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Intercellular Junctions in the Full Term Human Placenta

II. Cytotrophoblast Cells, Intravillous Stroma Cells and Blood Vessels

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Summary. Intercellular junctions within the villous stroma and the cytotrophoblastic layer of the human full term placenta were investigated using thin sectioning and freeze-fracturing. Numerous maculae adherentes (desmosomes) were found between the cytotrophoblast cells and the syncytiotrophoblast. This junction type was also seen connecting adjacent cytotrophoblast cells. Large gap junctions were frequently observed in contact areas of perikarya or at processes of adjacent fibroblasts. They often exhibited a peculiar pattern of their particles on the P-face of the membrane. Small rows of junctional particles were found on the P-faces of interconnected smooth muscle cells and gap junctions frequently bridged myoendothelial and interendothelial contact zones.

The significance of the junctional complexes is discussed in relation to functional systems within the villous stroma of the human full term placenta.

Key words: Placenta (human) – Villous stroma – Intercellular junctions – Endothelium – Ultrastructure – Freeze-fracturing.

Introduction

In early pregnancy, mesenchymal cells first appear within the villous stroma which are assumed to differentiate from the cytotrophoblast (Hertig, 1935). It has also been proposed that several stromal constituents derive from these undifferentiated cells such as fibroblasts, Hofbauer cells, and fetal blood vessels (Hertig, 1935; Vacek, 1970; Dempsey, 1972). The ultrastructure of the fibroblasts (Kaufmann et al., 1977), of Hofbauer cells (i.e. Enders and King, 1970; Vacek, 1970), and of large and small fetal blood vessels within chorionic villi (Becker and Seifert, 1963; Nikolov and Schiebler, 1973; Heinrich et al., 1976) has been

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studied; however, the data available on their intercellular junctions are incomplete and with one exception do not include freeze-fracture studies.

The occurrence of intercellular junctions within the syncytiotrophoblast and in intervillous contact areas is described in detail in the preceding paper (Metz and Weihe, 1979). Intercellular junctions which, until now, were found within the chorionic villi of the human full term placenta are: (1) maculae adherentes and focal tight junctions between the syncytiotrophoblast and cytotrophoblast cells (e.g. Tighe et al., 1967; Cavicchia, 1971), (2) maculae adherentes between the cytotrophoblast cells and (3) zonulae occludentes and gap junctions connecting endothelial cells (Heinrich et al., 1976).

The present investigation examines the intercellular junctions within the villous stroma in more detail using thin sections and freeze-fracturing.

Material and Methods

Within few minutes after separation, the human placentas were fixed by vascular perfusion through the arteriae umbicales using rinsing and fixative solutions according to Forssmann et al. (1977). Five placentas were processed according to this routine method, and in five placentas the tracer lanthanum chloride was introduced into the arterial system prior to fixation according to Weihe et al. (1977). For thin section electron microscopy, tissue blocks were subsequently rinsed several times in cacodylate buffer (pH 7.3; 0.1 mM), postfixed in ferrocyanide-reduced osmium tetroxide (Karnovsky, 1971), dehydrated in ethanol and embedded in Epon after Luft (1961). "En bloc" staining was carried out after osmium postfixation using uranyl acetate buffered in sodium maleate. For freeze-fracturing, tissue blocks were equilibrated with 30% glycerol solution and frozen in liquid nitrogen slush. Subsequently they were fractured and replicated in a Leybold Heraeus EPA 100 apparatus. Thin sections and freeze-fracture replicas were examined with a Zeiss EM 10.

Results

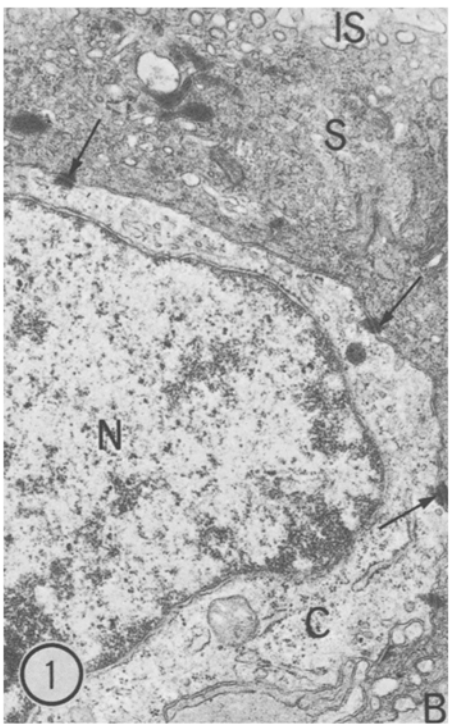
Within the chorionic villi of the human full term placenta, intercellular junctions are analyzed of (1) cytotrophoblast cells, specifically intercytotrophoblastic junc-

Fig. 1. Electron micrograph of a part of a chorionic villus of a human full term placenta. Numerous maculae adherentes (*arrows*) connect the syncytiotrophoblast (*S*) to a cytotrophoblast cell (*C*). Nucleus (*N*); basal lamina (*B*); intervillous space (*IS*). × 15,000

Fig. 2. Freeze-fracture of the appositional area between syncytiotrophoblast and cytotrophoblast. Maculae adherentes (*Ma*) are distributed on the E-face of the membrane. × 65,000

Fig. 3. Part of a chorionic villus after lanthanum chloride perfusion through the umbilical vascularization. Precipitates of the tracer (*arrows*) are continuously distributed in the interspace between the cytotrophoblast cells (*C*) and the syncytiotrophoblast (*S*). Nucleus (*N*); intervillous space (*IS*). × 65,000

Fig. 4. Maculae adherentes (*arrows*) are situated between the cytotrophoblast (*C*) and syncytiotrophoblast (*S*) and also between cytotrophoblast cells (*double arrows*). × 40,000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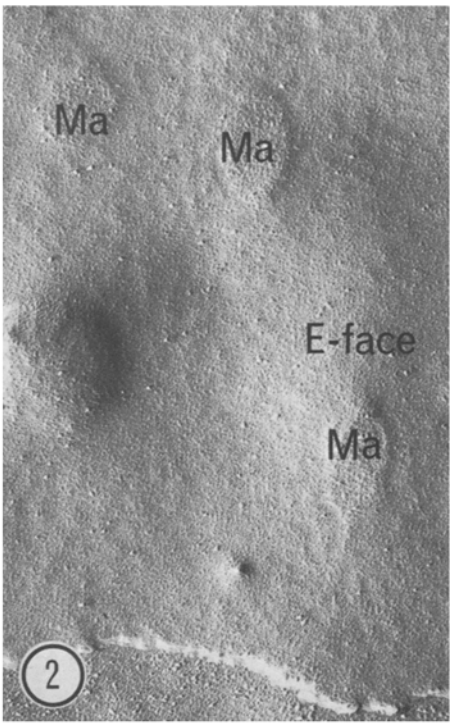
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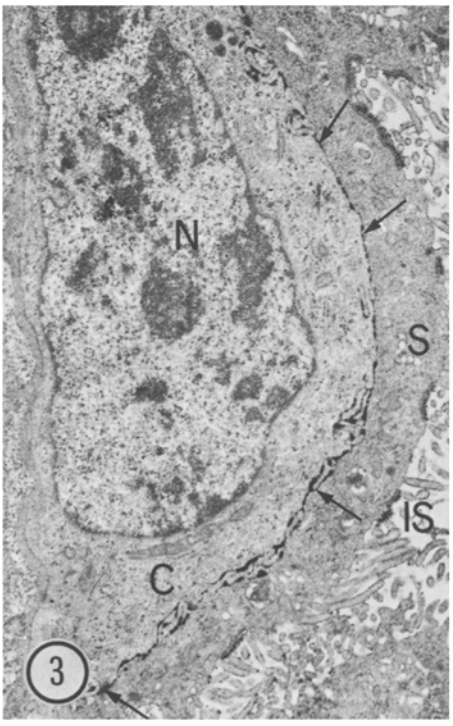
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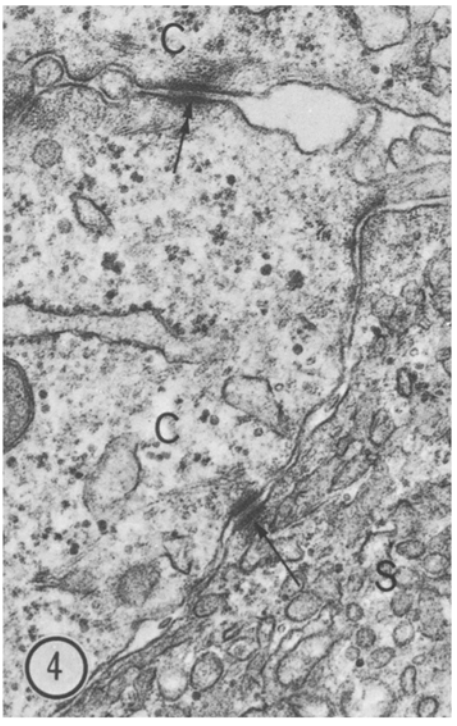
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tions and junctions between cytotrophoblast cells and syncytiotrophoblast, (2) fixed connective tissue cells, particularly fibroblasts, (3) endothelial cells, pericytes, and smooth muscle cells of the fetal blood vessels.

1. Cytotrophoblast Cells

The cytotrophoblast cells form a layer that is discontinuous and irregularly arranged adjacent to the intravillous surface of the syncytiotrophoblast (Fig. 1). Long flat processes and small as well as large projections often extend from the perikarya of these cells and protrude into the syncytiotrophoblastic layer. A basal lamina is interposed between the cytotrophoblast cells and the other stromal constituents (Fig. 1). Maculae adherentes frequently connect the syncytiotrophoblast to cytotrophoblast cells (Figs. 1 and 2) and are also regularly seen between neighbouring cytotrophoblast cells (Fig. 4). In thin sections, point-like membrane fusions which can be interpreted as tight or small gap junctions are rarely observed between these cellular structures. However, goniometric analysis and freeze-fracturing did not support this interpretation.

2. Fixed Connective Tissue Cells

The villous stroma of the human placenta consists of different connective tissue cells such as mesenchymal cells, fibroblasts, reticulum cells and Hofbauer cells. In the present investigation only the interconnections between fibroblasts are considered. Fibroblasts are variously distributed within the villous stroma. They often exhibit long stretched perikarya and some cytoplasmic projections (Fig. 5). Within their cytoplasm, granular endoplasmic reticulum is well developed and intracellular filaments are found. Numerous contact areas exist between perikarya and processes of neighbouring fibroblasts (Figs. 5–7). In thin sections a narrowing of the intercellular cleft is seen within these contact areas of the perikarya (Fig. 7). In freeze-fracturing particle aggregations are found which are typical of gap junctions (Figs. 8–10). Occasionally, several particle aggregations, different in size and shape, are situated closely together (Fig. 10). They often exhibit patterns of particle aggregations and particle-free regions on the P-face of the membrane (Fig. 9). Large membrane particles which are more numerous on the P-face of the membrane than on the E-face are randomly distributed over the cell surface (Figs. 9 and 10). Micropinocytotic vesicles are rarely found on the cell surface of the fibroblasts (Figs. 5, 8, 11).

Further studies were performed using lanthanum chloride as a tracer. A continuous staining of the intercellular cleft between the cytotrophoblast cells and the syncytiotrophoblast occurs (Fig. 3). Furthermore, an unrestricted distribution is found within the intravillous interstitium between the several cellular and fibrillar stroma constituents.

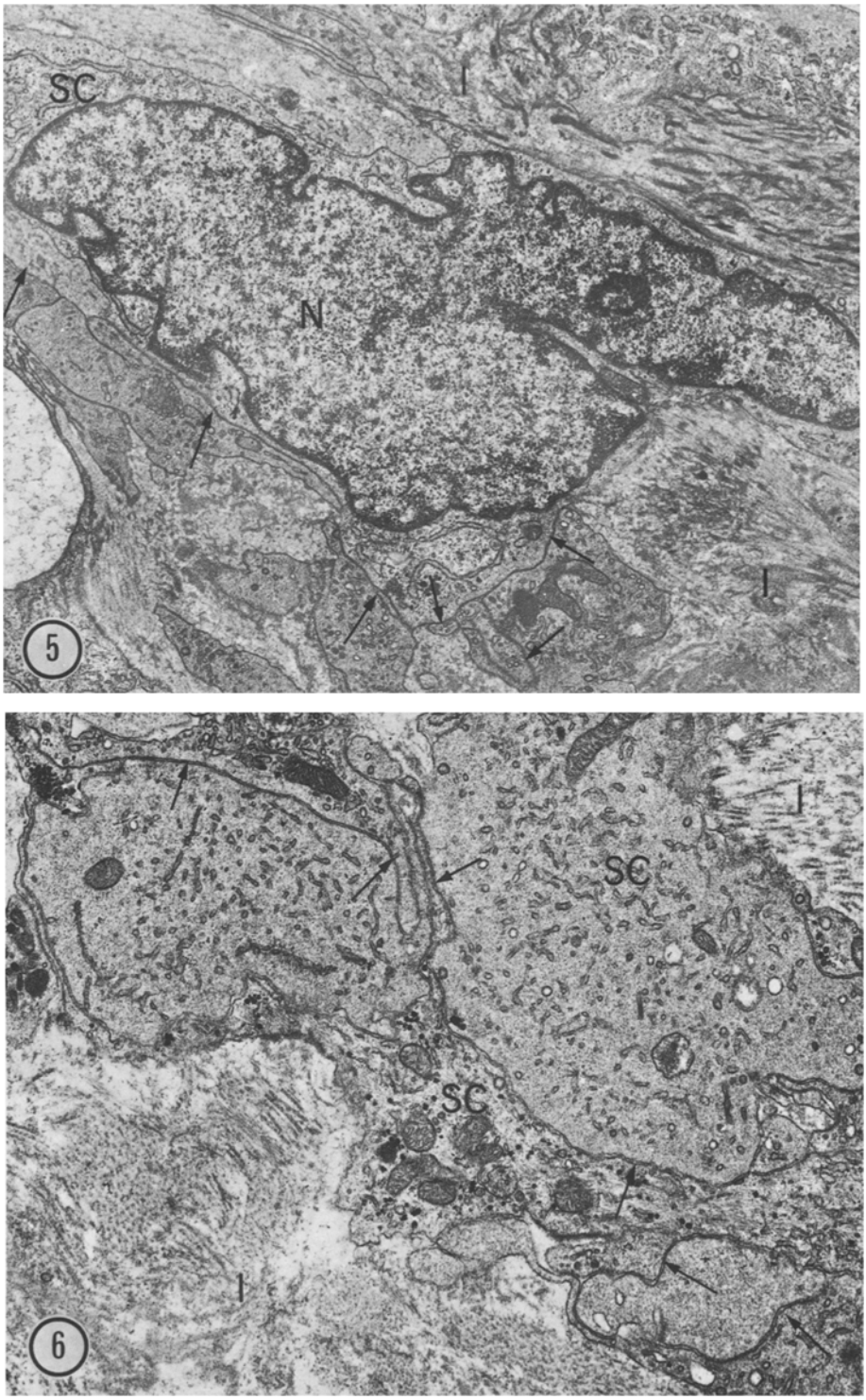
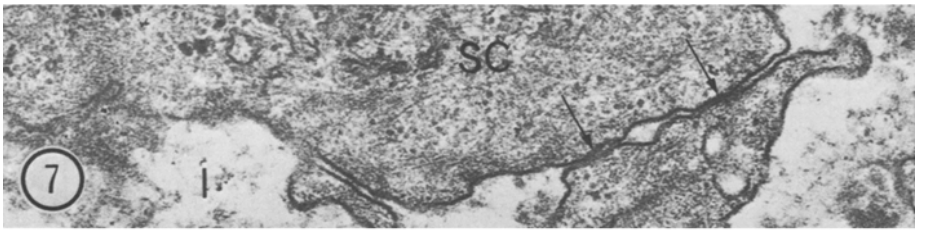
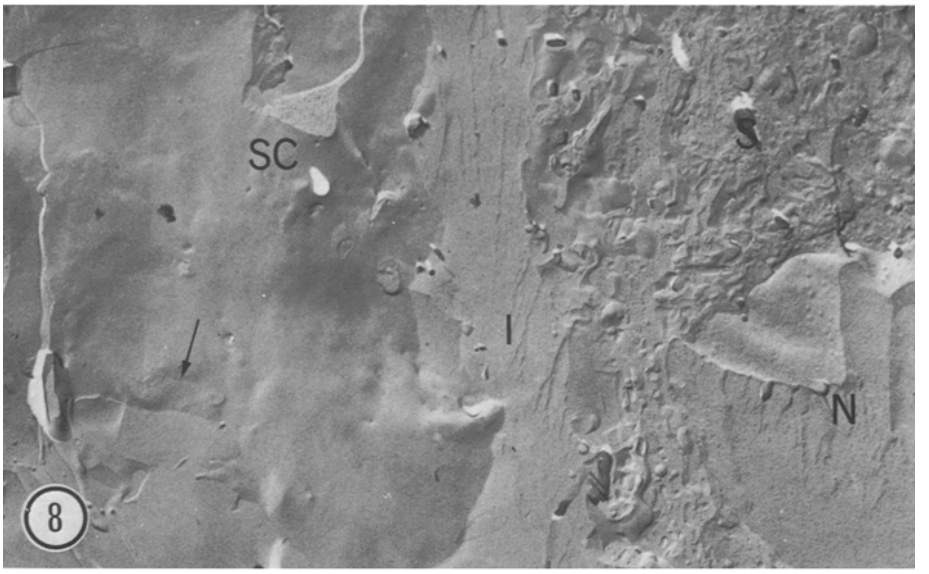


Fig. 5. Numerous appositional areas (*arrows*) are arranged between a perikaryon and several processes of stromal cells (*SC*), e.g. fibroblasts. Intravillous space (*I*); nucleus (*N*). ×9,000

Fig. 6. Appositional areas (*arrows*) are situated between large and small processes of fixed stromal cells (*SC*). Intravillous space (*I*). ×11,000





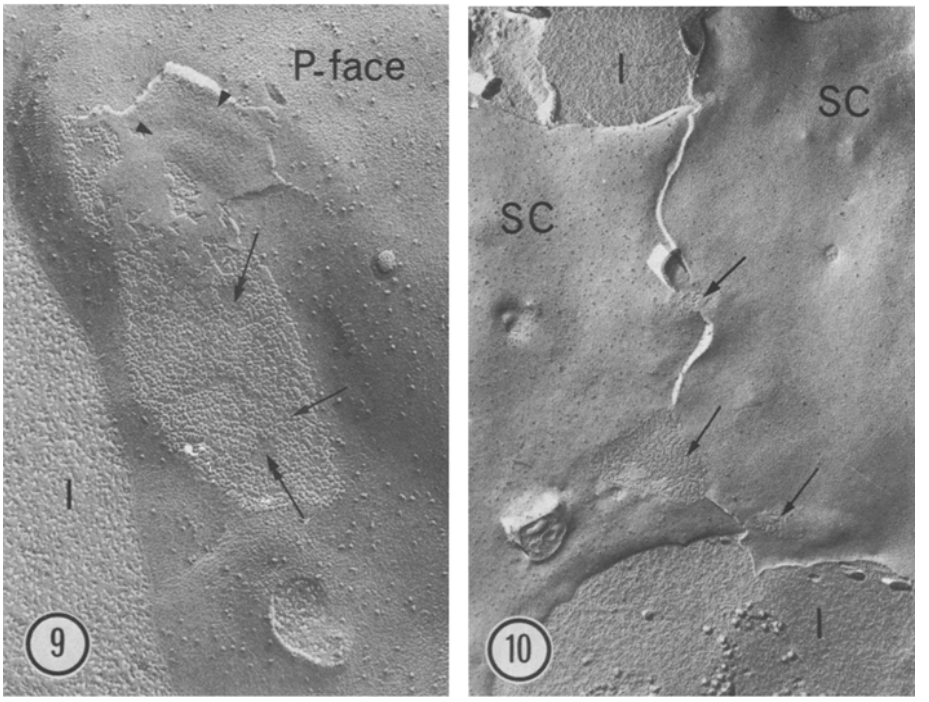
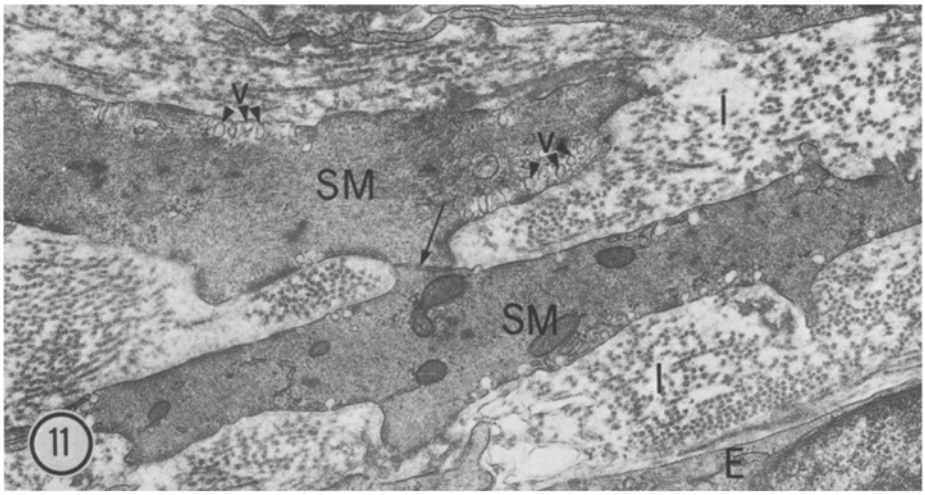


Fig. 7. A narrowing of the intercellular cleft (*arrows*) can be seen within an appositional area of villous stromal cells (*SC*), which is typical of gap junctions. Intravillous space (*I*). ×40,000

Fig. 8. In freeze-fracturing gap junctions (*arrows*) are identified on the membrane interface between stromal cells (*SC*). Syncytiotrophoblast (*S*); nucleus (*N*); intravillous stroma (*I*). ×8,000



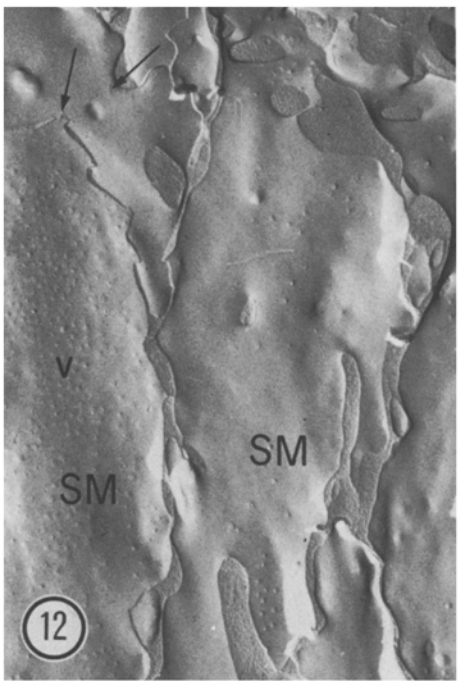


Fig. 9. Higher magnification from figure 8, showing a large particle aggregation (*arrow*), on the P-face and pits (*arrow heads*) on the E-face of the membrane of the stromal cell. The particle aggregation exhibits an irregular pattern of particle-free regions (*double arrows*). Intravillous space (*I*). ×65,000

Fig. 10. Two adjacent stromal cells (*SC*) are in contact by means of several gap junctions (*arrows*). Intravillous space (*I*). ×25,000

Fig. 11. An appositional area (*arrow*) between smooth muscle cells (*SM*) is located in the perivascular region within the villous stroma. Intravillous space (*I*) with collagen fibers; surface vesicles (*V*); capillary endothelium (*E*). ×18,000

Fig. 12. Freeze-fracture of smooth muscle cells (*SM*) situated in the intravillous space. Small particle aggregations (*arrows*) are seen on the surface of the cells exhibiting marked differences in the number of vesicles (*V*). ×14,000

Fig. 13. Higher magnification of figure 12 showing two small particle aggregations (*arrows*) on the P-face of the membrane. ×70,000

3. Endothelial Cells, Pericytes and Smooth Muscle Cells

Adjacent to the fetal blood vessels, smooth muscle cells and pericytes are situated; collagen fibers and basal laminas are also present (Fig. 5).

In many regions of the surfaces of smooth muscle cells, numerous micropinocytotic vesicles are seen (Figs. 11 and 12). Contact areas exist between neighbouring smooth muscle cells (Figs. 11–13) and between smooth muscle cells and endothelial cells of fetal blood vessels (Figs. 14–17). In thin sections, small point-like narrowings of the intercellular clefts are identified within the contact areas (Fig. 16). With freeze-fracturing, small rows or macula-shaped aggregations of particles are rarely found on the P-face of the membrane of smooth muscle cells (Figs. 12 and 13). Within the myoendothelial contact zones, larger particle aggregations are regularly observed (Figs. 15 and 17).

In the microvasculature of the placenta (capillaries and venous sinuses) adjoining endothelial cells overlap extensively (Fig. 18). Along their abluminal surfaces (facing the intravillous space) pseudopodial extensions are frequently arranged (Figs. 18 and 19). In thin sections, narrowings of the intercellular clefts are observed within the indentations formed by these pseudopodial processes (Fig. 20) which correspond to numerous small macula-shaped aggregations of particles seen on the P-face of the endothelial membrane in freeze-fracturing (Figs. 19 and 21).

Discussion

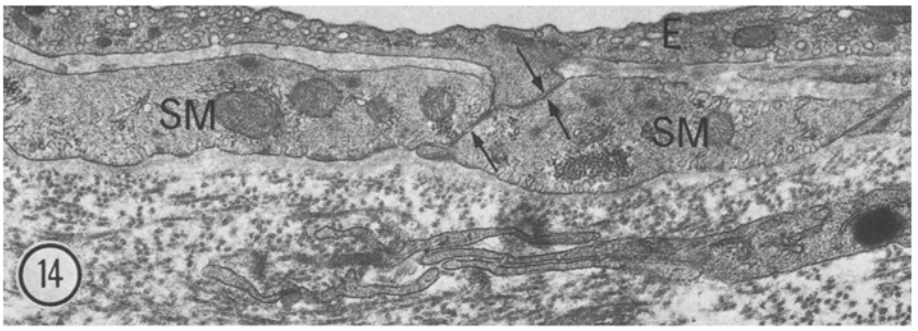
The villous stroma in the human placenta is derived from mesenchymal cells which differentiate into various constituents during placental development (Hertig, 1935). The cytotrophoblast is considered to represent the stem cell for all other connective tissue cells and for the endothelial cells within the villous vasculature (Dempsey, 1972). Some findings also indicate a syncytial fusion of the cytotrophoblast cells with the syncytiotrophoblast (Enders, 1965; Boyd and Hamilton, 1966). Intercellular junctions which have been identified between the cytotrophoblast cells and the syncytiotrophoblastic layer are maculae adherentes and point-like membrane appositions, which have been interpreted as focal tight junctions (Cavicchia, 1971). Adjacent cytotrophoblast cells are con-

Fig. 14. Appositional areas are seen (*arrows*) between a fetal capillary endothelial cell (*E*) and adjacent smooth muscle cells (*SM*). ×18,000

Fig. 15. Freeze-fracture of an endothelial cell (*E*) of a small fetal vessel. On the P-face of the endothelial cell membrane a particle aggregation (*arrow*) is seen. Capillary lumen (*L*). ×25,000

Fig. 16. Higher magnification of the appositional area of figure 14. Several narrowings (*arrows*) of the clefts between the endothelial cell (*E*) and the smooth muscle cell (*SM*) and also between both smooth muscle cells are seen. Intravillous space (*I*); vesicles (*V*). ×92,000

Fig. 17. Higher magnification of figure 15. On the P-face of the endothelial surface a gap junction (*arrows*) is identified. Capillary lumen (*L*). ×85,000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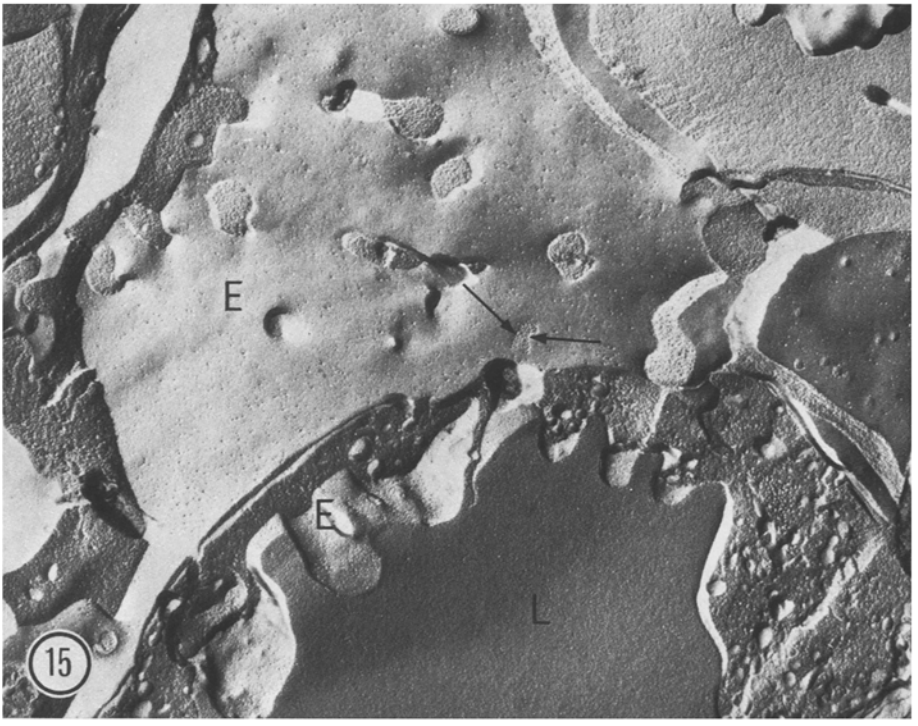
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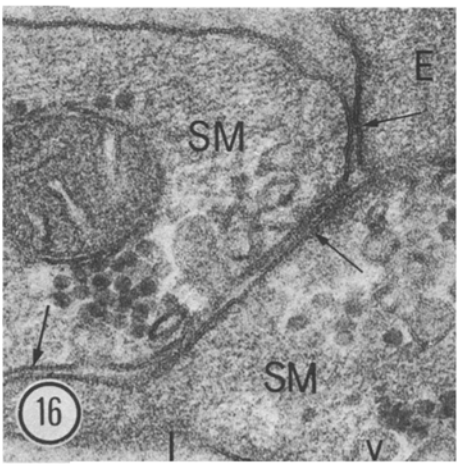
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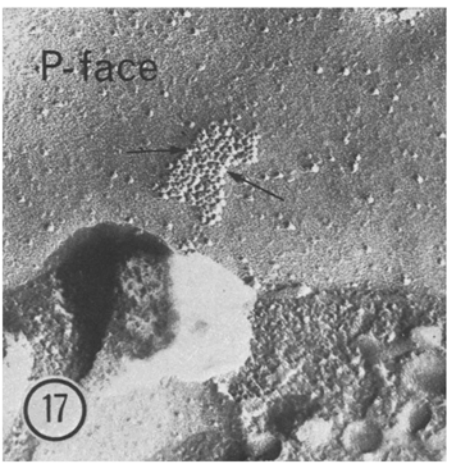
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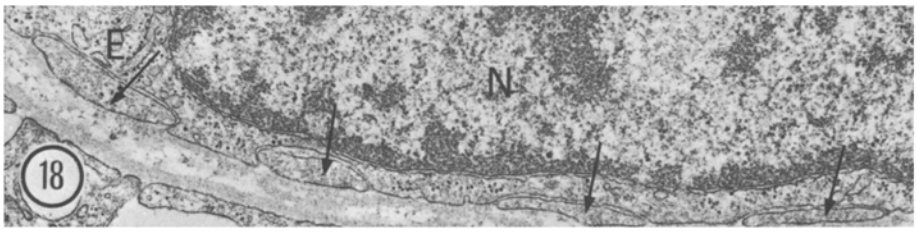
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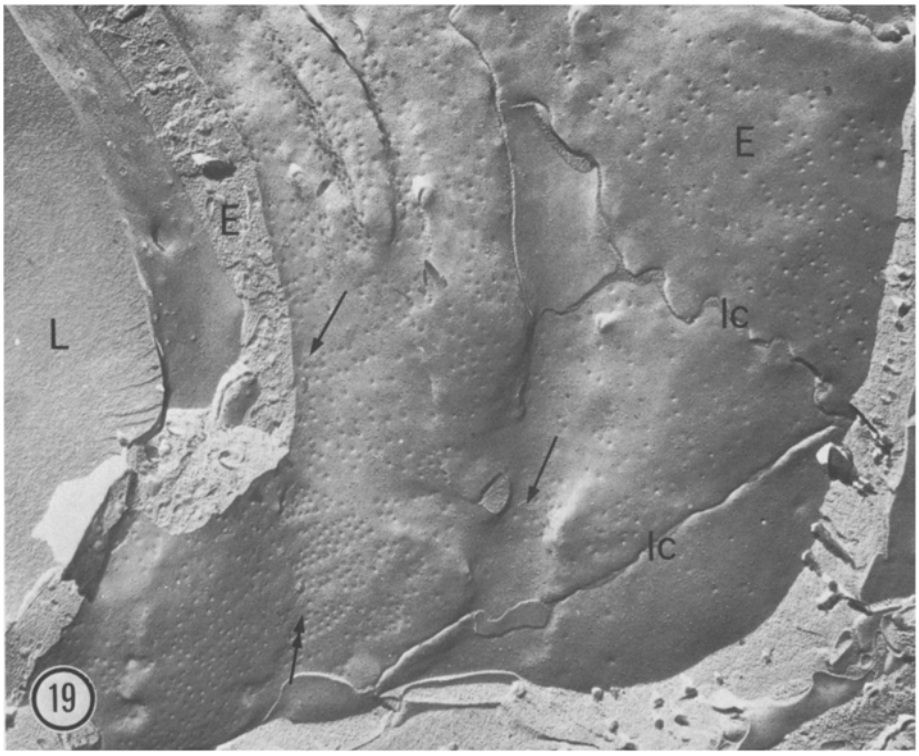
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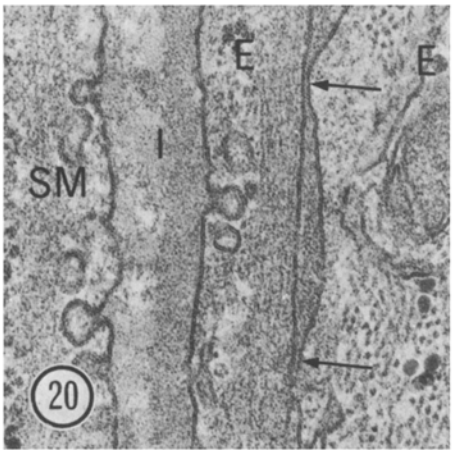
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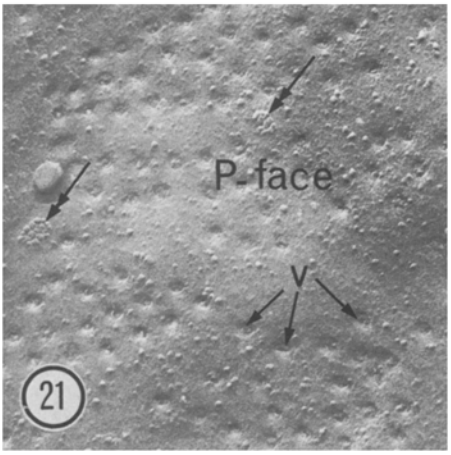
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nected by maculae adherentes. In the present investigation a functional coupling via gap junctions, or a compartmentalization of the membrane surface by tight junctions have not been found between the syncytiotrophoblast and cytotrophoblast cells or between them and the other stromal cells. However, we have observed that gap junctions interconnect some of the interstitial cells, particularly fibroblasts, within the villous stroma. A metabolic coupling of these cells is conceivably mediated by these structures (Gilula et al., 1972).

Intercellular contact areas frequently exist within the vasculature of the chorionic villi. Gap junctions interconnect the smooth muscle cells which are situated along parts of the fetal vasculature. Within myoendothelial and interendothelial contact areas the same junction type has also been identified. Gap junctions, located within those contact areas, are probably not restricted to the placenta but commonly occur throughout the vascular system. They are of special interest because they may contribute to the functional unity of these cells. Considering the human placenta as a model, the following observations support this hypothesis. The endothelial cells of the capillaries often exhibit contractile elements and this suggests a mechanism for individual cells to alter the size of the vascular lumen (Heinrich et al., 1976). The endothelial wall of the pre- and postcapillaries and of the larger vessels is surrounded by smooth muscle cells which frequently exhibit myoendothelial contacts (Nikolov and Schiebler, 1973). The gap junctions could contribute to a coordinative contraction coupling between (1) the endothelial cells, (2) endothelial cells and smooth muscle cells, and (3) adjacent smooth muscle cells. Previous investigations have failed to show evidence of innervations in the placenta (e.g. Boyd and Hamilton, 1970; Nikolov and Schiebler, 1973) and assuming humoral (metabolic) agents are involved in the regulation of the fetal blood flow, the endothelial cells may represent the first step in such a regulation system. Then, by functional coupling of endothelial cells to adjacent smooth muscle cells further control of the blood flow could be effected. We therefore propose that gap junctions are significant in their contribution to the control of placental vascular dynamics.

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Fig. 18. Several pseudopodial extensions (*arrows*) are seen on the abluminal surface of an endothelial cell (*E*) of an fetal capillary. Nucleus (*N*); lumen (*L*). ×17,000

Fig. 19. Several belt-like indentations, arising from pseudopodial extensions, are located on the abluminal surface of endothelial cells (*E*). In the indentations numerous particle aggregations (*arrows* and *double arrows*) are seen. Interendothelial cleft (*IC*); capillary lumen (*L*). ×19,000

Fig. 20. Narrowings (*arrows*) of the intercellular cleft between two adjacent endothelial cells (*E*). Intravillous space (*I*); smooth muscle cell (*SM*). ×70,000

Fig. 21. Higher magnification of figure 19, showing small particle aggregations (*double arrows*) among numerous micropinocytotic vesicles (*V*) on the P-face of the abluminal endothelial membrane. ×65,000

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