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THE ULTRASTRUCTURE OF FREE INTESTINAL CELLS ISOLATED WITH EDTA

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The preparation of single epithelial cells is a logical extension of in vitro studies (*Wilson* 1962). The first method for removal of intestinal mucosal cells was digestion with trypsin (*Harrer et al.* 1964, *Stern & Reilly* 1965). According to unpublished observations, these cells seemed to be morphologically intact under the electron microscope.

During the investigation on the effect of calcium-binding substances on intestinal absorption, a fragmentation of the intestinal epithelium caused by the calcium chelator EDTA was noted (*Søgnen* 1965, 1967).

Glucose metabolism in these cells was almost identical compared to the normal intestinal utilization (*Søgnen* 1965), which suggested that the EDTA-preparation might yield only moderately biochemically and morphologically altered cells.

The scope of this investigation was to study the structure of these cells and to compare them with the normal intestinal cell.

MATERIALS AND METHODS

According to the method described by *Søgnen* (1967), everted loops of the rat small intestine (*Wilson & Wiseman* 1954) were filled with 20 ml Ca^{++}/Mg^{++}-free Krebs Ringer phosphate solution containing 2 mM EDTA and incubated at 0°C for 1 hr. Thereafter the epithelium was detached by manual shaking; the outer fluid, now a suspension of cells, was spun down. After double washing with ice-cold physiological saline containing twice the

iso-ionic amount of Ca^{++} and Mg^{++} (to restore equilibrium), the cells were resuspended in a Krebs Ringer phosphate glucose solution, where they would be metabolically active for at least 1 hr. A pellet for microscopic examination was obtained after spinning down with 3 % glutaraldehyde.

The reference material was taken from the intestine of perfused rats (3 % glutaraldehyde in Millonigs phosphate buffer (with glucose) and 3 % Macrodex, pH 7.3). Further fixation was done with a 2 % solution of osmium tetroxide in Millonigs phosphate buffer for 2 hrs. After dehydration in acetone series, infiltration and embedding in araldite followed. Sections were stained with aquous uranyl acetate and lead citrate. Finally, the sections were examined under the electron microscope (Siemens Elmiscope Ia).

OBSERVATIONS

The normal cell

It is well-known that normal intestinal epithelial cells of the rat (Fig. 1) are of the tall columnar type, with basically located oval nuclei containing denser nucleoplasm at their periphery (*Trier & Rubin* 1965). Apically are located the characteristic microvilli. As has been pointed out, there is a difference in length

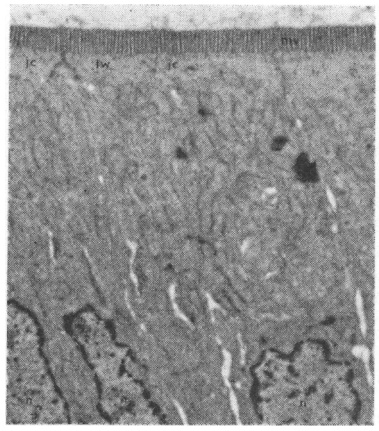

Figure 1. Normal rat intestinal cells. The microvilli are here quite short. n: nucleus; tw: terminal web; mv: microvilli; jc: junctional complexes. × 4.800

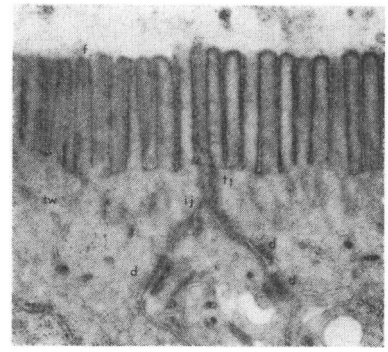

Figure 2. Junctional complexes of the rat intestinal cell. Note the three-laminated structure of the microvillous membrane. tj: tight junction; ij: intermediate junction; d: desmosomes; tw: terminal web; f: fuzz. × 18.000

and width of the microvilli depending on the location on the macrovilli. Most mitochondriae appear in the apical portion of the cells. A well developed endoplasmic reticulum is seen.

At a greater magnification (Fig. 2) the microvilli are seen as tightly spaced, finger-like processes clearly limited by a three-laminated membrane and covered by "fuzz". The central filamentous structures of the microvilli are seen to penetrate down into the terminal web.

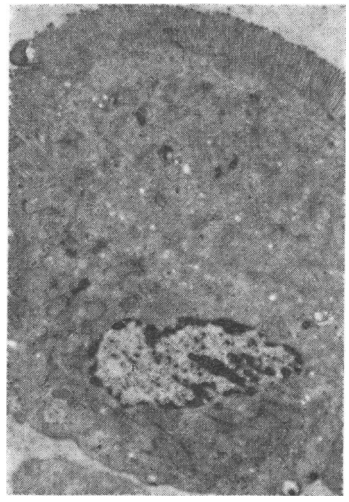

Figure 3. A free intestinal epithelial cell with basically located nucleus surrounded by many mitochondriae. Note the microvillous filaments dipping into the terminal web. n: nucleus; mi: mitochondriae; mv: microvilli. × 5.200

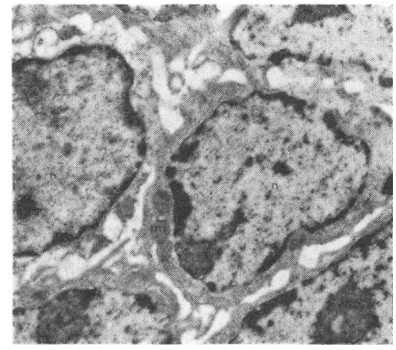

Figure 4. Free intestinal cells cut horizontally through their nuclei. The cells touch each other at cytoplasmatic protrusions. n: nucleus; mi: mitochondriae. × 5.400

Desmosomes (*Fawcett* 1966), believed to be specialized junctional attachments of the cells, are visible in their different modifications (Fig. 2), i.e. tight and intermediate junctions, desmosomes. The lateral plasma membranes may show laminated foldings.

The free cell

The free intestinal cell (Figs. 3 and 4) has a more spherical appearance; a logical alteration for a structure with an elastic

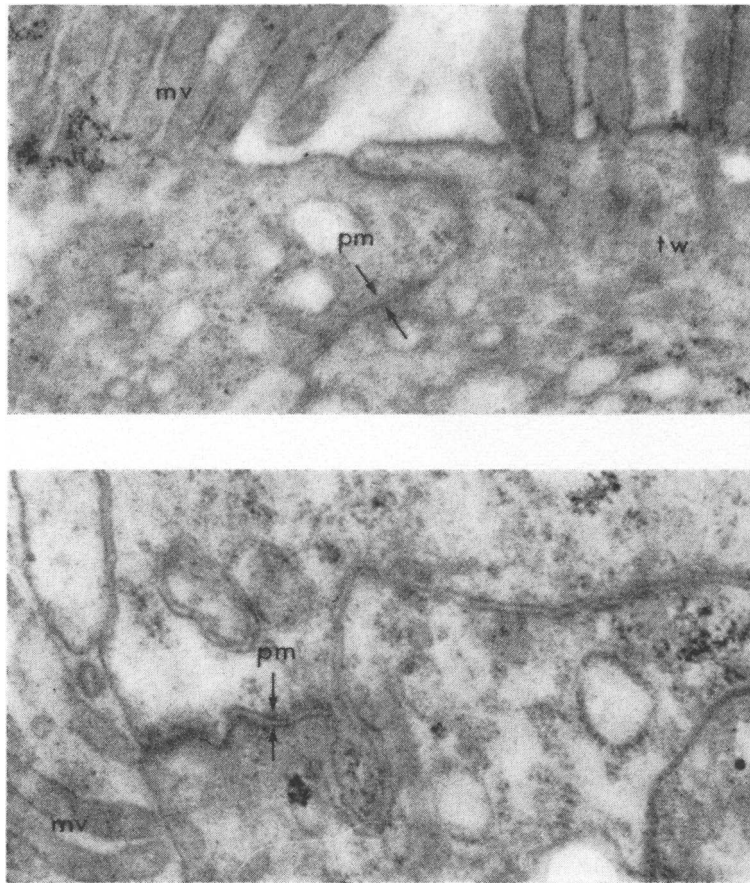

Figure 5 a, b. Cell borders after EDTA-preparation. The junctional complexes have almost disappeared, the cells seem to stick together only by adhesional forces. mv: microvilli; tw: terminal web; pm: plasma membranes. × 46.400

surface. Also the cell nuclei show a rounder form and are surrounded by more mitochondriae than usual; other prominent cytoplasmic changes are not visible. Owing to the modification of the cell outline, the microvilli become wider spaced, "fanned out". The structure of the plasma membranes seem to be morphologically intact, and they are clearly outlined.

The most apparent change is seen where cells lie adjacent to each other, not totally dispersed (Fig. 5a, b). The junctional complexes previously mentioned are mostly not seen or at least have greatly diminished in visibility. At a greater magnification

the intermediate junctions and the desmosomes can hardly be seen, whereas the tight junctions mostly have kept their structural characteristics. The adjacent cell membranes near the free border of the cells have developed marginal foldings as if produced by adhesional forces.

Cell counts averaged 135—205 $\times$ 10^6 per ml packed cells. Mean nigrosin-negativity (*Kaltenbach et al.* 1958), expressing living cellular membranes, was 61—86 %. Differential counts showed mostly intestinal cells (75—85 %), but also naked nuclei (10—15 %) and leucocytes (5—10 %).

DISCUSSION

The described method for the isolation of free intestinal cells with the calcium chelator EDTA, yields cells that are only moderately changed morphologically. Although a quantitative estimation by the electron microscope is difficult, the impression was that most cells were intact. This was confirmed by the nigrosin-staining procedure and earlier biochemical results.

The fact that during the process of isolation, the junctional complexes of epithelial cells were modified or lost, leads to the question of the structural and functional importance of desmosomes and the role of calcium in such intercellular attachments. With the use of calcium chelators further information on this point might be gained.

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SUMMARY

A brief electron microscopic study of free rat intestinal cells, isolated with the calcium chelator EDTA is given. The cells appeared morphologically intact, as was shown by nigrosin-staining. The junctional complexes were modified or lost.

ZUSAMMENFASSUNG

Die Ultrastruktur von freien Darmzellen isoliert mit Hilfe von EDTA.

Eine kurzgefasste elektronmikroskopische Untersuchung von freien Darmzellen von Ratten isoliert mit Hilfe von dem Kalziumbinder EDTA ist vorgenommen worden. Die Zellen schienen bei Nigrosinfärbung morphologisch intakt, jedoch waren die Verbindungszonen — die Desmosomen — meistens verschwunden.

SAMMENDRAG

Ultrastrukturen hos frie tarmceller isolert ved hjelp av EDTA.

Det er foretatt en kortfattet elektronmikroskopisk undersøkelse av frie rottetarmceller isolert ved hjelp av kalciumbinderen EDTA. Cellene syntes morfologisk intakte ved nigrosinfarging, men forbindelseskompleksene — desmosomene — var for det meste borte.

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