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Uptake of tracer by the epiplexus cells via the choroid plexus epithelium following an intravenous or intraperitoneal injection of horseradish peroxidase in rats

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ABSTRACT

Rapid passage of horseradish peroxidase (HRP) from the blood circulation to the cerebrospinal fluid was demonstrated in postnatal rats. At 30 min–1 h after an intravenous (i.v.) injection of HRP, the extravasated tracer from the blood vessels entered the connective tissue of the choroid plexus to reach the epithelial intercellular spaces where it was retarded by the apical tight junctions. The HRP which accumulated in widened intercellular spaces was readily endocytosed by the epithelial cells, notably at their lateral surfaces. This was especially pronounced 3 h after the injection. The endocytosed HRP was either routed to lysosomes or discharged apically by exocytosis into the CSF via membrane-bound vesicles by the epithelial cells. After longer survival periods, i.e. 6 h after injection, the intercellular spaces were relatively clear of tracer. HRP-labelled vacuoles or vesicles had diminished with a concomitant increase in the number of lysosomes containing HRP reaction product. In the course of HRP injection, the epiplexus cells residing on the choroid epithelium progressively accumulated HRP by endocytosis so that in rats killed 6 h after injection, the cells were heavily loaded with HRP incorporated into massive lysosomes. The labelling pattern of epithelial and epiplexus cells in rats injected intraperitoneally followed that observed in those receiving i.v. injections. These results suggest that the epiplexus cells together with lysosomal activity by the choroid epithelial cells serve as a protective line of defence for the blood–CSF barrier which appears to be inefficient.

INTRODUCTION

Several studies in mammals have shown that protein tracers such as ferritin, horseradish peroxidase (HRP) and microperoxidase (MP) could be taken up by the choroid plexus when they were administered intraventricularly (Brightman, 1965, 1968; Becker & Almazon, 1968; van Deurs, 1976, 1978*a*, *b*). Thus tracers such as HRP and MP were localised in the subepithelial connective tissue after ventricular perfusion (Becker & Almazon, 1968; van Deurs, 1976, 1978a, b), indicating a transepithelial transport from the cerebrospinal fluid (CSF) to the blood circulation. Ultrastructural studies have shown the removal of protein tracers by the choroid epithelium from CSF by micropinocytosis (Becker & Almazon, 1968; van Deurs, 1976, 1978*a*, *b*). While the passage of tracers from CSF into the connective tissue stroma seems unequivocal, the uptake of tracers (e.g. lanthanum, cytochrome C, HRP, MP) from the blood by the choroid plexus epithelium remains a debatable issue (Becker et al. 1967; Brightman, 1968; Reese & Brightman, 1968; Castel et al. 1974; Davis & Milhorat, 1975; van Deurs, 1978*a*, *b*). Protein tracers introduced intravascularly would readily pass through the blood vessels of the choroid plexus into the extracellular spaces to be taken up subsequently by the choroid epithelial cells by micropinocytosis (Becker et al. 1967; Davis & Milhorat, 1975; van Deurs, 1978*a*, *b*). The ultimate fate of the ingested tracer remains to be further explored, although the study by Davis & Milhorat (1975) showed that it is degraded by lysosomal enzymes.

Relevant to the present study is the enigmatic role of the epiplexus cells known to be phagocytes residing on the surface of the choroid epithelium (Ariëns-Kappers, 1953; Carpenter et al. 1970; Hoyosa & Fujita, 1973; Sturrock, 1978, 1979, 1983; Ling, 1979,

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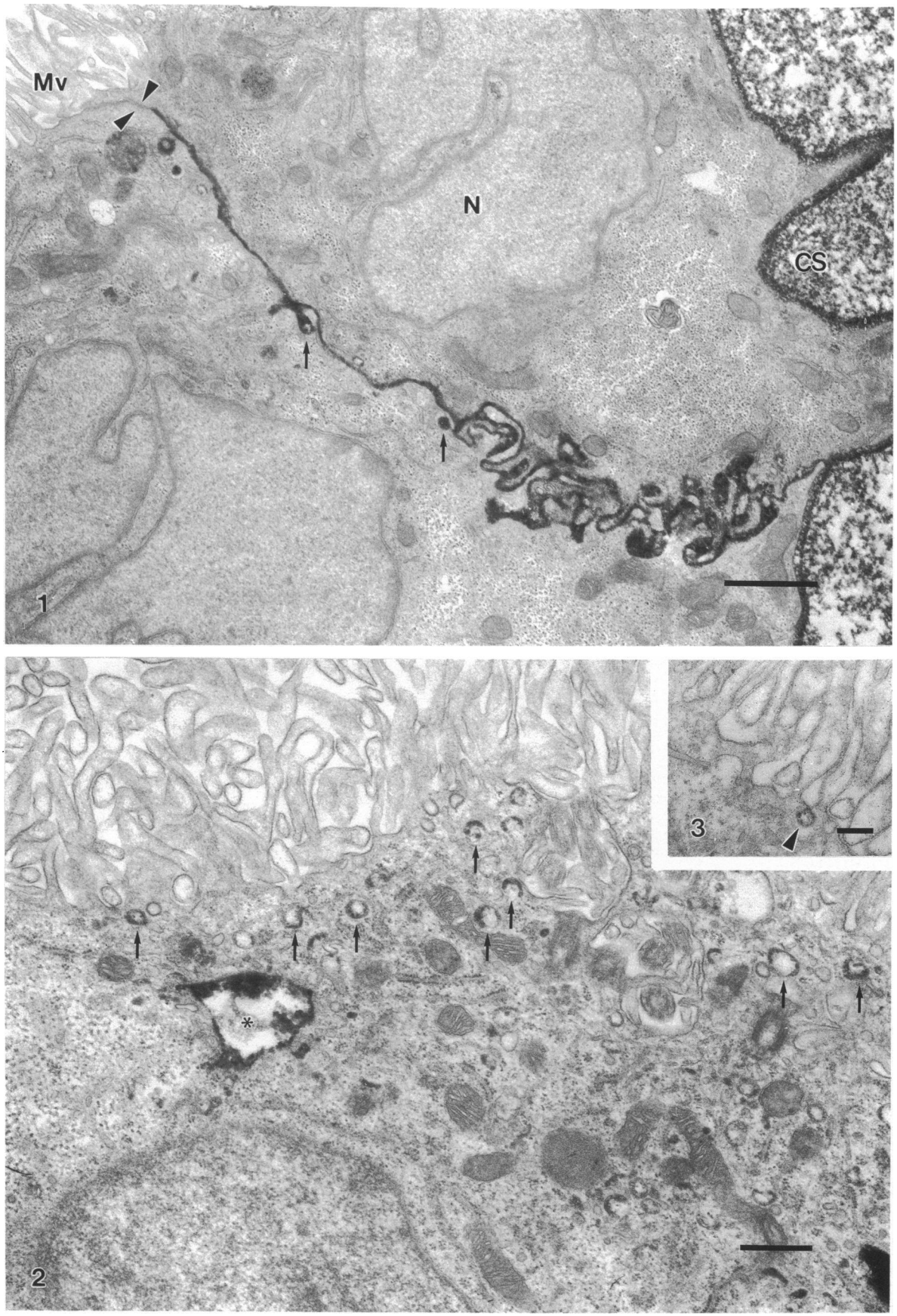

Fig. 1. Choroid epithelial cells 1 h after an i.v. injection of HRP. The subepithelial connective tissue space (CS) is filled with HRP. The tracer has seeped through the basal lamina to reach the intercellular space where it is stopped by the apical tight junction (arrowheads). Arrows indicate possible endocytosis of HRP along the lateral surface. Mv, microvilli; N, nucleus. Bar, 1 μm.

1985; Ling et al. 1988; Kaur et al. 1990). The involvement of these cells in the function of the blood–CSF barrier and their relation to the choroid epithelium is not fully understood. Recently there has been some indication that they may serve as scavenger cells to filter off any exogenous materials that may have gained access into the ventricular system. It has been demonstrated by Lu et al. (1993) that the fluorescent dye, rhodamine isothiocyanate (RhIc), was concentrated in the choroid plexus after intraperitoneal administration in rats of different ages, but was subsequently detected in the epiplexus cells. The labelling of the epiplexus cells by RhIc by the i.p. route suggests the possibility of an active transport of massive exogenous material across the choroid epithelial cells. One way of elucidating how this might be effected would be to administer an electron-dense tracer, e.g. HRP, by either an i.p. or intravascular route and to follow its passage to the epiplexus cells.

The objective of the present study was to ascertain whether injected HRP could cross the epithelium into the CSF and, if so, whether it would be taken up by the epiplexus cells. The information obtained would help to clarify the functional relationship between the epiplexus and the choroid epithelial cells, which is important for the understanding of the function of the blood–CSF barrier.

MATERIALS AND METHODS

Injection of HRP

The observations were made on 17 Wistar rats aged 1 d of either sex. They were divided into 3 groups for intravenous (i.v.) and intraperitoneal (i.p.) injections. The 1st group, consisting of 8 rats, was anaesthetised with ether and given an i.v. injection of HRP (type VI, Sigma) via the left external jugular vein with a Hamilton syringe; 0.5 µl per g bodyweight HRP in normal saline (8 mg HRP in 50 µl saline) was administered. The rats were killed in pairs at the following intervals by perfusion while anaesthetised: 30 min, 1, 3 and 6 h after the HRP injection. The 2nd group consisted of 3 rats, each receiving a higher dosage of HRP (3 µl per g bodyweight). They were killed at 30 min, 1 and 3 h after the injection. The 3rd group consisted of 6 rats which were given a single i.p. injection of HRP at 3 µl per g bodyweight under ether anaesthesia and killed in pairs at 1, 3 and 6 h after the HRP injection.

Perfusion and tissue processing

Under ether anaesthesia, the rats were perfused with Ringer's solution for a few minutes until the liver and lungs were clear of blood. This was followed with a mixed aldehyde solution composed of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 30 min. After the perfusion, the brain was removed and postfixed in the same fixative for 2 h and then stored overnight in 0.1 M phosphate buffer containing 10% sucrose at 4 °C. Coronal sections of the brain (100 µm) containing the lateral ventricles and choroid plexuses were cut with a vibratome (Oxford Instruments). The tissue sections were preincubated in 0.1 M Tris-HCl buffer (pH 7.6) for 5 min. They were then transferred to a similar solution, to which 0.5% $CoCl_2$ was added, for another 5 min before the final incubation in a medium containing 5 mg diaminobenzidine, 25 ml of 0.1 M phosphate buffer and 0.75 ml of 1% H_2O_2 for 1 h. The tissue sections were rinsed in 0.1 M phosphate buffer, and postfixed in 2% osmium tetroxide in phosphate buffer. They were then dehydrated in an ascending series of alcohol and embedded in Araldite. Ultrathin sections stained with lead citrate were viewed in a Philips 400T electron microscope.

RESULTS

Intravenous injection of HRP

At 30 or 60 min after the HRP injection, the interstitial spaces of the connective tissue underlying the epithelium of the choroid plexus contained HRP. The area between the basal lamina of the choroid epithelium and the vascular endothelium was filled with HRP reaction product (Fig. 1). The lumen of the fenestrated microvessels, however, was often devoid of the tracer. Connective tissue stromal macrophages laden with HRP were a common feature. The HRP had apparently diffused through the basal lamina of the choroid epithelium to be localised in the intercellular spaces (Fig. 1), but had failed to penetrate the apical tight junctions between the adjacent epithelial cells (Fig. 1). Plasmalemmal invaginations indicative of endocytosis of the extravasated HRP were observed along the basal as well as the lateral surfaces of the epithelial cells (Fig. 1). HRP-labelled vacuoles ranging from 400 to 1500 nm in diameter

Figs 2, 3. Apical cytoplasm of epithelial cell 1 h after an i.v. injection of HRP. HRP-labelled vesicles (arrows) and vacuoles (asterisks) are in close approximation to the apical plasma membrane. One of the HRP-labelled vesicles appears to communicate with the CSF of the ventricular lumen (arrowhead, Fig. 3). Fig. 2, bar, 500 nm; Fig. 3, bar, 250 nm.

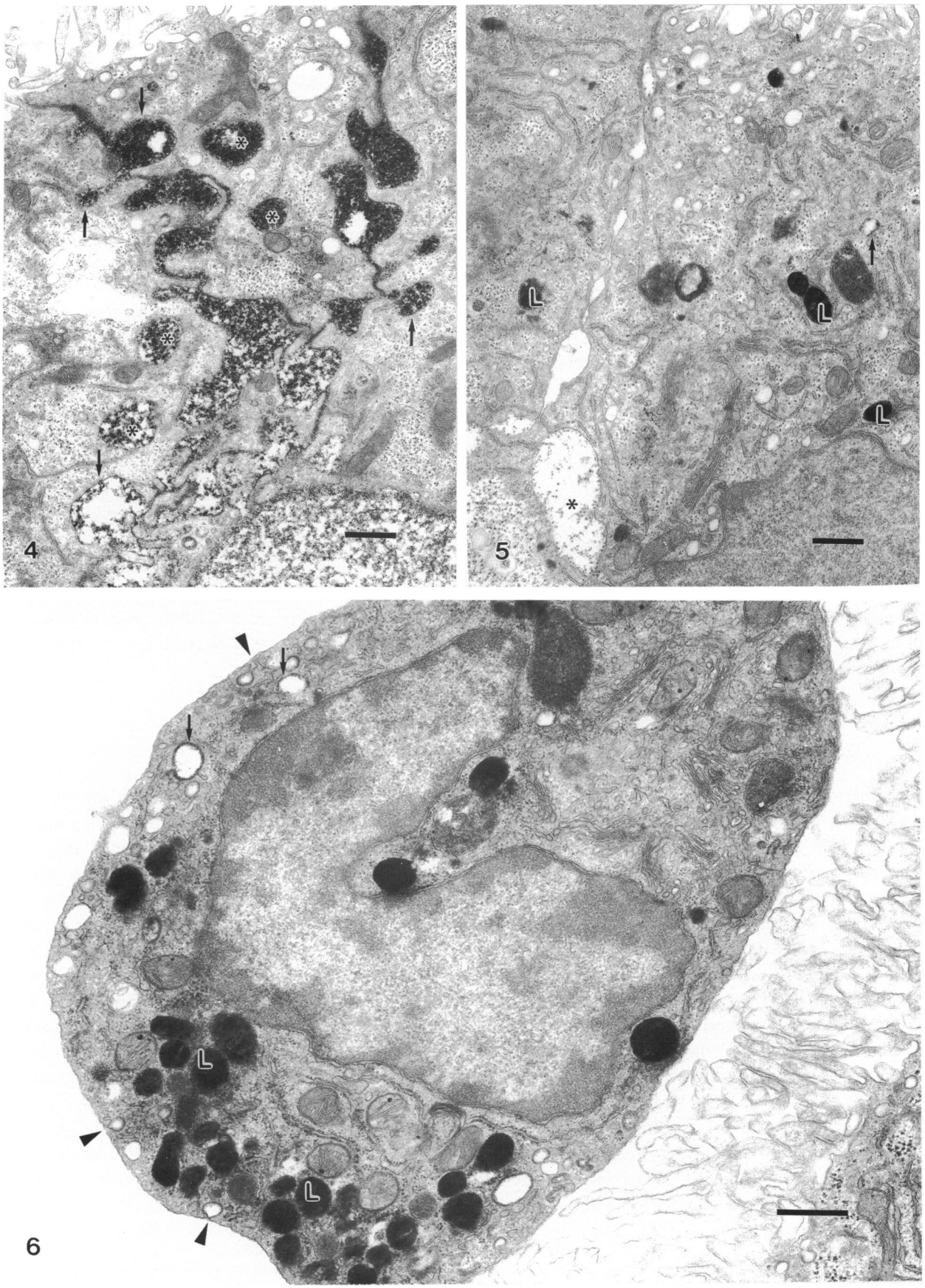

Fig. 4. Massive accumulations of tracer in widened intercellular spaces 3 h after an i.v. injection of HRP. Arrows indicate active endocytosis. Vesicular profiles (asterisks) contain a varying amount of internalized HRP. Bar, 500 nm.

and some smaller vesicles which measured 50–230 nm in diameter were distributed throughout the cell but preferably in the supranuclear region especially in rats given a high dosage of HRP (Fig. 2). Often, the HRP-labelled vesicles were seen in close approximation to the apical plasma membrane of the epithelial cells (Fig. 2) and sometimes communicated with the ventricular lumen (Fig. 3). Occasional lysosome-like dense bodies devoid of HRP reaction products were seen near the Golgi saccules. At 3 h after an i.v. injection of HRP, a large amount of tracer accumulated in the wide intercellular spaces related to the extreme convolutions of the lateral walls (Fig. 4). Endocytotic activity at the lateral surfaces of the epithelial cells was greatly enhanced. On the other hand, hardly any noticeable endocytotic activity was observed at the basal plasma membrane. As a result of the active endocytotic activity at the lateral walls, there was a concomitant increase in the number of HRP-labelled vacuoles and vesicles in the epithelial cytoplasm, many of them being aggregated in the apical cytoplasm. At 6 h after injection, the intercellular spaces were almost free of HRP (Fig. 5). The lateral cell walls became less convoluted, there was a simultaneous reduction in the number of HRP-filled vacuoles and vesicles, and heavily HRP-labelled lysosome-like dense bodies were widely distributed in the epithelial cells (Fig. 5).

At 1 h after an i.v. injection of HRP, labelled vacuoles and vesicles were observed in the epiplexus cells residing on the microvilli of the epithelial cells. Endocytosis of HRP was observed at the cell surface. Intracellular accumulation of HRP, however, was less common when compared with the epithelial cells or the stromal macrophages. At 3 h after the injection, epiplexus cells with more HRP-labelled vesicles and vacuoles and a few HRP-labelled lysosome-like dense bodies were observed. The epiplexus cells were clearly more fully loaded with HRP reaction product incorporated in the numerous lysosomes (Fig. 6), 6 h after the injection.

Intraperitoneal injection of HRP

A minimum of 1 h survival interval was allowed before the rats injected intraperitoneally were killed because of the longer time required for the HRP to gain access to the choroid plexuses. Such was the case in our previous study (Lu et al. 1993) with the fluorescent tracer, RhIc, in which the epiplexus cells after i.p. injection took a longer time to be labelled. The temporal sequence of the labelling pattern for both the epithelial and the epiplexus cells, however, concurred with that in the i.v.-injected animals.

At 1 h after an i.p. injection of HRP, the tracer was localised in some of the blood vessels of the choroid plexus and the connective tissue spaces. The passage of HRP into the intercellular spaces was again impeded by the apical tight junctions between the adjacent epithelial cells. Only a few HRP-labelled endocytotic vacuoles and vesicles were observed in the cytoplasm of the epithelial cells. At 3 h after the injection, the labelling of the apical cytoplasm of the epithelial cells was pronounced, showing many HRP-labelled vesicles and vacuoles. The microvilli of these cells were either weakly labelled or heavily coated with HRP. In the latter, HRP-labelled vesicles were sometimes seen to communicate with the ventricular lumen between adjacent microvilli (Fig. 7). The microvilli with which the epiplexus cells were associated were invariably devoid of HRP coating. By 6 h after an i.p. injection, most of the endocytosed HRP was sequestered in lysosome-like dense bodies in the perinuclear region. HRP-labelled vacuoles and vesicles were seldom observed.

Epiplexus cells were not labelled 1 h after an i.p. injection of HRP, but after 3 h the cells showed HRP labelling in a variable number of small vesicles, large vacuoles and lysosomes (Fig. 8). In rats killed 6 h after an i.p. injection, HRP reaction product was concentrated mainly in numerous lysosomes in the epiplexus cells.

DISCUSSION

The present study has demonstrated a rapid uptake of HRP by the choroid epithelium and the epiplexus cells after the tracer was administered either by the i.v. or i.p. route into newborn rats. The injected HRP entered the connective tissue stromal spaces between the choroid epithelium and the endothelium of the blood vessels. The intercellular spaces of the epithelial cells became considerably widened, probably in response to the incoming tracer. These would form an ideal

Fig. 5. Epithelial cells 6 h after an i.v. injection of HRP. The intercellular space (asterisk) is almost free of HRP. The lateral cell walls are relatively less convoluted. Lysosome-like dense bodies (L) labelled with HRP are widely distributed in the cytoplasm. Tracer-labelled vesicles (arrow) and vacuoles are less frequent. Bar, 500 nm.

Fig. 6. An epiplexus cell 6 h after an i.v. injection of HRP possessing numerous lysosomes (L) containing HRP reaction product. HRP-labelled vesicles (arrows) are distributed in the subsurface region. Arrowheads indicate sites of endocytosis. Bar, 500 nm.

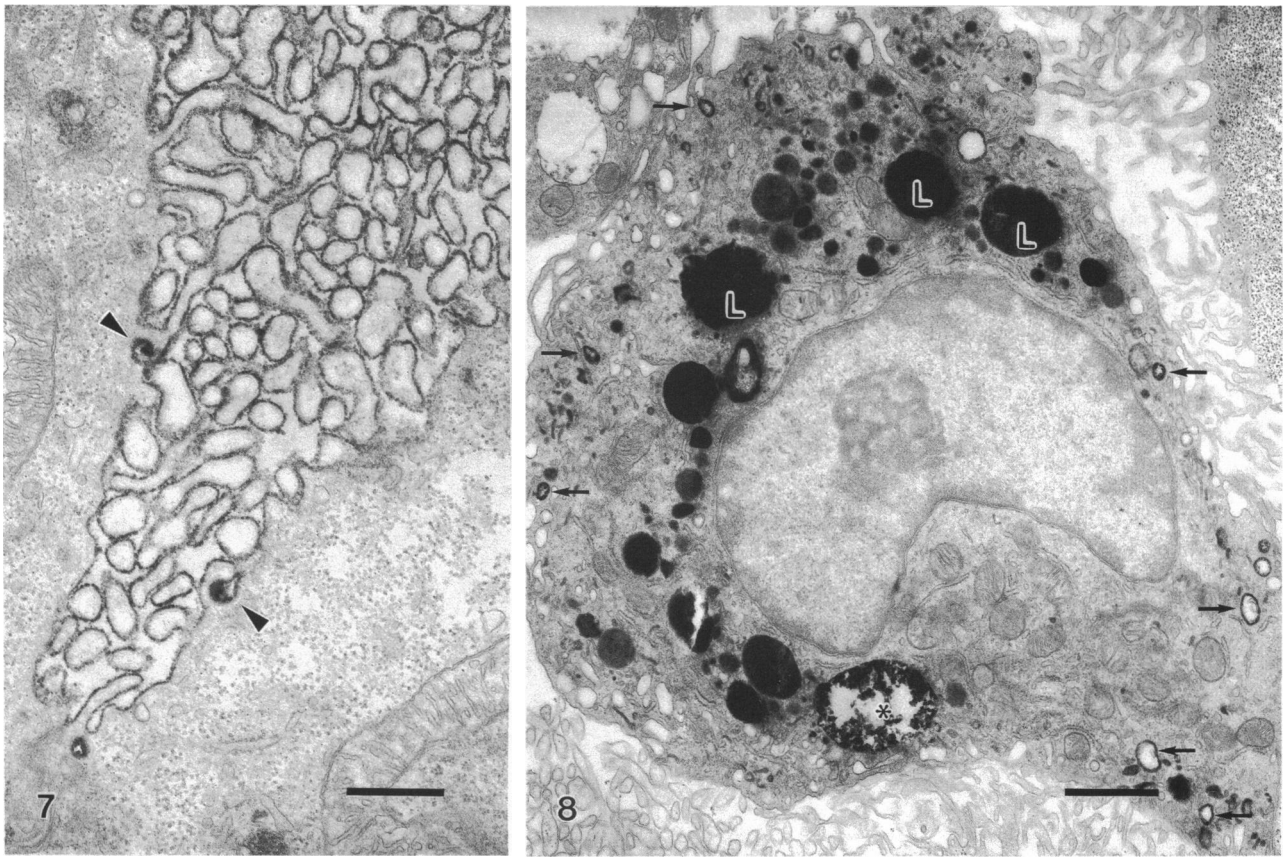

Fig. 7. Apical portion of an epithelial cell 3 h after an i.p. injection of HRP. The microvilli are coated with a layer of HRP reaction product. Arrowheads indicate 2 HRP-labelled vesicles communicating with the ventricular lumen between the adjacent microvilli. Bar, 500 nm.

Fig. 8. An epiplexus cell 3 h after an i.p. injection of HRP. Its abundant cytoplasm contains massive HRP-labelled lysosome-like dense bodies (L) of different sizes. The subsurface region shows numbers of HRP-filled vesicles (arrows) and a large vacuole (asterisk). Bar, 1 μm.

depot for the extravasated HRP. A remarkable feature was the avid uptake of the tracer notably at the lateral surfaces of the epithelial cells. The endocytosed HRP was either channelled into lysosomes or released into the ventricular lumen where it was ingested by the epiplexus cells residing on the surface of the epithelium. Figure 9 summarises the sequence of events at the choroid plexus following the injection of HRP.

It is apparent that the passage of the extravasated HRP in the intercellular spaces was impeded by the epithelial tight junctions, a phenomenon similar to that described by other authors in rodents (Becker et al. 1967; Brightman, 1968; Reese & Brightman, 1968; Brightman et al. 1970; van Deurs, 1978*a*; Tauc et al. 1984). Castel et al. (1974), on the other hand, described the penetration of the junctions by ionic lanthanum in the cat, thus suggesting that the epithelial tight junction may be permeable to ions.

The capability of uptake of HRP from the connective tissue stroma via micropinocytosis by the choroid epithelial cells has been reported in previous studies (Becker et al. 1967; van Deurs, 1978*a*). The present study confirms this and further extends the fact that the endocytotic activity occurs primarily at the lateral walls. The extremely corrugated lateral cell walls and the widened intercellular spaces would facilitate a rapid and massive transport of the accumulated exogenous materials derived from the blood. The engulfed HRP was sequestered either in small micropinocytotic vesicles or in large endocytotic vacuoles.

Although tracer-labelled vesicles were occasionally found close to the apical surface of the choroid plexus epithelial cells (Brightman, 1968), there was no evidence of the protein tracers being discharged into the CSF at the ventricle surface (Becker, 1967; Brightman, 1968; David & Milhorat, 1975; van Deurs, 1978*a*, *b*). On this basis, it was suggested that vesicular blood to CSF transport of macromolecules does not take place (van Deurs, 1980). In the present study, HRP-labelled vesicles appeared to communicate with the ventricular lumen. This provides a strong support for a transepithelial transport of the tracer from the connective tissue stroma to the CSF. Since the apical tight junctions were impervious to the tracer, it is justifiable to assume a vesicular-bound

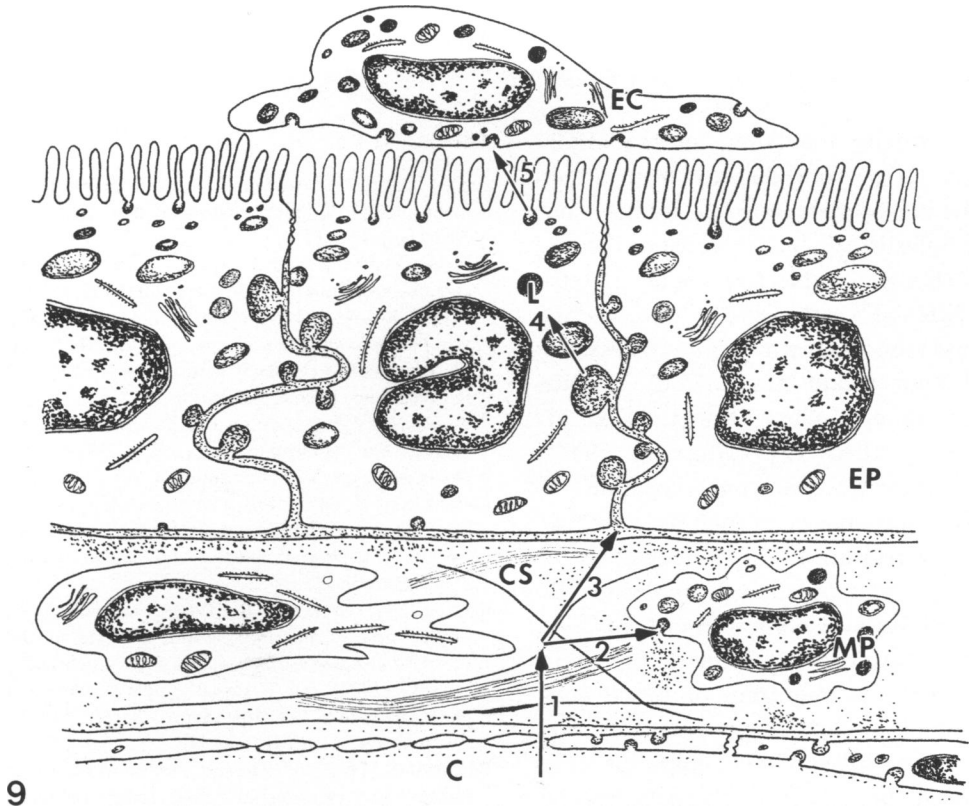

Fig. 9. HRP injected i.v. or i.p. is circulated to the fenestrated capillaries (C) in the choroid plexus where it is extravasated (1) into the connective tissue spaces (CS). The tracer is taken up (2) by the stromal macrophages (MP) or penetrates the intercellular spaces to be endocytosed (3) by the epithelial cells (EP). The endocytosed HRP is routed (4) to lysosomes (L), or released apically into the CSF to be phagocytosed (5) by the epiplexus cells (EC).

blood-to-CSF pathway for the transport of HRP across the choroid epithelium. Brightman and Reese (1969) and van Deurs (1978*b*), on the other hand, described the occurrence of a 'functional leak' at the base of the choroid plexus, where the ependymal cells are not sealed by the tight junctions and the intercellular spaces. They speculated that this may provide a route for exchange of molecules between CSF and choroid blood vessels. The present results failed to verify this feature.

The present study showed that with advancing time, the ingested HRP was incorporated into lysosome-like dense bodies (see also van Deurs, 1980). Relevant to this is the observation of Davis & Milhorat (1975) who reported a significant increase in lysosomal activity of the choroid epithelial cells when compared with controls following an i.v. injection of cytochrome C. It is speculated from our observation that the transepithelial vesicular transport of HRP may serve as an additional means allowing a rapid transportation of any endocytosed exogenous materials. It seems likely that the HRP endocytosed by the choroid epithelial cells would follow one of two pathways: (1) incorporation into the lysosome to be degraded, or (2) transepithelial vesicular transport into the CSF.

In the present study, HRP-labelled vesicles and vacuoles were observed near the ventricular surface of the epithelial cells. So far, only the smaller HRP-labelled vesicles have been observed to communicate with the CSF. The failure to observe any HRP-filled microvesicles communicating with the CSF in studies by other authors (Becker et al. 1967; Brightman, 1968; van Deurs 1978*a*) is probably due to either a rapid process of exocytosis which is not readily detected by electron microscopy or a different dosage of HRP. The higher dosage of HRP used in this study would probably facilitate a large amount of HRP to be discharged into CSF by the choroid epithelium.

The release of HRP into the CSF by the choroid epithelial cells is further demonstrated by its deposition on the surface of microvilli. In this instance, the rapid endocytosis of HRP by the overlying epiplexus cells provides further evidence of their phagocytic nature. This corroborates our earlier fluorescent labelling study (Lu et al. 1993) that after an i.p. injection of rhodamine isothiocyanate, all the epiplexus cells were subsequently labelled, indepen-

dent of age. It therefore seems likely that the strategically located epiplexus cells would serve as an effective system scrutinising a 'leaky' blood–CSF barrier.

Finally, the possibility that some of the HRP-labelled epiplexus cells were derived from other sources should be considered. Previous studies have suggested that epiplexus cells originate from macrophages in the choroid connective tissue stroma (Biondi, 1934; Ariëns-Kappers, 1953, 1958) or are derived from blood monocytes which are extravasated through the choroid blood vessels and migrate through the choroid epithelium (Carpenter et al. 1970; Merker, 1972; Allen, 1975; Sturrock, 1978). Ling (1981) observed the phenomenon of 'emperipolesis' of macrophages through the epithelial cells in monkeys. The recruitment of monocytes via an intercellular route to the population of epiplexus cells after experimental head acceleration injury was also observed by Maxwell et al. (1992). Remarkably, there is so far no evidence of migration of blood cells through the choroid epithelium in postnatal rats (Ling, 1979). In the present study, epiplexus cells clearly endocytosed the HRP discharged by the epithelial cells. Furthermore, the loading of these cells by HRP is progressive with time. Because we have not observed any HRP-labelled cells in transit through the choroid epithelium or the ependyma, it can therefore be safely concluded that the labelling of epiplexus cells was due to the phagocytosis of HRP by the intrinsic epiplexus cells residing on the surface of the epithelium. The recruitment of HRP-labelled blood monocytes or choroid connective tissue stromal macrophages to the population of epiplexus cells, if any, would be extremely rare.

Finally, it needs to be emphasised that only 1-d-old rats were examined in this study. It remains to be ascertained whether a similar pattern of HRP uptake by epiplexus cells occurs in adults. However, in our recent study (Lu et al. 1993), we have shown the labelling of epiplexus cells in both postnatal and adult rats after an i.p. injection of the fluorescent dye, rhodamine isothiocyanate. It therefore seems possible that a similar pattern of uptake of HRP by epiplexus cells would be seen in the adults.

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