2

Many non-enzymatic, electron-opaque tracers have been used to study capillary transport at the ultrastructural level. In particular, ferritin has been successfully used to delineate certain aspects of transendothelial exchange in a variety of tissues (Farquhar et al., 1961; Bruns & Palade, 1968; Clementi & Palade, 1969). There are many reasons why ferritin may be considered an ideal tracer (Bruns & Palade, 1968) especially for strial capillaries. They include: (1) ferritin (mol. wt. 460 000; mol. diam 110 Å) is the size required for a probe molecule of the large pore transport system, which is reportedly the only system in strial capillaries; (2) it is a biological substance, similar in nature and in size to most plasma proteins; (3) high blood concentrations are well tolerated by experimental animals; (4) individual molecules can be identified within ultrathin sections and therefore a direct measure of transport is possible; (5) ferritin is resistant to lysosomal hydrolases (Drysdale & Munro, 1966; Coffey & DeDuve, 1968) and therefore may be useful in pathologic conditions.

The advantage which the electron opaque tracer molecule ferritin may provide over enzymatic tracers warranted its evaluation. In addition, because the iron dextran results reported by Yamamoto & Nakai (1964) are in conflict with other strial capillary permeability studies, a reevaluation was judged necessary. Therefore, it was the purpose of this study to evaluate strial capillary permeability with the electron-opaque tracer molecules ferritin and iron dextran.

MATERIALS AND METHODS

Tracer substances

Iron dextran (Imferon, Lakeside Labs, Milwaukee, Wis.) was dispersed in physiological saline with 0.5% phenol as a preservative, and contained the equivalent of 50 mg elemental iron per ml.

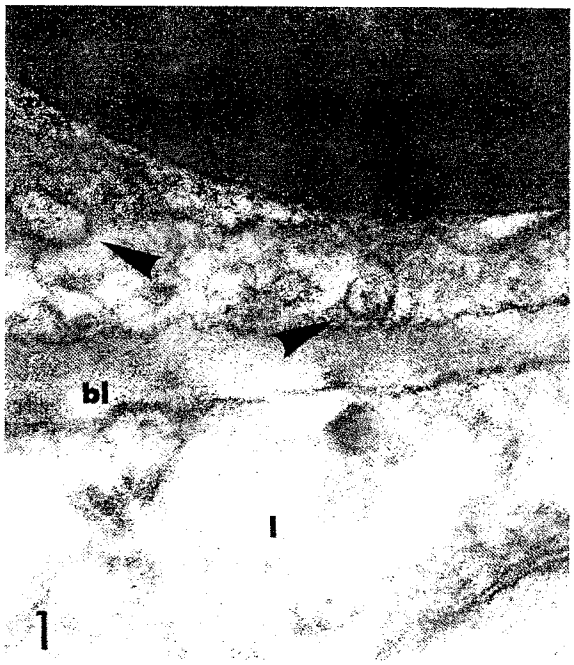
A 10 ml stock solution of 100 mg of cadmium crystallized ferritin per ml of physiological saline was obtained (Nutritional Biochemicals, Cleveland, Ohio). To remove the cadmium, the solution was dialysed against 3.7% ethylenediamine tetraacetic acid (EDTA) in 0.2 M phosphate buffer, pH 7.0, at 0–4°C for two changes (48 h each). Following two additional changes in buffer alone (24 h each), the ferritin solution was dialysed against physiological saline (24 h) and then passed through a 0.45 micrometer millipore filter into a sterile 10 ml injection vial.

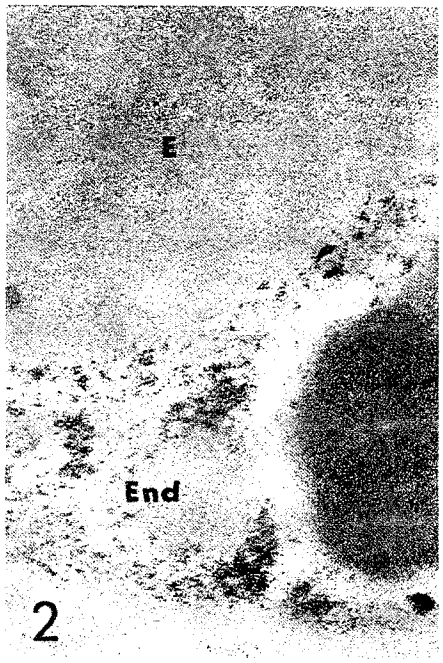
Animals

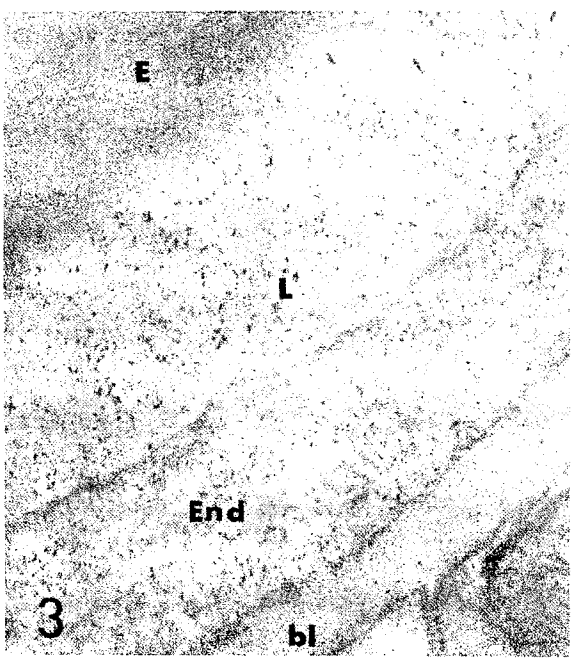
Young adult male mice, BALB c/J (Jackson Labs, Bar Harbor, Maine) ranging in weight from 15 to 20 g and showing positive Preyer reflexes to finger snaps were used. The animals were screened for middle ear infection by direct examination during temporal bone removal.

Methods

All animals received subcutaneous injections of sodium pentobarbital 2 mg/20 g body weight, prior to injection of tracer. Anesthesia was reached within 15 min, and lasted for 2–3 h for those animals allowed to recover. Under anesthesia, either the jugular vein or the inferior vena cava was exposed and was injected with 1 ml per 100 g body weight of ferritin or iron dextran solution. The injection was delivered through a 30 gauge needle over a period of 1–2 min. Animals were sacrificed by decapitation 1/2, 1, 2, 5, or 24 h after injection. Twelve control animals received anesthetic injection only and were sacrificed immediately after induction. The number of







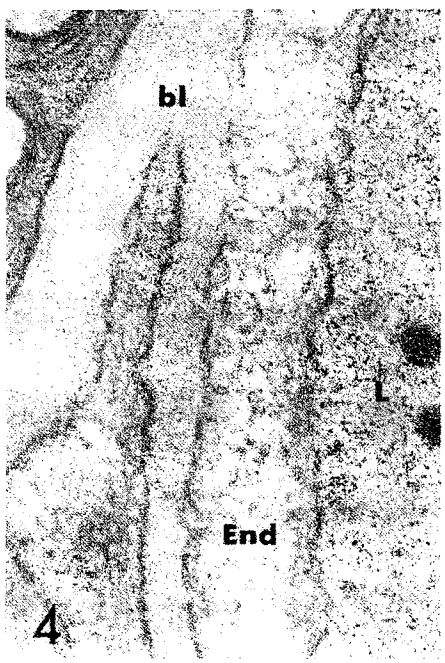


Fig. 1. Arrows indicate iron dextran within pinocytotic vesicles only of endothelial cell. ½ h post injection. Glutaraldehyde and osmium fixed. Unstained. ×78 380.

Fig. 2. Iron dextran within cytoplasm of endothelial cell. 1 h post injection. Glutaraldehyde fixation only. Unstained. ×69 215.

Fig. 3. Iron dextran within vesicles and free in cytoplasm of endothelial cell. 5 h post injection. Glutaraldehyde and osmium fixed. Unstained. ×80 000.

Fig. 4. Note absence of iron dextran in endothelial cytoplasm. 24 h post injection. Glutaraldehyde and osmium fixed. Unstained. ×83 300.

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bl, basal lamina; *E*, erythrocyte; *End*, endothelial cell; *I*, intermediate cell; *L*, capillary lumen; *R*, reticuloendothelial type cell.

animals per experimental condition is noted in Table 1.

Cochleae were fixed either in 1% osmium tetroxide or 2% glutaraldehyde in 0.2 M Cacodylate buffer, pH 7.4. Within 1 min after decapitation, the right temporal bone of each animal was excised, fractured open and immersed in about 5 ml of primary fixative. After 2 h of primary fixation at 0–4°C, all cochleae were briefly washed in buffer. Those ears fixed in glutaraldehyde were then osmicated for an additional 2 h. However, from each cochlea of those animals injected with iron dextran, pieces of stria were removed while in buffer and were not osmicated. At the completion of osmication, the specimens were again briefly washed in buffer. In ferritin injected animals, specimens were selected from basal, mid and apical portions of the cochlear duct. After the striae were microdissected free from the cochleae in 70% acetone, dehydration was completed in graded acetone, and the tissue embedded in Epon at 60°C for 24 h.

Ultra-thin sections were cut on glass and diamond knives using either the Sorvall MT 1 ultramicrotome or an LKB Ultratome. Section interference colors ranged around silver. Sections were picked up on copper grids and most were examined unstained so that electron dense tracer particles could be unequivocally identified. Some sections were stained with lead citrate (Reynolds, 1963). Contrast was significantly enhanced by the use of projector field diaphragms with the Zeiss 9S-2 EM.

RESULTS

Those animals allowed to recover from anaesthesia had positive Preyer reflexes. None of the experimental ears showed evidence of middle ear infection upon direct examination using a dissecting microscope.

The ultrastructural appearance of the stria vascularis was typical of the fixation procedure employed (Merck et al., 1974; Santos-Sacchi, 1978*a* and *b*). One noteworthy difference between the two fixation procedures was the decreased concentration of tracer particles within capillary lumina in primary osmium fixed specimens as compared to primary glutaraldehyde fixed tissues.

Iron dextran

The plasma concentration of iron dextran decreased over time. In the 1/2 h specimens, capillaries were observed which contained iron dextran only within endothelial pinocytotic vesicles (Fig. 1). However, more frequently, and in all animals beyond 1/2 h up to 5 h, the iron dextran appeared free as well as in vesicles within the cytoplasm of endothelia (Figs. 2 and 3). In fact, the tracer was occasionally observed within the nucleoplasm of some endothelia. By 24 h after injection, however, the iron dextran was absent from endothelia (Fig. 4). The passage of the tracer to the basal lamina was found to be almost nil, since rarely were any electron dense particles found in the basal lamina at any time point after injection. No tracer was observed within strial cells.

Ferritin

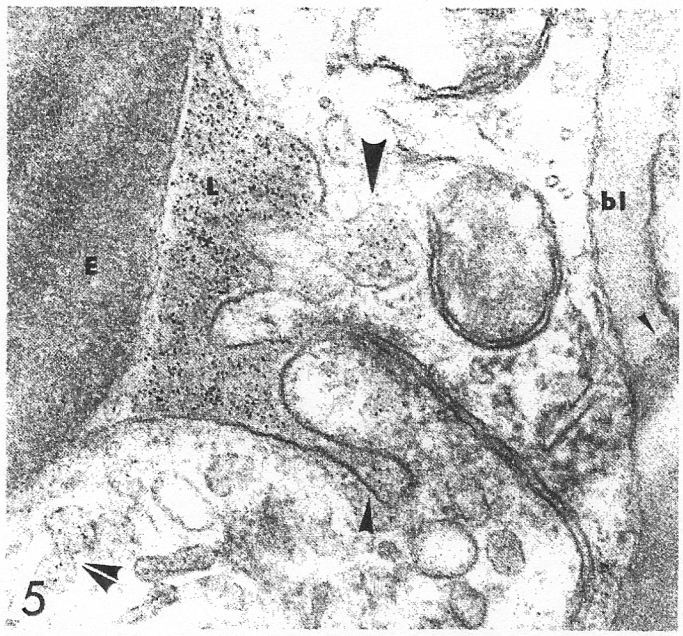
The appearance of ferritin within various spaces of the tissues studied was indicative of its passage from the circulatory system into the stria vascularis. The results are reported in terms of compartments within which ferritin was observed.

Capillary lumen

From 1/2 to 24 h following injection, ferritin was present within the capillary lumina of the stria in all areas studied. However, the concentration of ferritin within the blood plasma varied with time. By 24 h after ferritin administration the plasma concentration was considerably lower than its initial level. Ferritin was never present within capillary lumina of control animals.

Endothelial tunic

From the earliest to the latest time points after administration, ferritin labeled pinocytotic



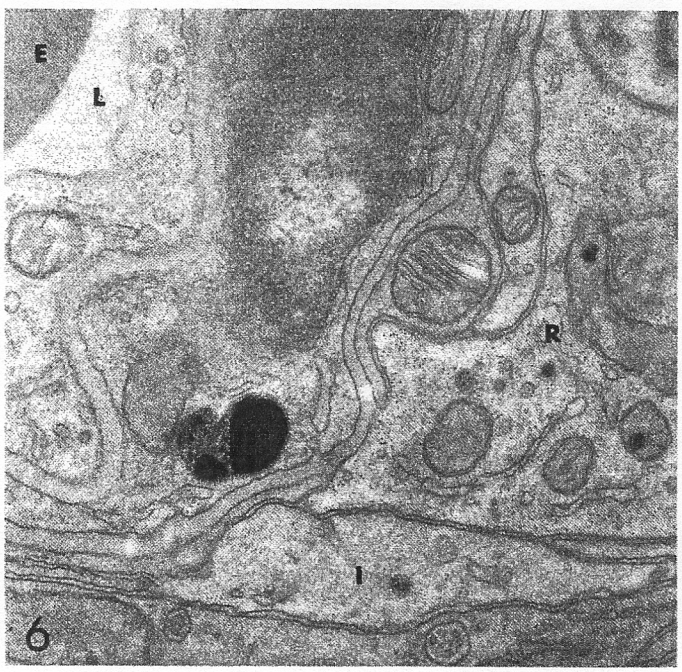


Fig. 5. Large arrow indicates open-mouthed vacuole containing large amount of ferritin on luminal front. Medium arrow indicates luminal tubular invagination containing ferritin. Double arrow indicates fusion of two labeled pinocytotic vesicles. Ferritin molecule can be seen in basal lamina (small arrow). ½ h post injection. Glutaraldehyde and osmium fixed. Unstained. Mid portion of cochlear duct. ×90 025.

Fig. 6. Control animal (uninjected) showing light cell (*R*) with abundant supply of endogenous ferritin-like granules. Osmium fixed. Unstained. ×39 600.

vesicles were observed within endothelial cells (Figs. 5, 7, 8 and 9). Labeling of any pinocytotic vesicles was limited to no more than a few molecules of ferritin. In any section, the number of such labeled vesicles per endothelial cell varied from none to a few, with no discernible relation to time after injection or area of cochlear duct. At times, the number of unlabeled vesicles was great. Although endothelial vesicles opening to the tissue front were

often empty, on occasion ferritin could be visualized within the basal lamina either just in front of the opening or not far away (Fig. 9). Nevertheless, there were direct observations of ferritin within vesicles opened upon the luminal and tissue fronts (Figs. 7, 8 and 9), as well as in those within the endothelial cytoplasm. Labeled vesicles within the endothelial cytoplasm were encountered more frequently. It cannot be ruled out that ferritin within open-mouthed vesicles diffused out during fixation.

Another occasional phenomenon was the appearance of tubular invaginations from luminal and tissue surface membranes of endothelial cells (Fig. 5). These structures were only encountered in thick portions of endothelia. Ferritin was found within some luminal invaginations, but direct connections from luminal to tissue fronts were never observed.

There appeared to be a relation between the time after injection and the accumulation of ferritin within vacuoles of endothelia. At the earlier time points, vacuoles containing ferritin in fairly large amounts were occasionally observed (Fig. 8). Rarely large vesicles with high concentrations of ferritin were seen open to the plasma front as if in the process of loading (Fig. 5). With increasing time, larger and denser cytoplasmic vacuoles were noted within which individual ferritin granules could be observed (Fig. 11). Pinocytotic vesicles labeled with ferritin were seen fused with large ferritin containing vacuoles (Fig. 8) or with each other (Fig. 5). Multi-vesicular bodies were also seen, that is, single membrane vacuoles within which were found labeled as well as unlabeled pinocytotic-like vesicles (Fig. 10).

Ferritin was neither seen in pinocytotic or other structures within endothelia of control animals, nor was it observed free in the endothelial cytoplasm of experimental or control animals.

Basal lamina and intercellular spaces

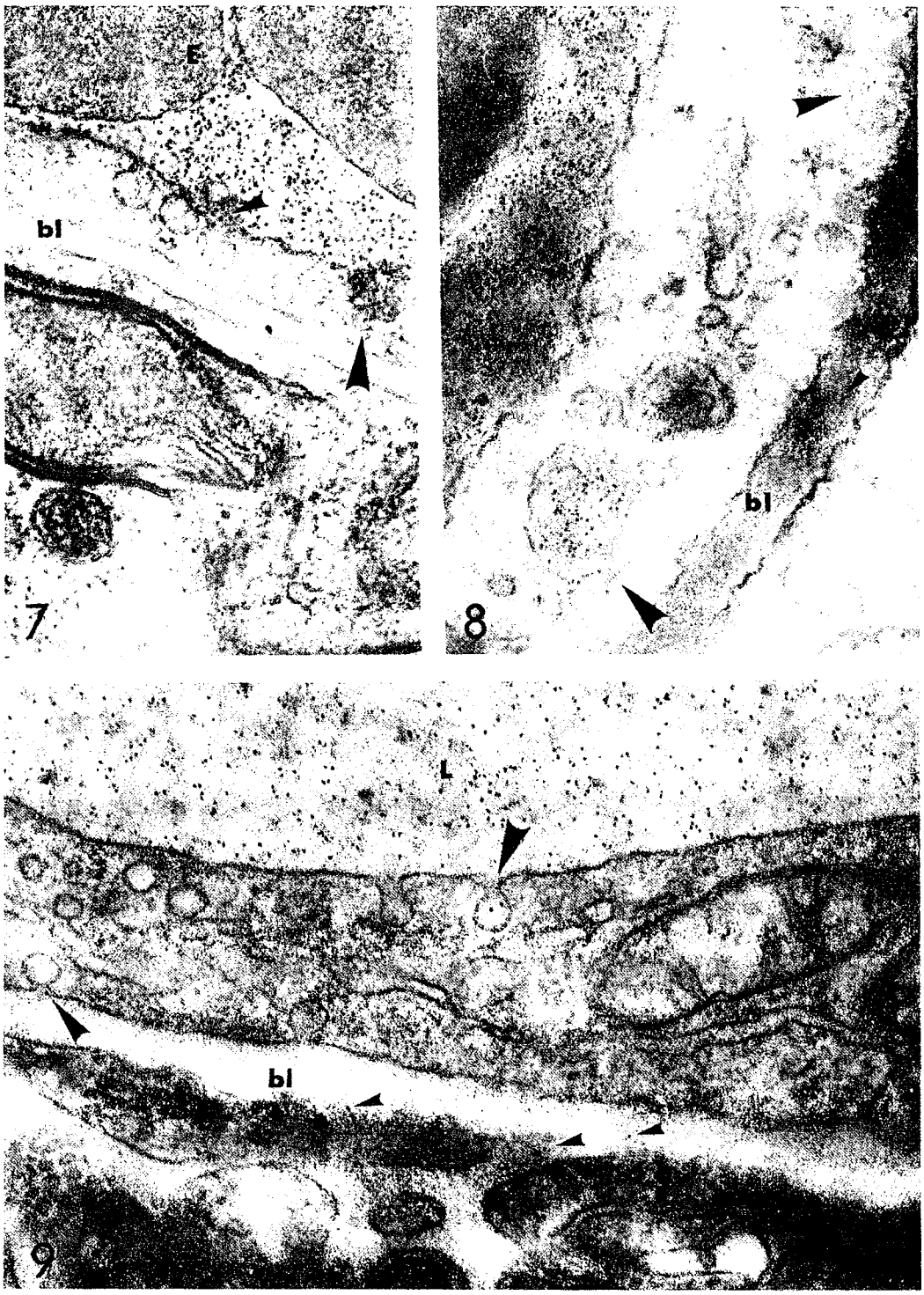
The basal lamina was not heavily infiltrated with ferritin molecules at any time point after injection. In the earlier specimens observed, molecules were either absent, or present at extremely low concentrations, singly scattered about the perimeter of the capillary (Figs. 5 and 8). No accumulation of ferritin within the basal lamina at particular points around the capillary was noted, save one instance. This occurred in a capillary of an animal sacrificed 1/2 h after injection (Fig. 12). An apparent leak within a discrete portion of a single capillary allowed a relatively large quantity of ferritin to escape into the basal lamina. This was the only occurrence of this kind in all specimens studied, and adjacent strial capillaries within the same section did not exhibit this profuse transendothelial passage of ferritin. This phenomenon was clearly not typical of normal strial capillary function.

In the 5 and 24 h specimens, accumulation of ferritin appeared slightly greater around some capillaries than in earlier specimens. However this was not the rule since other capillaries at the same time periods showed little or no ferritin present within the basal lamina. The overall picture revealed that ferritin labeling of the basal lamina was variable at all time points studied and that labeling even at its greatest was scant.

Fig. 7. Large arrow indicates numerous labeled pinocytotic vesicles within endothelium. Smaller arrow indicates open-mouthed vesicle upon luminal front which appears to have ferritin molecules within neck portion of vesicle. 1 h post injection. Osmium fixed. Stained with lead citrate. Mid portion of cochlear duct. ×92 325.

Fig. 8. Note numerous open-mouthed pinocytotic vesicles upon tissue front. Medium arrow indicates a ferritin labeled one. A ferritin molecule can be seen in the basal lamina (small arrow). Within the endothelial cell a pinocytotic vesicle can be seen fused with a large vacuole containing numerous ferritin molecules (large arrow). 5 h post injection. Glutaraldehyde and osmium fixed. Unstained. Basal portion of cochlear duct. ×88 635.

Fig. 9. Large arrow indicates open-mouthed pinocytotic vesicle containing two ferritin molecules on luminal front. A ferritin molecule can be seen at the opening of a pinocytotic vesicle at the tissue front (medium arrow). Small arrows indicate ferritin molecules within basal lamina. 2 h after injection. Osmium fixed. Unstained. Mid portion of cochlear duct. ×92 470.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Ferritin molecules within the intercellular spaces of the stria vascularis were not observed, and presumably the size of the molecule limited its penetration into such spaces. Ferritin was not seen within the basal lamina of control animals.

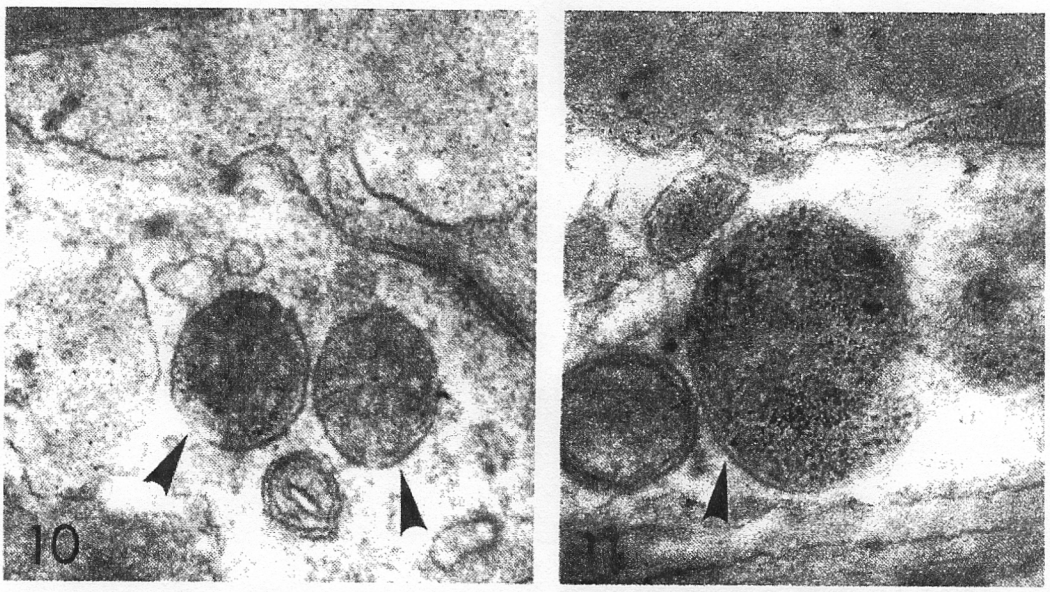
Strial cells

Ferritin was not seen within the marginal cells. However, large accumulations of electron dense particles closely resembling ferritin in size and shape were often found in specific cell processes of light cells (Fig. 6). In fact, the particles within these cells and the injected ferritin molecules appeared indistinguishable. The particles were found in high concentration free in the cytoplasm often accompanied by lysosomes containing the same particles. This phenomenon was found throughout all time periods studied, and *in control animals as well*. Further description and analysis of these cells will be the subject of another report (Santos-Sacchi and Marovitz, in preparation). No pinocytotic uptake of injected ferritin by these light cells was seen, although light cells occasionally possessed open-mouthed vesicles on the basal lamina. Also, no spectacular accumulation of ferritin within vacuoles was ever observed in these cells. There were no apparent differences in cytoplasmic ferritin-like concentration between experimental and control animals.

DISCUSSION

Iron dextran

The iron dextran results were perplexing. Iron dextran was present within strial endothelial cytoplasm from 1/2 to at least 5 h, but was apparently absent by 24 h. This did not seem to be a fixation artifact. It was concluded that the tracer initially entered the endothelia by means of pinocytotic uptake, since in some 1/2 h specimens and in the endothelia of a control animal (killed immediately after injection) iron dextran was present within vesicles and absent within the cytoplasmic matrix. The occurrence of iron dextran within the cytoplasmic matrix of endothelia is difficult to explain, since it is generally acknowledged that macromolecules have no route by which to enter the "membrane protected" cytoplasm. Although molecules the size of iron dextran are unable to pass through intact membranes, similar observations of free intracytoplasmic iron dextran following administration have been reported. Richter (1959), after injecting iron dextran intraperitoneally, studied the fate of that substance within liver and spleen. After 1 h he found accumulations of iron dextran within macrophages and sinusoidal endothelia of both organs. There were non-membrane bound aggregates of iron dextran within the cytoplasmic matrices, as well as numerous free particles. This is similar to the results of the present study. However, in Richter's study, by the fouth hour following injection, aggregates were enclosed by limiting membranes. Further, iron dextran was discernible within the cells up to 6 days after administration. In this regard, Richter's results differ from the present study, because iron dextran was absent from 24 h. However, it should be recalled that in the only previous study of iron dextran permeability in strial capillaries (Yamamoto & Nakai, 1964) the molecules remained within endothelia, reportedly within vacuoles, for 5 h, but by 24 h and beyond iron dextran was absent from endothelia. It seems that strial endothelia have an ability to remove or possibly metabolize iron dextran, so that by 24 h very little or none remains observable. Yamamoto and Nakai reported no transendothelial passage of iron dextran into the stria. Although this appears to be the case for the present study as well, the possibility exists that passage of some degraded form of the original molecule occurred which could not be visualized electronmicroscopically. Richter's experiment suggests that mouse cells may be able to metabolize the partially depolymerized dextran sufficiently to abolish the colloidal stability of the iron dextran preparation.



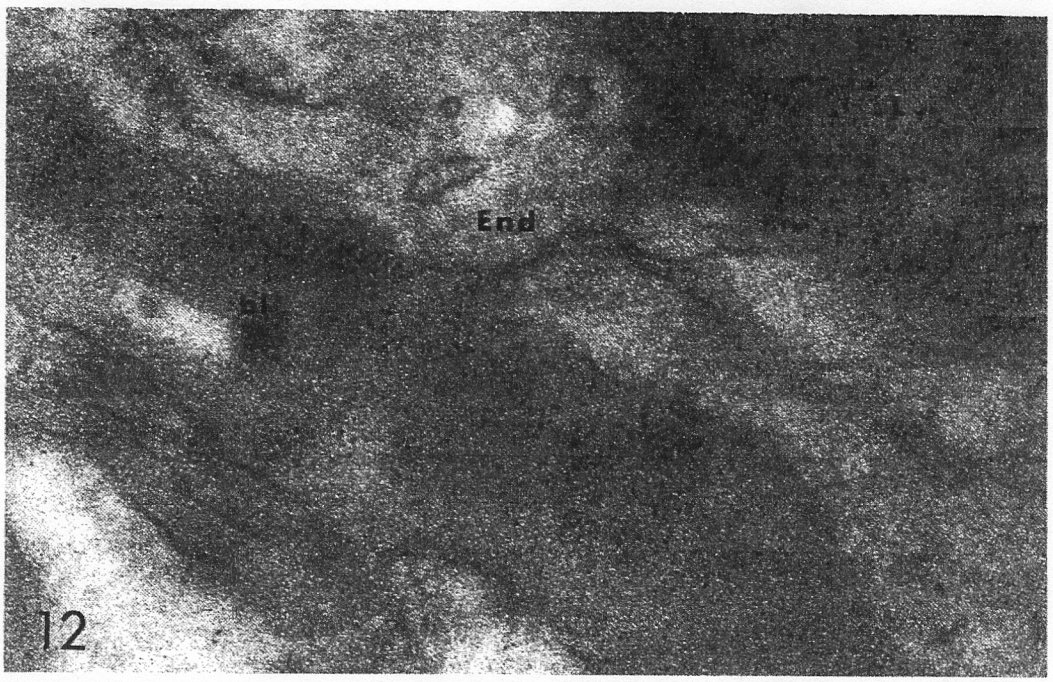


Fig. 10. Arrows indicate multivesicular bodies of labeled and unlabeled pinocytotic-like vesicles within endothelial cell. 24 h post injection. Glutaraldehyde and osmium fixed. Unstained. Apical portion of cochlear duct. ×85 500.

Fig. 11. Large lysosome containing ferritin within an endothelial cell (arrow). 5 h post injection. Glutaraldehyde and osmium fixed. Unstained. Basal portion of cochlear duct. ×88 065.

Fig. 12. Note large accumulation of ferritin within basal lamina. This was the only occurrence of its kind throughout all specimens. It is not typical of normal strial capillary transport, but highlights the possibility of such leakage. ½ h post injection. Glutaraldehyde and osmium fixed. Unstained. Basal portion of cochlear duct. ×118 000.

Of course, diffuse iron in ionic form or bound in low concentrations to carrier substances cannot be seen (Muir & Goldberg, 1961).

Clearly then, the use of iron dextran as an electron opaque tracer molecule for strial capillary transport is obviated by the possibly unique treatment it receives by strial endothelia which apparently results in the intracytoplasmic sequestration and eventual removal of iron dextran.

Ferritin

Although ferritin transport across strial capillaries had not been evaluated prior to the present study, HRP transport had been examined. The transport of the enzyme through strial vessels is very rapid. Within a few min after HRP injection, the basal lamina and intercellular spaces are densely stained with reaction product (Duvall et al., 1971; Winther, 1971). These results are more similar to those obtained with muscle capillaries than to those obtained with CNS capillaries, except that intercellular cleft transport of HRP is reportedly absent in strial capillaries. However, the results of the present study, which susgest minimal capillary transport, indicate that the permeability function of the strial capillaries is more similar to that of the CNS vessels. Thus, even by 24 h post injection, transport of ferritin across endothelia is scanty as determined by basal lamina labeling. Although transport of lipid insoluble substances certainly increases as molecular weight decreases, the evidence evaluated by Renkin (1964) suggests that transport rates of molecules in excess of about 60 000 Daltons remains constant. If the micropinocytotic transport system is the sole means of transendothelial passage of lipid-insoluble material within the stria, then the passage of HRP (whose molecular weight is close to Renkin's cut off size) should not be drastically different from that of ferritin. This may especially be true if, as some researchers believe (Balint & Nagy, 1971), HRP binds to B-globulin and IgG within plasma, thereby increasing the particle size and bringing it closer to the size of ferritin. Thus by size comparison it is difficult to explain the similarity of HRP results and the dissimilarity of ferritin results between strial and muscle capillaries. Certainly, the very small number of labeled strial capillary pinocytotic vesicles could account for the smaller amount of ferritin transport in strial as compared to muscle capillaries. Moreover, the dissimilarity of transport suggests that some other factor may play an important role in the rapid transport of HRP across strial endothelia. It is here that the established ototoxicity of HRP may come to bear (Ross et al., 1977). Perhaps the transport of HRP is rapid in strial capillaries because the HRP *itself* alters strial capillary permeability. Such a hypothesis would explain the similarity of HRP results in muscle and strial capillaries, and would not conflict with the ferritin results.

Research on the permeability of continuous type capillaries in other tissues also leads to the conclusion that the strial HRP results are unusual, i.e. in the light of this study's findings with ferritin. Raviola & Karnovsky (1972) evaluated thymic capillary transport using a variety of enzymatic tracers, including HRP. They also used the tracer molecule ferritin, so that it is possible to compare the permeabilities of both HRP and ferritin in the same capillary system. One must note however that the thymus contains both continuous and fenestrated capillaries. For the present purpose, only the continuous capillaries' permeability functions are of significance, since these capillaries, are structurally comparable to strial capillaries. Interestingly, these continuous capillaries, located within the cortical areas of the thymus, present a blood-thymic barrier. As in the stria and CNS, circumferential tight junctions halt extravascular leakage through endothelial intercellular clefts. At all time periods, from 1–24 h after ferritin injection, minimal amounts of the electron opaque tracer had traversed the thymic capillary endothelia. Labeling of pinocytotic vesicles was variable, but represented a very

small fraction of the whole vesicle population. Within the basal lamina only isolated or small groups of 2–3 ferritin molecules were scattered along the circumference of the capillaries. These findings are surprisingly similar to those of the present study. However, despite the similarities in capillary structure and ferritin transport, the transendothelial passage of HRP is vastly different between strial and cortical thymic capillaries. Raviola and Karnovsky (1972) demonstrated that the amount of HRP transport across cortical thymic capillaries is comparable to that of ferritin. That is, from 2–20 min post injection only limited vesicular transport of the enzymatic tracer occurred through endothelia; vascular adventitia was free of peroxidase reaction product. Apparently then, the transport of both ferritin and HRP is similar for a given capillary bed, provided that the micropinocytotic transport system is the sole means of transendothelial passage. The similar transport characteristics of HRP and ferritin agree well with the conclusions of Renkin (1964).

Another blood-tissue barrier has been reported which is similar to the thymic one (Gershon & Bursztajn, 1978). The myenteric plexus is supplied with continuous-type capillaries. Using HRP, Gershon & Bursztajn (1978) demonstrated minimal transport of the tracer, presumably by the pinocytotic pathway, since intercellular cleft transport was impossible due to tight junctional seals. Extravascular accumulation of the HRP reaction product in the myenteric plexus was undetectable, as Raviola and Karnovsky reported for cortical thymic tissue. However, in both tissues—thymus and myenteric plexus—it was concluded that some transport has occurred because perivascular phagocytes contained vacuolated tracer substance.

In the present study, the ferritin results appear to indicate the existence of a blood-strial barrier which is closer in nature to the blood-thymic and blood-myenteric plexus barriers than to the blood-brain barrier.

Another phenomenon which might account for the observed minimal transport of ferritin across strial capillaries is the phagocytotic activity of strial endothelial cells. Within these cells were observed large vacuoles and multivesicular bodies containing ferritin similar to those reported by Raviola & Karnovsky (1972). Apparently, one means of such vacuole formation was by pinocytotic vesicular fusion, since with increasing time, larger and denser ferritin containing bodies (presumably lysosomes) were present. It appeared that once ferritin conglomerates formed, the ferritin was immobilized within endothelia until degraded. In essence then, the phagocytotic activities of strial endothelia in conjunction with minimal pinocytotic transport and endothelial tight junctions contribute to the formation of a blood-strial barrier at least for molecules the size of ferritin.

Previous studies have suggested that intermediate cells may serve as macrophages within the stria. Such cells have been found to have open-mouthed pinocytotic vesicles and intracytoplasmic vacuoles labeled with HRP reaction product after a sufficient interval of time following HRP administration (Duvall et al., 1971; Winther, 1971). Also at the light microscopic level, Nomura (1961) found that intravenously injected trypan blue was phagocytized by certain strial cells scattered about the capillaries. Similarly, transported tracer substances within cortical thymic and myenteric plexus blood-tissue barrier systems were incorporated within perivascular phagocytic cells (Raviola & Karnovsky, 1972; Gershon & Bursztajn, 1978). The possibility exists that in this study some ferritin-like particles within the light cells of experimental animals may be exogenous ferritin; that is, perhaps the cytoplasm of these cells serves as a sink for transported exogenous ferritin. However, the fact that such particles were located, for the most part, free within the light cell cytoplasmic matrix argues against an exogenous source, since macromolecules of this size are not able to enter free cytoplasm. Further, it should be noted that there were no obvious differences

between control and experimental animals in regard to concentration of intracytoplasmic ferritin-like particles. Nevertheless within the cytoplasm of liver cells there is de novo apoferritin production within a few h following administration; subsequently, iron sequestration by apoferritin occurs, producing ferritin (Drysdale & Munro, 1966). In the present study, since the injected tracer contains iron, the de novo production of endogenous ferritin by experimental strial light cells might occur if indeed they phagocytized transported exogenous ferritin. The problem is made more difficult because the horse spleen ferritin tracer is very resistant to lysosomal breakdown (Coffey & DeDuve, 1968). Thus, although ferritin may accumulate within lysosomes, elemental iron may not be able to enter the cytoplasm because of the hydrolytic resistance of the protein. On the other hand, there is some evidence that the iron may enter and leave the apoferritin shell without protein disintegration (Mazur & Carleton, 1963). In this regard, it is noteworthy that in other permeability studies using ferritin as a tracer (Bruns & Palade, 1968; Raviola & Karnovsky, 1972), after 24 h, perivascular macrophages did exhibit intracytoplasmic ferritin granules. Presumably, they were endogenously produced, after the phagocytes accumulated the ferritin tracer within lysosomes. In the present study, since numerous lysosomes containing ferritin were found in control animals, a normal turnover mechanism may be deduced. However, it cannot be totally dismissed that the select light cells in experimental animals also contain exogenously injected ferritin within lysosomes. Finally, it should be noted that although light cells often exhibited open-mouthed vesicles upon the basal lamina, no ferritin labeling of these vesicles in experimental animals was found. Certainly, the scanty accumulation of ferritin within the basal lamina offered little probability of finding ferritin within such open-mouthed vesicles.

The central nervous system of mammals appears to require an unusually stable internal environment in order to function effectively (Katzman, 1972). Clearly, the blood-brain barrier affords such an environment. In a like manner, thymic and myenteric plexus blood-tissue barriers serve their own physiologic purposes. The finding that the transport of ferritin across strial endothelia is scanty may indicate that the internal milieu of the stria must be carefully regulated. The probable existence of strial phagocytic cells may also be important in this regard. Normal strial function may very well depend upon an intact blood-strial barrier.

The ferritin labeling technique used in this study of normal animals may be useful in determining altered strial capillary transport possibly associated with various auditory pathologies. It is known that under pathologic conditions of inflammation, extravascular leakage of high protein content fluid occurs (Florey, 1964). Indeed, this is known to occur in brain tissue, and involve a breakdown of the blood-brain barrier (Katzman, 1972). Certainly, under some deleterious influences, strial tissue becomes edematous, e.g. in response to ethacrynic acid or noise exposure. In the present study, only one instance of abundant ferritin accumulation within the basal lamina was observed. Since adjacent capillaries within the same section, and all other capillaries did not exhibit such abundant transport, the phenomenon was judged atypical of strial capillary transport. However, this occurrence indicates that the tracer molecule ferritin may be useful in determining altered strial transport. Ferritin is highly resistant to lysosomal hydrolases (Coffey & DeDuve, 1968). Therefore, if large amounts did traverse the endothelial tunic they would no doubt be detected within some "compartment" of strial tissue, assuming, of course, that the tight junctional seal delimiting the stria vascularis remains intact. However, the possibility of light cell incorporation of exogenous tracer, and the difficulty in differentiating between endogenous and exogenous electron dense particles should be kept cognizant.

ZUSAMMENFASSUNG

Enzymatische Tracermethoden für die Erforschung normales und pathologisches Kapillartransports der Stria stellen verschiedene Probleme vor. Die Verwendung opaker Elektronentracer kann viele von diesen Problemen verhüten. Eisendextran (mol. Dm. 20–70 Å) und Ferritin (mol. Dm. 110 Å) wurden intravenös eingespritzt, und die Mäuse wurden nach Zwischenzeiten von ½, 1, 2, 5 und 24 Stunden geopfert. Die Eisendextran-Ergebnisse waren ungewöhnlich, weil der Tracer von ½ bis 5 Stunden nach der Einspritzung in der zytoplasmatischen Matrize der Endothelen zugegen war, aber gegen die 24. Stunde war er ausgeräumt worden. Kein transendothelialer Austausch wurde bemerkt. Die Ferritin-Ergebnisse widersprachen vorhergehenden Ergebnissen, die Meerrettichperoxidase benutzten. Der Transport von Ferritin war ungeachtet der verstrichenen Zeit minimal. Nur ein paar Molekeln wurden um die Basalkapillarlamina bestreut. Die quer durch Kapillaren übergeführten Molekeln wurden anscheinend mittels des mikropinozytotischen Systems übergebracht. Die Ergebnisse deuten eine Blut-Stria-Sperre an, die den Blut-Thymus- und Blut-Myenterus-Sperren ähnlich ist. Versuchs-sowie auch Kontrolltiere wiesen Lichtzellen der Stria auf, die Ferritinähnliche Partikeln in ihren zytoplasmatischen Matrizen enthielten. Diese Lichtzellen sind wahrscheinlich Zellen des reticuloendothelialen Types. Ferritin könnte nutzbar sein, um die auf Gehörpathologien bezogenen Änderungen des Kapillartransports der Stria abzuschätzen.

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J. Santos-Sacchi, Ph.D.
Center for Biomedical Education
City College, Science Bldg. Rm. 915
The City University of New York
New York, NY 10031
USA