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Junctional complexes in the trophoblast of the human full term placenta

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INTRODUCTION

Two layers, namely, the cytotrophoblast and the syncytium, are recognized in the trophoblast of the full-term human placenta. Numerous authors have studied its ultrastructure (Dempsey & Wislocki, 1953; Boyd & Hughes, 1954; Bargmann & Knoop, 1959; Rhodin & Terzakis, 1962; Lister, 1963*a*, *b*; Ender, 1965; Anderson & McKay, 1966; Burgos & Rodríguez, 1966; Boyd & Hamilton, 1966, 1967; Tighe, Garrod & Curran, 1967; Wynn, 1967; Boyd, Boyd & Hamilton, 1968; Boyd, Hamilton & Boyd, 1968), describing both the cyto-syncytiotrophoblast contact surface and the one between the cytotrophoblast cells.

Desmosomes are the only union devices mentioned. They were found in shallow infoldings of the cell surface in the syncytium which resemble incomplete intercellular spaces (Burgos & Rodríguez, 1966).

In the present work, we thought it would be interesting to analyse in more detail the types of union in the full-term human placenta and their possible meaning. We have been able to prove that, in addition to the typical desmosome, the trophoblast contains other types of union not previously described.

MATERIAL AND METHODS

Full-term human placentas were obtained immediately after delivery. All the placentas studied were normal. Small fragments were cut into strips 1 mm thick and fixed in 1·33 % osmium tetroxide, 0·067 M s-collidine, 0·25 M sucrose and 0·01 M-$CaCl_2$

After fixation some blocks were treated with a freshly prepared 1 % aqueous solution of uranyl acetate for 2 h.

The specimens were dehydrated through graded ethanols and embedded in a mixture of Epon-Araldite (Mollenhauer, 1964). Thin sections were stained with lead citrate and uranyl acetate (Reynolds, 1963) and examined in a Siemens Elmiskop I.

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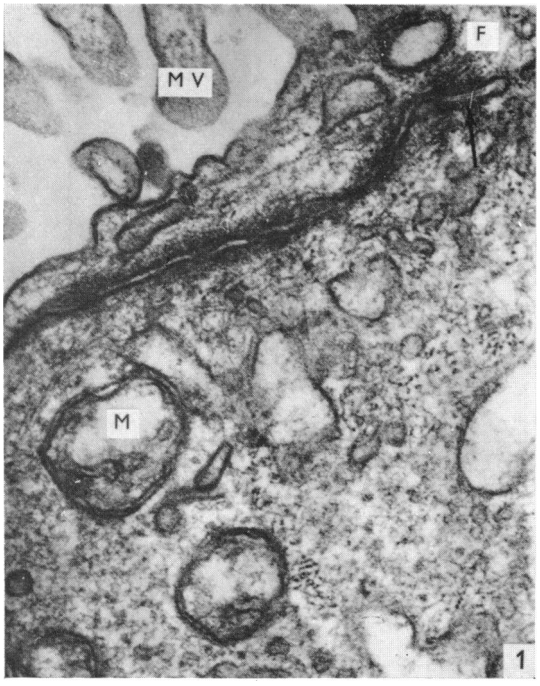
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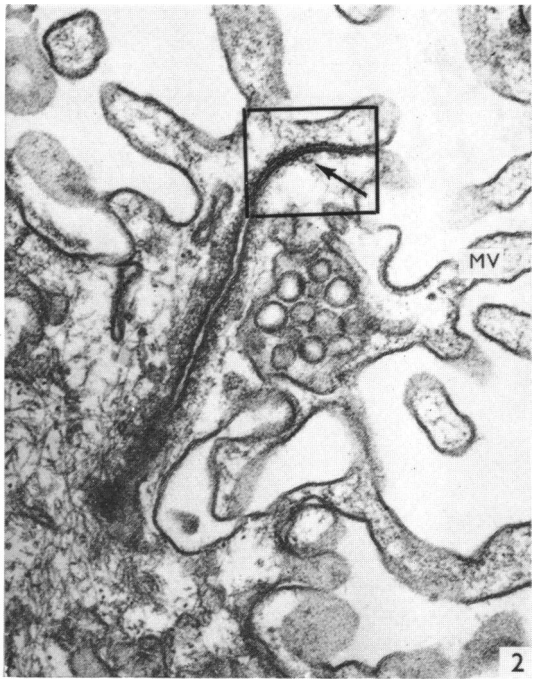
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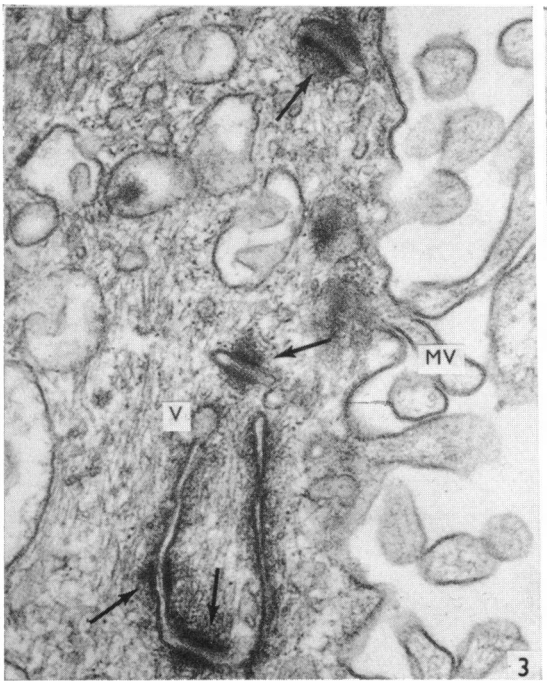
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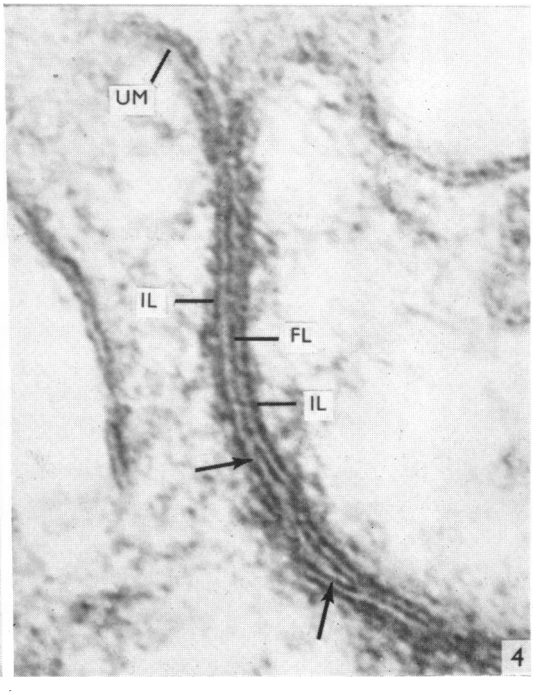
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RESULTS

The trophoblast which covers the free chorionic villi presents a continuous cellular layer, the syncytium, and a discontinuous layer of clear cells, the cytotrophoblast. A typical basement membrane separates it from the connective tissue of the villus which contains the foetal blood vessels.

The junctional structures found in the present work are:

(1) The desmosome (macula adhaerens) is a condensation 0·1–0·6 μm long made up of two dense plates 5 nm thick parallel to the plasmalemma; a clear band 4 nm thick separates each plate from its cell membrane. The intercellular space measures here 15–28 nm (average 22 nm) and contains a dark central lamina of 3 nm, equidistant from both cell membranes. Bundles of cytoplasmic filaments converge at an acute angle on to the inner aspects of the desmosomal apparatus (Fig. 5).

(2) The tight junction (zonula occludens) is a region of close apposition of the cell membranes with fusion of their outer leaflet (*c.* 3 nm thick). In the placenta the length of this type of union varies between 0·1 and 0·25 μm. It is a pentalaminar structure about 20 nm thick, made up of a central lamina (fusion line) of 3 nm; on both sides there is a clear area (3 nm thick) separating the central line from the two lateral layers, which are about 4 nm thick (Figs. 2 and 4).

(3) The adhering band (zonula adhaerens) is more difficult to identify. It is about 0·2 μm long, though occasional ones as long as 1 μm can be found. The cell membranes approximate each other in a characteristic manner, limiting a space 13 nm to 18 nm thick which contains an amorphous intercellular material of low electron density. This type of device owes its typical dense appearance to the intense staining of the plasmalemma and to the presence of oblique and convergent intracytoplasmic filaments (Fig. 6).

The distribution of the different structures is as follows:

(A) Intrasyncytial. Along the free surface of the trophoblast there are invaginations with tight junctions just below the free surface. An occasional splitting of the intervening leaflets interrupts the fusion line (Fig. 4, arrows); the resulting space, 12 nm wide, contains a homogeneous substance of moderate electron density. The invagina-

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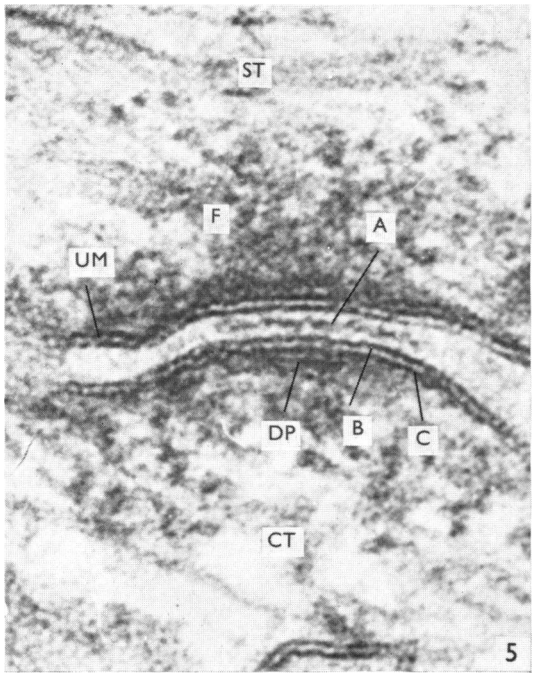
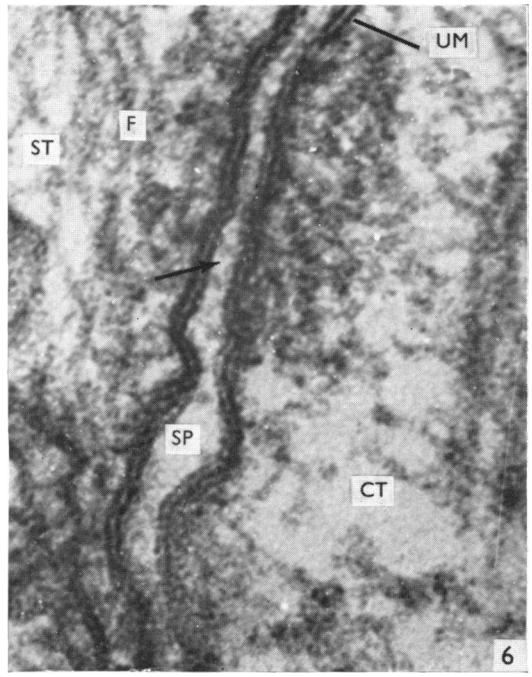
ABBREVIATIONS USED IN ALL FIGURES

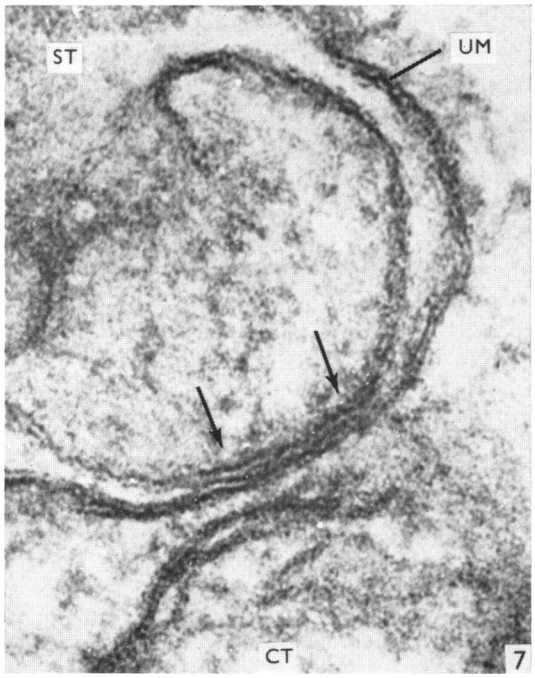
BI, basal interdigitations; BM, basement membrane; CO, collagen; CT, cytotrophoblast; F, filaments; FL, fusion line; IL, inner leaflet; M, mitochondrion; MC, mesenchymal foetal cell; MV, microvilli; R, polyribosome; SP, intercellular space; ST, syncytiotrophoblast; UM, unit membrane; V, vesicle; Va, vacuole.

Figs. 1, 2 and 4 illustrate invaginations of the free surface of the syncytium showing different union devices. Fig. 1. Desmosome (arrow) at the bottom of an invagination. × 50000. Fig. 2. Tight junction (arrow) just below the free surface. × 50000. Fig. 4. View of the area enclosed in the rectangle in Fig. 2. A space of moderate electron density (arrows) interrupts the fusion between the two outer leaflets of the tight junction. × 247000.

Fig. 3. Desmosomes (arrows) linking short-paired membranes. Note vesicle near each end of desmosome. × 45000.







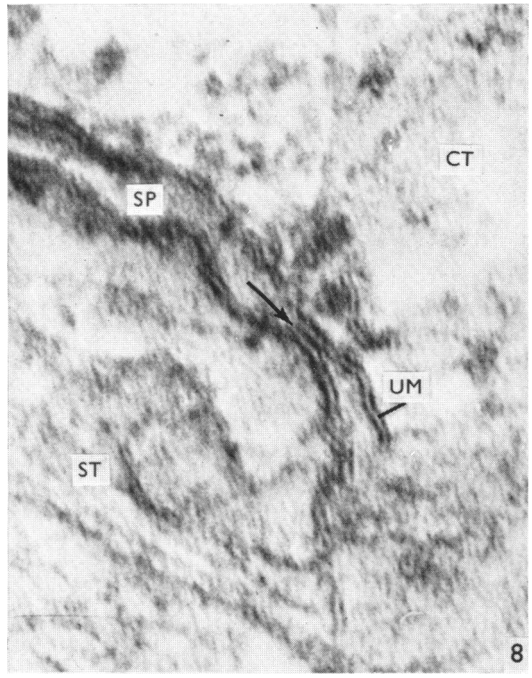
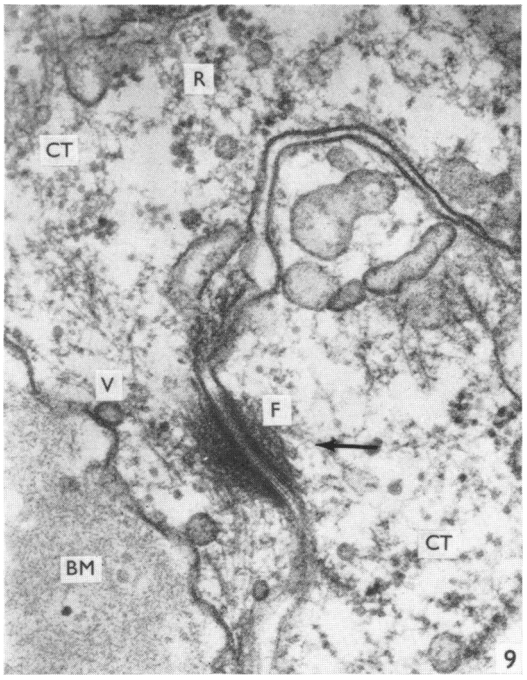


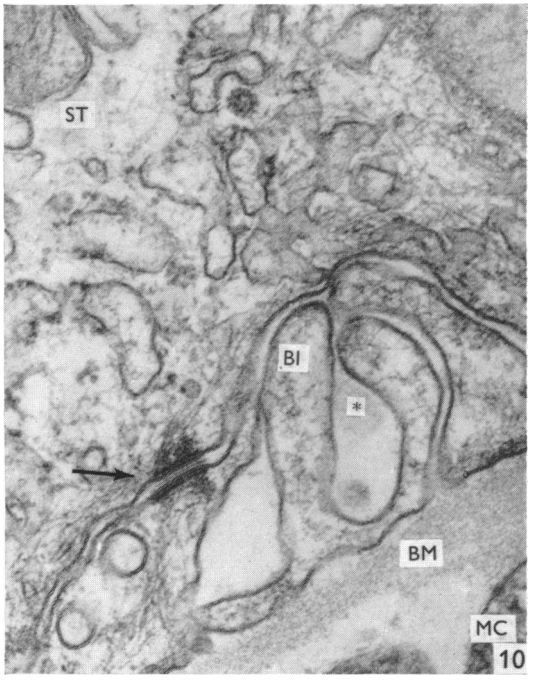
Fig. 5. Typical desmosome displaying a dense central lamina (A), the outer leaflet (B) and the inner leaflet (C) of the cell membranes. DP, desmosomal plaque. ×230000.

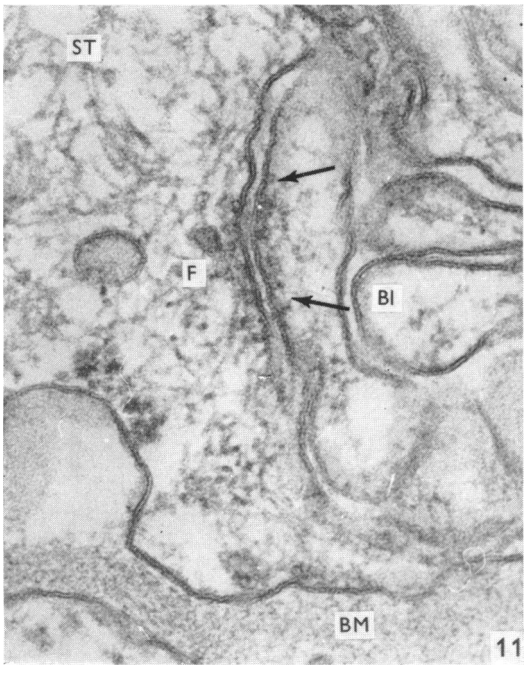
Fig. 6. Adhering band between cytotrophoblast and syncytiotrophoblast. The arrow shows a material of moderate electron density between the two cytoplasmic membranes. ×232000.

Fig. 7. A tight junction. ×230000.

Fig. 8. A focal tight junction (arrow) is seen between the cytotrophoblast and the syncytium. ×240000.







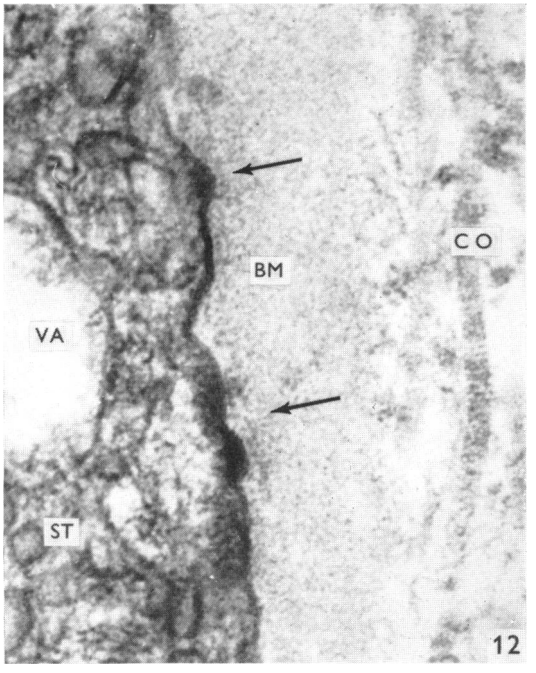


Fig. 9. Two adjoining cells of the cytotrophoblast contain bundles of filaments which converge toward a typical desmosome (arrow). × 50000.

Fig. 10. A desmosome (arrow) fastens basal interdigitations of the syncytium. The space between interdigitations contains a material of moderate electron density (asterisk). × 48000.

Fig. 11. Adhering band (arrows) between basal interdigitations of the syncytium. × 93000.

Fig. 12. The cytoplasm of the syncytium contacting the basement membrane shows focal basal condensations (arrows). × 86000.

tions also display typical desmosomes at the bottom (Fig. 1); desmosomes can also be found scattered in the cytoplasm linking two short parallel segments of membranes apparently not connected with the syncytial surface (Fig. 3). Next to the basement membrane, desmosomes (Fig. 10) and adhering bands (Fig. 11) link the interdigitations protruding from the syncytium.

(B) Syncytium-cytotrophoblast. The space between syncytium and cytotrophoblast is irregular; it displays widenings, microvilli projected by both cell types, desmosomes, adhering bands (Fig. 6), and occasional tight junctions between both limiting cells (Fig. 7). Some tight junctions, such as that illustrated in Fig. 8, are very short and belong to the so-called focal tight junction (Hay, 1968).

(C) Cytotrophoblast-cytotrophoblast. The cytotrophoblast is generally represented, at term, by isolated cells, but in the sections where two or more cells are in contact, desmosomes and interdigitations can be observed (Fig. 9).

(D) Trophoblast-basement membrane. Well-circumscribed thickenings of the cell membrane are frequent in the zone of contact between syncytium and basement membrane (Fig. 12). Such condensations, as far as it has been observed, are not present at the sites of contact between cytotrophoblast and basement membrane.

DISCUSSION

Desmosomes are the only type of union device which has been described so far in the trophoblast of the human placenta (see Tighe *et al.* 1967). In the present study, several junctional complexes, such as tight junctions and zonula adhaerens, have been found in the human placenta. According to Kanno & Lowenstein (1966), the tight junctions are areas highly permeable to the transport of certain ions and large molecules. The fact that these junctions are present between syncytiotrophoblast and cells of cytotrophoblast could indicate that some kind of transport takes place between these two compartments of the trophoblast. The interdigitations and microvilli projected by both trophoblasts have also led Burgos & Rodriguez (1966) to postulate the existence of a functional interrelationship between the syncytio- and cytotrophoblast. On the other hand, the lack of tight junctions and the scarce number of interdigitations between cells of the cytotrophoblast could indicate that transport between these cells is not as active as that between the cytotrophoblast and the syncytium. It seems reasonable to assume that the tight junctions at the apical invaginations of the syncytium do not play any role in transport, since most or all the invaginations do not appear to divide the syncytium into different compartments. However, it seems possible that the apical invaginations, tight junctions and desmosomes represent vestiges of the non-syncytial state of the syncytium.

The adhering bands observed in the syncytium are similar to those described by Farquhar & Palade (1963) in several epithelia. The fact that these junctions have not been observed previously in the trophoblast could be due to the different techniques used in the present study, specially the staining *en bloc* with uranyl.

The focal tight junctions resemble very much the types of junction found by Hay (1968) in embryonic tissues.

In summary, in the trophoblast of the full-term human placenta there are several types of junctional structures, the functional significance of which should be further studied.

SUMMARY

Block staining of trophoblast of the human full-term placenta with uranyl acetate has revealed the presence of several types of union devices:

1. Tight junctions localized in apical invaginations of the syncytium and also between the syncytium and cells of the cytotrophoblast; 2, zonula adhaerens found between the syncytium and cytotrophoblast; 3, desmosomes localized to apical invaginations of the syncytium, in paired membranes apparently isolated within the syncytial cytoplasm, between syncytio- and cytotrophoblast, and between cells of the cytotrophoblast.

The probable functional significance of some of these formations is discussed.

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