

## Acid mucopolysaccharide layer of the surface nasal epithelium

*Kensuke Watanabe, Yozo Saito, Isamu Watanabe, Tokyo, Japan*

### SUMMARY

*In the anterior tip of the inferior turbinate, the epithelium has microvilli which are covered by a mucopolysaccharide layer. This layer is about 0.8  $\mu$  in depth, well stained by ruthenium red and with a high density. The spaces between these microvilli are filled with this dense layer; therefore the movement of microvilli are suppressed, but this layer is a strong defensive line against ambient air. It is known that most inhaled particles are deposited in the anterior nares. So virus, microorganisms and antigens etc. will easily penetrate the epithelium if this layer becomes thin. Two centimeters behind the anterior tip where the epithelium is ciliated, the mucous layer over the cilia is less stained by ruthenium red and has a lower density than the anterior tip. This layer does not insert itself between the cilia. The fine filamentous mucopolysaccharide which seems flexible binds the cilia and the mucous layer. While the beat of cilia is not suppressed the inhaled particles cannot insert between cilia. In conclusion, the deep mucopolysaccharide layer over the anterior tip of the inferior turbinate and ciliary beat are important defensive mechanisms against the trauma of the ambient air.*

The epithelium of inferior turbinate consists of ciliated cells, cilia free cylindrical cells, intermediate cells with both cilia and stereocilia, goblet cells and squamous cells. Under the basement membrane the tunica propria is found. Nasal glands and capillaries are found in tunica propria. The epithelial cells of nasal mucosa eliminate bacteria, viruses and antigens etc., and also absorb and secrete. It is known that the form of epithelial cells is not the same everywhere on the inferior turbinate. In the anterior tip, the epithelium is nonciliated and farther back on the inferior turbinate, the epithelium becomes ciliated (Mygind, 1975; Hilding, 1963; Lenz, 1973). Under ordinary circumstances the nose is the first target and the first defensive line against the ambient air which has unphysiological temperature and humidity and furthermore microorganisms and allergens. In particularly the anterior third of the nasal cavity is charged by these influences to such a degree that it differs from the rest of the mucous membrane

of the respiratory tract because it is known that most of the inhaled particles are deposited in the anterior third (Hilding, 1963; Proctor, Andersen and Lundquist, 1973). Therefore, it must play a different role in the defensive mechanism between the anterior tip of the inferior turbinate and the area more posteriorly. It is very interesting to study since the layer which covers the surface of the anterior epithelium is different from that of the posterior area of the inferior turbinate. In the present investigation, cytochemical technics at the ultrastructural level have been used to study the acid mucopolysaccharide of nasal epithelium and to discuss the relationship between morphology and the function of the nasal epithelium.

#### MATERIAL AND METHODS

With a local anesthesia (0.5% procain 0.5cc) biopsies were taken with small forceps, from 2 parts of a normal person's inferior turbinate. One is taken from the anterior tip of the inferior turbinate, the other is taken 2 cm behind the anterior edge. Small blocks were fixed for 1 hour by immersion in ice cold 1.5% glutaraldehyde buffered with 0.067M cacodylate, PH7.3 and containing 500 p.p.m. purified ruthenium red (Luft, 1964). Following fixation with glutaraldehyde-ruthenium red, the tissue blocks were immersed in 0.1M cacodylate for 10 minutes and then postfixed for 3 hours at room temperature, with 2%

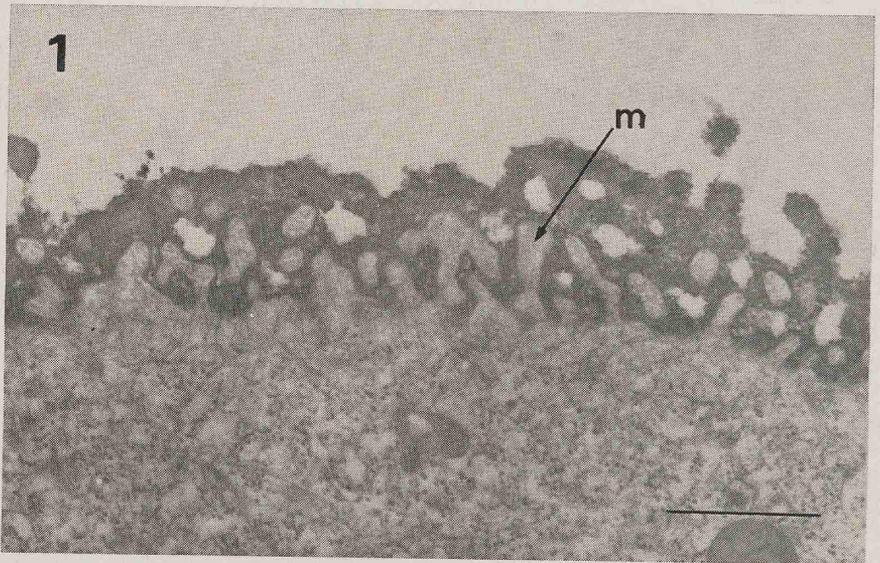


Figure 1. In the anterior tip of the inferior turbinate, the epithelium has microvilli which are covered by the mucopolysaccharide layer, which looks like viscid. M: microvilli.  $\times 20,000$ .

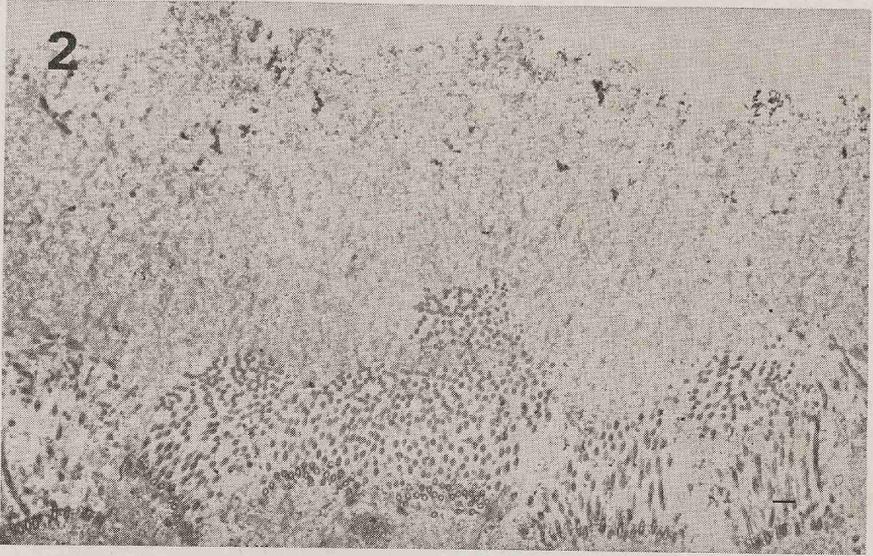


Figure 2. The mucopolysaccharide layer in 2 cm behind the front edge of the inferior turbinate is deeper than the anterior tip. And it is less stained by ruthenium red and has a lower density than the anterior tip.  $\times 3,000$ .

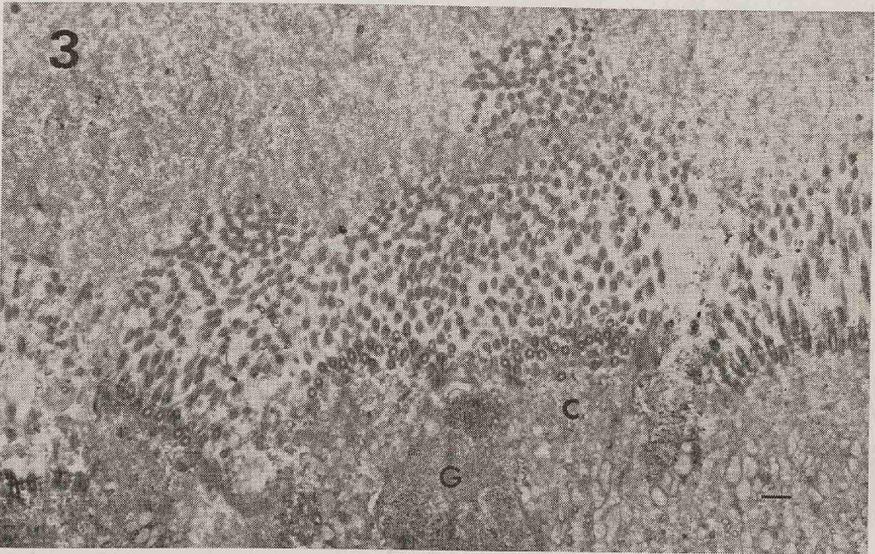


Figure 3. The ciliated cells are lined and goblet cells are found sporadically. The mucopolysaccharide layer does not insert between cilia. G: goblet cell. C: ciliated cell.  $\times 4,000$ .

$O_5O_4$  buffered with 0.067M cacodylate, PH7.3 and containing 500 p.p.m. purified ruthenium red. The blocks were next dehydrated with ethanol and embedded in Epon 812. Ultrathin sections were treated with uranyl acetate and lead to enhance contrast for electron microscopy.

#### RESULTS

Anterior tip of the inferior turbinate: The epithelium has microvilli which are covered by the mucopolysaccharide layer. This layer is about  $0.8 \mu$  in depth, well stained by ruthenium red, with high density and appears viscid. The space between microvilli are filled with this viscid layer (Figure 1).

Two centimeters behind the anterior edge of the inferior turbinate: The ciliated cells are plentiful while the goblet cells are found sporadically (Figure 3). The depth of the mucopolysaccharide layer over the epithelium is not uniform, but it is deeper than that over the anterior tip. This layer is less stained by ruthenium red and has a lower density than the anterior tip, and appears to

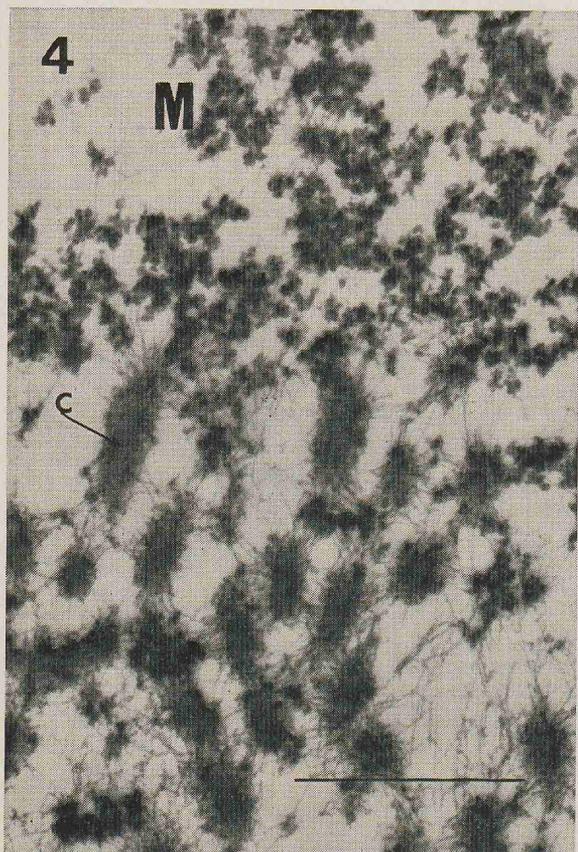


Figure 4.

The fine filamentous mucopolysaccharide which looks flexible binds cilia and this fiber extends to the mucous layer. M: mucous layer.

C: cilia.  $\times 30,000$ .

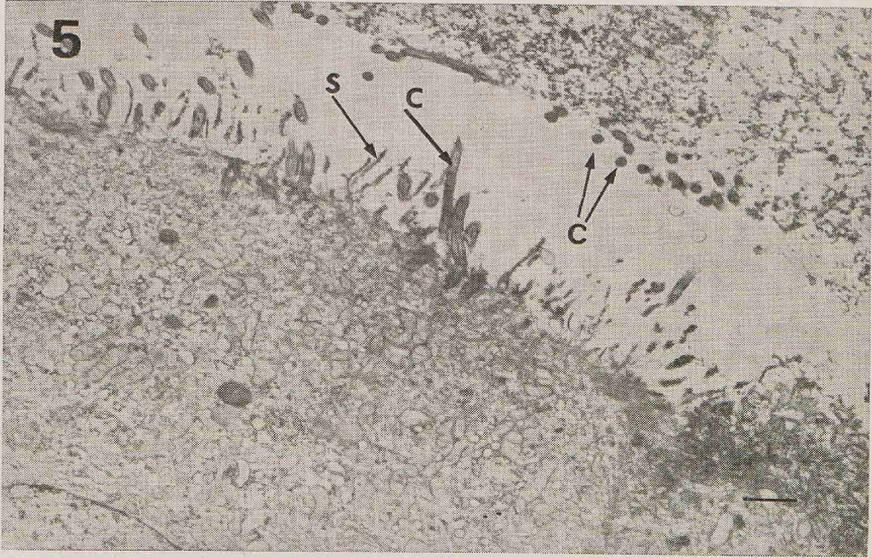
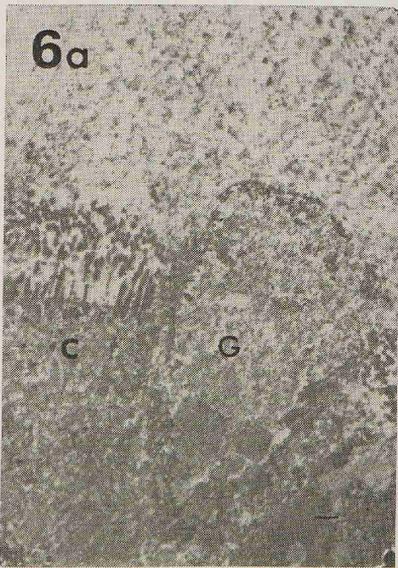


Figure 5. An intermediated type cell which has both cilia and stereocilia. The mucopolysaccharide layer is blocked only by cilia. C: cilia, S: stereocilia.  $\times 7,000$ .



$\times 3,000$



$\times 3,000$ .

Figure 6a and 6b. The surface of the goblet cell is gradually swollen and mucous is secreted to the nasal cavity. G: goblet cell, C: ciliated cell.

have a low viscosity. This layer does not insert between the cilia but is carried to the nasopharynx by ciliary beat (Figures 2 and 3). The fine filamentous mucopolysaccharide which looks flexible, binds cilia and extends to the mucous layer (Figure 4).

An intermediate type cell between the ciliated cell and the nonciliated cell is found, which has both cilia and stereocilia (Figure 5). It is considered that the cilia bear the main function of this cell, since the mucous layer is found only over the cilia and does not touch the stereocilia.

The goblet cell is one of the chief producers of the mucous blanket. This is true because the electron density of the goblet cell granules is very similar to that of the mucous blanket (Figure 7). The surface of the goblet cell is swollen (Figure 6a and 6b) and the mucous is secreted to the nasal cavity (Figure 7).

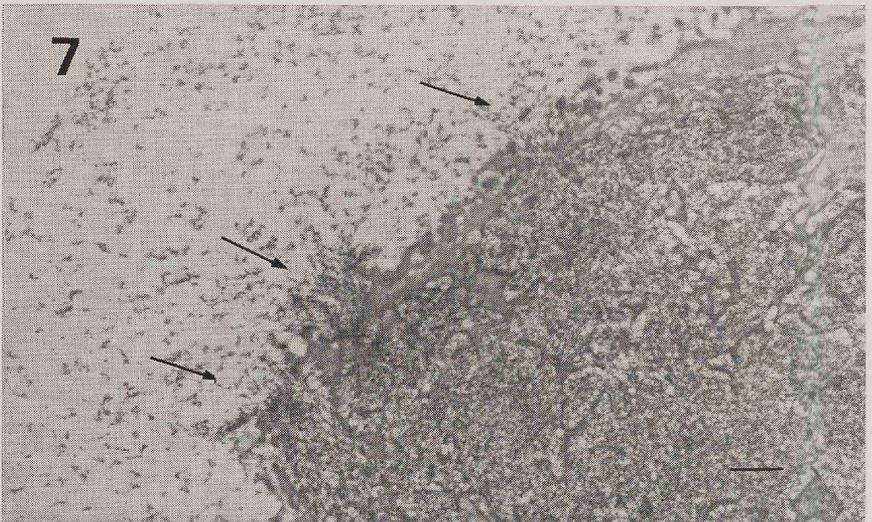


Figure 7. Mucous is secreted from goblet cell to nasal cavity.  $\times 7,000$ .

## DISCUSSION

The nose is both the first target and first line of defense against the ambient air. According to Morrow (1960), the factors controlling deposition of particles from an air-stream are gravity, inertia, velocity, diffusion and the viscosity of the medium. Proctor (1973) reported that at the anterior nares (1.5 cm to 2 cm posterior to the nostrils) the linear velocity of air stream is greater than in any other portion of the respiratory tract. Hounam, Black and Walsh (1971) found that nasal particle deposition is related to the individual's nasal resistance to air flow. As high velocity coincides with an elevated nasal resistance to air flow, the likelihood for impaction of inhaled particles in respiratory tract is

in the anterior nares. As the epithelium is nonciliated in the anterior nares, mucous flow rate in this region is low (Bang, Mukherjee and Bang, 1967; Ewert, 1965; Hilding, 1963; Proctor and Wagner, 1965). Many particles which are deposited in the anterior nares remain for a long time.

A surface complex is known to cover the epithelium of the respiratory tract. Lipid, protein and mucopolysaccharide have been identified in surface-active extracts of epithelial cells of the respiratory tract. And some attempts have been made to demonstrate these components at the epithelial surface, especially on the lung epithelial surface (Brooks, 1969; Adamson and Bowclen, 1970). This time, the authors attempted to demonstrate the acid mucopolysaccharide at the epithelial surface of the inferior turbinate.

The anterior tip of the inferior turbinate where maximum deposition of inhaled particles are observed is covered by a deep layer of acid mucopolysaccharide. As the space between the microvilli are filled with a viscid layer, the mobility of the microvilli is suppressed, but this layer is a strong defense line against ambient air. This mucopolysaccharide layer involves IgA (Mygind and Bretlau, 1974), antigen-antibody reactions (Weiss, 1969) and provides specific cellular receptor sites for the binding of virus particles (Burnet, McCrea and Ston, 1946). As mentioned previously, the anterior part of the nasal cavity is a non-ciliated area and the velocity of particles which deposit at the tip of the inferior turbinate is very low, and inhaled particles remain for a long time. If this mucopolysaccharide layer becomes thin, then viruses, antigens and microorganisms, etc. can penetrate the layer and easily attach to the plasma membrane of the epithelial cells. So the deep layer of mucopolysaccharide at the tip of the inferior turbinate is a most important defense against the ambient air.

Adamson and Bowden (1971) reported that in oxygen poisoning the pulmonary cells with the thinnest layer of mucopolysaccharide (type 1 epithelium) were highly susceptible to injury and cells with a thick layer (type 2 epithelium) were more resistant. They suggested that the mucopolysaccharides served to protect the lipoprotein complex of the plasma membrane.

Much inhaled matter is deposited in the anterior tip of the inferior turbinate whence it is carried posteriorly. In the main airstream the epithelium is ciliated and the mucous flow rate is high (Hilding, 1963; Proctor, Andersen and Lundqvist, 1973). Cilia is covered by a mucous blanket. This layer is less stained by ruthenium red and has a lower density than the layer over the anterior tip of the inferior turbinate. The fine filamentous mucopolysaccharide binds the cilia but ciliary beat is not suppressed. The mucous blanket does not insert between the cilia. If ciliary action stops, mucous blanket easily inserts between the cilia.

It has been reported that a deterioration in mucociliary function of the respiratory epithelium appeared in some cases: i.e.: connections with naturally

occurring common colds and in experimentally induced rhinovirus infections (Sakakura et al., 1973; Bang and Bang, 1976), colling, excessive dryness (Hilding, 1963) and absence of enough mucous (Sade, Eliezer, Silberberg and Nevo, 1970). We speculate that when the deep mucopolysaccharide layer which covers the anterior tip of the inferior turbinate becomes thin for whatever reason, that viruses, micro-organisms and antigens can easily penetrate the mucopolysaccharide layer to the plasma membrane of the epithelium. Then the direct effect of air containing particulate matter to the epithelium of the anterior turbinate tip can secondarily stop the ciliary beat, which inturn causes the deterioration of the defensive function of the nasal epithelium.

#### ZUSAMMENFASSUNG

Am Kopf der unteren Nasenmuschel besitzt das Epithel sog. Mikrovilli, die von einer Mukopolysaccharid-Schicht bedeckt sind. Diese Schicht ist etwa  $0,8 \mu$  tief und lässt sich mit Ruthenium Rot gut anfärben. Sie ist auch von hoher Dichte. Der Raum zwischen den Mikrovilli ist mit dieser dicken Schicht angefüllt. Dadurch wird die Bewegung der Mikrovilli gebremst. Es wird angenommen, dass die Mikrovilli wie auch die bedeckende Schicht eine wichtige Funktion in der Abwehr von Mikroorganismen spielen. Bekanntlich ist die vordere Nase mit inhalierten Partikeln und Mikroorganismen wie auch Antigenen angereichert, die in diese Schicht eindringen können, sofern sie dünn ist. Das Epithel der Schleimhaut etwa zwei Zentimeter hinter dem Kopf der Muschel besitzt ebenfalls Mikrovilli. Hier ist aber die Dichte der Schicht niedriger, so dass sich die Mikrovilli freier bewegen können. Ferner wird ein Zwischenzelltyp beschrieben, der sowohl Mikrovilli als auch Stereocilien besitzt. Auch diesen Zellen wird eine Abwehrfunktion zugeschrieben.

#### ACKNOWLEDGEMENT

The authors wish to express their thanks to Prof. V. Mizuhira for his helpful suggestions and advices.

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K. Watanabe, M.D.

Y. Saito, M.D.

I. Watanabe, M.D.

Department of O.R.L.

Tokyo Medical and Dental University,

1-5-45 Yushima

Bunkyo-ku, Tokyo, Japan.