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The ultrastructure of the human antral mucosa as demonstrated by freeze-fracturing

A. Köling, R. Aust, H. Rask-Andersen and M. Hoffstedt, Uppsala, Sweden

SUMMARY

The fine structure of the normal mucous membrane in the human maxillary sinus was investigated by means of the freeze-fracture technique. Special interest was directed to membrane structures in the epithelial cells. The morphology of tight junctions could be analysed. According to morphological criteria these junctions might be classified as "very tight". So called ciliary necklaces were well distinguishable. Their morphology seemed to be in concordance with the structure reported in other mammalian respiratory epithelia. Abluminally the epithelial cells frequently displayed abundant caveolae or micropinocytotic vesicles. The present investigation was performed as a preface to later studies on pathologically altered antral mucosa.

INTRODUCTION

The mucous membrane of the upper airways provides a physiological permeability barrier and is responsible for the mucociliary transport mechanism.

In contrast to the nasal mucosa, there has been few investigations devoted to the ultrastructure of the epithelium of the normal human paranasal sinuses (Vidić and Tandler, 1976; Toppozada and Talaat, 1980; Addicks et al., 1982). After the introduction of freeze-fracturing as a preparation technique for electron microscopy the nasal mucosa in various animals has been studied using that method (For a review, see Menco, 1980). No corresponding investigation of the antral mucosa seems to have been performed.

In the present study we used the freeze-fracture technique in order to obtain more information about the ultrastructure of the normal epithelium of the human maxillary sinus with special reference to the cell membranes and their specializations. The present investigation was performed as a preface to later studies of pathologically altered sinus mucosa.

MATERIAL AND METHODS

Samples of macroscopically normal antral mucous membranes were collected from patients with no history of allergic rhinitis or sinuitis. The specimens were

obtained with permission from the patients during surgical procedures for maxillary fractures.

The tissue pieces were immediately placed in a solution containing 2% v/v glutaraldehyde in 0.1 M of Sörensens phosphate buffer pH 7.2–7.4. After rinsing with buffer the specimens were transferred into a solution of 25% buffered glycerol for 30 min and then mounted on goldcup specimen holders. The specimens were subsequently frozen in supercold nitrogen slush at approximately – 190 °C and fractured in a Balzers 360 M or 301 freeze-etching apparatus with double replica technique (Sleytr and Umrath, 1974). The resultant replicas were mounted on mesh copper grids and examined in a JEOL-100B or Philips 301 electron microscope at 80 kV.

It is generally agreed that during freeze-fracturing the cell membranes are split along their hydrophobic central zone. Thus, preparations may show an internal, protoplasmic face (P-face) and an external exoplasmic fracture face (E-face) (Branton et al., 1975).

OBSERVATIONS

General morphology

The freeze-fracture replicas of the human antral mucosa often exposed considerable areas of epithelium. A good comprehension of the general structure of the epithelial cells was obtained (Figure 1). As a rule the cells were fractured along their long axis, but in a few instances the random fracture laid bare numerous cross-fractured cells.

Tight junctions

In the apical part of the epithelial cells typical tight junctions appeared either as continuous belt-like mesh-works of branching and anastomosing thin ridges on the E-face or as corresponding furrows on the P-face (Figure 2). The entire depth of the tight junctions was 0.4–0.6 µm. It generally consisted of 5 to 11 strands. (Gap junctions were not observed within the tight junctions, nor in isolated position.)

Ciliary necklaces

In the neck region of the cilia intramembranous particles could be seen (Figure 3). They were organized into strands and they surrounded the base lying in parallel. The distance between single strands was 0.02–0.04 µm. The total width of the ciliary necklaces was about 0.2 µm.

Micropinocytotic vesicles

Abluminally the epithelial cells frequently displayed abundant caveolae or micropinocytotic vesicles. Their diameter averaged 60 nm. Not seldomly they

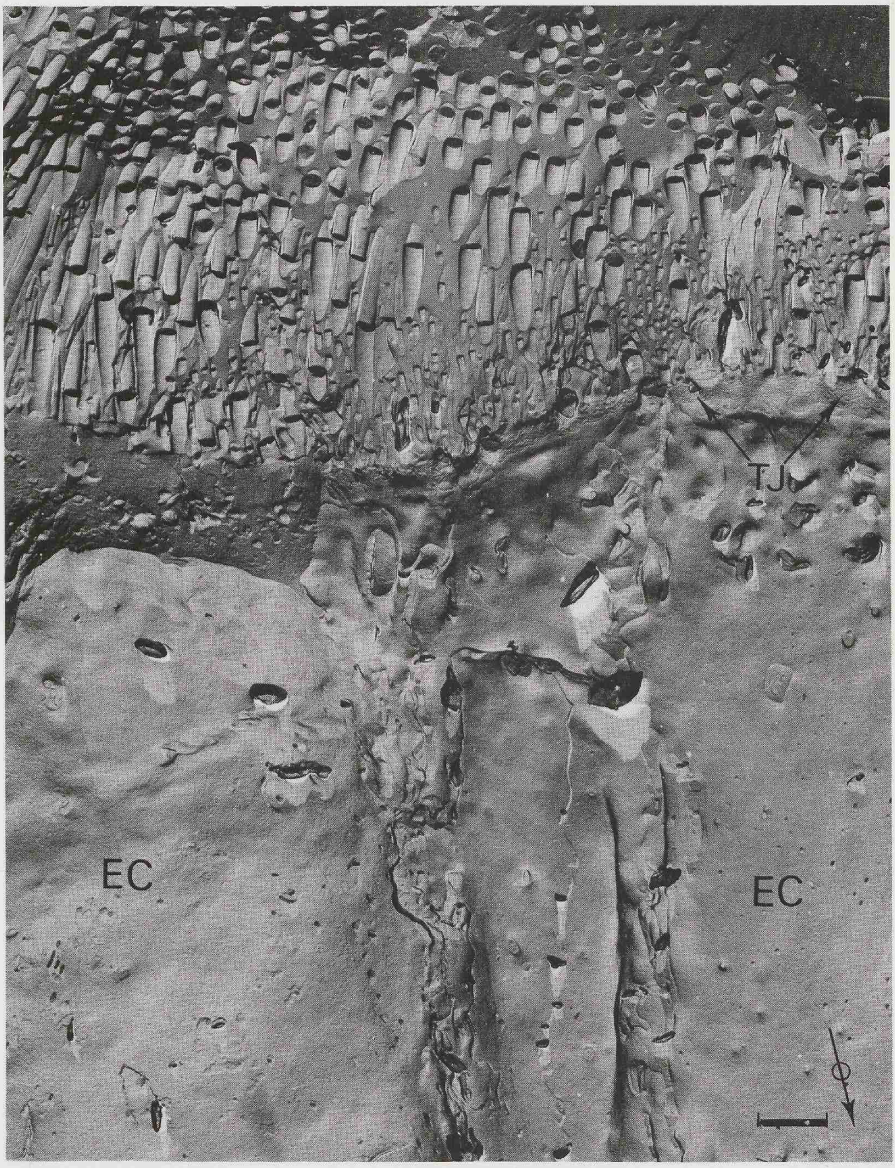

Fig. 1. Low-power micrograph of a freeze-fracture replica from the mucous membrane of the human maxillary sinus. Epithelial cells (EC) are seen. The tight junctional complex (TJ) is visible at the apical part of the cell bodies. Abundant kinocila and microvilli are seen at the top. Scale bar 1 µm. Arrow at bottom right indicates direction of platinum shadowing.

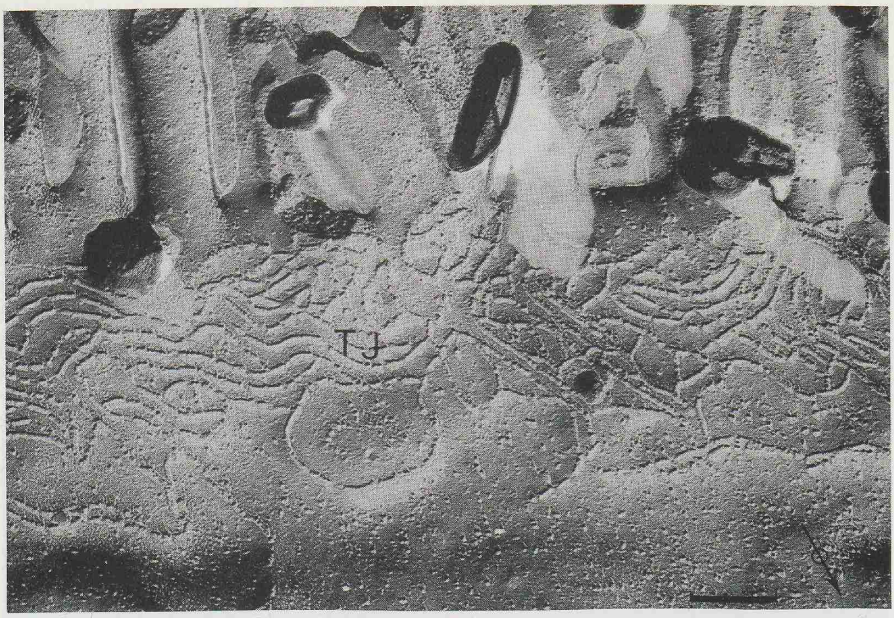

Fig. 2. Tight junction (TJ) at higher magnification. A continous belt is formed of ridges and grooves frequently anastomosing. Scale bar 0.2 µm.

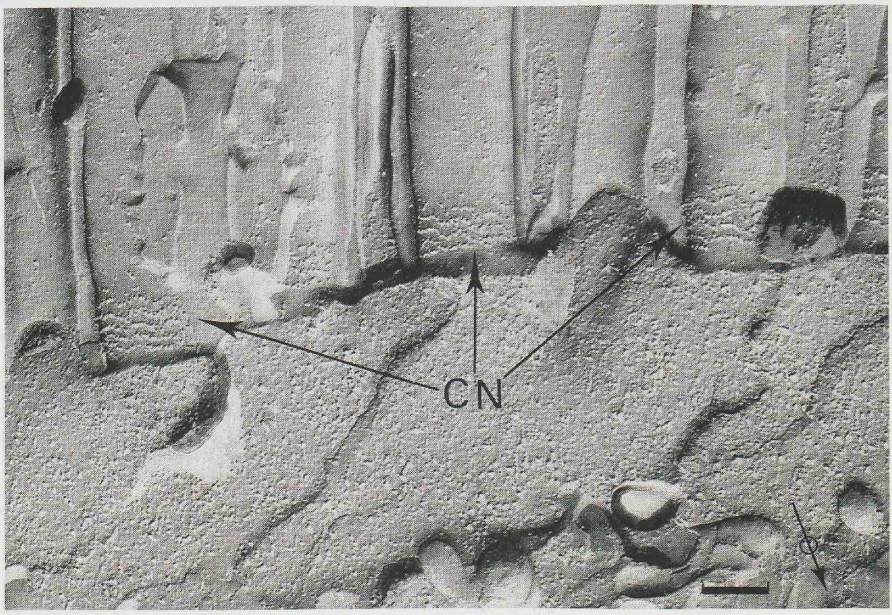

Fig. 3. Higher magnification of the basal part of some kinocilia. Intramembranous particles are organized in parallel strands forming so called ciliary necklaces (CN). Scale bar 0.2 µm.

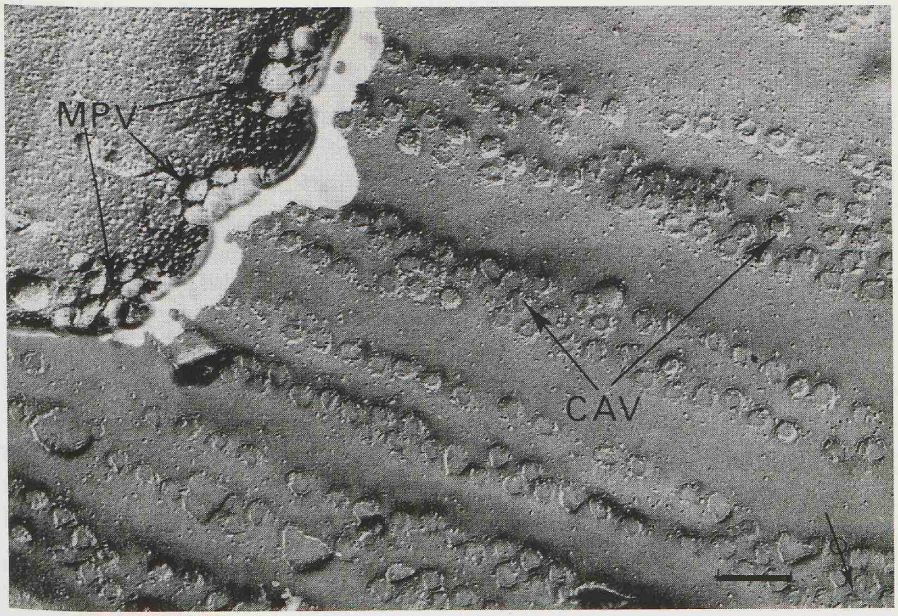

Fig. 4. Freeze-fracture of the basal part of maxillary sinus epithelium. Linear arrangements of caveolae (CAV) representing the surface stomata of micropinocytotic vesicles.

were arranged linearily or in a geometrical pattern. Abundant caveolae, could be ascertained on cross-fracturing to represent the surface opening of micropinocytotic vesicles.

DISCUSSION

The cellular architecture of the mucosa of the human paranasal sinuses was first described with the use of light microscopy by Messerklinger (1958) and Bauer (1960). Some reports about the ultrastructure of the human maxillary sinus have been presented earlier (Vidić and Tandler, 1976; Toppozada and Talaat, 1980). The freeze-fracture technique may give us additional information about the structure of this respiratory epithelium.

The total depth and number of tight junctional strands were analyzed. Claude and Goodenough (1973) correlated junctional morphology and transepithelial electrical resistance. According to their data the number of junctional strands is related to the transepithelial resistance so that an increased number of strands is often found in epithelia showing a high electrical resistance. In the present study we found a mean number of nine intercellular junctional strands indicating that this epithelium is "very tight". It must however be borne in mind that there are instances where a poor correlation between tight junction structures and para-

cellular permeability has been reported (Martinez-Palomo and Erlij, 1975; Möllgard et al., 1976).

Desmosomes have been demonstrated in the junctional complexes between epithelial cells in the respiratory mucosa (Farquhar and Palade, 1963). No attempt was made to investigate them further since freeze-fracturing is not an ideal method for examination of such junctions. This may be due to the fact that the structural elements of desmosomes are not closely associated with the central hydrophobic region of the contributing membranes exposed by the freeze-fracturing (Staehelin, 1974).

It has been speculated that tight junctions in respiratory epithelia undergo morphological changes through allergy and other inflammatory diseases. A study is in progress where we will collect samples of the maxillary sinus mucosa from patients suffering from allergic rhinitis-sinuitis.

Another interesting structure, well distinguishable on freeze-fracture replicas, is the so called ciliary necklace. The morphology of ciliary necklaces found in the present study was largely consistent with that found in other mammalian cilia (Gilula and Satir, 1972). The function of the ciliary necklace is not known. Firstly, it has been suggested that it may provide a barrier to diffusion of compounds constituting the membranes and it would, in this respect, resemble the tight junction (Gilula and Satir, 1972). Secondly, these necklaces have been believed to take part in the mechanical attachement of cilia to the cell soma. This theory has not gained much support and in fact olfactory cilia in the frog have been found to have fewer necklace strands than those in mammals despite the fact that they are longer (Reese, 1965). Another suggestion is that the ciliary necklaces may be involved in mechanisms regulating ciliary function including movement. These structures may thus be important to study during pathological conditions. Decreased ciliary activity in the antral mucosa has been reported in association with chronic sinuitis (Reimer et al., 1978).

ZUSAMMENFASSUNG

Die Ultrastruktur der normalen Schleimhaut in der Kieferhöle wurde mit der Gefrierfrakturtechnik undersucht. Die Verfasser haben sich besonders für die Membranstruktur in den Epithelzellen intressiert. Die Morphologie der "tight junctions" wurde analysiert. Morphologische Kriterien zeigten dass man diese "junctions" als "sehr dicht" klassifizieren kann. Sogenannte ziliare Halsbänder waren gut sichtbar. Abluminal in der Epithelzellen hat man oft eine grosse Mänge von caveolae oder mikropinocytotische Vesikeln gefunden.

Die Verfasser sehen ihren Aufsatz als eine Vorstudie für kommende Untersuchungen über patofysiologische Veränderungen der Schleimhaut.

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A. Köling, M.D.
Dept. of Otorhinolaryngology
University Hospital
S-75185 Uppsala
Sweden