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Epithelium of the human chorion laeve in diabetes mellitus

Light and electron microscopic examination

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Summary. The epithelium of human chorion laeve from pregnancies complicated by diabetes mellitus was examined under the light and electron microscopy.

In comparison with normal chorion laeve, the epithelium of chorion laeve in diabetes mellitus shows the following morphological changes:

1. The trophoblast cells display more microvilli and cell processes on their surface.

2. Trophoblast cells with different cytoplasmic characteristics can be seen. Some display few organelles and large glycogen depositions; other are rich in organelles. Furthermore, some cells with electron dense filaments and few cell organelles can be found.

3. Necrotic cells are frequently present in the outer epithelial layers.

4. The intercellular spaces in the epithelial layers near the basement membrane are narrower.

Since vasculopathy of the decidual arteries with disturbance of blood circulation is often found in pathological pregnancies, we assume that the morphological changes found are due to deficiency in nutrient and oxygen supply.

Key words: Epithelium – Chorion laeve – Diabetes mellitus – Electron microscopy

Although many investigations have been carried out on the fine structure of the epithelium of human chorion laeve during the course of normal pregnancy (Petry 1963; Lister 1968; Thliveris and Speroff 1977; Minh et al. 1980, 1981; Schmidt et al. 1982; Bartels and Wang 1983; Wang and Schneider 1983), there are no ultrastructural investigations of this fetal membrane as yet, in pathological states other than toxaemia (Thliveris and Speroff 1977).

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In this paper the fine structure of the epithelium of chorion laeve from pregnancies complicated by maternal diabetes mellitus has been examined. Morphological changes in comparison with the normal chorion epithelium will be discussed.

Material and method

Chorion laeve was taken, immediately after birth, from the placentae of six patients with clinically manifest and insulin-dependent diabetes mellitus.

The length of pregnancy in these patients ranged from 37 to 40 weeks after the last menstruation.

Tissue was divided into small pieces and subsequently fixed in a mixture of glutaraldehyde (2.5%) and formaldehyde (2.0%) in 0.1 M sodium cacodylate buffer (pH 7.3) for one hour at 4° C, and after rinsing in same buffer, fixed once again in 1% buffered osmium tetroxide solution for one hour at room temperature.

The tissue was then dehydrated in graded alcohols and embedded in epon. Semithin section (1 μm thick) and thin sections were prepared with a LKB-4800 Ultrotome. The semithin sections were stained in an alkaline toluidine blue solutin; the thin sections were contrasted with uranyl acetate and lead citrate.

Electron microscope: Siemens Elmiskop 1a.

Results

Light microscopic examination

The epithelium of chorion laeve in diabetes mellitus, as in normal pregnancy, usually consisted of four layers of trophoblastic cells. The intercellular spaces between the neighbouring cells were narrower than in normal epithelium of chorion laeve in the layers close to the basement membrane. However, they were wider in the outer layers (Fig. 1a, b). The nuclei were either polygonal or round and located in the central region of the cell body. Lipid droplets were seldom seen in the cytoplasm. The basement membrane was easily recognized. Necrotic cells were numerous in the outer epithelial layers. They were easily differentiated from the darkly stained trophoblast cells because they pick up less stain. Their nuclei were not present or difficult to identify.

Electron microscopic examination

In comparison with normal epithelium of chorion laeve (see Wang and Schneider 1983), the trophoblast cells in all layers of the epithelium in diabetes mellitus showed an increase in microvilli and wide cell processes on their surface. In the epithelial layers adjacent to basement membrane, the cell processes of the neighbouring cells were closely interlocked (Fig. 2), so that the width of intercellular spaces was greatly reduced (ca. 20 nm to 80 nm wide). In the outer epithelial layers, the intercellular spaces were frequently wide and filled with microfibrils (10 nm in diameter) and base-

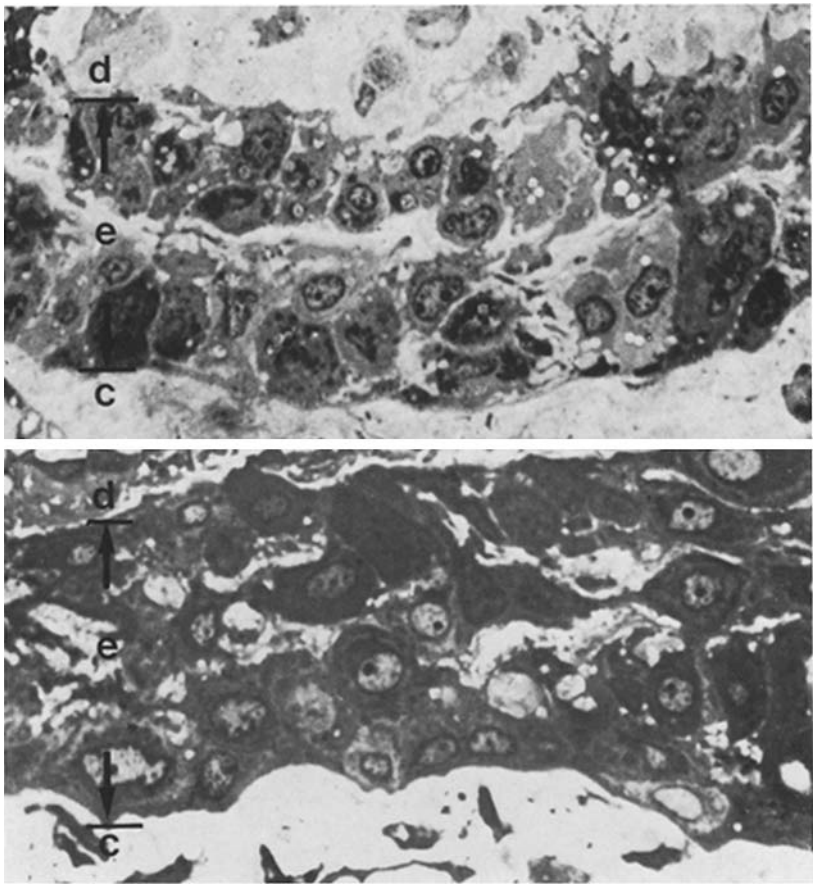


Fig. 1 a, b. The epithelium of chorion laeve (*e*) in normal pregnancy (**a**) and in diabetes mellitus (**b**). In the epithelial layers close to basement membrane, the intercellular spaces of neighbouring cells in diabetes mellitus are narrower than those in normal pregnancy. *d* decidua; *c* connective tissue. × 625

ment membrane-like material (Fig. 3). Desmosomes were often found, especially in the outer epithelial layers. Gap junctions and tight junctions were only rarely found. While many trophoblast cells, as in normal epithelium of chorion laeve, were characterized by the scarce occurence of rough endoplasmic reticulum and mitochondria as well as large depositions of β-glycogen particles, some trophoblast cells showed the opposite: abundant rough endoplasmic reticulum and Golgi complexes, and scattered glycogen particles only (Figs. 2, 4). Occasionally, trophoblast cells containing heavily electron dense filaments (10 nm in diameter) and few cell organelles were seen in the outmost layer adjacent to the decidua (Fig. 5). The polygonal nuclei, containing many marginally located chromatin masses, occurred mainly in trophoblast cells with fewer organelles. However, rounded nuclei with scanty chromatin were often found in the trophoblast cells rich in

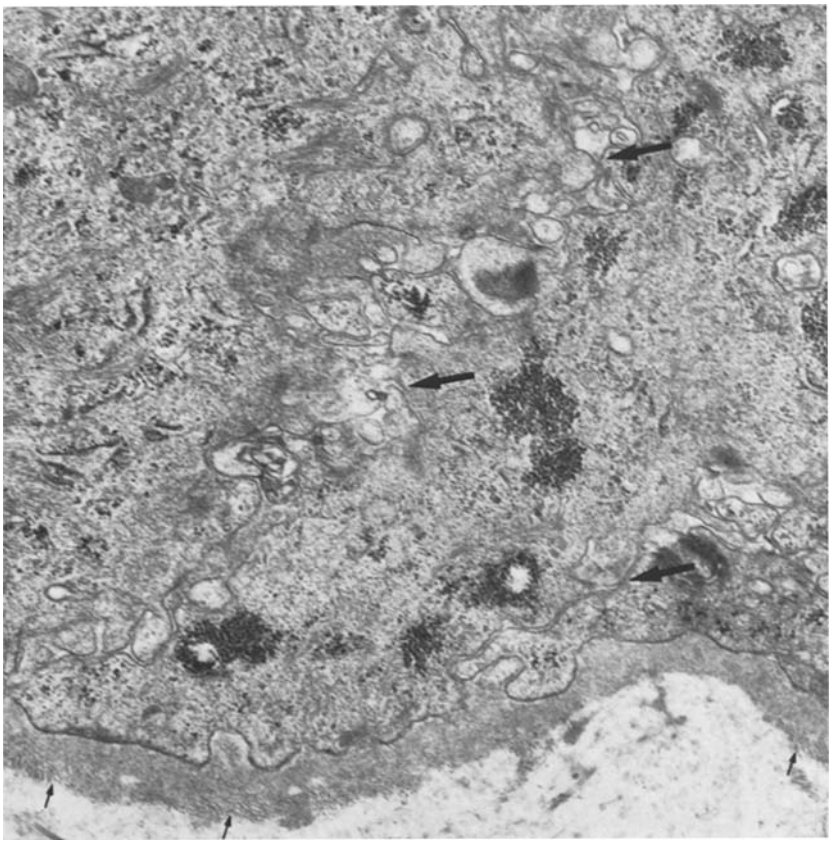


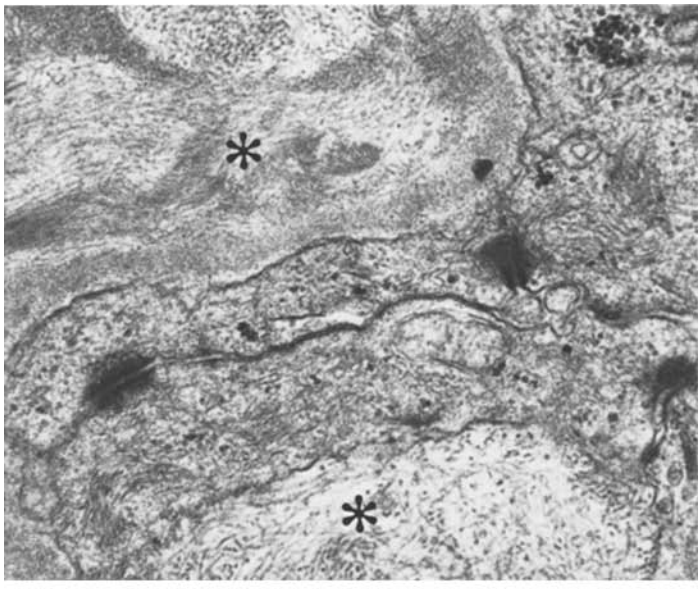
Fig. 2. The cell processes of the neighbouring cells are closely interlocked in the epithelial layers adjacent to the basement membrane (*large arrows*). *Small arrows* show microfibrils in the vicinity of basement membrane. ×18,000

organelles. Lipid droplets were rarely observed. The basement membrane was discontinuous and stratified.

Microfibrils were encountered in the vicinity of and between the basement membrane layers (Fig. 2). In the outer epithelial layers, necrotic cells were numerous. They contained a fine granular substance, a lot of vacuoles, in which dense bodies could frequently be seen, and remnants of organelles (Fig. 5). The plasma membrane was incomplete. The nuclei were either no longer recognizable or only appeared as small chromatin clumps.

Discussion

According to Thliveris and Speroff (1977) the epithelium of the chorion laeve from pregnancies complicated by toxaemia is similar in all aspects to that from normal pregnancy. In contrast to these observation, our find-



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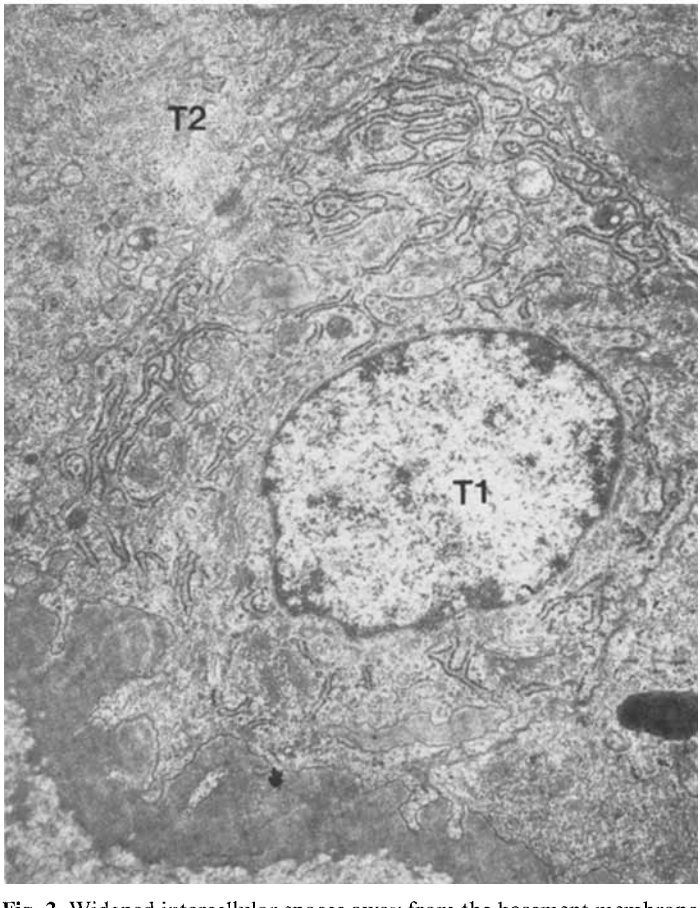

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Fig. 3. Widened intercellular spaces away from the basement membrane containing microfibrils and basement membrane-like material (*asterisk*). × 34,000

Fig. 4. Different epithelial cells of the chorion laeve in diabetes mellitus. *T1* A trophoblast cell with abundant organelles, particularly numerous Golgi complexes and extended rough endoplasmic reticulum. *T2* Part of a trophoblast cell with scarce organelles but abundance of filaments. × 10,200

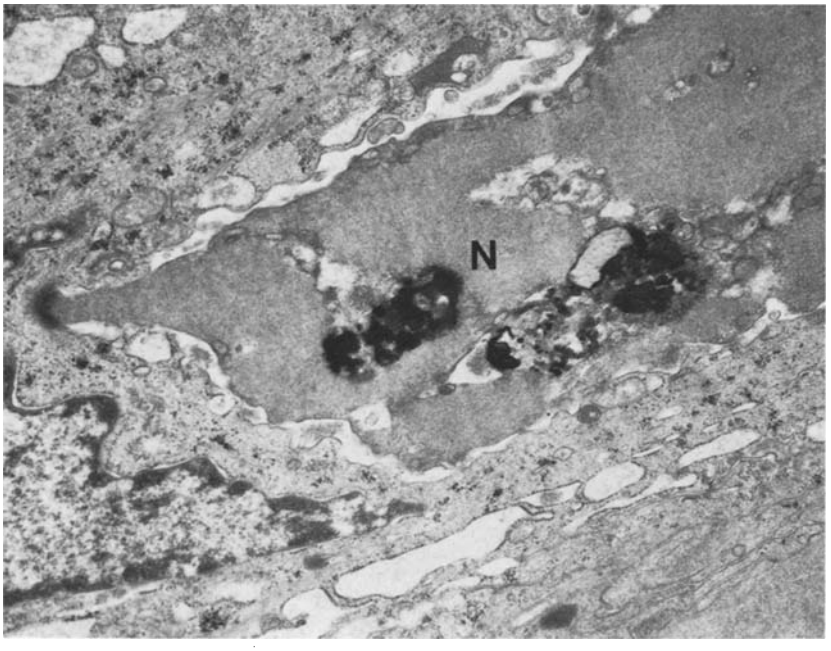

Fig. 5. A necrotic cell (*N*) containing a fine granular substance, vacuoles, in which dense bodies can be seen, and remnants of organelles. × 12,000

ings show that the epithelium of chorion laeve in diabetes mellitus has the following morphological changes when compared with normal chorionic epithelium:

1. The trophoblast cells show increased microvilli and cell processes on their surface.

2. Trophoblast cells with different cytoplasmic characteristics can be seen. Some display few organelles and large glycogen depositions; other are rich in organelles, especially rough endoplasmic reticulum and Golgi complexes. Furthermore, some trophoblast cells with electron dense filaments and few cell organelles can be found.

3. Necrotic cells are frequently present in the outer epithelial layers.

4. The intercellular spaces in the epithelial layers close to the basement membrane are narrower.

Bourne (1962) reported that following the death of the fetus, the amnion slowly degenerates whilst the chorion remains. In late pregnancy, the amnion and chorion laeve contain no blood vessels (Bourne 1962; Hoyes 1971). These observations indicate that the chorion laeve obtains its oxygen and nutrients predominantly from the decidua. In many pathological pregnancies, vasculopathy such as arteriosclerosis, swelling of intima and necrosis of the vessels walls in the decidual arteries with disturbances of blood circulation have been observed (Robertson et al. 1967 and 1975; Emmerich et al. 1975; Robertson 1976; Sheppard and Bonnar 1976; De Wolf et al. 1980). Consequently, the placenta as well as the chorion laeve are directly affected

by the deficiencies of oxygen and nutrients. In order to survive these disadvantages, the cells may enlarge their resorptive surface. Therefore, increase in the microvilli and cell processes of the trophoblast cells in diabetes mellitus may be regarded as a survival attempt of the chorionic epithelium in the phase of deficient oxygen and nutrient supply.

Kaufmann (1972) observed that the cytotrophoblast cells (Langhans cells) of the human placenta in pathological pregnancies are richer in organelles than those in normal pregnancy. In diabetes mellitus, trophoblast cells rich in organelles are often seen in the chorion laeve. Because the placenta and the chorion laeve are affected by oxygen and nutrient deficiency in pathological pregnancies (see above), we are of the same opinion as Kaufmann (1972) that the appearance of higher activity of the trophoblast cells is due to chronic ischaemia. The trophoblast cells of the chorion laeve are often regarded as the production site of numerous hormones such as steroids (Beneditti et al. 1973; Murphy 1977), renin (Symonds et al. 1970; Poisner et al. 1981), prostaglandin (Keirse and Turnbull 1976), placenta protein 5 and pregnancy associated protein 1 (Ho et al. 1982). The epithelium of chorion laeve shows an increase in necrotic cells in diabetes mellitus and thus the rationale for this greater activity of trophoblast cells may be seen as a compensatory phenomenon.

As in the normal epithelium of the chorion laeve (Bartels and Wang 1983; Wang and Schneider 1983), desmosomes, gap junctions and tight junctions are present as cellular junctions in this epithelium in diabetes mellitus. Physiologically, the desmosomes play an important role in adhesion and gap junctions in the charge related and metabolic coupling of neighbouring cells (McNutt and Weinstein 1973; Staehelin 1974). As has been established in other epithelia (Farquhar and Palade 1963; Brightman and Reese 1969), they do not have any barrier function against protein diffusion. The rare tight junctions occurring in the epithelium of chorion laeve, as established in freeze-fracture studies (Bartels and Wang 1983), occur only in the form of maculae occludentes. They have apparently no influence on the permeability of this epithelium. These structural characteristics suggest that the intercellular spaces together with the discontinuous basement membrane, which can hardly function as a diffusion barrier, represent a paracellular pathway for the passage of proteins through the chorion laeve.

The permeability of an epithelium is determined not only by its physicochemical properties but also by the geometrical construction (length and width) of the paracellular pathway (Claude 1978). In diabetes mellitus, the intercellular spaces of the epithelium of chorion laeve in the layers near the basement membrane are narrowed by the strong interlocking of the cell processes. Therefore, one might expect that transepithelial transfer of proteins through the intercellular spaces of the chorion laeve in diabetes mellitus would be more difficult than in normal pregnancy.

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