# Peculiar ruthenium red staining pattern of the human early trophoblast

M. Sideri, G. de Virgiliis, R. Rainoldi and G. Remotti

*Section of Obstetrical and Gynecological Pathology of the First Clinic of Obstetrics and Gynecology of the University of Milan and CNR Centre of Cytopharmacology, Via Commenda 12, I-20122 Milan (Italy), 9 July 1981*

*Summary.* This paper describes the ruthenium red staining (RRS) pattern observed on the 2 sides, maternal and fetal, of the human early trophoblast. The possibility of a different plasma membrane composition of the 2 sides of the human early trophoblast is discussed.

Ruthenium red has been recommended by Luft[1] as an electron stain for cell surfaces as it binds the anionic groupings of mucopolysaccharides of the external cell coat[2,3]. In the human placenta, ruthenium red staining (RRS) was used by Reissig[4] to reveal the glycocalyx at the plasmalemmal infoldings between microvilli and the syncytial micropinocytotic activity. This paper reports more detailed observations on the RRS pattern of the early placenta concerning the composition of both the syncytial and cytotrophoblastic plasma membranes.

*Materials and methods.* Biopsies of 3 human placentas of 10–13 weeks into pregnancy, were obtained from voluntary interruption of pregnancy performed by dilatation and curettage. They were fixed by immersion for 2 h in a solution of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (PH 7.4) containing 0.1% ruthenium red at 4 °C. Biopsies were then rinsed overnight at 4 °C in a solution of cacodylate buffer 0.1 M (PH 7.4) containing 0.1 ruthenium red. Postfixation was carried out in a solution of 1% osmium tetroxide in 0.1 M cacodylate buffer (PH 7.4) containing 0.1% ruthenium red. Biopsies were dehydratated in ascending alcohols and embedded in Epon 812. Thin sections were cut with a diamond knife, stained with lead citrate and finally observed by means of a Philips EM 200 electron microscope.

*Results.* The syncytial plasma membrane facing the intervillous space (SPMm) appeared as a dark layer stained by ruthenium red. The dye did not bind homogeneously to the external cell coat but in some areas (mostly on the microvillar surface), it formed little clumps of electron dense material, quite uniformly distributed. RRS marked the inner plasma membrane of the coated vesicles opening at the syncytial surface and also that of some vesicles enclosed in the syncytial cytoplasm (fig. 1).

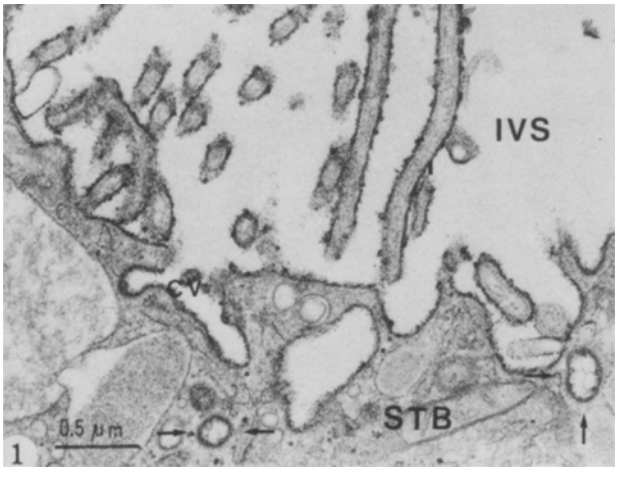


Figure 1. First trimester placenta. The figure shows the non-homogeneous binding of ruthenium red to the syncytial plasma membrane bathed by the maternal blood. Some coated vesicles are also evident in the syncytial cytoplasm (arrows). IVS, intervillous space; STB, syncytiotrophoblast; CV, coated vesicles.

In contrast to what was observed on the SPMm, both the syncytial and the cytotrophoblastic plasma membranes facing the fetal capillary were homogeneously contrasted by RRS (fig. 2). Finally, the trophoblastic basement membrane appeared as a uniform, thick, and dark layer (fig. 3).

*Discussion.* RRS uniformly marks the external cell coat[1,3,5]. The presence of ruthenium red clumps on the SPMm is the most interesting finding in our report, and suggests a nonhomogeneous composition of this plasma membrane. This interpretation is in agreement with the cytochemical study by Martin et al.[6] that demonstrated the heterogeneity of the SPMm. Even though the nonspecific binding of the RRS does not allow an evaluation of the biochemical constitution of the trophoblastic plasma membrane, it nevertheless demonstrates a difference between the 2 sides, maternal and fetal, of the early human trophoblast. It is possible that the peculiar staining pattern of the SPMm is

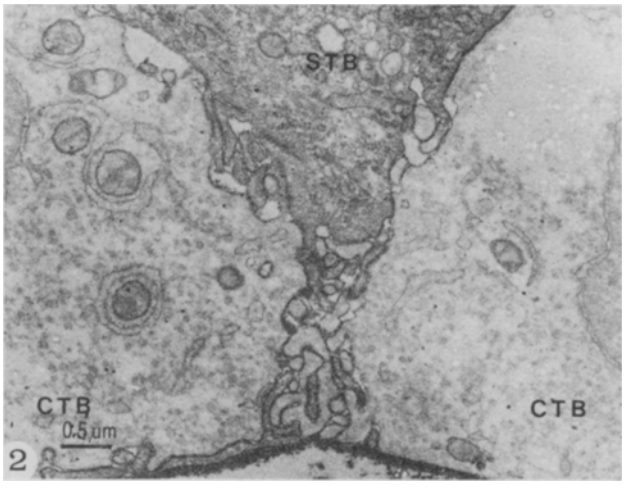


Figure 2. First trimester placenta. The figure shows the intertrophoblastic space as it appears after ruthenium red staining. Note the homogeneity of the staining. STB, syncytiotrophoblast; CTB, cytotrophoblast.

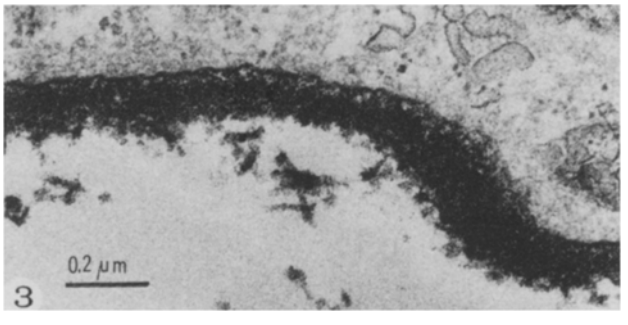


Figure 3. First trimester placenta. The figure shows a detail of the trophoblastic basement membrane after ruthenium red staining. The dark and uniform staining pattern is evident.

related to the variety of transport, enzymatic, and receptor activities which are likely to be properties of this membrane[7,8].

The presence of the dye on the inner plasma membrane of some coated vesicles enclosed in the syncytial cytoplasm, may be due to the section plane, or can be interpreted as the final step of an endocytotic process started at the time of fixation and not suddenly blocked by the fixative. The latter is a problem of general experimental cell biology which reaches far beyond the scope of the present investigation, and deserves further discussion.

Finally the RRS pattern of the trophoblastic basement membrane may be related to the presence of acidic glycoproteins since they were demonstrated by a previous biochemical study in the basement membrane of the human trophoblastic epithelium[9].

1 J.H. Luft, Anat. Rec. *171*, 347 (1971).
2 B.A. Fowler, Histochemie *22*, 155 (1970).
3 K. Yamada, Histochemie *23*, 13 (1970).
4 D. Reissig, Verh. anat. Ges. *71*, 499 (1977).
5 A.G. Everson Pearse in: Histochemistry, vol. 2, p. 1275, 3rd edn. Churchill and Livingstone, Edinburgh and London 1972.
6 J. Martin, S.S. Spicer and N.M. Smythe, Anat. Rec. *178*, 796 (1974).
7 L.K. Kelley, B.F. King, L.W. Johnson and C.H. Smith, Exp. Cell Res. *123*, 167 (1979).
8 M. Sideri, G. de Virgiliis, G. Fumagalli and G. Remotti in: Electron microscopy, vol. 2, p. 30. Eds P. Brederoo and W. De Priester. 7th Eur. Congr. Electron Microsc. Found., Leiden 1980.
9 M. Ohno, R.B. Nagle, E. Meezan and K. Brendel, J. Supramolec Struct. *12*, 457 (1979).

## Stereotaxic technique for transplantation of neural tissues in the brain of adult rats[1]

G.D. Das and D.T. Ross

*Department of Biological Sciences, Purdue University, West Lafayette (Indiana 47907, USA), 19 October 1981*

*Summary.* A technique of neural transplantation in the brains of adult animals, using stereotaxic apparatus, is described. It facilitates transplantation of neural tissues of small volumes in precisely defined structures of the host brain, and yields a high percentage of successful transplantations.

The successful transplantation of neural tissues in the brains of laboratory mammals depends heavily upon the technique of transplantation. A sound technique aids in keeping the neural transplants viable for long durations, inducing minimal or no pathological reaction in the host brain, causing minimal bleeding at the site of transplantation, and achieving a very high percentage of success in the survival, growth and integration of the transplants. The technique described in our earlier publications[2,3] meets these requirements satisfactorily. That technique requires the use of a glass tuberculin syringe with a glass needle sealed into it for the injection of neural transplants into the host brain. The injection is done with one hand, while the host animal is held firmly in the other hand. That technique has the advantage of freedom of holding the host animal in any position and of injecting the neural transplants from any angle. But, it does not aid in injecting the transplants in precisely defined structures of the host brain.

Neuroendocrinologists have conventionally used stereotaxic apparatus for implanting non-neural tissues[4] or chemical substances[5] in the hypothalamus of the experimental animals. In recent years the same technique has been used for implantation of neural tissues in the brains of the host animals[6]. In all these studies the investigators have used trocar, or metalic cannulae or needles of 1 mm or more in diameter for carrying the implant inside the host brain and expelling it at the desired site. With this instrumentation one has the advantage of using the stereotaxic apparatus, but disadvantages of metalic cannulae or needles. During the past few years, in some studies, we have employed a modified procedure by adapting the glass syringe with a glass needle to a stereotaxic apparatus for transplantation. In the following some details on the assembly of instrumentation and technical advantages of it are presented.

*Instrumentation and its assembly.* The setup for transplantation includes 3 glass syringes and a stereotaxic unit. The 3 syringes include a transplantation syringe (Luer-Slip, 0.5 ml), a carrier syringe (Luer-Lok, 5.0 ml), and a hydraulic syringe (Luer-Lok, 5.0 ml). The relationship between these syringes is shown in figures 1 and 2. The carrier syringe, to start with, is used for holding the transplantation needle in position and for lowering or raising it with the aid of the hydraulic syringe. In order to achieve these functions the syringe is held upside down, and attached firmly to an electrode holder with appropriate clamping device. Into its Luer-Lok socket, which is at its top, an 18-gauge metal needle is attached. This needle is connected to the hydraulic syringe, which also has an 18-gauge metal needle in its Luer-Lok socket, with the aid of a 60–70 cm long clear polyethylene tubing (PE-60). These 2 syringes and the polyethylene tubing are filled with deionized water. By moving the plunger in the hydraulic syringe to-and-fro the plunger in the carrier syringe can be moved synchronously up-and-down.

The outer end of the plunger of the carrier syringe, which faces downward, has a Luer-Lok socket attached at its head with the aid of epoxy cement. Luer-Lok sockets which are used in this setup can be readily extracted from discarded syringes. Before gluing this Luer-Lok socket a large hole, 3-4 mm in diameter, is made on its side. It is important to note that the Lue-Lok socket is attached to the head of the plunger in such a fashion that the central vertical axes of both are coincidental.

The transplantation needle (fig. 2 inset), that is affixed to the Luer-Lok socket, consists of a bevelled glass capillary needle, a 17-gauge metalic needle hub, and 60–70 cm long polyethylene tubing (PE-60). The glass needle is made from a capillary tubing (0.8 mm outer diameter, 0.6 mm inner diameter, 3.0 cm long), and it is beveled to a smooth tip at one end as described in earlier publications[2,3]. The other end of the glass needle is inserted into the polyethylene tubing and their junction sealed with epoxy cement. The glass needle is then inserted into the metalic needle hub, sealed with epoxy cement, and allowed to dry overnight. It