An ultrastructural study of the mucosal surface of the human inferior concha. I. Normal appearances

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INTRODUCTION
The nasal airway is mainly lined by ciliated pseudostratified columnar epithelium. Many ultrastructural studies of this type of epithelium have been performed since the study of the mammalian trachea by Rhodin & Dalhamn (1956); these have largely been of the lower respiratory tract or of mammals other than man. Early work by Mygind & Bretlau (1973) outlined the basic morphology of the human nasal epithelium but used a freeze-drying technique for scanning electron microscopy which causes marked artefactual change. Since then Mygind, Pedersen & Nielsen (1982) and Petruson, Hansson & Karlsson (1984) have examined the ultrastructure of nasal mucosa.

The aim of the present study was to re-examine the normal appearance of the nasal mucosa at a precisely defined site and to discuss the functions of some of the structures seen. In further studies we will describe the changes seen in cigarette smokers and in subjects with altered airflow due to deviation of the nasal septum.

MATERIAL AND METHODS
Nasal mucosa was obtained from 17 subjects (8 males, 9 females; age range 19–65, mean 35.9 years). They had no clinical evidence of nasal or ear pathology including septal deviation and were on no medication. Biopsies were obtained during a general anaesthetic administered for an unrelated purpose. Informed consent was obtained in all cases according to ethical committee stipulations.

The biopsies were taken with a sharp cup forceps, from the medial surface of the inferior concha, 1.5 cm behind its most antero-inferior point. This site was precisely defined to maintain uniformity. Specimens were immediately placed in 2.5% glutaraldehyde in 0.05 M phosphate buffer and shaken to dislodge excess mucus from the tissue surface. After fixation, suitably sized specimens were cut in two for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) on the same subjects. Those for SEM were dehydrated in 70% ethanol, then graded concentrations of acetone, and finally in a critical point dryer (Emscope CPD 750). They were then sputter-coated with platinum (Emscope SC500) to a thickness of roughly 30 nm. For TEM, specimens were transferred to 1% osmium tetroxide in 0.05 M phosphate buffer, dehydrated in graded concentrations of ethanol and cleared in propylene oxide before embedding in Araldite resin as modified by Mollenhauer (1964). One μm thick sections were collected on Formvar-coated copper grids and stained with Reynolds lead citrate and 30% uranyl acetate in methanol.
All microscopy was performed with a JEOL 100Cx Temscan electron microscope. Measurements were taken directly from electron micrographs. Linear measurements were calculated according to scale; surface areas were measured using an image analyser (Reichert MOP AMO2). In each case dimensions were taken from at least 25 cells or organelles, and the mean and range presented.

**RESULTS**

**Scanning electron microscopy**

With SEM the epithelial surface showed three types of cell: ciliated and non-ciliated columnar cells and goblet cells in which mucus secretion was occurring at the surface. Goblet cells in other phases of activity were indistinguishable from non-ciliated columnar cells. The cells formed a mosaic pattern at the surface, visible where there were few or no ciliated cells.

**Ciliated columnar cells**

At this site on the nasal mucosa there was wide variation in the proportion of ciliated cells (7–96% of the surface area, mean 27%). The mean surface area of cells that could be individually distinguished was 133 μm² (range 65–271 μm²). These cells were densely covered with cilia (Figs. 1, 2); microvilli could be seen at their margins. Nearly all cilia were of the same length; however, cells with short developing cilia were seen occasionally. No abnormal cilia were identified and examination of cilial tips under high power showed no evidence of ‘cilial crowns’ (see below).

**Non-ciliated columnar cells**

These cells were more varied in surface area than the ciliated cells (mean 186 μm², range 18–795 μm²). All cells were densely covered with microvilli (Fig. 1). Their length was uniform on individual cells but varied a little from cell to cell. The tips of the microvilli measured 0·3–0·46 μm in diameter.

At the cell membranes the intercellular space was crossed by numerous cytoplasmic strands of constant thickness (0·19–0·21 μm) (Fig. 6). The significance of these strands is not clear, but it seems most likely that they are an induced artefact.

**Goblet cells**

These were identifiable when a convex mucus droplet was forming on the surface (Fig. 5). Cells in this state varied greatly in number between subjects (mean 10·6 per field at ×1500, range 0–41), and occurred most frequently in densely ciliated areas. The droplets varied in size (up to 11 μm in diameter). They had a smooth or slightly rough surface and occasionally bore residual microvilli (Fig. 6). The cell membrane was generally intact; rarely a goblet cell was observed with a hole in the surface membrane (Fig. 5) presumably representing the ‘stoma’ through which Tos (1982) suggested that mucus is secreted. A few cells were seen in which several small droplets protruded from an otherwise flat surface bearing microvilli, indicating extrusion of discrete secretory granules rather than the shedding of the whole distal part of the cell.

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**Fig. 1.** SEM. Normal nasal mucosa showing ciliated cells (c), non-ciliated cells (n) covered with microvilli and secretion droplets (d). The intercellular junctions between non-ciliated cells are clearly visible.

**Fig. 2.** SEM. More extensively ciliated mucosa.
Ultrastructure of human nasal mucosa
Transmission electron microscopy

With transmission electron microscopy the three surface cell types were seen; basal and intermediate cells were present deeper in the epithelium but will not be discussed in this paper. Lymphocytes were also seen apparently migrating through the epithelium.

Columnar cells, ciliated and non-ciliated

These cells were approximately 55–80 μm in length and 9–14 μm in width. Human nasal cilia have been described in detail elsewhere (Smallman & Gregory, 1986). In the present study, cilia were approximately 5·5–7 μm long and 0·35–0·4 μm in diameter.
Ultrastructure of human nasal mucosa

Fig. 5. SEM. Secreting goblet cells with discharging mucus droplets (d), largely intact. Holes (h) in the cell membrane suggest discharge of individual secretory granules.

Fig. 6. SEM. Secreting goblet cells. Residual microvilli (m) are seen on the surface of one droplet. Cytoplasmic strands (cs) cross the intercellular junctions.
They contained the typical axoneme composed of microtubules with a 9 + 2 arrangement. Very occasionally a 'crown' was seen, composed of discrete filaments projecting from the ciliary tip (Fig. 7). Ciliary abnormalities were rare and consisted of small cytoplasmic protrusions on the side of the ciliary shaft.

Ciliated and non-ciliated columnar cells were covered with microvilli. These were usually straight but roughly one in ten microvilli branched up to six times (Fig. 10). Fine electron-dense filaments were identifiable in the cytoplasm lying parallel to the

Fig. 7. TEM. Vertical section through tips of cilia showing ciliary crowns (arrowheads).
Fig. 8. TEM. Surface of goblet cell bearing microvilli (m) with prominent glycocalyx (gx).
Ultrastructure of human nasal mucosa

Fig. 9. TEM. Microvilli of non-ciliated columnar cell, demonstrating branching and glycocalyx (gx).

Fig. 10. TEM. Microvilli of ciliated cell, showing occasional branched structure, and filaments (f) in cytoplasm running parallel to long axis.

long axis (Fig. 10). Microvilli differed in detail between the two cell types. Those on ciliated cells were 2.6–3.3 μm long and 0.075–0.1 μm in cytoplasmic diameter (or 0.24–0.36 μm in total diameter including the surface coat). They lay parallel to the cilia. Those on non-ciliated cells were of similar length (2.5–3.4 μm) but rather thicker than those of the ciliated cells (0.09–0.15 μm in diameter) (Fig. 9). They were also more randomly arranged. All microvilli bore a prominent surface coat or glycocalyx (Figs. 8–10), consisting of a loose network of electron-dense fibrils lying in a radial fashion, up to 0.18 μm thick. The glycocalyx was denser and thicker on microvilli of the non-ciliated cells but was also clearly defined on those of ciliated cells.

Goblet cells

Goblet cells could be observed in all phases of activity (Figs. 3, 4) and therefore appeared more numerous with TEM than SEM (up to one third of surface cells). They were approximately 65–100 μm in length, and 9–15 μm in width. Their size and ultrastructure varied with their physiological state. Large numbers of mucus granules within the apical cytoplasm gave the cell its characteristic shape. These granules were electron-lucent and membrane-bound. Near the cell surface the granules frequently
coalesced. In general, it appeared that the distal part of the cell was shed with an intact cell membrane in an apocrine fashion (Fig. 4). However, occasionally granules were seen to discharge individually (see SEM).

The goblet cells also bore microvilli. These were less densely packed than those of the columnar cells (Fig. 8) and were shorter and thicker (length 1.2–1.8 μm, width 0.15–0.18 μm). They bore a similar glycocalyx, up to 0.18 μm thick.

**DISCUSSION**

Any study of normal man must take into account the effects of the environment. This is true especially of the nasal lining, the function of which is to modify inspired air by filtration, humidification and temperature control. The epithelium shows a graded structural change from the front to the back of the nose. The ratio of its principal component cells is modified by such environmental factors as altered airflow (Moore Gillon, 1985), atmospheric pollutants (Ballenger, 1981) and virus infections (Winther, Brofeldt, Christensen & Mygind, 1984); individual exposure to these factors varies greatly. Previous studies of normal mucosa (Mygind & Bretlau, 1973; Petruson et al. 1984) lack precise definition of the site sampled. In the present study we biopsied a constant site to demonstrate the normal variation in structure of this active epithelium.

The nasal mucosa is capable of rapid functional adaptation in response to environmental factors. In the SEM part of this study, the numbers of secreting goblet cells varied greatly. Some specimens showed many mucus droplets being released, perhaps due to an immediate neurosecretory response to intranasal stimulation by the biopsy forceps.

Nasal mucosa is also continually exposed to antigens. The migration of lymphocytes through the mucosa emphasises the active immunological role of normal nasal epithelium (Mygind, 1986). Lymphocytes were found in the respiratory tract by light microscopy as long ago as 1885 (Jeffery & Reid, 1975) and their ultrastructure has been described in detail in rat trachea by Rhodin & Dalhamn (1956). It is possible that these lymphocytes are migrating through the epithelium to sample antigen on the mucosal surface.

**Goblet cells**

The ultrastructure of goblet cells has been widely studied (Florey, 1960; Freeman, 1962; Hafez, 1977) and their structure and function in the nasal mucosa described by Mygind & Bretlau (1974) and Tos (1982). Mygind & Bretlau (1974) showed goblet cells with a wide rupture in the apical cell membrane through which could be seen septa between individual granules; Tos (1982) described this as a ‘stoma’. These features were possibly artefactual due to prolonged glutaraldehyde fixation or freeze drying of specimens; in the present study the goblet cell appeared to maintain an intact cell membrane until the whole distal part of the cytoplasm was shed in an apocrine fashion. Only rarely was a ‘stoma’ seen. It is notable in the SEM micrographs that the emerging mucus droplets remained intact until they protruded above the surrounding ciliary tips into the lumen. This may help to minimise mixing of mucus and periciliary fluid.

**Cilia**

In the present study, cilia showed the classic ‘9 + 2’ pattern on TEM. Local extrusions of ciliary cytoplasm were occasionally seen. These have previously been described by Dalen (1981) and are thought to be fixation artefacts. A study by one of
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cells of mucociliary clearance, abnormalities (Smallman & Gregory, 1986). They have also been observed in various disease states (Herzon, 1981, 1983; Takasaka, Sato & Onodera, 1980). The biological significance and mode of their formation is largely unknown.

An inconstant feature observed in this study was the ciliary crown. This appeared as a cluster of four to six projections extending out from the membrane at the ciliary tip. This crown was first observed in 1972 by Dirksen & Satir in mouse oviduct, and has also been seen in thymic cysts of nude mice (Cordier, 1975) and rat trachea (Jeffry & Reid, 1975). Kuhn & Engleman (1978) studied its relationship to the underlying axoneme in mammalian respiratory mucosa; the crown was found to consist of transmembranous filaments bound to the disc at the tip of the axoneme. To our knowledge this structure has not been described in cilia in man. Its function is unknown. Sleigh (1974) assumes that during the effective stroke only the tips of the cilia protrude from the periciliary fluid to ‘claw’ the mucus forward. The identification of the crown lends weight to this hypothesis.

Microvilli

The microvilli of all three cell types were broadly similar in structure. Those on the goblet cells were more disorganised in orientation, perhaps because of the continually changing shape of the surface of these cells during their secretory cycle. On non-ciliated cells, the microvilli varied in length between cells but were of uniform length within the cell. This suggests that cells are at differing stages of development, although no cells were seen without microvilli. About 10% of the microvilli branched. Branching microvilli have not been previously described, although they can be seen in published micrographs of epithelium from the trachea of rat (Iravani, Melville & Horstman, 1977) and guinea-pig (Dalen, 1983).

Like those of the trachea (Dalen, 1983), nasal microvilli have a central core of filaments which may represent actin fibres. Actin has been demonstrated in intestinal microvilli (Mooseker & Tilney, 1975) and also in the apical region of the columnar cells of human nasal mucosa (Petruson et al. 1984). Intestinal microvilli are motile (Sandstrom, 1971), as are those of renal proximal tubules (Thuneberg & Rostgaard, 1969). Dalen (1983) has suggested that tracheal microvilli may have a motile function, assisting flow of periciliary fluid; perhaps this also occurs in nasal mucosa.

A further possible function of respiratory microvilli is fluid transfer. For efficient mucociliary clearance, the level of periciliary fluid must be maintained just below the tips of the extended cilia (Proctor, Adams, Andersen & Man, 1977); reduction of this level reversibly slows ciliary activity (Iravani et al. 1977). Intestinal microvilli bear a surface coat, or glycocalyx, which has been implicated in their absorptive function (Fawcett, 1965). A similar layer is found outside the cell membrane of many cell types (Janssen et al. 1985). The glycocalyx consists of a loose network of complex carbohydrate molecules (Carlson, 1977). It has a number of properties; in particular it is permeable to water while filtering out larger molecules (Bennett, 1963). Kilburn (1968) and Dalen (1983) suggest that the glycocalyx on tracheal microvilli may play such a role in fluid absorption. The present work has demonstrated that nasal microvilli bear an elaborate glycocalyx. Another of their functions, therefore, may be the maintenance of low viscosity periciliary fluid around the proximal parts of the cilia.

The glycocalyx can also affect surface adhesion (Janssen et al. 1985). This property varies with cell type and is due, on red blood cells, to an electrostatic charge (Eylar, Madoff, Brody & Oncley, 1962; Grimes, 1980). The glycocalyx on nasal microvilli may therefore also help prevent adhesion and tangling of both microvilli and cilia,
thus maintaining motility of these structures. Further study is needed both of this surface coat and of the nasal microvilli in general, as they may well play an important part in mucociliary transport.

**SUMMARY**

A study is presented of normal human nasal mucosa. Tissue was taken from a defined site on the inferior concha to minimise individual variation and was studied using scanning and transmission electron microscopy.

Three cell types were found at the epithelial surface; ciliated and non-ciliated columnar cells and goblet cells. The distribution of cell types varied greatly between specimens, perhaps owing to environmental factors.

All cells bore microvilli which showed minor structural differences between the cell types. Two important features were common to all microvilli. Cytoplasmic filaments were seen running parallel to the long axis of the structure; these may be involved in microvillar motility. Also found was a prominent surface glycocalyx. This may help maintain the volume and viscosity of periciliary fluid; it may also prevent adhesion and tangling of microvilli and cilia.

A notable feature of the cilia seen on transmission electron microscopy was the presence of occasional ciliary crowns; these claw-like projections from the ciliary tips may be involved in the propulsion of mucus.

The goblet cells showed apocrine secretory droplets which were extruded intact into the nasal lumen. This may help to preserve the integrity of the mucous and periciliary fluid layers.

We conclude that the mechanism of mucus secretion and transport in the nose may involve several surface structures including ciliary crowns and microvilli. Their function is not yet clear and further study is indicated.

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**REFERENCES**


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