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Amniotic Epithelium in Diabetes Mellitus

Light and Electron Microscopic Examination

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Summary. The epithelium of human amnion was examined under the light and electron microscopes. Morphologically, the amniotic epithelium in diabetes mellitus differs from that of a normal term specimen by the following characteristics:

1. An increase in the number of β-shaped glycogen granules and lipid droplets;
2. The presence of epithelial areas with dead cells;
3. Thickening of the basal membrane.

Areas with dead cells could be identified in the amniotic epithelium of diabetic patients. These were not found in normal term pregnancies nor in other types of high-risk pregnancies, such as non-diabetic hydramnios, rhesus incompatibility, or pretoxaemia/toxaemia. These findings may indicate that the areas are specific to diabetes mellitus.

Key words: Amniotic epithelium – Diabetes mellitus – Electron microscopy.

Introduction

Although there have been numerous reports on the fine structure of the human amnion during the course of a normal pregnancy, there have been no studies as yet, as far as I know, regarding the amniotic epithelium in diabetes mellitus.

It is the object of this paper to describe the morphological structure of this epithelium more closely and emphasize how it may differ in certain respects from that of a normal term pregnancy (=normal amniotic epithelium). Furthermore, I hope to clarify the question still open from my previous investigation, whether or not the areas of epithelium with dead cells are specific to diabetes mellitus (Wang et al., 1979).

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Material and Methods

Amniotic tissue was acquired, immediately after birth, from the placentae of 2 patients following normal pregnancies and from 6 patients with clinically manifest and insulin-dependent diabetes mellitus.

The length of pregnancy of the patients with diabetes ranged from 37 to 39 weeks.

The tissue was separated into small pieces, fixed in a mixture of glutaraldehyde (2.5%) and formaldehyde (2.0%) in 0.1 M sodium-cacodylate buffer (ph 7.3) for 1 h at 4° C, rinsed briefly in the same buffer, and fixed once again in a 1% osmium tetroxide solution (Millonig, 1962) for 1 h at room temperature. The tissue was thereupon dehydrated in alcohol and embedded in epon. Semithin and thin sections were made in a LKB-4800 III ultrotome. The semithin sections were stained in an alkaline toluidine blue solution; the thin sections were contrasted in uranyl acetate and lead acetate, and examined in a Siemens elmiscope 1a.

Results

Light Microscopic Examination

The amniotic epithelium in diabetes mellitus consists of a layer of cuboidal cells with centrally located nuclei, resting upon a clearly visible basal membrane. In certain places where the stain seems faint or flaky, the epithelium appears to be 2 to 3 cell layered. The number and size of these areas and their form differ in different parts of the epithelium (Fig. 1). Nevertheless, they are less distinct than in the amniotic epithelium of hydramnios-complicated diabetes mellitus (see Wang et al., 1979). The cytoplasm contains many lipid droplets. Microvilli are present on the free cell surfaces. One can clearly recognize the intercellular spaces between adjacent cells.

Electron Microscopic Examination

The amniotic epithelial cells in diabetes mellitus display both simple and braching microvilli on their free surfaces; the microvilli are densely packed together

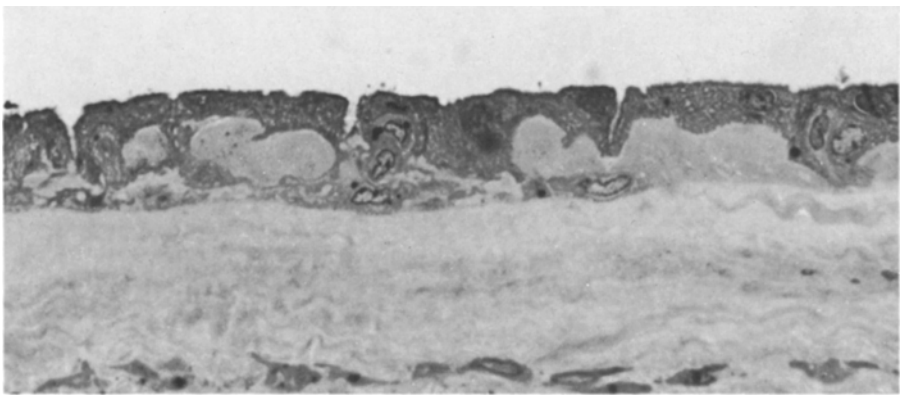

Fig. 1. Amniotic epithelium in diabetes with faintly stained and flaky areas. ×625

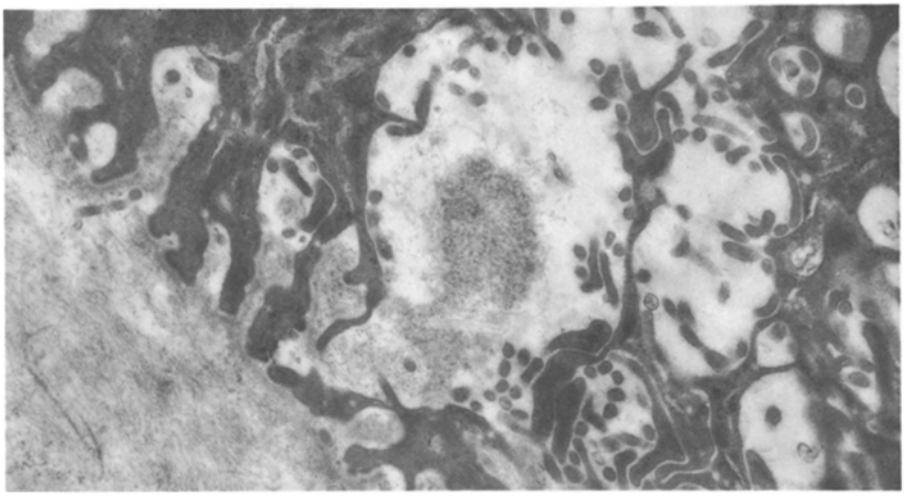

Fig. 2. Widened intercellular spaces at the cell base containing flocculent maternal. × 16,000

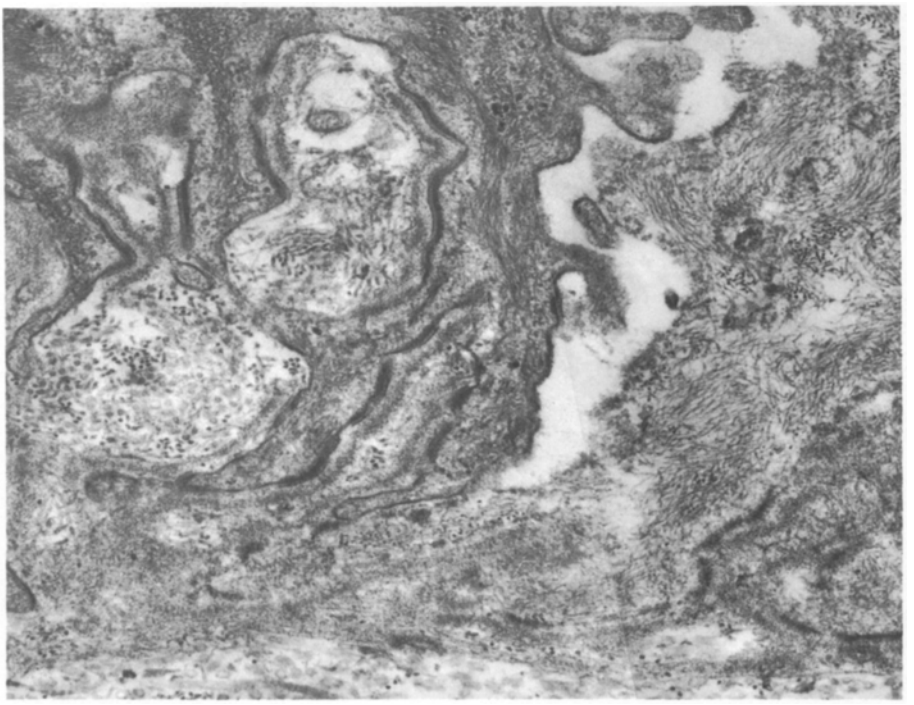

Fig. 3. Epithelial areas consisting of weakly contrasted, densely packed and often whirling bundles of filaments, with the rest of basal membrane. × 16,000

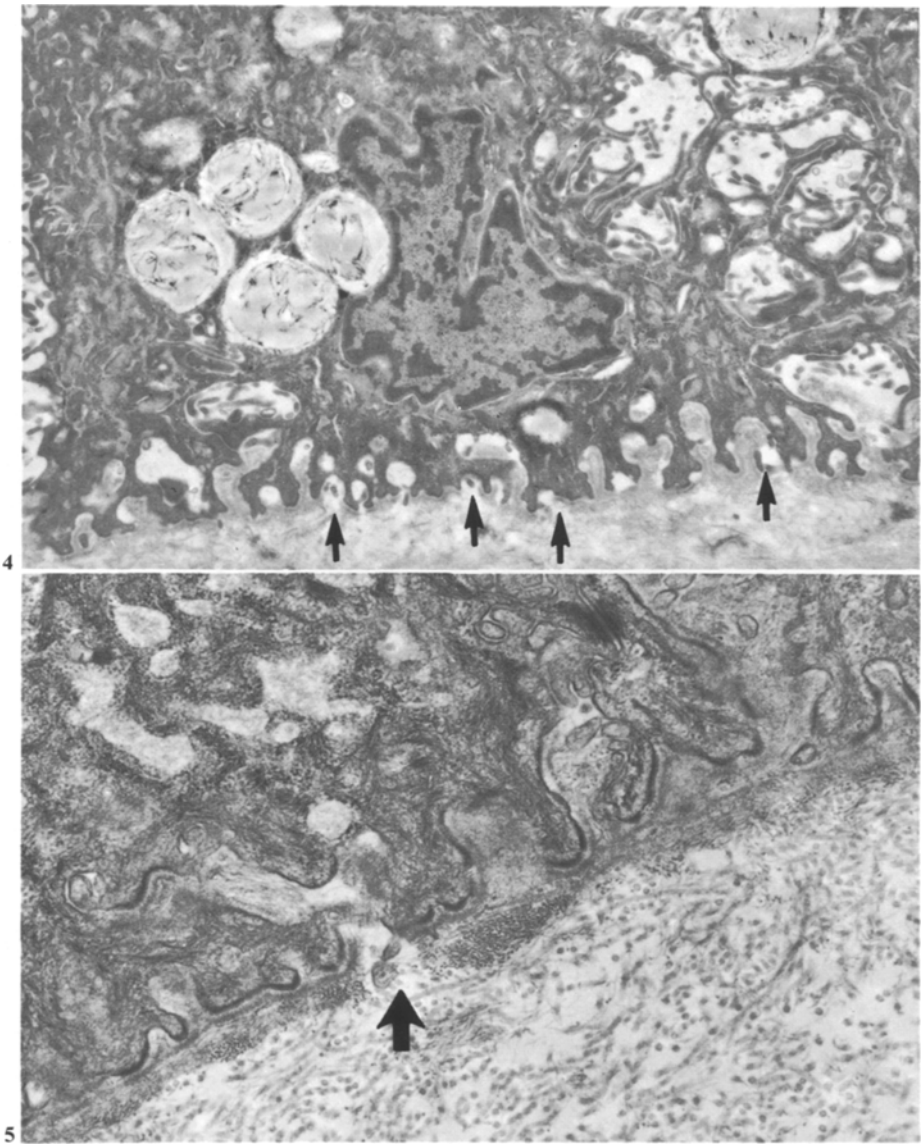

Fig. 4. Lipid droplets and discontinuous segments in the basal membrane (↑) ×6,000

Fig. 5. Discontinuous segments in the basal membrane at higher magnification. (↑) ×19,000

and are somewhat longer than those in the normal amnion (ca. 0.4 μm normal; ca. 0.5 μm in diabetes mellitus).

The lateral cell wall exhibits numerous processes with extend into the intercellular space, and are connected to adjacent cells with similar processes through desmosomes, etc. Large numbers of microvilli emanate from these lateral processes. In comparison with normal amnion or hydramnios, a more frequent

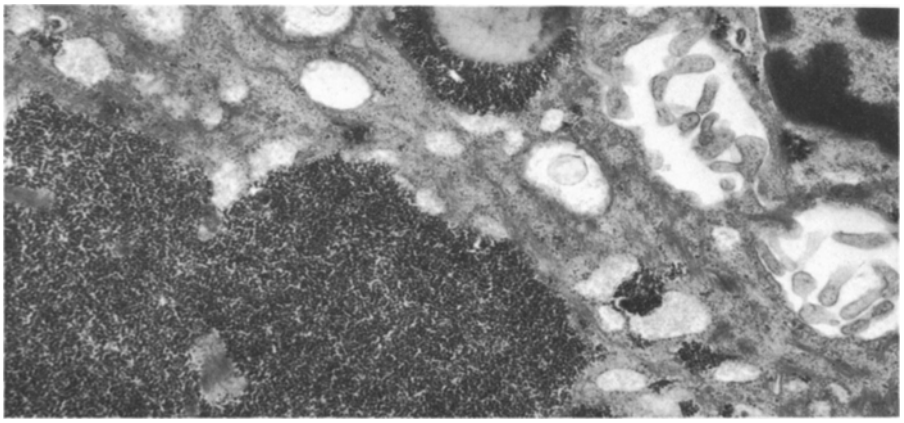

Fig. 6. β-shaped glycogen granules, located round the lipid droplets and densely packed in cyteplasm. $\times$ 16,000

and wider expansion of the intercellular spaces at the cell base is observed (Wang et al., 1979).

The long processes of several neighbouring cells, which appear to be meshed together, build an enormous basal labyrinth-like structure, similar to that of the proximal segment of the nephron. One can frequently observe a flocculent material inside the intercellular spaces (Fig. 2). Between the cells of the amniotic epithelium are areas consisting of weakly contrasted, densely packed, and often whirling bundles of fine filaments.

Optically vacant vesicles and small dense bodies as well as thicker, heavily-contrasted filaments appear inside of and around these bundles (Fig. 3). The areas correspond to the faintly stained and flaky segment seen under the light microscope, and probably represent the remains of dead cells (Wang et al., 1979).

The basal plasma membrane exhibits deep folds, which are, in most cases closely followed by the basal membrane. Unlike normal amnion, the basal membrane from patients with diabetes is often thicker and discontinuous. Hemi-desmosomes can be seen between the basal folds of the plasma membrane and the basal membrane (Figs. 4 and 5).

β-shaped glycogen granules, which are located around the lipid droplets or occasionally densely packed in certain sections of cytoplasm, are found to be more abundant in the cytoplasm of these epithelium cells than in normal amnion. Fatty droplets are observed in diabetic amniotic epithelium markedly more often than in normal term specimens. Their diameter measures up to 3.0 μm and they are situated mostly near the nucleus. Numerous droplets may be present in a single cell (more than 10 droplets of differing size), which give the cell its sievelike appearance.

In addition to the bundles, filaments and numerous pinocytotic vacuoles – found for the most part at the base of the microvilli near the lateral cell wall – the amniotic epithelium cells in diabetes mellitus exhibit the typical

cell organelles. The Golgi apparatus and the granular endoplasmic reticulum, which is filled with a moderately electron-dense substance, both appear increased in number and complexitiy.

Discussion

The findings indicate that the amniotic epithelium in diabetes mellitus differs morphologically from normal amniotic epithelium by the following characteristics: 1. an increase in the number of β-shaped glycogen granules and lipid droplets; 2. the existence of epithelial areas with dead cells; 3. thickening of the basal membrane.

Numerous apical and laterally-located microvilli together with basilar folds and lateral and basal processes which extend into the widened intercellular spaces, produce a considerable enlargement of the epithelial cell surface. This increase, together with the fact that the concentration of glucose in amniotic fluid in maternal diabetes mellitus at the end of pregnancy is higher than in normal pregnancy (Pedersen, 1954; Spellacy et al., 1973; Archimaut et al., 1974; Cassady et al., 1977, and others) leads to an improved intake of glucose into the amniotic epithelium.

Biochemically, the effect of insulin on carbohydrate metabolism in peripheral tissue is well known. The increase in β-shaped glycogen granules and fatty droplets in diabetic amniotic epithelium could be interpreted as a result of insulin action on absorbed glucose.

The areas of epithelium with dead cells, frequently observed in the amniotic epithelium in diabetes mellitus, have been seen neither in normal pregnancy nor in other high-risk pregnancies, such as non-diabetic hydramnios, rhesus incompatability or toxaemia. These findings could imply that this change is specific to diabetes mellitus. The number and size of these areas vary, but they are both larger and more abundant in hydramnios-complicated diabetes. The concentration of glucose in fetal serum is directly dependent upon the concentration in maternal serum (Whalley et al., 1966; Coltart et al., 1969; Spellacy et al., 1973; Dranzančić and Kuvačić, 1974; Wood and Sherline, 1975). Fetal hyperglycemia leads to an increased excretion of glucose through the fetal kidneys into the amniotic fluid. These factors, together with the suggestion that hydramnios could arise from poorly stabilised diabetes mellitus through osmotic diuresis, support the hypothesis that the appearance of these areas corresponds to the degree of severity of the disease. Vrako and Benditt (1970 and 1972) assumed that the thickening of the capillary basal membrane in diabetes mellitus was due to the fact that the membrane persisted following death of the endothelial cells. The new cells, fused new membrane with the old, creating a lamellar thickening.

The amniotic epithelium in diabetes mellitus appears stratified in places where epithelial areas of dead cells are found. In agreement with Vrako and Benditt (see above), I would assume that a similar process has taken place in this epithelium. Following dissolution of these areas, the remaining basal membrane sticks to the basal membrane of the overlying cell layers, resulting in a compound thickening of the basal membrane in amniotic epithelium.

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