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 fetomaternal 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