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SCANNING ELECTRON MICROSCOPIC STUDIES OF THE HUMAN NASAL MUCOSA IN NORMAL PERSONS AND IN PATIENTS WITH PERENNIAL RHINITIS

I: Cilia and Microvilli

By

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The upper respiratory tract is under constant influence of the inspired air, which has an unphysiological temperature and humidity and furthermore contains microorganisms, other particles and potential allergens. 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. When this part of the mucous membrane in patients with perennial rhinitis has to be examined, it is therefore important to be aware of the fact that it is not possible to compare this nasal mucous membrane with a perfectly normal one. It is only possible to compare it with mucous membranes, which have been exposed to one injurious factor less.

Lenz (5, 6) recently published papers on scanning electron microscopy of the normal human nasal mucosa and of the mucous membrane on the inferior concha in patients with perennial rhinitis of such a severe character that conchotomia was necessary. We have preferred to take biopsies from the anterior part of the inferior concha, which has given less tissue

for examination, but on the other hand possibility for examining a larger part of the patients with perennial rhinitis. Scanning electron microscopy (SEM) is a method of investigation, in which the results depend on the method used for preparation, and in which there is a constant risk of artifacts. We therefore examined specimens from different methods of preparation and a series of corresponding specimens in transmission electron microscope (TEM) and in anoptal contrast (AC). As other authors have given detailed descriptions of the human nasal mucous membrane in TEM (3, 4, 11), it is intended in this work to use TEM only as a supplement to SEM investigations and, if possible, to disclose artifacts.

As the surface of the mucous membrane in normal persons and in patients with perennial rhinitis is very similar with only small differences, as will be seen later, this work will not be divided into a normal and a pathological section, but into two papers of which the first deals with cilia and microvilli and the second with structures, which have connection with the formation of the secretion.

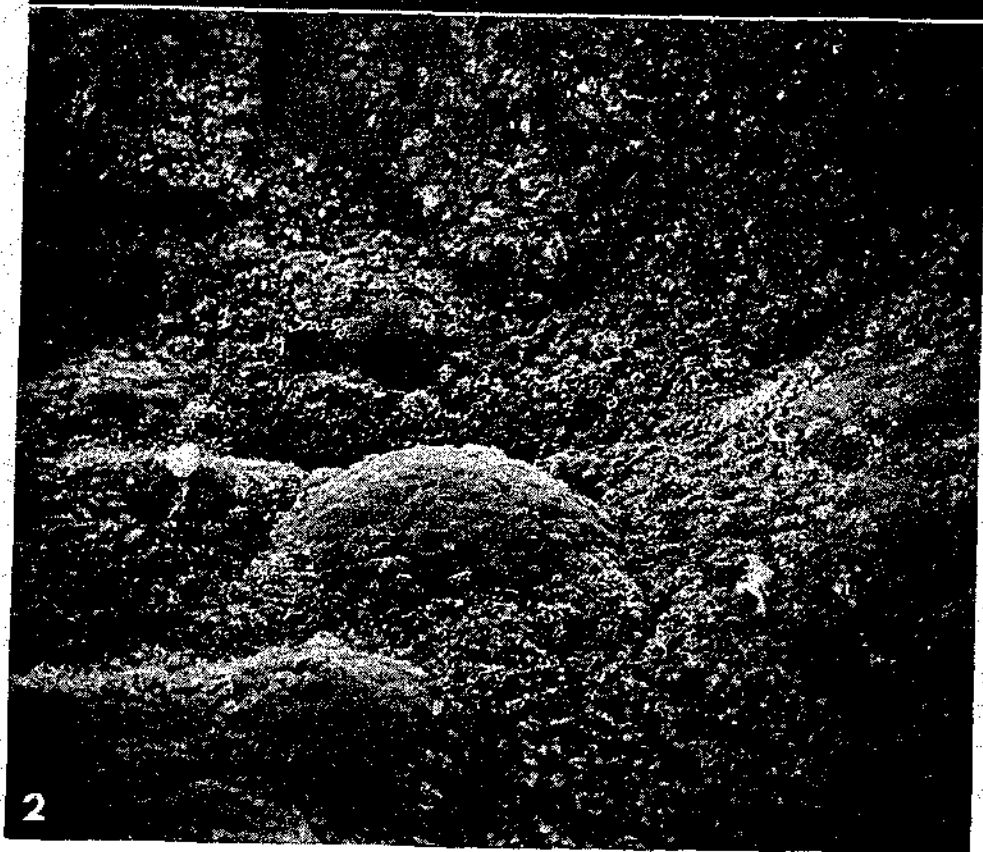
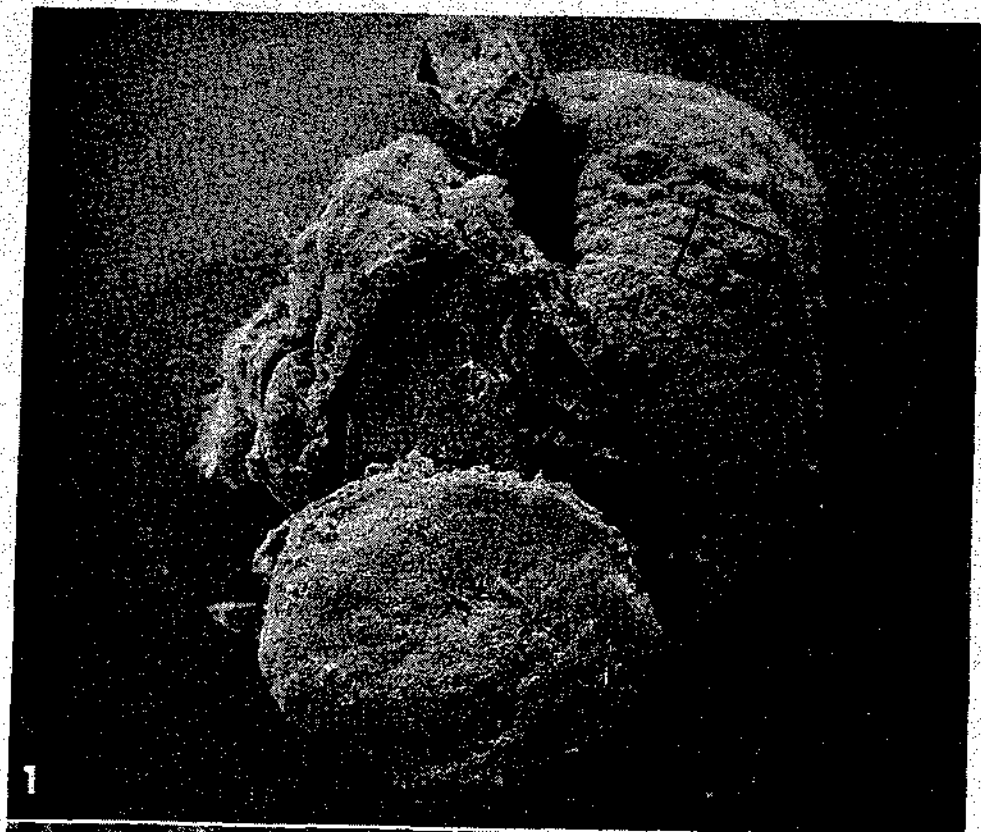
In this paper it is of special interest to examine, whether an allergen-induced destruction of the cilia takes place in the human chronic allergic mucosa as it has been found in sensitized animals after an allergen challenge (2).

Fig. 1.

× 28. Air-dried specimen. The total biopsy from the anterior part of the inferior concha. The biopsy is 2 × 3 mm. In the middle part of the biopsy is seen a mark after the biopsy-forceps. This part is useless, but as the picture shows, there are parts of intact mucous membrane suitable for microscopy, especially in the right, upper corner of the biopsy.

Fig. 2.

× 225. Air-dried specimen. Corresponds to the area marked on Fig. 1. With this magnification of survey the cell-boundaries can be seen, and an impression is obtained of the ratio between non-ciliated cells (dark with regular surface) and ciliated cells (light with irregular surface).



MATERIAL AND METHODS

30 adult patients with perennial rhinitis were examined. They all had constant and distinct symptoms, but were included independent of the appearance of the mucous membrane. A similar number of persons without nasal symptoms and allergic diseases were used as controls. The mean age of the two groups was 27 years and 23 years respectively. One half of the specimens were embedded for anoptral contrast (AC) and occasionally used for TEM, while the other half was examined by SEM.

Biopsies of the size of $1\frac{1}{2}$ –3 mm were taken with a small forceps without local anaesthesia from the lower edge of the inferior concha $\frac{1}{2}$ –1 cm behind the front edge. The biopsies were immediately fixed in a cold 5 per cent solution of glutaraldehyde, buffered with a 0.2 M sodiumcacodylate (pH: 7.2). After 24 hours fixation the biopsies were stored in the buffer. The specimens for TEM were postfixed for two hours at 4° C with 2 per cent osmium tetroxide (buffered with sodiumcacodylate to pH 7.2–7.4). After dehydration in ethyl alcohol the blocks were embedded in Epon. Sections were cut by a Reichert ultra-microtome UM 2. Semithin sections were analysed in AC. Ultrathin sections were doublestained with lead citrate and uranyl acetate and transmission electron micrographs were obtained with a Zeiss EM 9A electron microscope.

The specimens for SEM were after dehydration in ethyl alcohol transferred to benzene. After air-drying or freeze-drying the specimens were mounted on stubs and a conduction coat of gold was deposited in vacuum*. The specimens were studied with a Cambridge Stereoscan S2.

A few specimens for SEM were postfixed in 2 per cent osmium tetroxide, others were fixed in isoosmolar 2.5 per cent glutaraldehyde solution and for other specimens again, the duration of fixation was altered. None of these

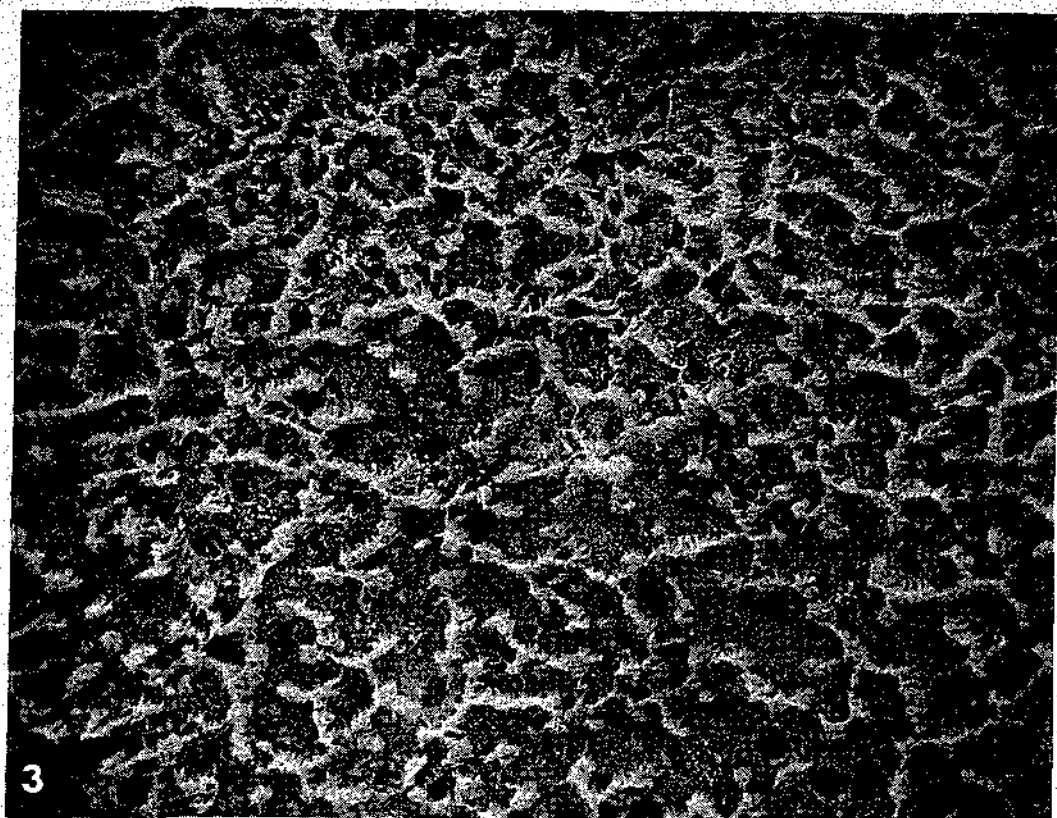
* We used the method of freeze-drying, which in details is described by Nørrevang & Wingstrand (9).

Fig. 3.

× 630. Freeze-dried specimen. In this place of the biopsy the cilia are seen as a honeycomb network. It is difficult here to see the boundaries of the cells. The scattered round grooves with elevated edges are goblet cells, which have expelled their secretion. In the upper part of the picture it is just possible to see other goblet cells with apocrine secretion.

Fig. 4.

× 1.200. Freeze-dried specimen. It is seldom that the cilia in the anterior part of the nose form a thick carpet, however, on the specimen presented they are found as very close rows separated by cells with microvilli.



changes in the procedures gave rise to any observable differences in the appearance of the specimens in SEM. A few of the SEM specimens were later embedded in Epon and examined in TEM. As seen from Fig. 8 the ultrastructures were poorly preserved, however, the sections showed that the layer of gold was thicker than expected, viz. about 100 nm on a flat surface of the specimen and somewhat thinner on non-flat facets of the specimen.

RESULTS

Although the specimens only were of the size of $1\frac{1}{2}$ –3 mm, 2/3 of them were fit for use. The rest had to be rejected either because the epithelium had been squeezed or torn off, or because the biopsy during mounting on the stub had been turned in a wrong direction, or because the surface was covered by a blanket of mucous. However, it can be said, that if these small biopsies are treated very carefully, they do give good and usable specimens, although it is seldom that the whole surface can be used (Fig. 1).

With a magnification of 200 times it is easy quickly to look over the specimen and get an impression of its fitness for use. In addition information is obtained on the main lines of the surface structures and the ratio between cells with and without cilia (Fig. 2).

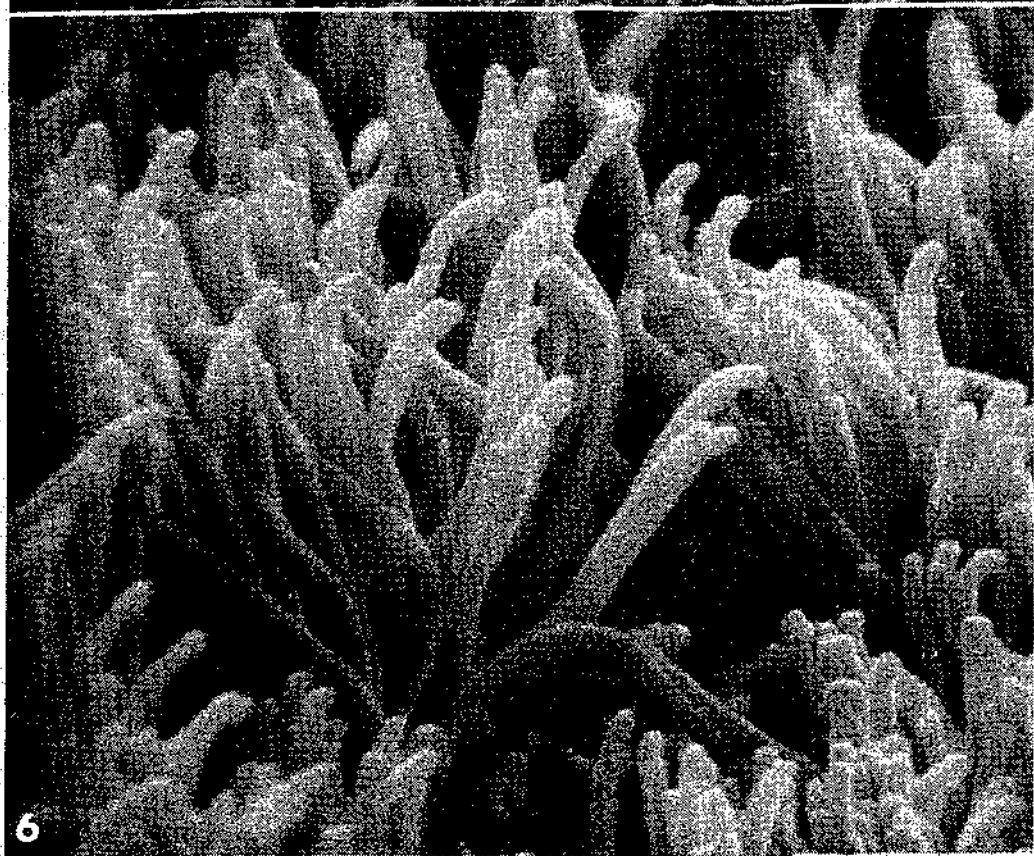
By higher magnification it is often possible to see the boundaries of the single cells distinctly. The surface area of the cells varies appreciably and the shape can be circular as well as

Fig. 5.

× 2,400. Freeze-dried specimen. The cilia are found in groups, and each group, which correspond to one cell, consist of about 50 cilia. The cilia are erect, but not quite free-standing in this specimen, although it has been freeze-dried. Between the groups of cilia irregular bunches of long, matted microvilli are found.

Fig. 6.

× 12,000. Freeze-dried specimen. The single cilia are erect and as free-standing as possible with the method employed. The upper tenth part of the cilia are bent 30° in the stroke-direction of the cilia. With this magnification the surface of the cilia appears smooth.



polygonal. While adjacent cells can have a surface of very different character, it is homogeneous for the single cell (Fig. 14).

In SEM it was found that 4/5 of the biopsies contained cilia and on an average 20 per cent of the surface was coated by cilia. There was no difference between normal persons and the patients with perennial rhinitis. In the anterior part of the nasal cavity it is seldom that the cilia form a thick carpet as is the case further back in the respiratory tract. Most often they are found in a honeycomb pattern, in rows or in separate islands where the single island corresponds to one cell (Fig. 3-5). If it is a ciliated cell, the cilia are spread evenly over the whole surface of the cell and there are 40-50 of them. This number of cilia applies to the majority of cells, however, the number may vary from 25 to 150 cilia per cell. A few free-standing cilia on a cell are not seen. While the cilia in the air-dried specimens are found in lumps and are somewhat flattened they are in the freeze-dried specimens more erect and free-standing (Fig. 6 and 13). By high magnification the surface is irregularly uneven in SEM while this is not the case in TEM (Fig. 7 and 9). The length of the cilia has in AC been measured to 4-6 μm , and their thickness in TEM to be 0,3 μm . In SEM the corresponding figures were 3-5 μm and 0,3 μm .

In 1/3 of the biopsies from patients with perennial rhinitis bunches of cilia were found which were totally flaccid, matted together and often of varying thickness, undoubtedly they were destroyed (Fig. 10-11). The majority of cilia from patients, however, had a quite normal appearance.

Fig. 7.

Original magnification $\times 6.360$. Transmission electron microscope picture of a ciliated cell. The cilia have a smooth surface and are of a homogenous size. Between the cilia thin microvilli are found, consisting of cytoplasm without structure and surrounded by a lengthening of the cytoplasm-membrane. The first part of the microvilli often has the shape of onions, but has otherwise a smooth surface and uniform thickness. It can be seen, that a few microvilli are bipartite.



In none of the specimens there was found any sign of increased ciliogenesis in the form of many cells with growing cilia of short length.

Thin microvilli, maximum length $2\ \mu\text{m}$, are seen in AC on some cilia-free surfaces (Fig. 12). In TEM it can be seen that also between the cilia $0.1\ \mu\text{m}$ thin microvilli are found (Fig. 7). These are often at their base onion-shaped with a diameter approaching that of the cilia. The rest of the microvillus has a constant diameter and a smooth surface. It is not seldom to find a single microvillus split into two. In SEM it is surprising to see that all cells, apart from goblet cells and squamous epithelial cells, are completely covered by microvilli close together (Fig. 14). It is characteristic that the length of the microvilli is different from cell to cell, but homogeneous on the single cell, which shows that microvilli develop synchronically on the cell.

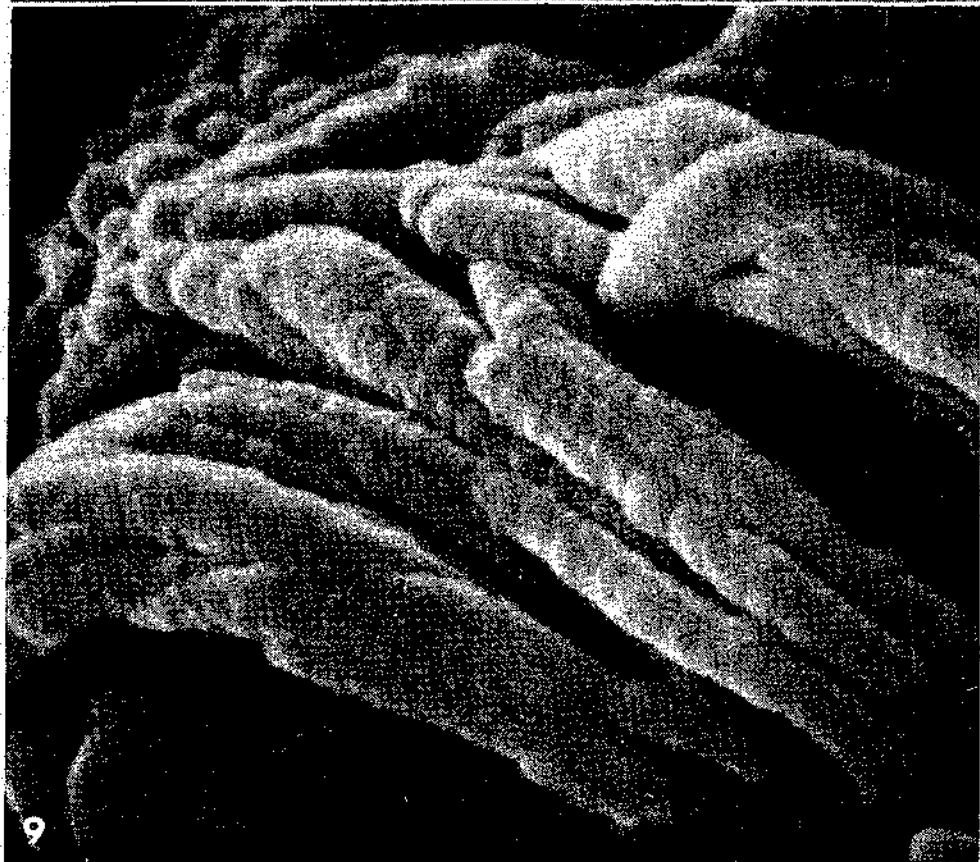
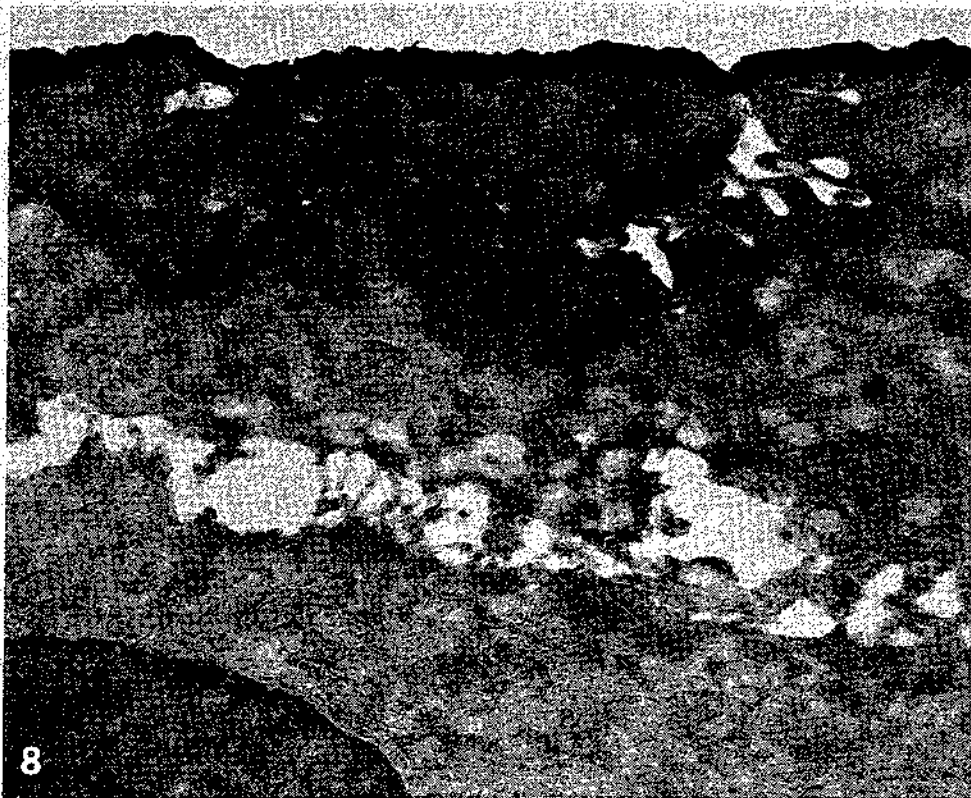
In non-ciliated cells several hundred microvilli are found and on the ciliated cells 3–4 times as many microvilli as cilia, however, this ratio varies considerably. In the air-dried SEM specimens the microvilli are thick and blunt, while in the freeze-dried they are more thin and discrete. In the gold-

Fig. 8.

Original magnification $\times 6,360$. Transmission electron microscopic picture of a biopsy, which after examination in the scanning electron microscope has been embedded in Epon. The semithin section can give some information in anoptal contrast, but for ultrastructural studies the preservation is poor. On plane surfaces the gold layer was found to be about $100\ \text{nm}$, but in the section shown it is somewhat thicker as it is sectioned oblique.

Fig. 9.

$\times 23,000$. Air-dried specimen. At high magnification the surface of the cilia is irregular, independent of the method of preparation, due to unevenness in gold coating as the surface of the cilia is smooth in the transmission electron microscope, even in high magnification. It should always be remembered that in the scanning electron microscopic specimens one does not see the "reality", but always "a snowcovered landscape".



coated SEM specimens they can appear drumstick-shaped and are not so thin as in TEM. The long microvilli are not erect and free-standing in SEM, but for every cell they form an irregular bunch (Fig. 5). On the ciliated cells microvilli are obscured by cilia.

DISCUSSION

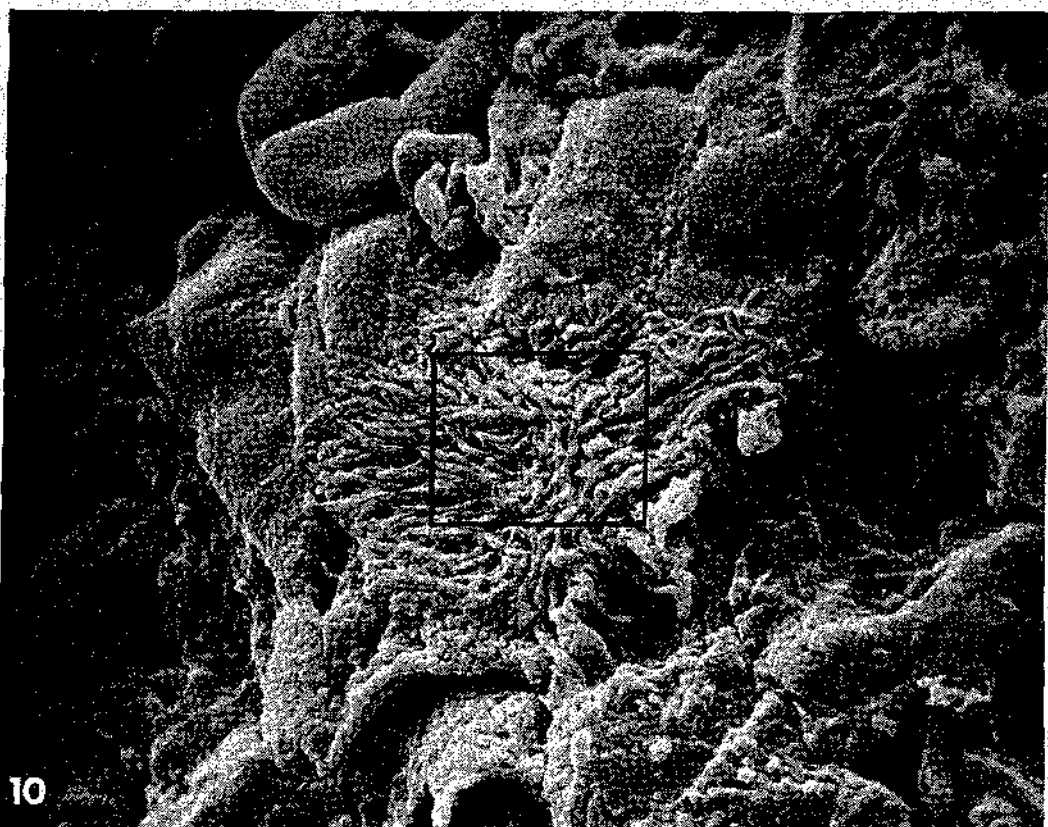
The fact that small biopsies of the mucous membrane can give specimens suitable for SEM, makes it possible to carry out corresponding examinations from other parts of the respiratory tract. It must, however, be remembered that the smaller the biopsies are made the more unsuitable specimens are obtained, furthermore, a larger number of specimens have to be examined before quantitative differences between the two groups can be shown. This may be part of the explanation, why it has been possible for Lenz (5, 6) on removed inferior concha in a higher degree to show differences between the normal and the allergic mucous membrane, as he found that in the allergic mucous membrane there was a reduced number of cilia and microvilli, and furthermore, increased ciliogenesis. It has been possible for us to a certain degree to verify some of these observations in nasal polyps (7). Another part of the explanation may be that the anatomical conditions are different on the inferior concha in total and on the place of our biopsy, and that Lenz's patients had so pronounced symptoms and a macroscopic abnormal mucous membrane that it was indicated to remove the inferior concha, while our patients were

Fig. 10.

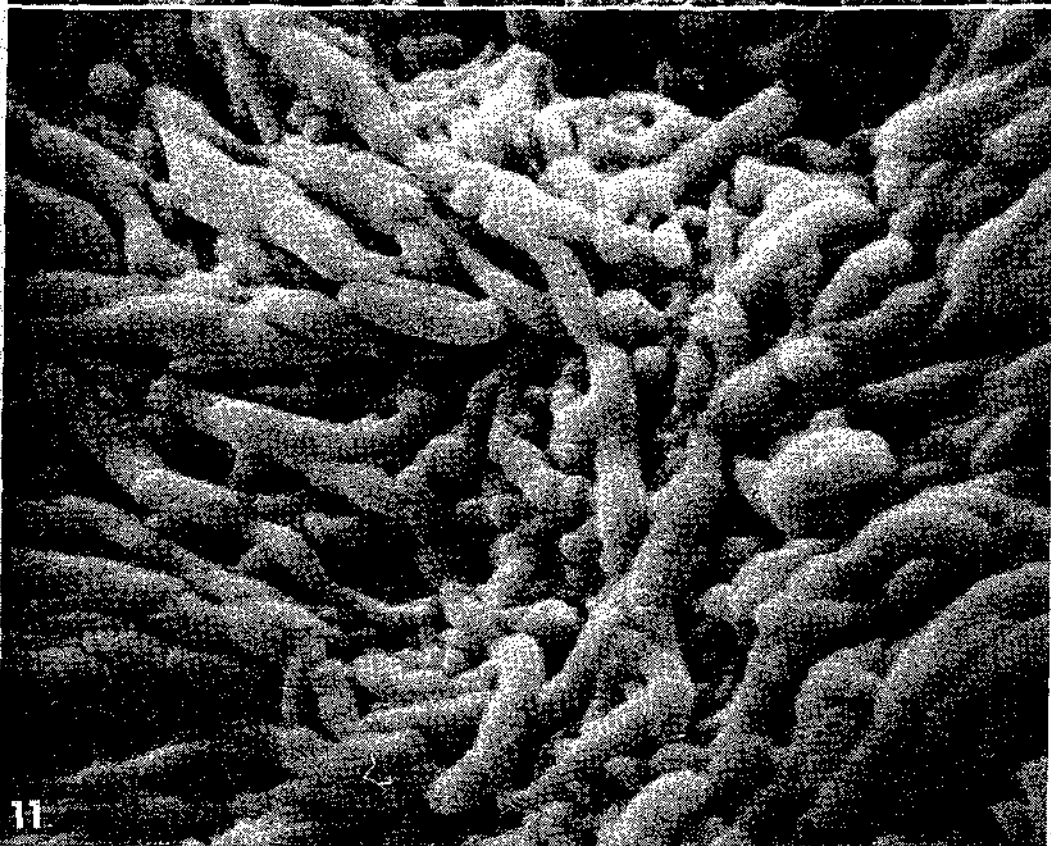
× 2,250. Air-dried specimen from a patient with perennial rhinitis. In part of the specimen the cilia lie flat and matted. Much secretion is seen especially in the lower half of the picture.

Fig. 11.

× 11,200. It seems that adjacent parts of the single cilia are of varying thickness in the specimen from a patient with perennial rhinitis.



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chosen only according to subjective symptoms. Lenz also mentions that he found microvilli short and thick in allergic patients. It has not been possible for us to confirm this, and we think that it is necessary to be very cautious in drawing such conclusions, when microvilli in SEM due to gold coating and shrinkage appear quite different than in TEM. Furthermore, their appearance varies to a great extent according to which drying method has been used.

SEM gives a good impression of the number and distribution of cilia. We have found that the number of cilia per cell is below what has been calculated in most of the investigations, where TEM has been used (10, 11).

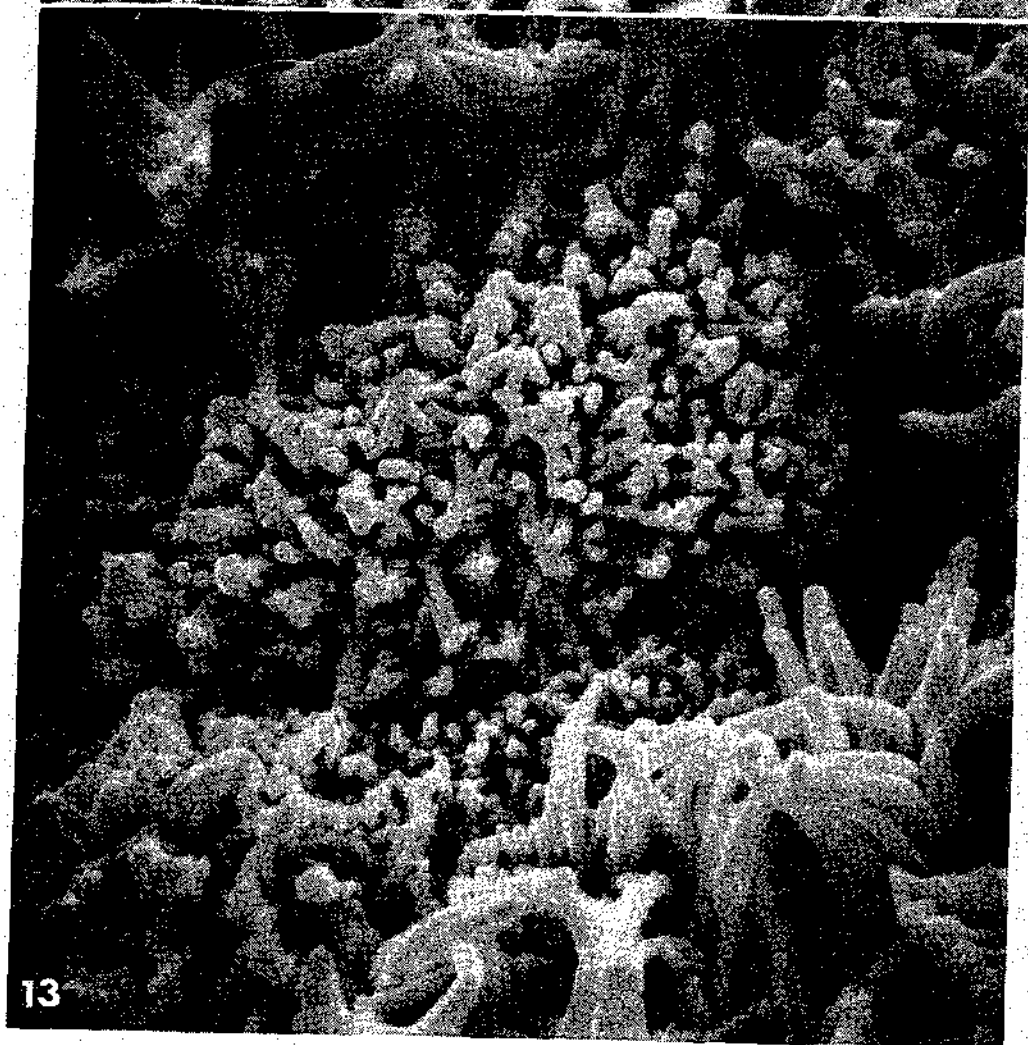
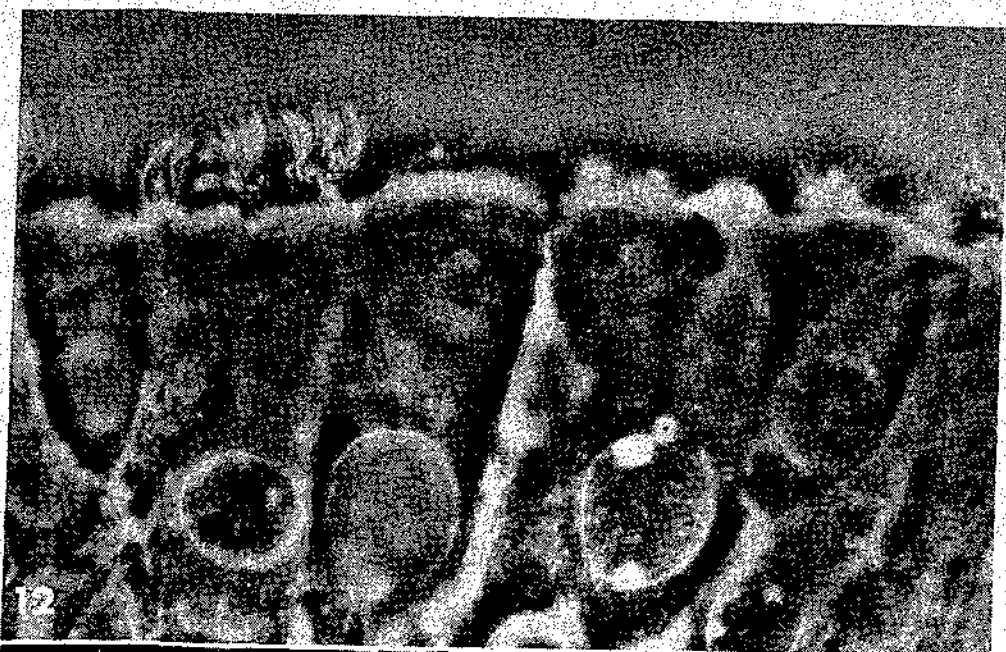
The destruction of cilia, as shown in some areas in 1/3 of the biopsies from the patients, cannot be explained immediately as a directly allergen-induced destruction. Against this it can be said that it has not been possible to find destroyed cilia on the surface of nasal polyps (7), and that the larger part of the epithelium in patients with perennial rhinitis had quite normal cilia. Another possible explanation of the occurrence of the destroyed cilia can be a change in amount and viscosity of the secretion and that the mucous membrane on the swollen inferior concha possibly has been pressed against the floor of the nasal cavity. It must, however, be stated that the majority of cilia have a normal appearance in normal persons as well as in patients with perennial rhinitis.

Fig. 12.

Original magnification $\times 800$. Anoptral contrast microscopy of semithin section of Epon-embedded specimen. On the surface of the epithelial cells is seen partly quite thin microvilli and partly thicker cilia.

Fig. 13.

$\times 7,400$. The cilia are erect and relatively free-standing, as they are in a freeze-dried specimen. In the lower left corner short, growing cilia are seen, which is seldom the case. In the middle of the picture a cell with microvilli of medium length is seen. In this specimen the microvilli are thin and discrete.



A correct picture is not obtained in SEM of the microvilli, as they due to the methods of preparation are thicker than in TEM. In spite of this SEM gives a good impression of the character of the cilia-free surface and especially of the dense covering with microvilli. This increase of the surface area must facilitate absorption through the mucous membrane to the extent in which it is not an intercellular process. Besides that the microvilli must be capable of holding moisture, so that the ability of the mucous membrane to resist dehydration is increased.

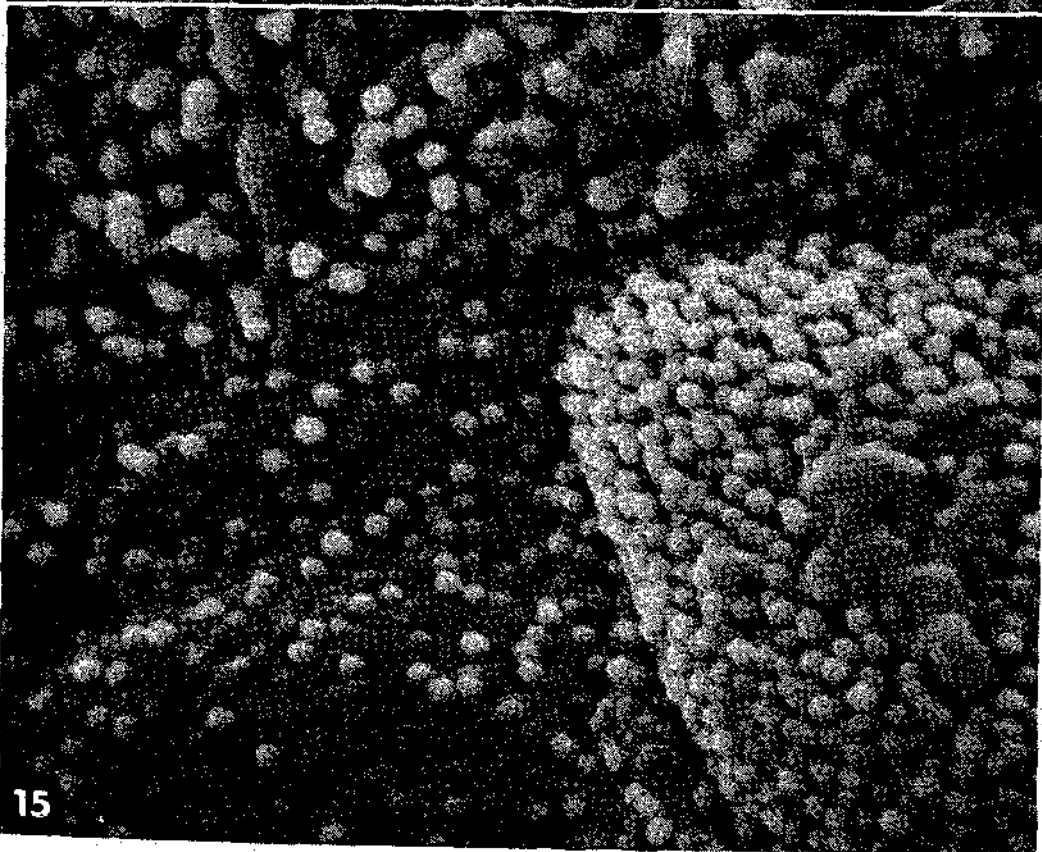
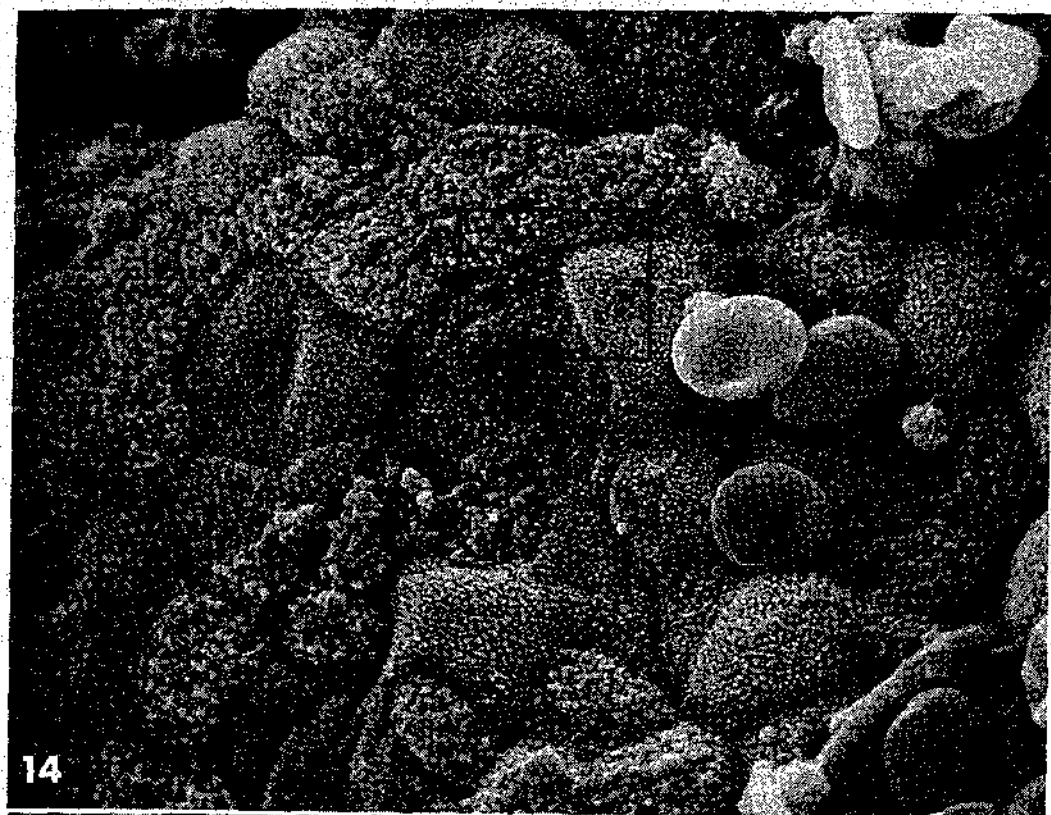
SEM investigations have shown how different results can be obtained by different methods of preparation (1, 8). It is therefore necessary to be cautious in interpreting results obtained by only one procedure, and although it is not possible on human biopsy material to perform larger investigations concerning the methods, it will be wise to try some different methods of preparations. In this work the time of fixation has not been critical, but the method of drying has been important, as the freeze-dried specimens have given the best presentation of cilia and microvilli.

Fig. 14.

× 2,320. Air-dried specimen. Non-ciliated epithelium with distinct cell boundaries. All cell surfaces are covered by microvilli close together. The surface of the single cell is homogenous, but there are differences in the appearance of the microvilli from cell to cell. In the right side of the picture some erythrocytes are seen.

Fig. 15.

× 11,600. Air-dried specimen. Three cells with microvilli of different length. Microvilli of the single cell are generally of the same length, which means that the growth of microvilli on the single cell is synchronized. The microvilli are thick and blunt as they are in air-dried specimens, but in scanning electron microscopic specimens they are never as thin as in the transmission electron microscope, as the thickness of the gold layer is of the same order as the diameter of the microvilli.



SUMMARY

30 biopsies from the anterior part of the inferior nasal concha from normal persons and from patients with perennial rhinitis were examined in the scanning electron microscope and an equal number were embedded in Epon and examined in an optical contrast and transmission electron microscope. 20 per cent of the total area was covered with cilia, which were found in a honeycomb pattern, in rows and in islands. The ciliated cells were covered by 40–50 cilia, which were 3–6 μm long and 0.3 μm thick. Every non-ciliated cell was covered by microvilli close together with a maximum length of 2 μm . There were marked differences between the appearance of adjacent cells, but the surface of the single cell was homogeneous. The only observable difference between normal persons and the patients with perennial rhinitis was the occurrence of destroyed cilia in 1/3 of the biopsies from the patients. It is concluded that in the scanning electron microscopic specimens there is always a risk for artifact and the results depend on the choice of preparation methods. Fixation in glutaraldehyde, ethanol dehydration and freeze-drying from benzene is a method of preparation, which is found suitable for the purpose.

ACKNOWLEDGEMENTS

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REFERENCES

1. Boyde, A. & Wood, C. (1969): *J. Microscopy* 90, 221–249.
2. Chevance, L. G. (1971): *Acta oto-laryng.* (Stockh.) 72, 121–133.
3. Jahnke, V. (1972): *Z. Laryng. Rhinol.* 51, 30–41.
4. Jahnke, V. (1972): *Z. Laryng. Rhinol.* 51, 152–162.
5. Lenz, H. (1972): *Z. Laryng. Rhinol.* 51, 618–632.
6. Lenz, H. (1972): *Arch. Ohr., Nas., u. Kehlk.-Heil.* 203, 353–359.
7. Mygind, N., Bretlau, P. & Sørensen, H. (1973): In preparation.

8. Nowell, J. A., Pangborn, J. & Tyler, W. S. (1972) : Proceedings of the fifth annual scanning electron microscope symposium. (Ed. by Om Johari & Irene Corvin), p. 306-312. IIT Research Institute. Chicago, Illinois.
9. Nørrevang, A. & Wingstrand, K. G. (1970) : Acta zool. (Stockh.) 51, 249-270.
10. Rhodin, J. & Dalhamn, T. (1956) : Z. Zellforsch. 44, 345-412.
11. Spoendlin, von H. (1959) : Pract. oto-rhino-laryng. (Basel) 21, 484-498.

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