Virchows Arch. A Path. Anat. and Histol. 379, 229–241 (1978)

*Virchows Archiv A*


# An Ultrastructural Study of the Placenta in Materno-Fetal Rhesus Incompatibility

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**Summary.** An electronoptical study has been made of eleven placentae from cases of materno-fetal rhesus incompatibility. The characteristic findings are focal, but sometimes quite extensive, syncytial necrosis, retention of normal pinocytotic and secretory activity in the non-necrotic syncytiotrophoblast, cytotrophoblastic hyperplasia, thickening of the trophoblastic basement membrane, immature-type endothelial cells in the fetal villous vessels and thickening or lamination of the capillary basement membranes. The pathogenesis of many of these changes is not clear but there is no evidence that they are immunologically mediated. It is suggested that the syncytial necrosis may be due to narrowing of the intervillous space as a result of increased villous size, that the cytotrophoblastic hyperplasia is a response to the syncytial damage and is responsible for the changes in the trophoblastic basement membrane and that the fetal capillary changes are indicative of endothelial cell damage due, possibly, to fetal anaemia. Despite the damage suffered by the placenta in materno-fetal rhesus incompatibility there is little evidence of impaired functional efficiency.

**Key words:** Placenta — Ultrastructure — Rhesus incompatibility.

## Introduction

Placental abnormalities in haemolytic disease of the neonate due to materno-fetal rhesus incompatibility have been fully documented at the light microscopic level (Hellman and Hertig, 1938; Javert, 1942; Henderson, 1942; Kline, 1948; Schmidt, 1949; Bichenbach and Kivel, 1950; King, 1951; Respetti and Pescetto, 1951; Martius, 1956; Chernyak and Rabtsevich, 1959; Thomsen and Berle, 1960; Becker and Bleyl, 1961; Marziale, 1961; Cecco et al., 1963; Calderon et al., 1963; Wentworth, 1967; Liebhart, 1969, 1973; Busch and Vogel, 1972; Hölzl et al.,

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0340-1227/78/0379/0229/$02.60

1975). Placentae from such cases have, however, rarely been subjected to electron microscopic examination and the resulting paucity of information as to their ultrastructural pathology has been compounded by the limitation of these few studies to the examination of only a very small number of placentae and by a failure to reach any measure of agreement as to their findings (Arnold et al., 1961; Zacks and Blazer, 1963; Widmaier, 1969; Liebhart, 1971). This is unfortunate, for it has been suggested that the placenta in this disease serves as a model both for immunologically mediated attack on trophoblastic tissue (Burstein et al., 1963) and for placental insufficiency (Bender, 1974). We therefore report here an ultrastructural study of a relatively large sample of placentae from infants with haemolytic disease due to materno-fetal rhesus incompatibility.

## Material and Methods

Eleven placentae were examined, all of which were from pregnancies in which the only complicating factor was materno-fetal rhesus incompatibility. Six of the pregnancies were terminated between the 29th and 35th week of gestation and five between the 35th and 30th weeks. Anti-D antibodies were present in the serum of all the mothers, the titre ranging from I: 32 to I: 4096. All the infants were considered to have haemolytic disease and nine required exchange transfusions; four of the babies died during the neonatal period from rhesus haemolytic disease but the series did not include any stillbirths.

The placentae were obtained immediately after delivery and a portion of villous tissue taken from the central area of the placenta at a point 1 cm deep to the basal plate. The selected pieces of tissue were diced on dental wax into fragments which were fixed at room temperature in 2.5 per cent gluteraldehyde in 0.1 M cacodylate buffer at pH 7.4. The tissues were then processed in a manner previously described (Jones and Fox, 1977) and examined in either an AEI EM6B or a Phillips 301 electron microscope.

## Results

Observations were limited to the terminal villi, no attempt being made to examine any other component of the placenta.

*Syncytiotrophoblast.* The microvilli on the syncytial surface were, in many areas, diminished in number (Fig. 1) or distorted into irregular, sometimes bizarre, shapes (Fig. 2); these microvillous abnormalities were often associated with focal degenerative or necrotic changes in the immediately underlying syncytiotrophoblast indicated by swelling and rarefaction of the mitochondria and vesiculation of the membrane systems. In most placentae these changes were largely confined to the superficial part of the syncytiotrophoblast but in two, both from severely affected infants delivered before the 30th week of gestation, there were extensive areas in which there was a full thickness necrosis of the syncytial tissue. In the non-necrotic syncytiotrophoblast of these two latter placentae there was vacuolation of the endoplasmic reticulum, swelling of the mitochondria and electron-lucent nucleoplasm in which the nuclear chromatin

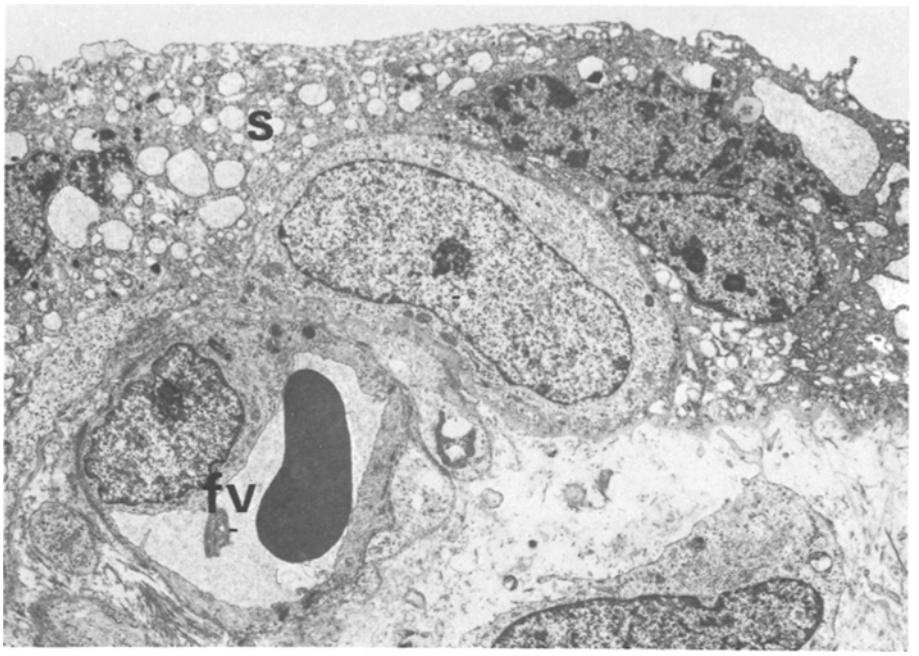


**Fig. 1.** An area of villous trophoblast in which there is almost complete loss of syncytial microvilli and in which the syncytiotrophoblast shows vesiculation of the endoplasmic reticulum and mitochondrial swelling. An abnormally small fetal vessel is present in the villous stroma (EM × 5000). *s* syncytiotrophoblast; *fv* fetal vessel

was coarsely aggregated against the nuclear membrane. In the other nine placentae, however, the non-necrotic syncytiotrophoblast, which comprised the vast bulk of this tissue, contained numerous electron-dense mitochondria of normal size, well formed Golgi bodies and abundant non-vacuolated endoplasmic reticulum; there was active pinocytotic activity and formation of multivesicular and dense bodies (Fig. 3). Secretory droplets were numerous and were, on occasion, unusually large. The syncytial nuclei in these placentae were generally normal with an irregular outline, moderately aggregated chromatin and electron-dense nucleoplasm. In one placenta, from a moderately affected infant delivered at 36 weeks, there were however, some nuclei which had broken down into coils of nucleoprotein with myelin body formation (Fig. 4). In several placentae there was evidence of oedema, particularly around the area of the basal plasma membrane which was frequently thrown into interdigitations of an unusual degree of complexity.

*Cytotrophoblast.* In the two placentae with very extensive syncytial necrosis the cytotrophoblastic cells formed an almost complete layer and were, in some places in which they were denuded of overlying syncytium, in contact with the maternal blood in the intervillous space; under these conditions the cyto-

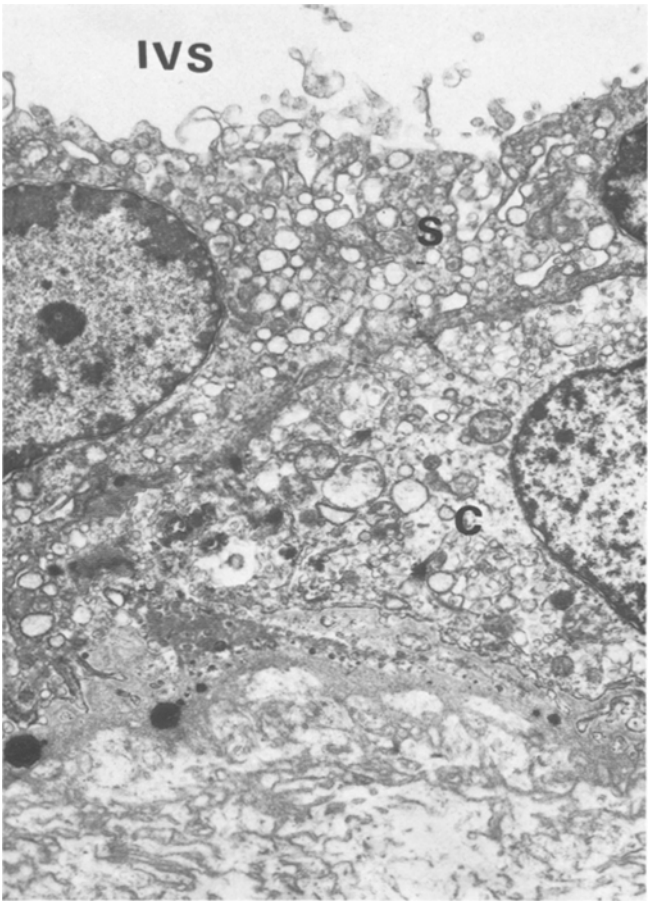


**Fig. 2.** Villous trophoblast: the microvilli are markedly abnormal in shape whilst degenerative changes are seen both in the syncytiotrophoblast and in a cytotrophoblastic cell (EM ×10,000). *ivs* intervillous space; *s* syncytiotrophoblast; *c* cytotrophoblastic cell

trophoblastic cells showed a tendency to develop microvilli on their apical surface (Fig. 5). Numerous cytotrophoblastic cells were also present in all of the remaining nine placentae (Fig. 6) and were often, even in specimens delivered at 38 or 39 weeks, seen to be undergoing mitotic division (Fig. 7). The cells were frequently of the metabolically active "intermediate" type (Fig. 8) with electron-dense, membrane bound granules and numerous Golgi bodies; moderately electron-dense mitochondria were often associated with narrow cisterns of rough endoplasmic reticulum, clusters of free ribosomes were numerous and microtubules, microfilaments and occasional nematosomes were also present. Coated vesicles, both in the region of the Golgi apparatus and in contact with the plasma membrane, were also found. The cytotrophoblastic nuclei were usually oval with evenly dispersed chromatin and at least one nucleolus.

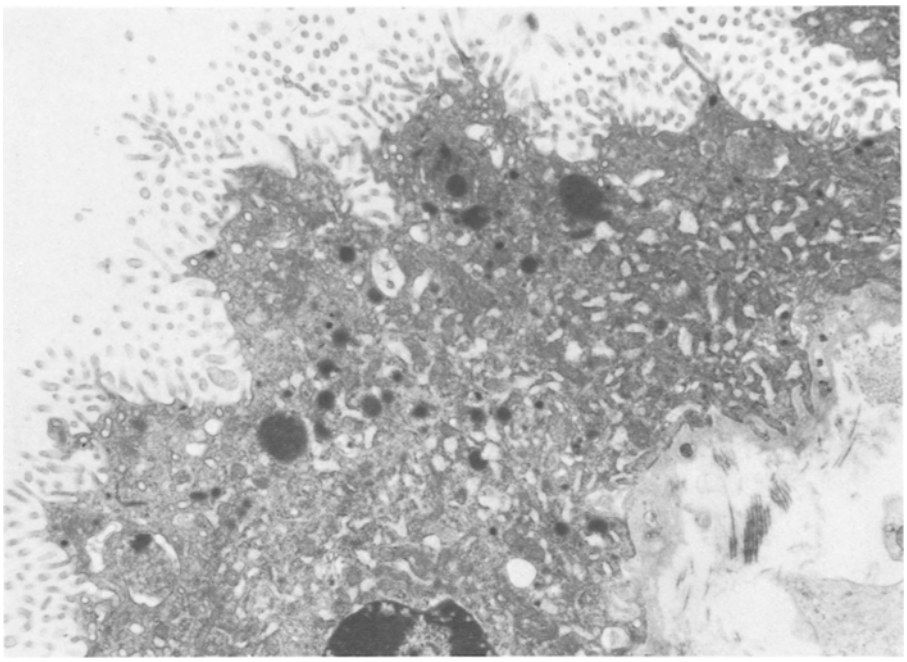

**Fig. 3.** A healthy area of villous trophoblast; the syncytiotrophoblast contains abundant secretory droplets, small dense mitochondria and a well formed Golgi apparatus whilst the syncytial microvilli are normal (EM $\times 10{,}000$)

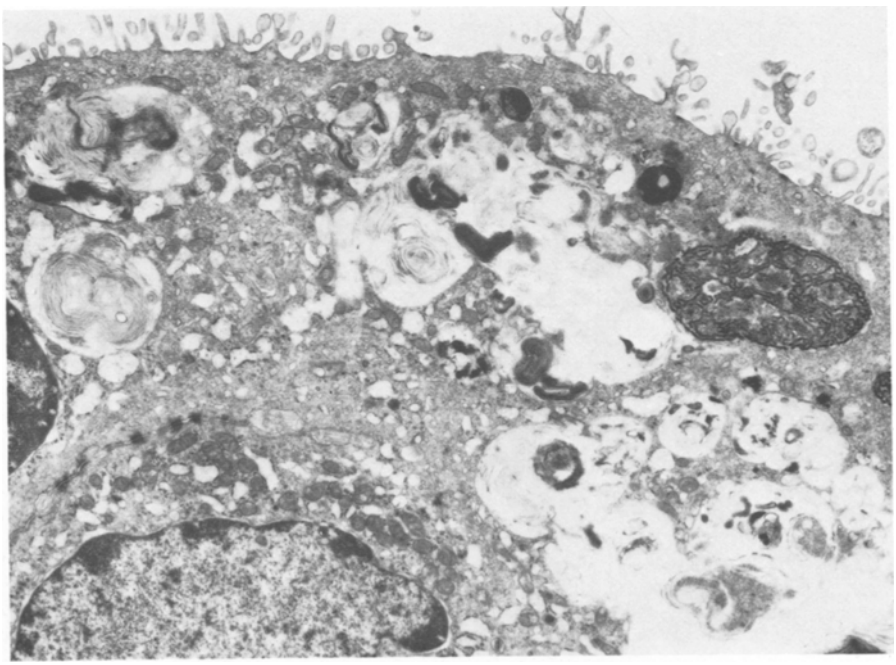

**Fig. 4.** An area of villous trophoblast in which the syncytial nuclei have broken down to coils of nucleoprotein (EM $\times 7500$)

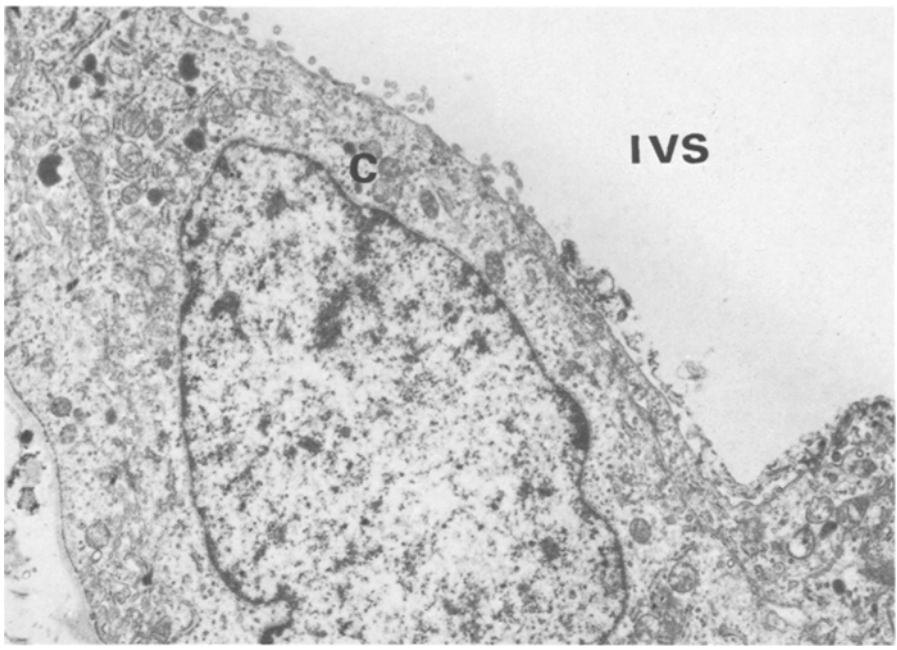


**Fig. 5.** A villous cytotrophoblastic cell in a placenta from an infant delivered at the 29th week of gestation; total syncytial necrosis has exposed the cytotrophoblast to the intervillous space and small microvilli are forming on its free surface. Some residual syncytial debris is present. (EM $\times$ 10,000). *ivs* intervillous space; *c* cytotrophoblastic cell

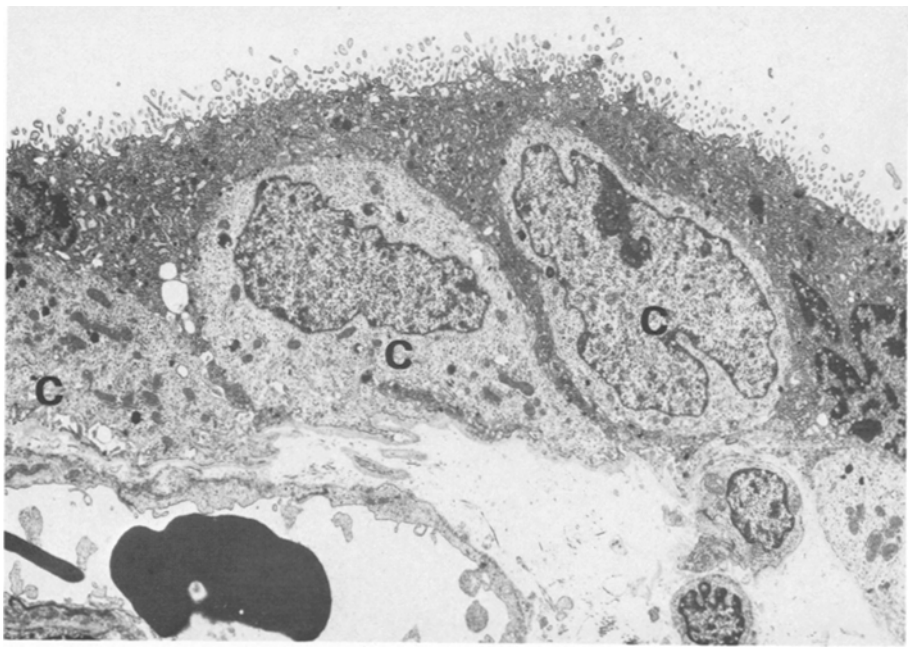


**Fig. 6.** Villous trophoblast in which a number of prominent cytotrophoblastic cells are present. (EM $\times$ 5000). *c* cytotrophoblast

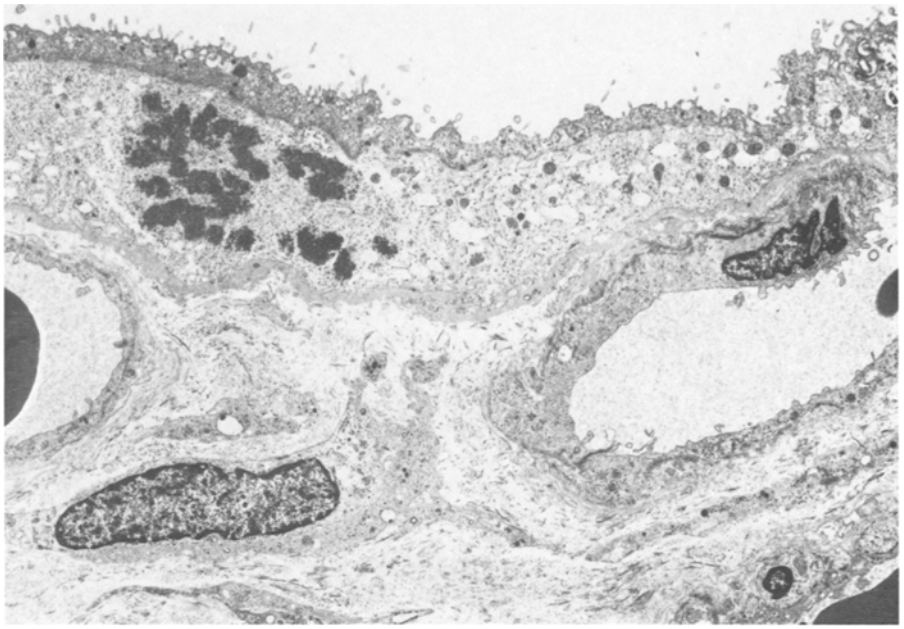

**Fig. 7.** A cytotrophoblastic cell undergoing mitotic division in a placenta from a 38 week gestation (EM $\times$ 5000)

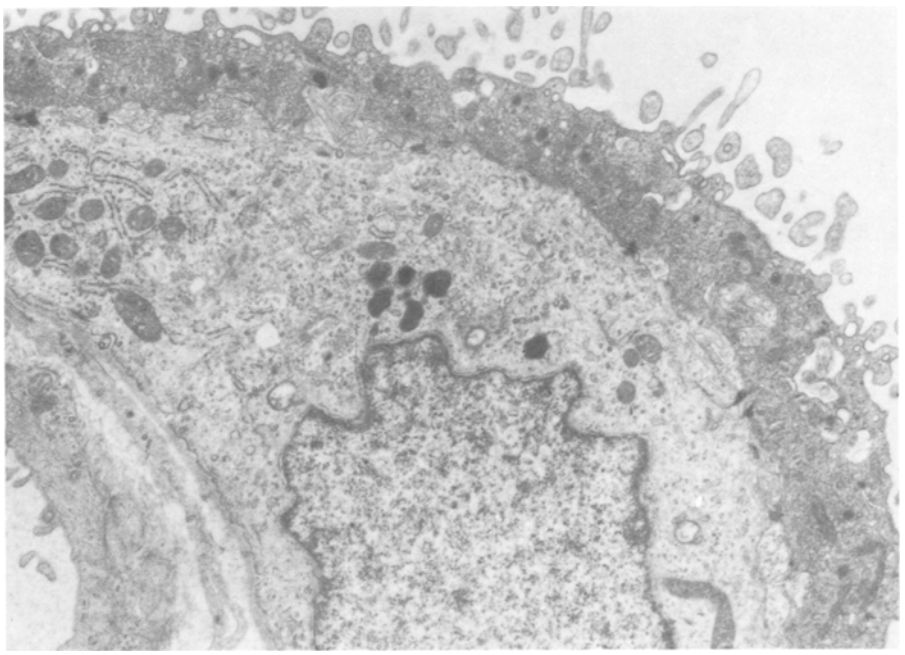

**Fig. 8.** A metabolically active cytotrophoblastic cell (EM $\times$ 10,000)

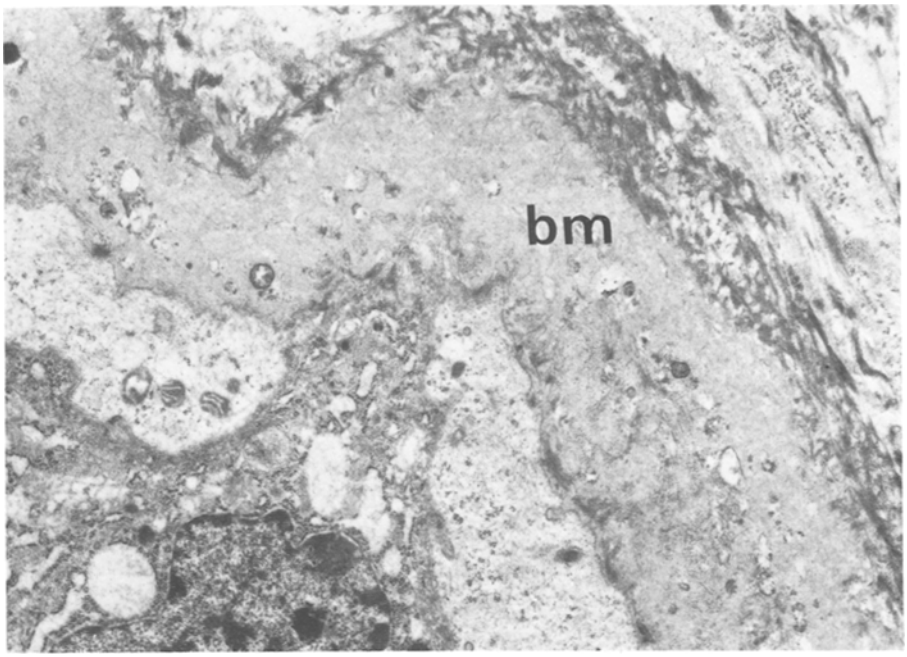


**Fig. 9.** Markedly thickened trophoblastic basement membrane (EM ×12,500). *bm* basement membrane

Very occasional cytotrophoblastic cells were encountered which showed degenerative changes such as swelling of the mitochondria and vacuolation of the endoplasmic reticulum (Fig. 2).

*Trophoblastic Basement Membrane.* This was, in seven placentae, excessively thickened and laminated and also contained small, or medium sized, electron-dense particles as well as a fibrillary component (Fig. 9). Occasionally, basement membrane thickening was associated with electron-dense material subjacent to it but there was no evidence of deposition of immune complexes.

*Fetal Stromal Capillaries.* These were generally small and non-dilated and many were lined by thick endothelial cells which often contained numerous organelles, dilated cisternae of rough endoplasmic reticulum and deposits of glycogen (Figs. 1 and 10). Cytoplasmic "blebs" from the endothelial cells were seen with some frequency and these appeared to be being extruded both into the stroma and into the capillary lumen. Pericytes were plentiful whilst the capillary basement membranes were sometimes extremely thickened or laminated (Fig. 11). No evidence of immune complex deposition was seen either in or around the fetal vessels.

*Villous Stroma.* This was frequently oedematous and often contained an excessive number of cells and of collagen; Hofbauer cells were frequently seen.

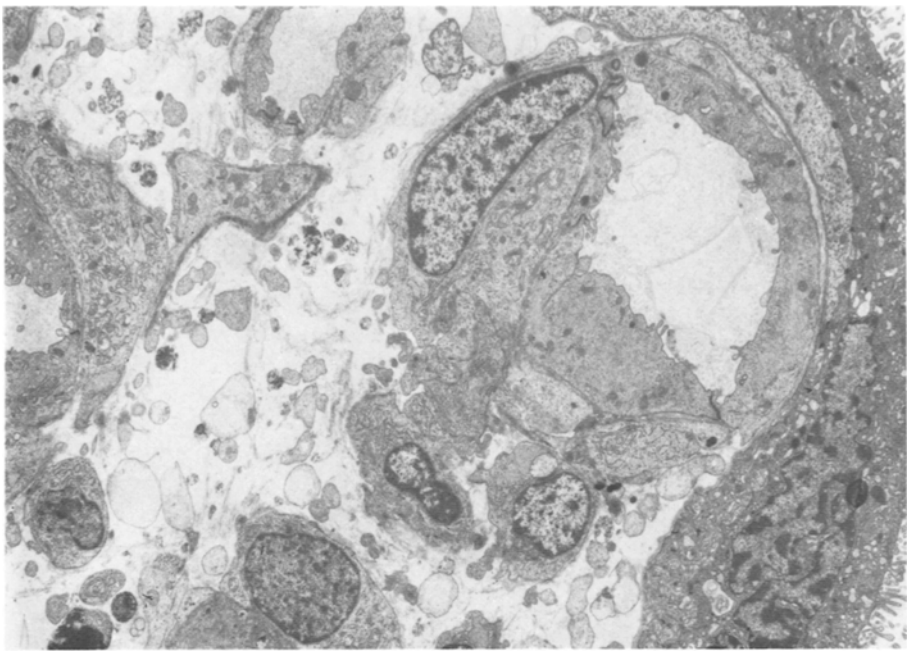

**Fig. 10.** A fetal stromal vessel is present in the stroma deep to the trophoblast; it is abnormally small and is lined by thick endothelial cells of immature type (EM ×5000)

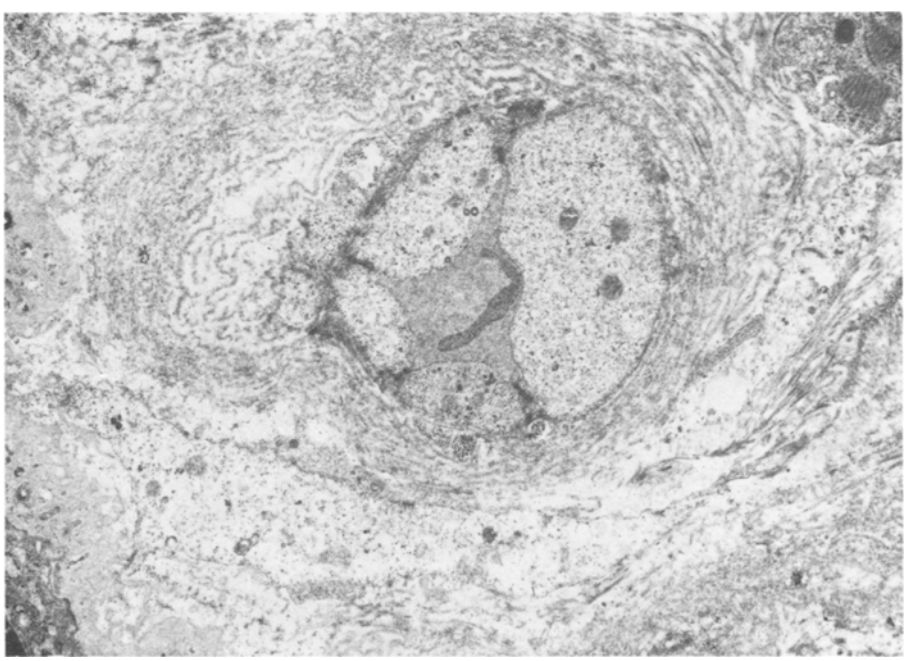

**Fig. 11.** An abnormally small fetal vessels with immature endothelial cells and a markedly laminated basement membrane (EM ×11,250)

## Discussion

Our results differ in many respects from those found in previous ultrastructural studies of placentae from cases of materno-fetal rhesus incompatibility. Thus Zacks and Blazer (1963), who examined four specimens, could find no morphological abnormality apart from slight vacuolation of the syncytial endoplasmic reticulum whilst Liebhart (1971), in her study of two placentae, described extensive cytotrophoblastic damage with little evidence of any syncytial abnormality. Arnold et al. (1961) examined only one placenta and noted widespread mitochondrial swelling, a change also found by Widmaier (1969) who further commented on a marked reduction in pinocytotic activity; this latter finding may have been due to the fact that one of the two placentae in his series was from a prolonged pregnancy, circumstances under which reduced pinocytosis is regularly found (Jones and Fox, 1978).

A striking finding in the present study was the focal, but sometimes quite extensive, necrosis of the villous syncytiotrophoblast, a feature that has also been commented on by a number of light microscopists (Hellman and Hertig, 1938; Kline, 1948; Thomson and Berle, 1960; Hölzle et al., 1975) and was noted in Widmaier's (1969) electronoptical study. The pathogenesis of this abnormality is not clear but, as rhesus haemolytic disease is clearly immunologically mediated, the possibility that the syncytial necrosis is due to immune attack merits consideration. There have, over the years, been a number of claims to have detected C-antigen in placental tissue (Boormann and Dodd, 1943; Käser, 1947; Preisler, 1958; Bazso and Gyongossy, 1959; Pozzi and Marzetti, 1962; Skorczynski and Skrzpulec, 1967) whilst Burstein and Blumenthal (1962) maintained that fluorescein-labelled anti-D localises on villous basement membranes and the walls of fetal stem arteries of placentae from rhesus positive infants of rhesus negative mothers. Martius (1956) and Dördelmann (1963) were, however, unable to demonstrate D-antigen in placental tissue and with the use of more specific techniques it has become widely accepted that rhesus factors are tissue specific alloantigens found only on erythrocytes (Bagshawe and Lawler, 1975). It is therefore unlikely that syncytial necrosis in these placentae is due to an immunological attack on a trophoblastic antigen but it could be argued that immune complexes from the fetal circulation may be deposited in, and thus damage, the trophoblast; however, a search for such complexes in our material has proved fruitless.

The syncytial necrosis is unlikely to be a consequence of fetal anaemia for it is well established that the trophoblast is dependent solely upon the maternal blood for its oxygen supply and survives well in villi that are avascular. A further possibility therefore is that the syncytial damage is due to a reduction in the maternal supply of oxygen to the trophoblast. Little is known about the state of the maternal uteroplacental vasculature in materno-fetal rhesus incompatibility but there seems no obvious reason why these vessels should be abnormal in this condition. Alvarez et al. (1972) have, however, suggested that in any condition in which the villi are unduly large the capacity of the intervillous space is reduced and the material blood flow through the placenta is thus decreased. This hypothesis appears rather mechanistic but, if true, would serve as an adequate explanation for the syncytial necrosis as the villi in these

placentae are, because of a combination of immaturity and oedema, usually unduly large; unfortunately, however, this concept has not been subjected to confirmation by haemodynamic studies and the true cause of the syncytial necrosis remains a matter for speculation.

The prominent cytotrophoblastic component of the villous trophoblast which is observed in these placentae is clearly due to hyperplasia of these cells which is almost certainly a response to the syncytial necrosis; the status of these cells as the "stem cells" of the trophoblast is now well established and in any condition in which syncytial damage occurs they undergo a compensatory hyperplasia in an attempt to repair and replace the injured tissue, a task which appears to be adequately fulfilled in most placentae from cases of materno-fetal rhesus incompatibility. It is likely that the thickening of the trophoblastic basement membrane is a non-specific side effect of the cytotrophoblastic hyperplasia for it is probable that this lamina is secreted by these cells and that their proliferation is accompanied by the secretion of excess basement membrane material, a situation akin to that seen in capillary vessels following endothelial cell proliferation (Vracko and Benditt, 1970); certainly no evidence was found to suggest that the basement membrane changes were due to immune complex deposition.

The endothelial cells in the fetal villous vessels appeared markedly immature; this could be due either to a delay in the process of normal maturation or to a continuing process of endothelial cell death and replacement by freshly formed immature cells. The latter would appear the more likely for thickening or lamination of the capillary basement membranes was commonly found, this suggesting that there had been endothelial cell proliferation and excess secretion of basement membrane material, the overall process being very similar to that which has been shown to occur in diabetic microangiopathy (Vracko and Benditt, 1975). The factors leading to endothelial cell death or damage are not known but again the possibility arises that this is immunologically mediated. These cells appear to contain Fc receptors (Johnson et al., 1976) which are selective for aggregated or complexed antibody and which could therefore bind immune complexes present in the fetal circulation; no morphological evidence of immune complex deposition in the endothelial cells was however seen in this study. It could, of course, be that the endothelial cell damage is secondary to the fetal anaemia and this, at the moment would appear the most likely explanation.

Overall, therefore, it is clear that the placenta in cases of materno-fetal rhesus incompatibility suffers a variety of lesions the pathogenesis of which is largely speculative. There is, however, no convincing evidence that the placenta is being subjected to immune attack in this condition whilst the degree and extent of the damage is, in most instances, unlikely to impair the functional efficiency of the placenta.

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Received April 3, 1978