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Studies on the Ultrastructure and Permeability of the Hemotrichorial Placenta*

II. Fetal Capillaries and Tracer Administration into the Fetal Blood Circulation

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Summary. The distribution of horseradish peroxidase and lanthanum chloride within the full term chorioallantoic placenta of the rat was examined after administration of these tracers into the umbilical artery. Both tracers rapidly traverse the capillary endothelium. Transendothelial channels, fenestrations and micropinocytotic vesicles provide the main pathways. Intercellular clefts which are either patent or interrupted by leaky intercellular junctions, also contribute to a rapid passage of low and high molecular weight substances. Deep channel-like invaginations, effecting an increase of the exchange area of layer III, are freely accessible to the tracers from the interspace between the capillary endothelium and trophoblastic layer III. The invaginations, however, are not in continuity with the interspace between layers II and III, verifying the syncytial character of layer III. Neither an uptake of the tracers nor a passage across layer III is observed. The main permeability barrier to feto-maternal transfer within the chorioallantoic placenta is localized in the syncytiotrophoblastic layer III. This layer controls the passage of low molecular weight substances and restricts the penetration of high molecular weight substances.

Key words: Placenta (rat) – Capillary – Permeability – Tracer – Ultrastructure.

The transfer of substances across the rat placenta from maternal to fetal compartments has been investigated by several authors (Tillack, 1966; Robertston et al., 1971; Fels and Themann, 1971; Metz et al., 1978). The main barrier controlling permeability in the hemotrichorial placenta of the rat, and likewise in hemodichorial and hemomonochorial placentas, is localized in the syncytiotrophoblastic layer investing the maternal blood circulation (Metz et al., 1978).

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Materno-fetal transfer across the chorioallantoic rat placenta appears to be restricted to low molecular weight substances.

The transfer of ferritin from fetal to maternal compartments in the rat placenta was studied by Tillack (1966). In ultrastructural investigations he found a rapid transport of ferritin across the placental barrier. However, Herms et al. (1974) found only a very small and slow transfer of radioactivity from the fetus to the mother after intraamnial or intrafetal administration of ^{14}C-tyrosine.

The purpose of the present study was to investigate the distribution of tracers within the placental labyrinth after their administration into the fetal blood circulation. Our experimental approach was different from that used by Tillack (1966). We applied peroxidase or lanthanum chloride as tracers into the umbilical artery followed by perfusion fixation. This technique ensured precise timing and improved the tissue preservation.

Materials and Methods

Adult female Wistar rats, weighing about 250 g, were used on the twentieth day of pregnancy.

Tracers. Horseradish peroxidase Types II and IV from Sigma (Munich), or Type II from Boehringer (Mannheim), suspended in phosphate buffer at a concentration of 1 mg/ml, were perfused for 1 to 15 min through an umbilical artery prior to fixation. Lanthanum chloride was diluted to a concentration of 5–10 mM in 0.1 M cacodylate buffer. The tracer solutions were saturated with oxygen before perfusion. Both tracers were applied after briefly clearing the placenta of blood with a buffer solution.

Fixation by Perfusion Through the Umbilical Artery

In this procedure the lower abdominal cavity of anesthetized pregnant rats was opened. The fetus and the umbilical cord were exposed after the uterine wall and fetal membranes had been carefully incised far enough from the attachment site of the placenta to avoid major vessels of the uterus and the fetal membranes. Under a dissecting microscope an umbilical artery was cannulated with a 25 gauge yale disposable needle from which the mouthpiece had been detached (Fig. 1). The needle was tightly fitted into a 25 cm long polyethylene tube (Clay-Adams, Intramedic P.E. 90) containing a volume of about 0.2 ml. This tubing was connected to a three-way valve (Fig. 2). The reservoirs for the perfusion solution were at a height of 60 cm. The perfusion was started with 0.2 ml saline solution and was followed by aldehyde fixative for 5 min. Upon initiation of the perfusion, the umbilical vein was sectioned to allow the egress of the fluids. Several placentas were fixed from one pregnant rat. The placentas were detached immediately after beginning the perfusion.

Further procedures used for thin sectioning and freeze-fracturing were essentially the same as described in the companion paper (Metz et al., 1978).

Results

The feto-maternal barrier in the placental labyrinth consists of the fetal capillary endothelium, an interstitial space with basal laminae and interstitial cells, and the trophoblastic layers III, II and I. The lumina of the capillaries, which are much smaller than the sinuses containing the maternal blood, are cleared after perfusion from the fetal side (Figs. 3–6).

Figures 3 and 4 are light micrographs of transverse sections through a control placenta (Fig. 3) and a placenta 5 min after perfusion of peroxidase into the

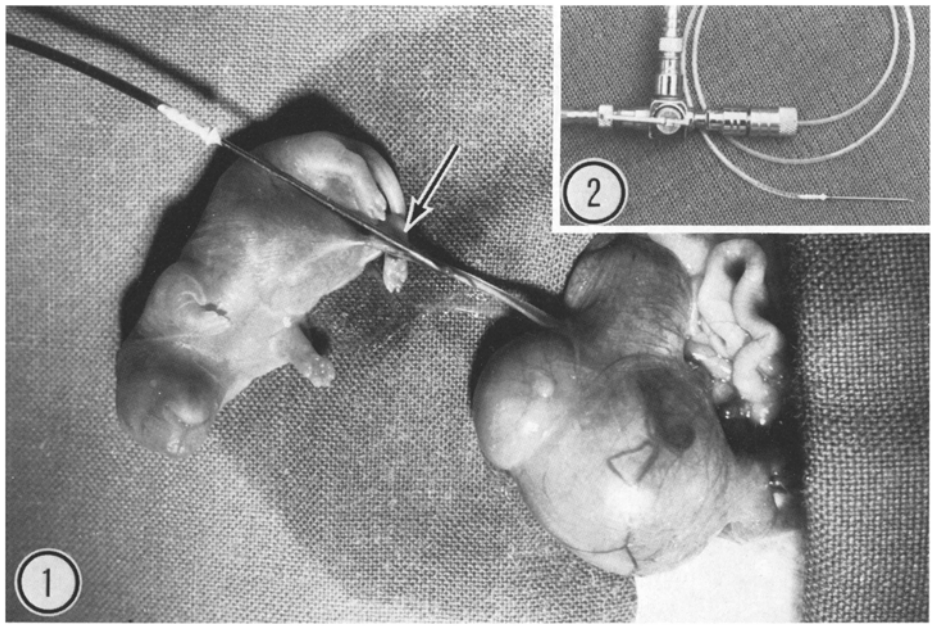


Fig. 1. The cannulation of the umbilical artery is illustrated. The umbilical vein is cut at the level of the arrow

Fig. 2. Perfusion line used for fixation through the umbilical artery. A 25 gauge disposable needle – the mouth piece detached – is fastened to the end of a 35 cm polyethylene tube. The other end is connected by an adaptor to the three way stopcock

umbilical artery (Fig. 4). At this magnification the capillaries appear to be surrounded by electron dense rings of a peroxidase reaction product which is not seen in control placentas (Fig. 4). The peroxidase is localized in the interstitial spaces between the capillary endothelium and trophoblastic layer III. Peroxidase is also seen in continuity with the irregular interstitium along the capillaries. No penetration of peroxidase into trophoblastic layer III or into the maternal blood is detected. The red blood cells in the maternal sinuses are intensely stained by DAB reaction product due to endogenous peroxidase.

The tissue preservation of placentas after perfusion of the umbilical artery with ionic lanthanum for 1–5 min is indinstinguishable from that of untreated controls. Lanthanum is recognized only as small clumps of filamentous material attached to the luminal surface of the capillaries at the light microscopic level. Most of the lanthanum, however, is usually washed away from the lumina of the blood vessels by the fixative that follows the tracer solution. The localization of this tracer within the primary lamellae cannot be identified clearly at the light microscopic level.

The distribution of the tracers after their administration into the fetal blood circulation is shown in low magnification electron micrographs (Figs. 5, 6). One minute after peroxidase (Fig. 6) or lanthanum perfusion (Fig. 5) the tracers are found in the interstitial space and at the basal lamina between the capillary endothelium and trophoblastic layer III. After lanthanum (Fig. 5), micropinocy-

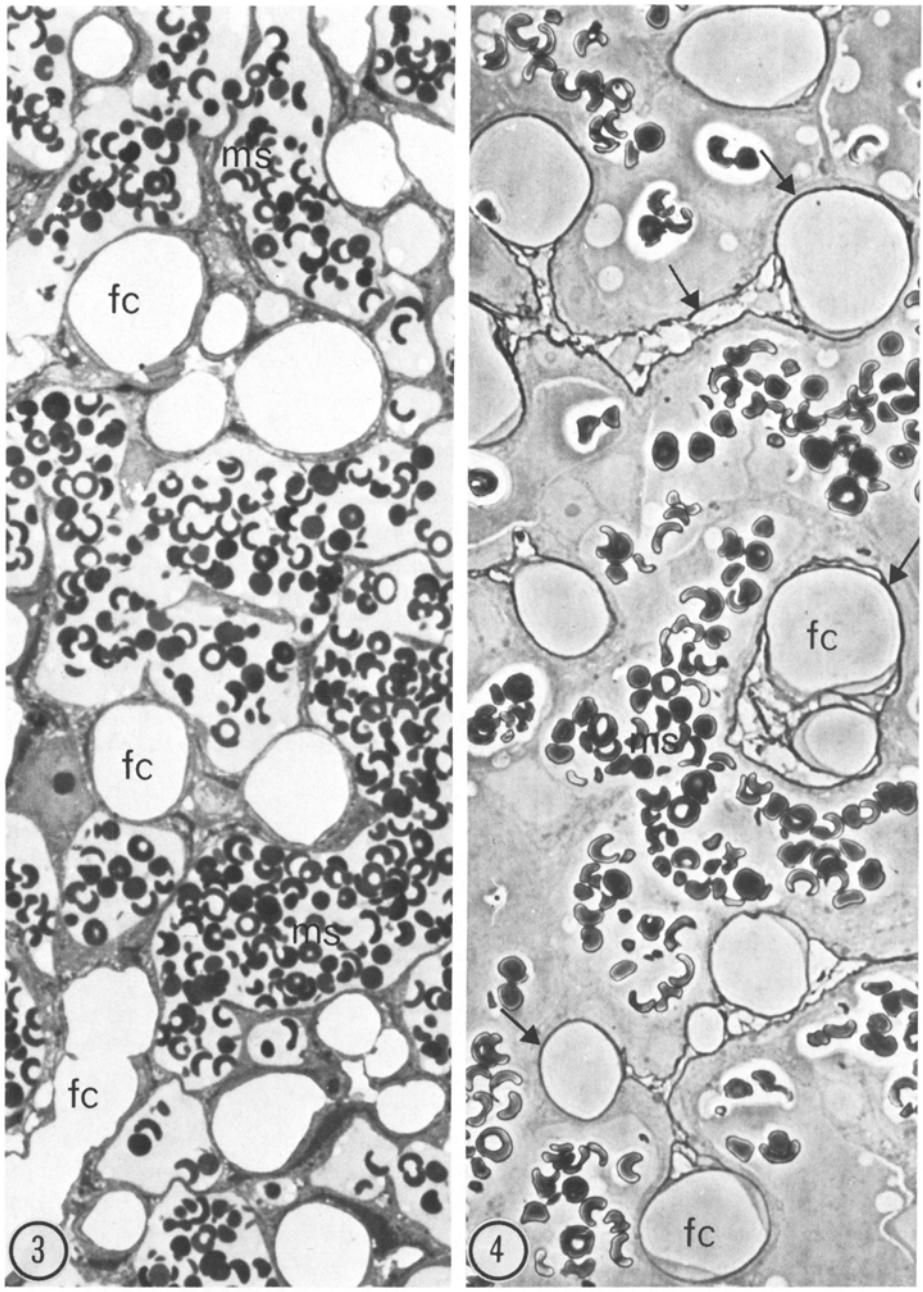


Fig. 3. Light micrograph of a control placenta fixed through the umbilical artery and sectioned transversely to the axis of the placenta. The fetal capillaries (*fc*) are empty whereas the maternal sinuses (*ms*) are filled with blood. × 240

Fig. 4. Light micrograph of a placenta fixed 2 min after perfusion with peroxidase. Reaction product (arrows) is seen in the interstitium around and between the fetal capillaries (*fc*). × 240

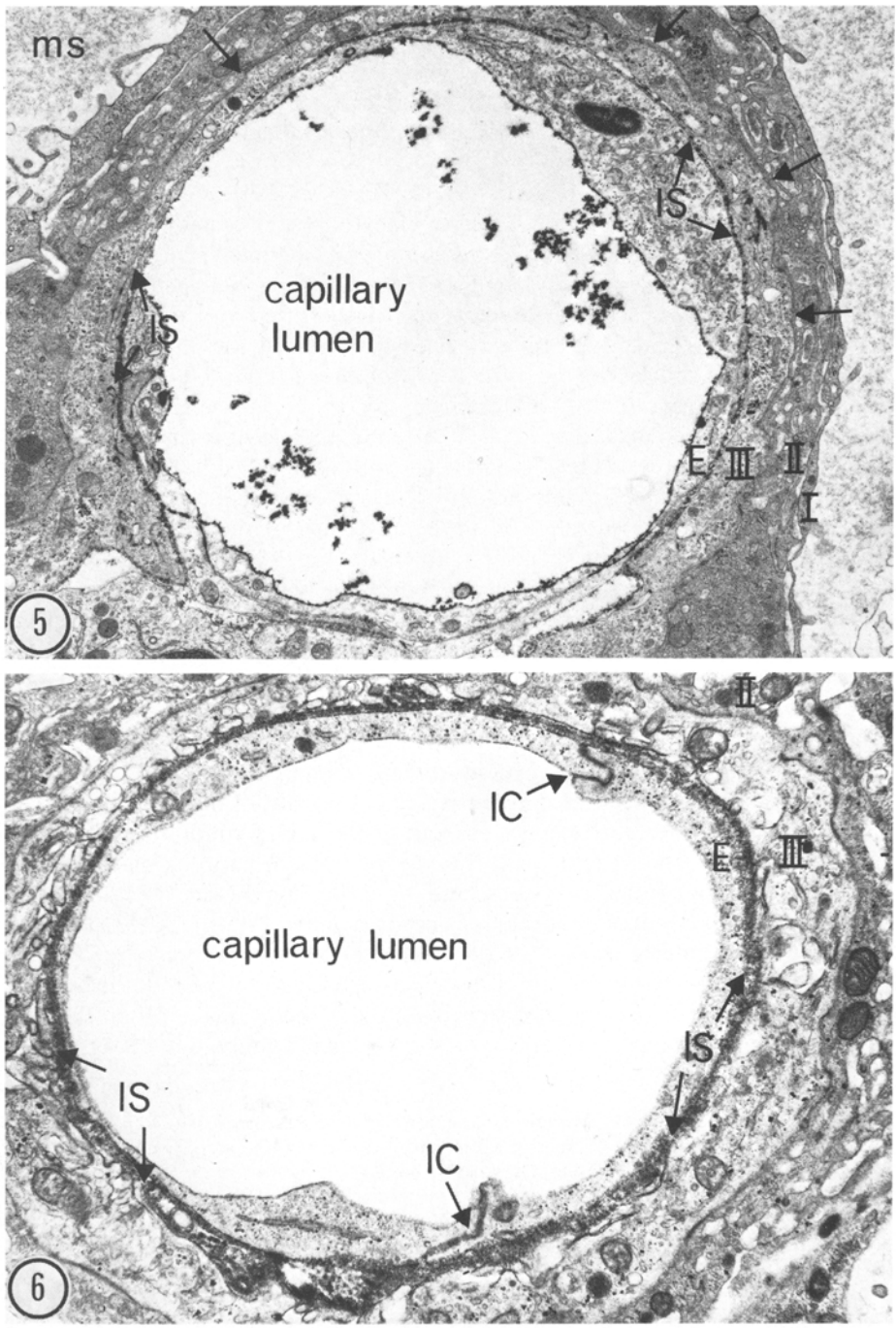


Fig. 5. Electron micrograph of a primary lamella 1 min after perfusion with lanthanum chloride. The tracer is seen in the capillary lumen, at the luminal side of the capillary endothelium (*E*) and within the intercellular space (*IS*) between the capillary and layer III. No tracer is detected beyond the border between layers III and II (arrows) or within the maternal blood space (*ms*). × 6500

Fig. 6. Part of a primary lamella 2 min after perfusion with peroxidase. Reaction product is seen all along the intercellular clefts (*IC*) between the capillary endothelial cells (*E*) and within the intercellular space (*IS*) between the endothelium and layer III (III). Layer II (II). × 15,000

totic vesicles are only rarely filled with tracer, but after peroxidase administration many labelled vesicles are found (Fig. 6). No tracer is observed within the cytoplasm of layer III, in the intercellular space between layers II and III, or beyond this border.

The passage of the tracers across the capillary endothelium is permitted by continuous channels, fenestrations, micropinocytotic vesicles and leaky or patent intercellular clefts (Figs. 7–13). Transendothelial channels, fenestrations and micropinocytotic vesicles are preferentially found in areas of the endothelial cells where the cell is very narrow; often not much thicker than one micropinocytotic vesicle (Figs. 5, 7, 8, 15). In the thicker parts of the endothelial cells, micropinocytotic vesicles are more frequently observed (Figs. 6, 17). The channels are sometimes in continuity with adjacent pinocytotic vesicles (Fig. 7). Thin diaphragm-like structures are seen at the luminal as well as at the interstitial opening of the channels (Fig. 7). The fenestrations also exhibit diaphragms in control placentas (Fig. 8). After a brief perfusion of lanthanum a much higher concentration of tracer is found on the luminal side of the diaphragms of the fenestrations than is seen on their interstitial side (Fig. 9). After longer perfusion periods similar concentrations are seen on both sides (Figs. 10 and 15).

The penetration of the tracers through the intercellular clefts between the endothelial cells of the capillary was further analyzed. The position of tracer within the endothelium depends on the length of perfusion time. Tracers are first seen partially filling the intercellular clefts from their luminal side. Both tracers penetrate into the interspaces between the membrane appositions of the intercellular junctions, which exhibit several membrane contacts (Fig. 11). Lanthanum and peroxidase are later observed in that part of the intercellular cleft abluminal to the intercellular junction, and finally are in continuity with tracer material within the interstitial space (Fig. 6). In freeze-fracturing, discontinuous ridges are found consisting mostly of strands or particulate rows on the P-face of the membrane (Fig. 12) and of discontinuous grooves on the E-face (Fig. 13). Particles sometimes form orderly arrays on the P-face of the membrane (Figs. 12, 13).

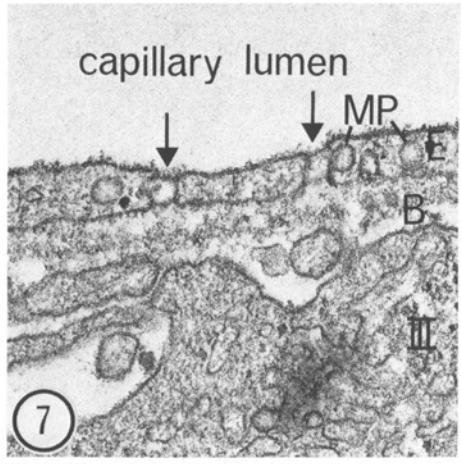
In continuity with the interstitial space between the capillary endothelium and trophoblastic layer III, a complex system of infoldings of layer III is observed which reaches deep into the cytoplasm (Figs. 14–16). These channel-like invaginations

Fig. 7. Part of a primary lamella of a control placenta: Patent channels (arrows) are seen along the flat process of an endothelial cell (*E*). Some micropinocytotic vesicles (*MP*) are in continuity with the channels. Basal lamina (*B*); layer III, capillary lumen. × 55,000

Fig. 8. Part of a primary lamella of a control placenta. Fenestrations with diaphragms (arrows) are seen in the endothelial cells (*E*) of the capillary. Micropinocytotic vesicles (*MP*); basal lamina (*B*); layer III (III). × 55,000

Fig. 9. 30 sec after lanthanum perfusion, high concentrations of the tracer are seen at the luminal side of the capillary endothelium (*E*) and the diaphragms of the fenestrations (arrows). Smaller amounts are found within the interstitial space (*IS*) between the capillary endothelium and layer III. × 40,000

Fig. 10. 2 min after lanthanum perfusion, the tracer is seen at the luminal side of the capillary endothelium (*E*), in fenestrations (arrows), in a few micropinocytotic vesicles (*MP*), and within the interstitial space between the capillary endothelium, the interstitial cells (*IC*) and layer III. × 80,000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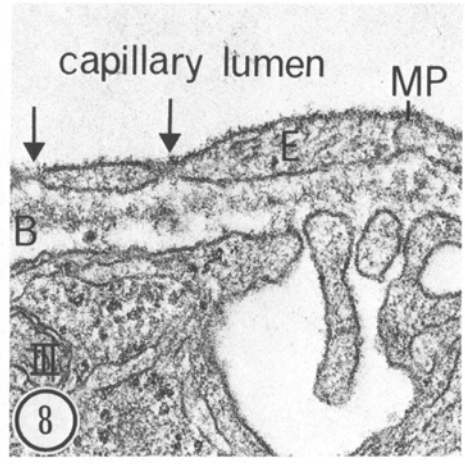
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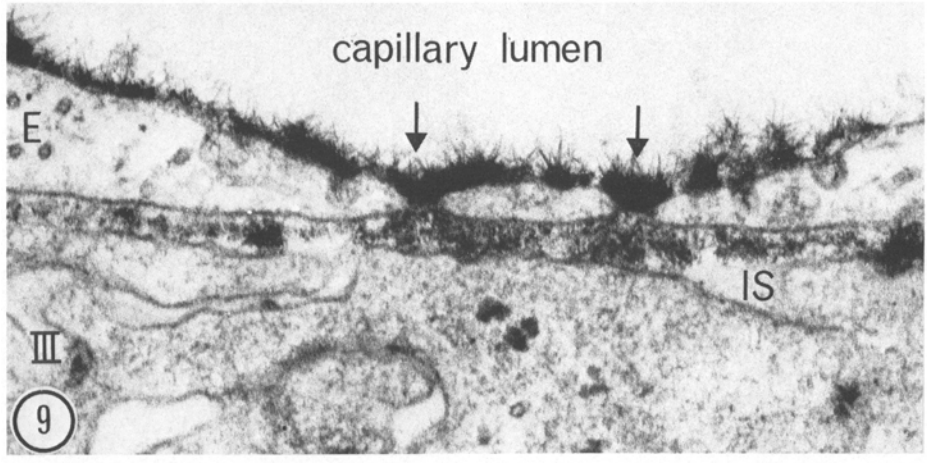
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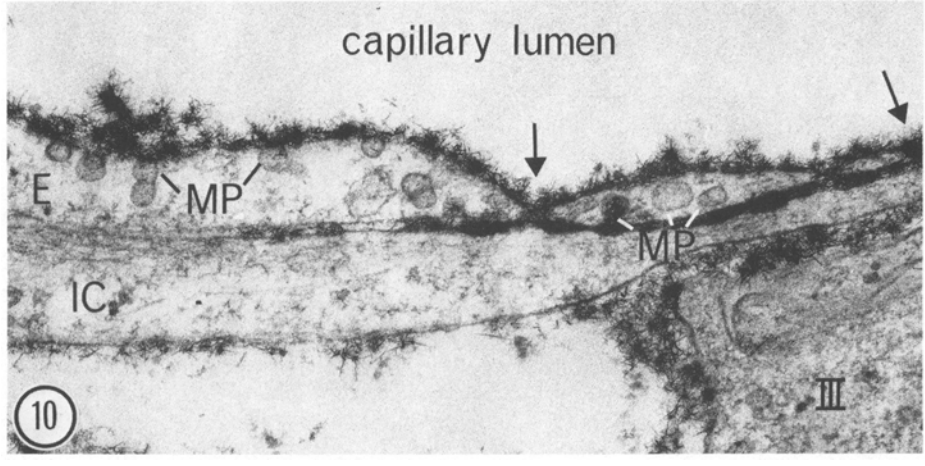
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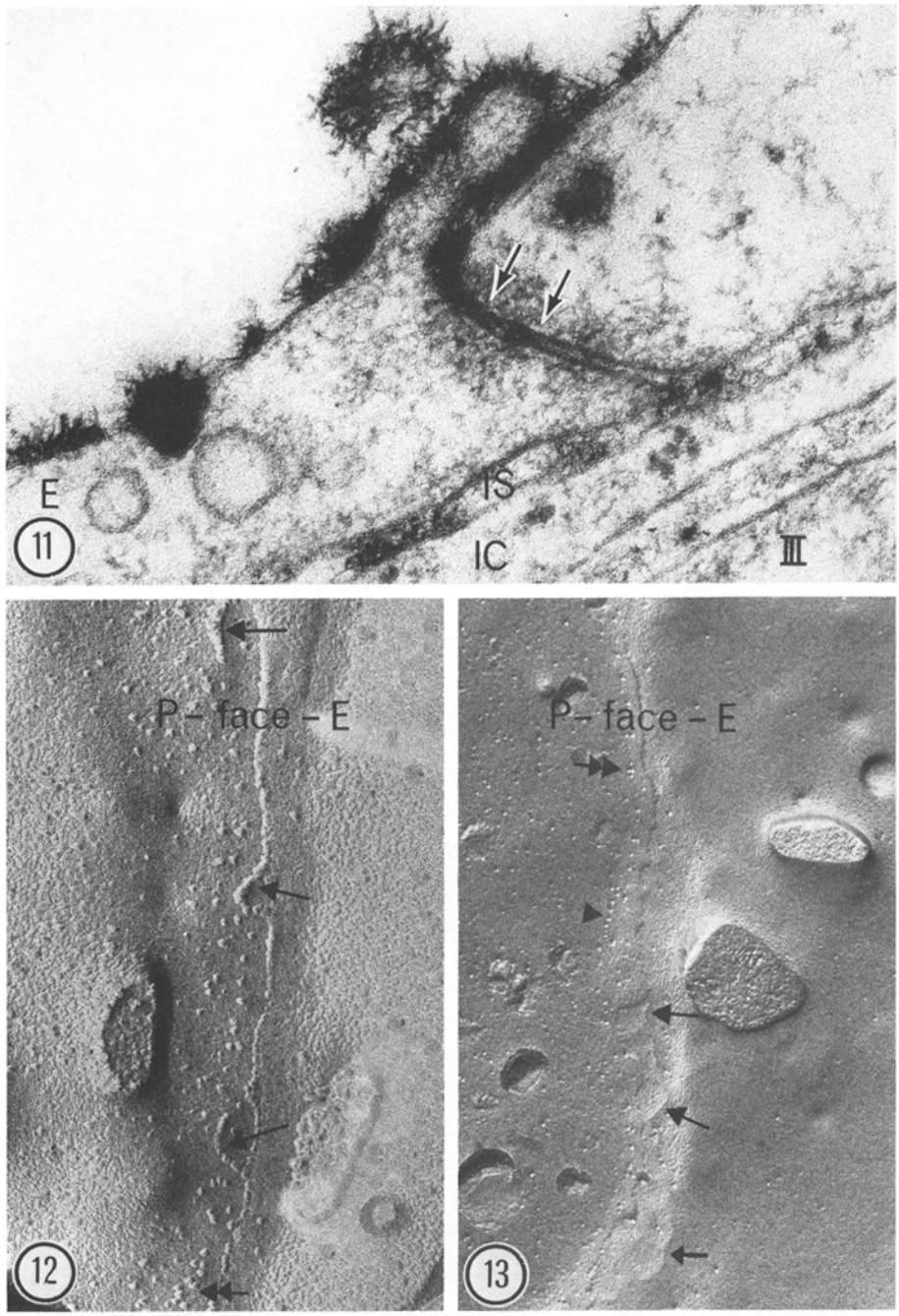


Fig. 11. 15 sec after lanthanum perfusion, the tracer is seen penetrating the junctional complex (arrows) within the intercellular cleft of the capillary endothelium (*E*). The main concentration of lanthanum is in the luminal part of the cleft. Minor concentrations are found within the intercellular space (*IS*). Interstitial cell (*IC*); layer III (III). × 70,000

Fig. 12. Freeze-fracture image of endothelial cells. In the junctional area discontinuous strands of fused particles (arrows) and groups of particles (double arrows) are seen on the P-face (*P*). E-face of the membrane (*E*). × 85,000

Fig. 13. Freeze-fracture image of endothelial cells. Irregularly interrupted grooves (arrows) are seen on the E-face in the junctional area. Groups of particles (double arrow) and rows of loosely oriented particles (arrowhead) are observed on the P-face of the membrane. × 76,000

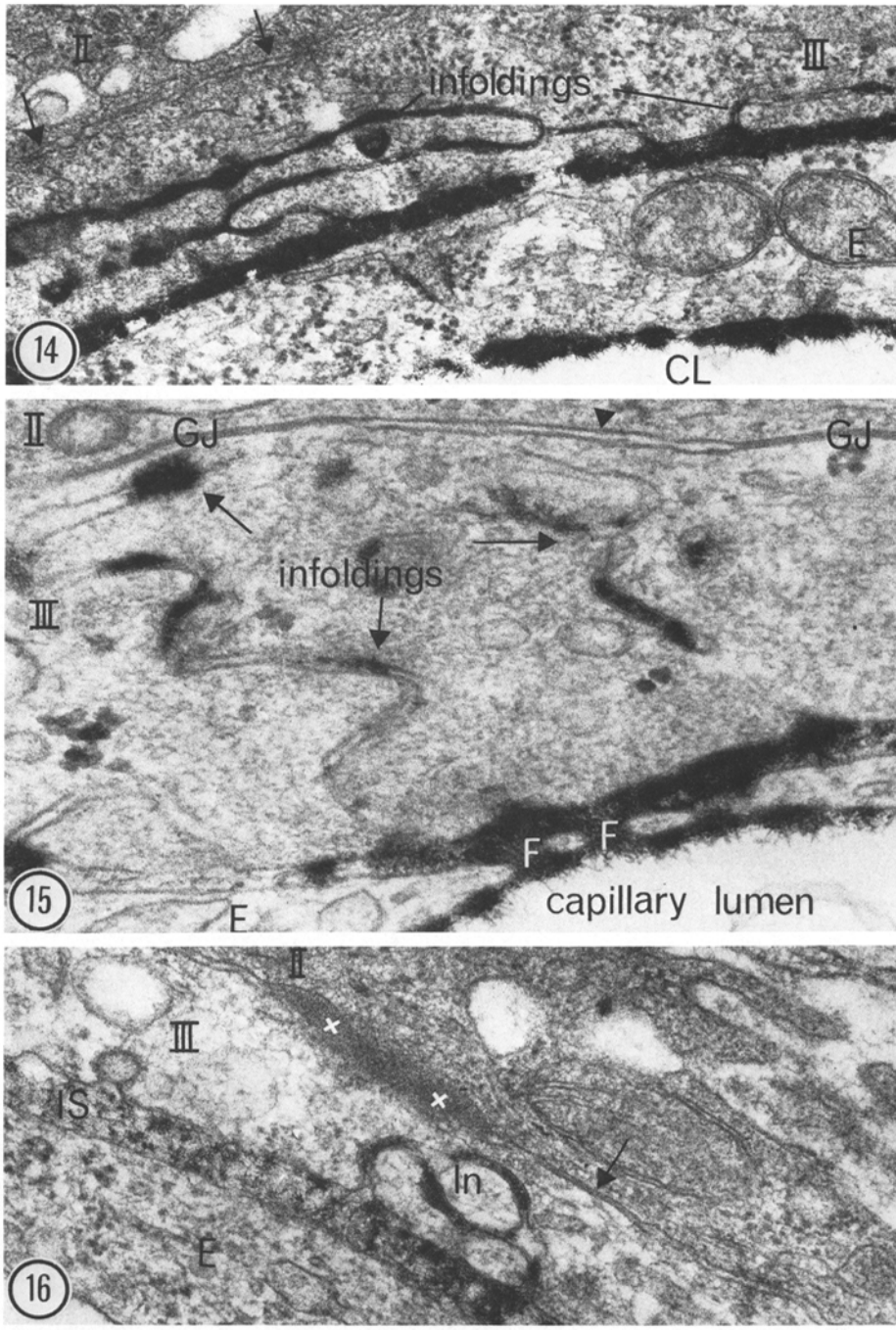
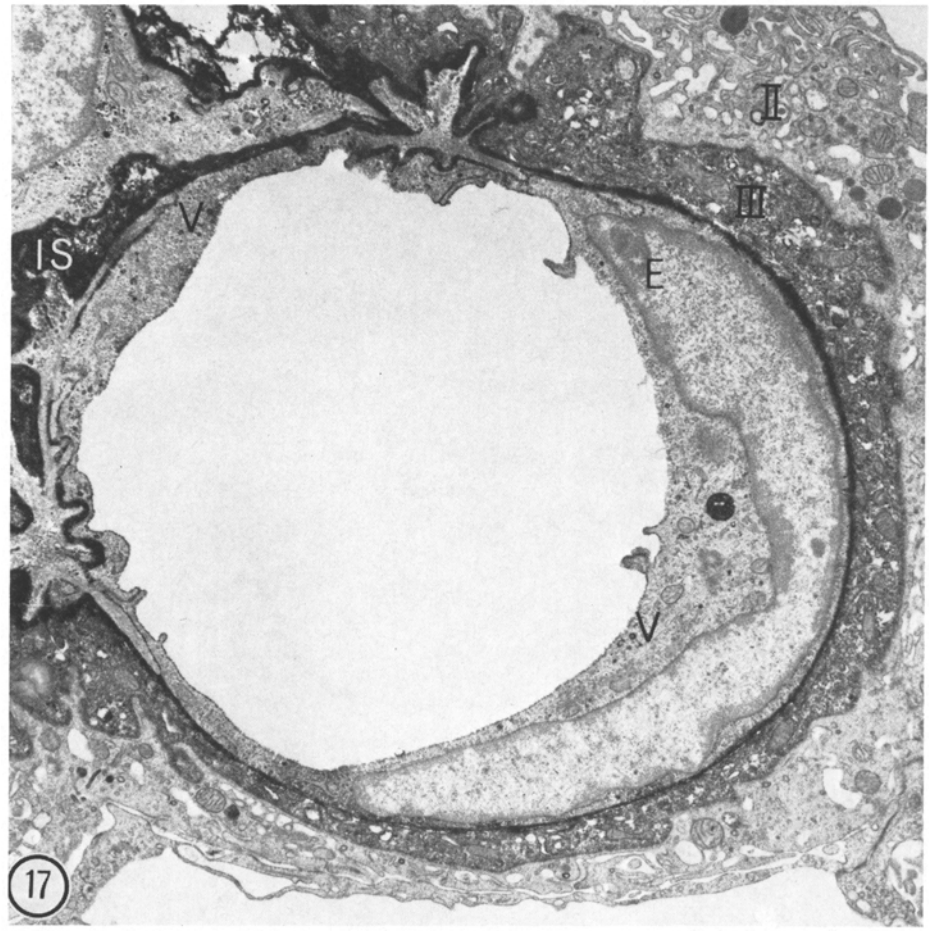


Fig. 14. 2 min after lanthanum perfusion, tracer is found in extensive infoldings of the surface of layer III, which are in continuity with the interstitial space between the capillary endothelium (*E*) and layer III. No tracer is observed within the cytoplasm, in the intercellular cleft between layers III and II (arrows) or beyond this border. Capillary lumen (*CL*). × 60,000

Fig. 15. Channel-like infoldings of layer III, containing tracer, reach close to the interspace between layers III and II (arrowhead): no tracer is observed within the interspace. Gap junctions (*GJ*); endothelial cell (*E*), fenestrations (*F*). × 100,000

Fig. 16. 2 min after lanthanum perfusion: electron dense material (x) between layers III and II is clearly different from the tracer material in the infoldings (*In*) and the interstitial space (*IS*). Endothelium (*E*); gap junction between layer III and II (arrow). × 72,000



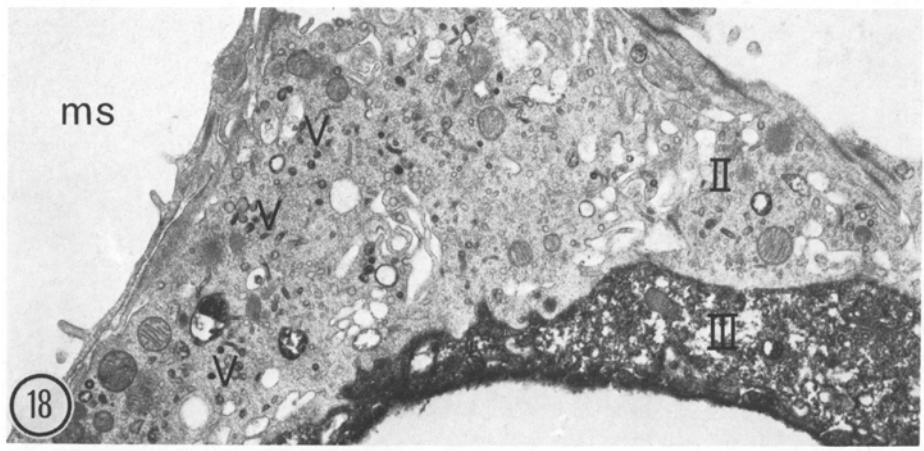


Fig. 17. 2 min after perfusion with type II peroxidase: the cytoplasm of layer III is flooded by the tracer and is more electron dense than the other layers. Tracer is also seen in vesicles within the cytoplasm of the endothelial cell (*E*), and in the interspace between the capillary endothelium and layer III. × 7000

Fig. 18. 2 min after perfusion with type II peroxidase. Reaction product is heavily concentrated in layer III and is also found in many vesicles within the cytoplasm of layer II. Maternal blood sinus (*ms*). × 12,000

follow a tortuous, branching and anastomosing course and are filled with tracers. They often terminate close to the interstitial space between layers II and III. Openings of the infoldings into this intercellular space are not found. The syncytial character of trophoblastic layer III at this developmental stage is confirmed. Furthermore, after lanthanum or peroxidase perfusion for longer periods of time no penetration of the tracers beyond the cell membrane of layer III is observed and in no case are they found in the space between layers II and III. No micropinocytotic vesicles are seen in layer III which would indicate a vesicular transport of the tracers.

A finding in our experiments that may lead to misinterpretation is the occasional presence of a material with an electron density indistinguishable from that of peroxidase. This material is located at the interface of layers II and III and is found in both control and tracer-injected animals (Fig. 16). Its electron density increases with ferrocyanide addition to osmium fixative. The use of lanthanum as a tracer, however, allows this endogenous substance to be clearly identified because of its different electron opacity (Fig. 16).

Perfusion of a high concentration of a less pure type II peroxidase occasionally leads to an uptake and to massive overfilling of the cytoplasm of layer III (Fig. 17). The tracer can be seen between the endothelial cells of the fetal capillary, in the interstitial space, and throughout the cytoplasm of layer III. Even under these conditions no significant penetration of the tracer into the space between layers II and III is observed.

After injury to layer III, which was found by chance in one of our specimens, peroxidase-positive material appeared in layer II (Fig. 18). In this case, however, no tracer is found in the intercellular space between layers II and III, whereas intracytoplasmic vesicles and lysosomes containing peroxidase are observed in the perikarya of layer II.

Discussion

In the rat the diaplacental transfer of substances occurs mainly in the labyrinth of the hemotrichorial placenta. The passage of substances is strictly controlled by the feto-maternal blood barrier and their direction is determined by different functional requirements existing on either side. The feto-maternal transfer of substances is not merely a reverse process of the passage from maternal to fetal compartments since the nature and kinetics of the materials transferred are different, e.g. nourishing substances from the mother and catabolites from the fetus. A structural polarization of the several layers in the primary lamellae of the placental labyrinth is also recognized. Extensive surfaces of the syncytiotrophoblastic layer II facing the maternal plasma space favour a large uptake of substances. This uptake occurs primarily by transmembranous transport processes and is therefore restricted to low molecular weight substances (Tillack, 1966; Metz et al., 1978). Gap junctions probably facilitate the transfer between the syncytiotrophoblastic layers II and III (Forssmann et al., 1975; Metz et al., 1976a, b). Capillaries on the fetal side provide a rapid transport into the fetus.

For the transfer of substances from the fetal to the maternal side the

permeability properties of the fetal capillary and of trophoblastic layer III must first be considered. The capillary wall exhibits species-dependent differences, which may correlate with the structural arrangement of the trophoblastic layers in hemochorial placentas (Becker and Seifert, 1965; Heinrich et al., 1976; Metz et al., 1976a).

In the hemomonochorial human placenta the capillary endothelium is continuous, exhibiting exceedingly scarce micropinocytotic vesicles and tight intercellular junctions (Heinrich et al., 1976). The transendothelial passage at the level of the fetal capillary is mainly controlled by the cytoplasm and the plasmalemma of the endothelial cells and by the permeability properties of the intercellular junctions. These features of the human placenta favour a predominant passage of low molecular substances. It could be shown that tracers administered into the fetal blood circulation are retained by the capillary endothelium (Weihe et al., in press).

The fetal capillary endothelium in the rat placenta is different from that in the human placenta, since it exhibits patent intercellular clefts, micropinocytotic activity, many fenestrations with diaphragms and continuous transendothelial channels (Metz et al., 1976a). Micropinocytotic vesicles, fenestrations and channels represent the large pore system (Pappenheimer, 1953) and are interpreted as different morphological images of related parts in a system of dynamic structures (Simionescu et al., 1972). The transfer of substances rapidly permeating across the capillary endothelium, is suggested to occur mainly across these structures. In contrast to peroxidase, virtually no vesicular transport of lanthanum is observed. Open intercellular clefts as well as clefts joined by leaky junctional complexes are rapidly penetrated by peroxidase and lanthanum (small pore system; Pappenheimer, 1953). Thus the endothelium of the rat fetal capillary is not a significant barrier to high and low molecular weight substances, although a regulatory function is conceivable.

No passage of peroxidase or lanthanum across the plasmalemma of layer III facing the interstitial space to the fetal capillary is found. Also no uptake for example into vesicles is observed, which could account for a vesicular transport of these substances across layer III. Deep infoldings of the plasmalemma, reaching far into layer III are easily filled by tracers. These infoldings approach the vicinity of the plasmalemma of layer III adjacent to layer II. This elaboration of the cell membrane is qualitatively rather similar to the surface development of layer II (Metz et al., 1976a, 1978). It represents a device for increasing the effective exchange area of the cell surface without augmentation of the cell volume. The observation that these infoldings are not in continuity with the intercellular space between layers II and III confirms earlier findings that trophoblastic layer III is a syncytium at this developmental stage (Jollie, 1964; Forssmann et al., 1975; Metz et al., 1976a).

The junctional complexes of the endothelial cells exhibit membrane appositions in thin sections and discontinuous small strands or oriented particles in freeze-fracture replicas. The freeze-fracture images are interpreted as leaky zonulae occludentes (Yee and Revel, 1971; Claude and Goodenough, 1973; Metz et al., 1977). Since the leakiness of the tight junctions is confirmed by the tracer studies, these findings also provide further evidence that freeze-fracture images, although representing intramembranous faces of the plasmalemma, nevertheless allow

deductions concerning the permeability properties of the intercellular contacts (Metz et al., 1977, 1978). We would like to stress, however, that it is only the discontinuity or continuity of tight junctional strands which determine whether or not this junction is leaky with respect to the passage of macromolecules (Yee and Revel, 1971; Claude and Goodenough, 1973; Wade and Karnovsky, 1974; Humbert et al., 1976; Metz et al., 1977, 1978).

Material with an electron density similar to peroxidase is found between layers II and III. It is identified as endogenous material because of its presence in controls and because its electron opacity is different than that of lanthanum. Although under our experimental conditions we do not find any uptake or permeation of tracers across layer III, it is possible that protein tracers are taken up by the trophoblast and degraded by the lysosomal system. If this were the case, the tracers would not be detected by histochemical or biochemical means. A distinct lysosomal activity is observed in the perikarya of layer II after floating layer III with peroxidase. This mechanism does not seem to represent a transport system which operates under normal conditions, but the fact that high molecular weight substances eventually cross layer III and are taken up by layer II seems to be very interesting and important. In spite of a lysosomal uptake and a degradation of these substances, transport may also occur into the maternal blood sinuses. This latter hypothesis would account for differences in the barrier functions of layer II and III with respect to the direction of transport.

In summary, we conclude that the main barriers within the full term hemotrichorial rat placenta consist of the syncytiotrophoblastic layers II and III. These layers control the transfer of low molecular weight substances and prohibit the passage of high molecular weight substances. The uptake of materials from maternal or fetal compartments occurs mainly by transmembranous transport processes of these layers.

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