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

II: Secretion

By

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The secretion of the respiratory tract consists of components produced in glands and in goblet cells, and, in some cases, of transudation from the blood vessels. Cellular components, especially leucocytes, are also found. The secretion is of great importance for the air-conditioning and for the function of cilia. These facts have been well-known for decades (4, 19). As it is possible to show the presence of antibodies in secretion, it has become more and more clear that it also serves as a medium for many different antigen-antibody reactions (7). The first contact between bacteria and vira in the inspired air and antibacterial and antiviral antibodies, as well as between allergens, IgE and blocking antibodies takes place in the secretion of the respiratory tract. It is still not quite clear how great the importance of these reactions taking place in the secretion is for protection against and development of diseases in the respiratory tract.

A detailed knowledge of the anatomy of the mucous membrane of the upper respiratory tract is, however, necessary for complete comprehension of the reactions taking place there.

The ultrastructure of the nasal mucosa has been described in several transmission electron microscopic studies (9, 11, 12, 19) and recently also in a scanning electron microscopic investigation (13). We shall give a scanning electron microscopic description of the surface structures in the human nasal mucosa which are connected with secretion in normal persons and in patients with perennial rhinitis. A corresponding study of cilia and microvilli was published previously (17). The scanning electron microscopic study is to be supplemented with light-microscopy and transmission electron microscopy to the extent that they can contribute to showing artifacts in scanning electron microscopy (SEM).

MATERIALS AND METHODS

Material and methods are described in detail elsewhere (17). Here it need only be mentioned that biopsies were taken from the anterior part of the inferior concha in 30 adult patients with perennial rhinitis and from a similar number of normal persons. The biopsies were fixed in 5 per cent glutaraldehyde buffered with 0.03 M sodiumcacodylate. After alcohol dehydration and air-drying or freeze-drying from benzene, one half of the biopsies were used for scanning electron microscopy. The other half were embedded in Epon. Semithin sections were investigated in the anoptral contrast microscope, and ultrathin sections in the transmission electron microscope.

RESULTS

As the amount and composition of the secretion depends on the type of epithelium, we shall first mention the different epithelial types found in the anterior part of the inferior concha. A squamous epithelium could be identified in only one of 23 suitable SEM specimens (Fig. 1), while this was the case in not less than 12 of the 30 light-microscopic specimens (Fig. 2). This means that several of the specimens, which in section are seen as typical squamous epithelium, cannot be identified as such in SEM, as the surface is covered by short microvillous-like projections. Typical squamous epithelium as well as intermediate types between squamous epithelium and pseudostratified cylindrical epithelium can be seen in the light-

microscope. However, the typical pseudostratified cylindrical epithelium is most frequent in the location investigated. In SEM no certain quantitative differences in the distribution of epithelial types between normals and patients with perennial rhinitis were observed. However, in another study, blinded examination of a number of biopsies in anoptral contrast microscope has shown—contrary to expectation—that normal persons had squamous epithelium and epithelium of the intermediate type on the anterior part of the inferior concha to a higher degree than patients with perennial rhinitis (16).

Glandular openings were observed, and there appeared to be more of these in patients with perennial rhinitis, but an exact quantitation is difficult because of the small size of the biopsies. Intraepithelial glands were found in biopsies from normals and more frequently in biopsies from patients with perennial rhinitis. It was, however, not possible to distinguish these with certainty from true subepithelial glands in SEM (Fig. 3).

While anoptral contrast microscopy, and especially transmission electron microscopy, are suitable methods for showing goblet cells in all phases of secretion, these cells as a rule cannot be identified in SEM. The empty or sparsely filled goblet cell is compressed apically and shows in SEM as a cilia-free cell with an irregular surface and a small surface area. Goblet cells in this phase of secretion are difficult to identify. On the other hand, one can sometimes recognise the filled goblet cell, which has a smooth surface, covered with only a few, short microvillous-like projections. If the cell is being emptied, the mucus protrudes hemispherically, or later polyp-like, with a typical irregular, granular surface (Figs. 4, 5, 7 and 12). If the cell has recently expelled its mucous content, a round or slightly irregular cavity is seen, often surrounded by a peripheral elevation. In the cavity, septal walls are seen which divide the mucus in the filled cell into various sections (Figs. 6 and 7). When pressure from the neighbouring cells reduces the cavity, the peripheral elevation protrudes inwards and closes the cell apically. The surface of the cell is then seen



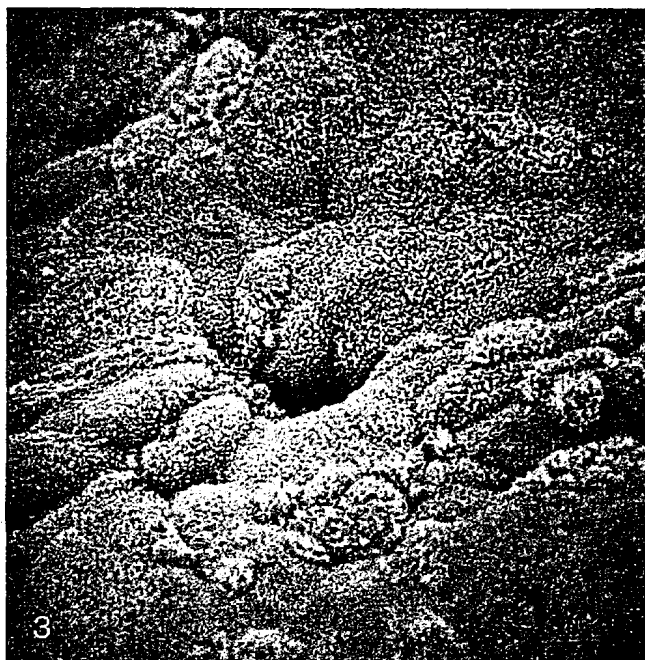


Fig. 1

$\times 1,100$. An epithelial surface without cilia, actual microvilli and goblet cells. As no desquamation of horny cells can be seen, it is probably a non-keratinized, squamous epithelium, as shown in Fig. 2. The nature of the granular, intercellular substance (arrows) is obscure, but it may be lamellar granules. All the figures in this paper are derived from biopsies of the anterior part of the human nasal mucosa.

Fig. 2

Original magnification $\times 320$. An anoptral contrast micrograph of a non-keratinized squamous epithelium. The surface appears smooth, and desmosomes are seen between cells in all layers.

Fig. 3

$\times 1,280$. An opening from a gland. The appearance and the dimensions correspond to that of an intracellular gland. All cells are covered by microvilli of different lengths.

to be elevated and irregular (Fig. 6). In some regions, especially in biopsies from patients with perennial rhinitis, there was a pronounced expulsion of mucus, while this was not the case in adjacent regions in the same biopsy. The mode of secretion from the goblet cells was mainly apocrine with expulsion of the whole or a large part of the mucous granules. Merocrine secretion with expulsion of single mucous granules from the filled cell was observed to a much lower extent.

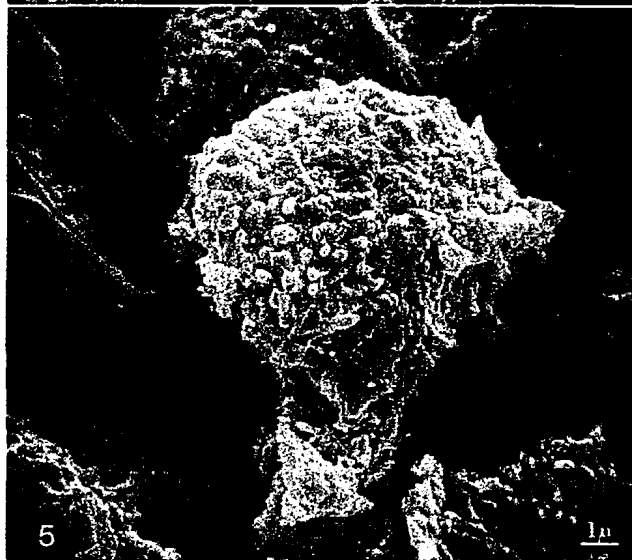
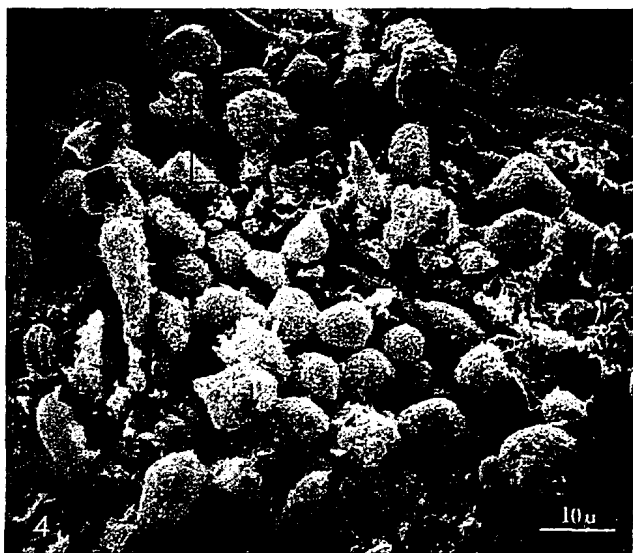
In anoptral contrast and in the transmission and scanning electron microscopes we quite frequently saw apical cytoplasm protuberances, which now and then are disconnected from the epithelial cells (Figs. 8 and 9). Other authors have designated this phenomenon, "apocrine secretion" (3, 8, 14). However, this expression is misleading as there are no morphological indications that it really is a secretion. Furthermore, the expression may easily be taken to refer to the genuine apocrine secretion from the goblet cells. In this paper therefore we prefer to use the descriptive expression, "cytoplasm protuberances". These structures are stalky and polyp-like on the ciliated cells (Fig. 9) and are spherical with a broader base on the microvillous-carrying cells (Fig. 8). As in the case with the secretion from the goblet cells, it is seldom that the activity is evenly distributed over the surface of the specimen; however, in smaller, well-defined areas it is homogenous. In section the cytoplasm protuberances have a perfectly smooth surface (Fig. 9), which may appear folded in SEM (Fig. 8) probably

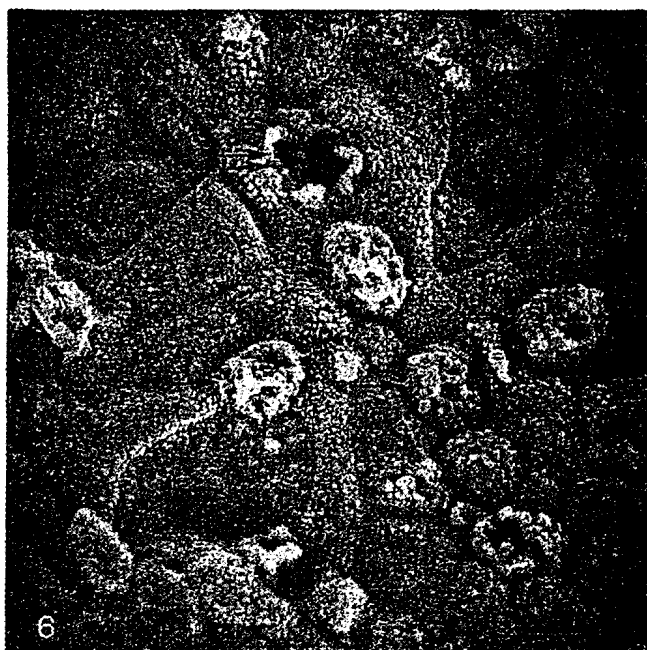
Fig. 4

× 1,140. Nasal mucous membrane from a patient with perennial rhinitis. The snowball-like formations are mucus expelled from the goblet cells. There is a very intensive secretory activity, and it is seen that the most of the goblet cells shown are in the same phase of the secretory process.

Fig. 5

× 5,700. Detail from Fig. 4, showing one goblet cell, which has expelled a polyp-like, viscid, granular mass of mucus. The lowest, slender part of the expelled mucus corresponds to the aperture of the goblet cell.





because of the shrinkage which accompanies preparation procedures. The size of the single cytoplasm protuberance varies considerably, but it does not reach the same dimension as the mucus which is expelled from the goblet cells as the base of the cytoplasm protuberance takes up only a part of the surface area of the cell. However, the total volume of the actual cytoplasm protuberances exceeds the total volume of the secretion actually being expelled by the goblet cells. It is not possible to decide how large a part of the total amount of secretion is contributed by these cytoplasm protuberances, but the transfer to secretion can be pronounced, and they can be identified in the secretion, at times in considerable quantities. Seen in the transmission electron microscope, the cytoplasm protuberances consist of a surrounding cytoplasm membrane and of a normal looking cytoplasm with scattered ribosomes, but without secretory granules (Fig. 9). The cells with cytoplasm protuberances on their surfaces do not differ morphologically in other respects from other cells, and in particular they show no sign of degeneration. There were no apparent differences between normal persons and patients with perennial rhinitis with regard to these structures.

Now and then a cell was seen leaving the surface of the epithelium. It is not possible to decide with certainty, whether

Fig. 6

$\times 2,300$. Several empty goblet cells. One cell (double arrow) has recently expelled its mucous content and appears with an empty cavity, in which septal walls are seen. The cavity is surrounded by a peripheral elevation. In other goblet cells (single arrows), which are in a later phase of the secretory cyclis, the aperture is closed, and the surface is irregular and elevated.

Fig. 7

Original magnification $\times 800$. Anoptal micrograph, showing ciliated cells and three goblet cells. One is distended by mucigen granules, which protrude hemispherically on the surface. Two cells have recently expelled their mucous content by an apocrine secretory process. The apical part of the cell is empty, but septal walls are seen (arrows).

it was an epithelial cell, a neutrophile leucocyte, an eosinophile leucocyte or a mast cell (Figs. 10 and 11).

The epithelium is usually even, without protruding cells and with intact terminal bars which may be on the same level as the cell surface, slightly raised or, more frequently, slightly depressed in comparison to it (Figs. 3 and 6). The surface of the epithelium may also, however, be irregular with many protruding goblet cells and penetrating leucocytes (Fig. 12). It can also appear irregular with widened intercellular spaces and intercellular oedema (Figs. 13, 14 and 15). This may be seen in normals as well as in patients with perennial rhinitis, but it is possibly more frequent in the latter.

DISCUSSION

The methods used for preparation of SEM specimens were found suitable for the purpose. There was no difference between the air-dried and the freeze-dried specimens as regards the structures described here. As freeze-drying gives much better presentation of cilia and microvilli (17), this procedure is to be preferred.

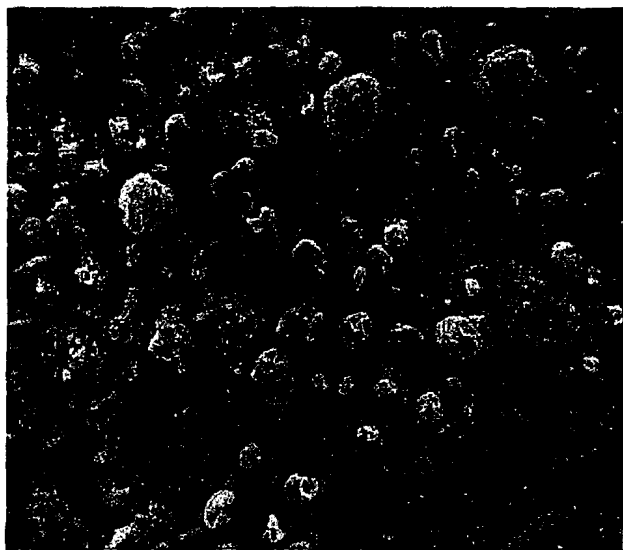
The total number of goblet cells cannot be quantified in SEM, but this method gives a good illustration of the mode of secretion. For the individual cell this is not continuous but cyclic with expulsion of all or a great part of the mucigen

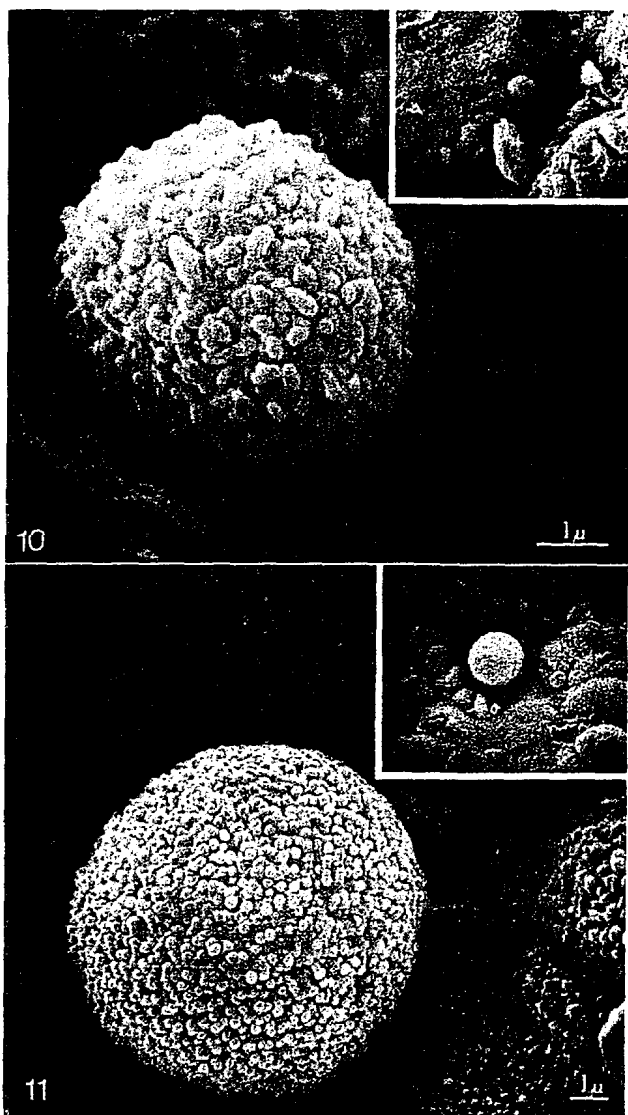
Fig. 8

$\times 1,140$. Several polyp-like cytoplasm protuberances protrude from the epithelial cells. The surface of the cytoplasm protuberances appears folded, but smooth in contrast to the mucus from the goblet cells (arrows), which have a granular surface and, in addition, a greater volume.

Fig. 9.

Original magnification $\times 6,360$. Transmission electron micrographs of a cytoplasm protuberance from an epithelial cell. At low magnification the formation is found to be stalky and polyp-like, and at higher magnification it can be seen that the cytoplasm does not contain secretory granules.





granules. Most of the secretion is formed by apocrine secretion, but merocrine secretion from the filled cells does occur. While it was impossible to observe any differences in the mode of secretion, there was an increased number of goblet cells in the specimens from patients with perennial rhinitis. This is not surprising as it has been shown in many investigations that chronic inflammatory reactions in the mucous membrane of the respiratory tract evoke an increased number of goblet cells (4). In the lowest part of declive areas of the specimens there was an accumulation of goblet cells. This observation is compatible with the fact that the number of goblet cells increases in nasal cavities which are not influenced by the normal air-change (nasal cavities, which have been closed off by operation in animals, laryngectomized patients) (9, 11). Thus several arguments support the assumption that a mucous membrane in the respiratory tract which is not influenced by the normal flow of respiratory air will react with an increased number of goblet cells. If, on the other hand, it is highly exposed to unconditioned inspired air, it will metaplasize in the direction of a squamous epithelium (4).

Clara (3) described light-microscopic "Keulen- oder Lapenartigen Fortsätzungen" from the epithelial cells in the bronchioli and called the phenomenon "apocrine secretion". Other authors (6, 8, 14) have mainly found these structures in the mucous membrane with various inflammatory reactions. In the present study, similar structures have been found in the

Fig. 10

× 11,000. A cell which has penetrated the epithelium and is now leaving the surface. The surface of the cell is compatible with that of a leucocyte. The small micrograph shows the surroundings in which the cell was found.

Fig. 11

× 5,700. This cell, which is also being expelled from the epithelium, has a surface ultrastructure which is not different from the microvillous-covered surface of the epithelial cells. Therefore it may be an epithelial cell. The small micrograph shows the surroundings in which the cell was found.

anterior part of the inferior concha both in normal persons and in patients with perennial rhinitis. However, it is preferable to call the phenomenon "cytoplasm protuberances". It is not certain what function these structures have. Ultrastructural investigations do not indicate that they are part of a proper secretory process, as they contain no secretory granules. The theory that they are part of a degenerative process in the cell cannot be substantiated electron microscopically as cells with cytoplasm protuberances on the surface do not seem to differ from those without in ultrastructure of cytoplasm and nucleus. Another possibility is that the cytoplasm protuberances contain substances which have a function in secretion. These substances might, for instance, be secretory component and IgA. Secretory component is synthesized in the epithelial cells, and IgA are transported through the epithelium intracellularly as well as intercellularly (7). Both substances have thus been demonstrated to be present in epithelial cells and must also be present in cytoplasm protuberances. It is possible that when the cytoplasm protuberances are formed the mucous membrane can actively regulate the transfer of secretory IgA to the secretion in this way.

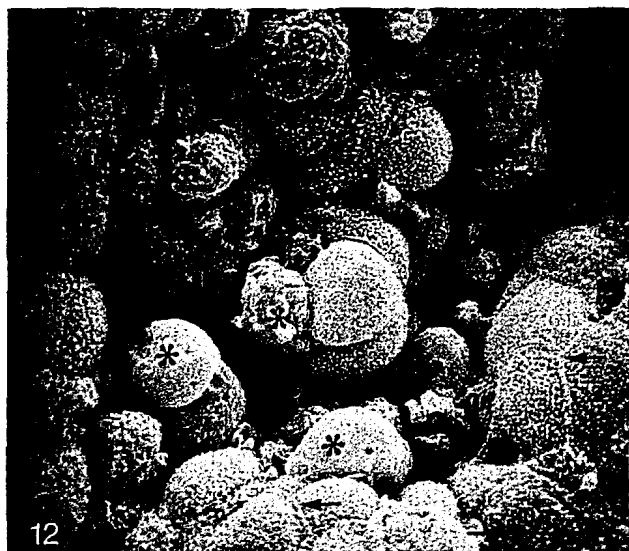
In the phase-contrast microscope it can be seen that dying cells form large bulgings of the cytoplasm, which can be expelled to the surrounding liquid (1, 15). If cells are in contact with hypotonic environment a similar phenomenon can be seen (2). Although the fixative used in the present study is not

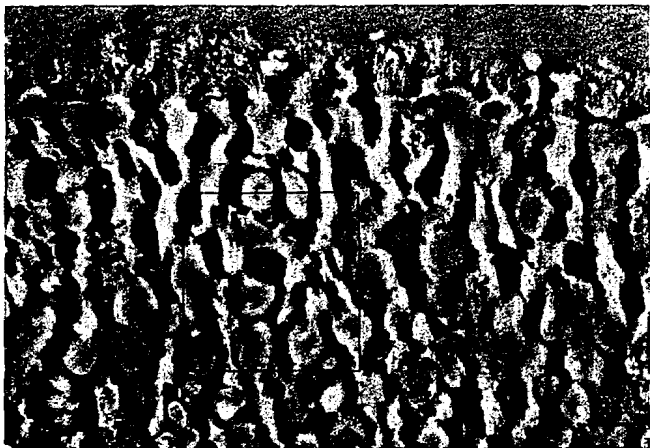
Fig. 12

× 2,200. Irregular epithelium from a patient with perennial rhinitis, showing prominence of epithelial and goblet cells. The latter are characterized by a surface with no or only few microvillous-like projections (*). The terminal bars are intact and appear slightly elevated (arrows).

Fig. 13

× 1,930. Epithelium from a patient with perennial rhinitis. The epithelial cells are prominent and the intercellular spaces widened. An epithelial cell has been expelled, leaving an empty hole in the epithelial surface (*).





hypotonic, and although the morphology of the cytoplasm bulgings of the dying cell does not quite correspond to that of the cytoplasm protuberances from the epithelial cells, it is not possible from the facts available, to state definitely that cytoplasm protuberances are entirely *in vivo* phenomena.

Nor is it possible from our investigations into the occurrence of widened intercellular spaces to conclude definitely which phenomena are *in vivo* and which are artifacts. We found widened intercellular spaces especially in specimens from patients with perennial rhinitis but also a few in normals. Several investigators using other methods have, however, unanimously shown that in allergic epithelium the following phenomena occur: (a) An increased intercellular transport (19). (b) Widening of the intercellular spaces (12, 13, 19). (c) An increased tendency to expel epithelial cells (18) or desquamation of entire pieces of epithelium (4). These investigations point to the assumption that the widened intercellular spaces, observed in SEM, are to some extent due to *in vivo* phenomena and are not merely technical artifacts. On the other hand, it is unlikely that the epithelium *in vivo* has the appearance shown in Figs. 13-15. Methodological studies have demonstrated a considerable shrinkage of tissues and cells under the preparation procedures (5).

From the present study, together with our other investigations (16, 17), we must conclude that we have shown only minor differences between the appearance of the mucous

Fig. 14

Original magnification $\times 320$. An anoptral contrast micrograph, which corresponds to Fig. 13. The intercellular spaces are widened, and the terminal bars appear to be defective. The surface is covered by cilia, and some cytoplasm protuberances from the epithelial cells are seen.

Fig. 15

Original magnification $\times 3,400$. Transmission electron micrograph of the marked area in Fig. 14. The most prominent phenomenon is a widening of the intercellular spaces which contain oedema fluid and interdigitating processes.

For the rest we shall refer to the text.

membrane in the anterior part of the nose in normals and in patients with perennial rhinitis. It is our opinion therefore that the changes induced by allergic reactions in the nasal epithelium are not as far-reaching as the changes caused by infection with, for instance, influenza virus which destroys the cilia and sometimes the entire epithelium (10). In allergic conditions, the major histological changes take place in the lamina propria, i.e. dilation of blood vessels, tissue-oedema, infiltration with eosinophile leucocytes and proliferation of the glands.

SUMMARY

Fifteen biopsies from the anterior part of the inferior nasal concha from normal persons and 15 from patients with perennial rhinitis were examined in the scanning electron microscope and an equal number were embedded in Epon and examined in anoptal contrast and a transmission electron microscope. The epithelium most often observed at the place investigated was of the pseudostratified, cylindrical type, but intermediate forms as well as a typical squamous epithelium were seen. In the scanning electron microscope it was sometimes difficult or impossible to differentiate between non-keratinized squamous epithelium and epithelium of the intermediate type, while a horny squamous epithelium and a ciliated epithelium were easily identified. Openings from glands were recognised and occurred in an increased number in patients with perennial rhinitis. The same was the case with goblet cells, which were very well represented, when they were expelling their mucous content by a mainly apocrine secretory process. Formation of cytoplasm protuberances in the epithelial cells was observed in normal persons as well as in patients with perennial rhinitis. There were no morphological indications that it really was a secretory process. The hypothesis is set forth that these cytoplasm protuberances are the structural base for the surrender of secretory immunoglobulin A to the secretion from the epithelial cells. Although an irregular epi-

thelium with widened intercellular spaces apparently occurred more often in patients with perennial rhinitis, it is concluded that the allergic reactions in the nasal mucosa only provoke minor changes in the epithelial surface, compared to what is evoked by, e.g. viral infections, where the total epithelium may be destroyed.

ACKNOWLEDGEMENTS

The Cambridge Stereoscan microscope was kindly placed at our disposal by the Institute for Historical Geology and Paleontology (H. J. Hansen, M. Sc.). We owe Mr. Jørgen Fuglsang many thanks for operating the microscope for us. We would also like to express our gratitude to Mrs. Inge Christiansen and Mr. Erik Jensenius for skilful technical assistance.

REFERENCES

1. Bessis, M. (1973): *Living blood cells and their ultrastructure*, p. 55. Springer, Berlin.
2. Boyde, A. & Vesely, P. (1972): In: Johari, Om & Corvin, I. (eds.): *Scanning Electron Microscopic Symposium*, p. 265-272. ITT Research Institute, Chicago.
3. Clara, M. (1937): *Zur Histologie des Bronchialepithels*. Z. mikr.-anat. Forsch. 47, 321.
4. van Dishoeck, H. A. E. & Majer, E. H. (1964): In: Berendes, J., Link, R. & Zöllner, F. (eds.): *Hals-, Nasen-, Ohrenheilkunde*, Vol. I, p. 365-412. George Thieme, Stuttgart.
5. Frederiksen, O. & Rostgaard, J. (1974): Absence of dilated lateral intercellular spaces in fluid-transporting frog gall bladder epithelium: Direct microscopic observations. J. cell. Biol. In press.
6. Giesekeing, R. (1968): Elektronenoptische Befunde zur Bronchialsekretion. Beitr. Klin. Tuberk. 138, 332-342.
7. Hansson, L. Å. & Brandzaeg, P. (1973): In: Stiem, E. R. & Fulginiti, V. A. (eds.): *Immunologic disorders in infants and children*. Saunders, Philadelphia.
8. Hentzer, E. (1972): Ultrastructure of the middle ear mucosa in secretory otitis media. Acta oto-laryng. (Stockh.) 73, 394-401.
9. Hilding, D. A. & Hilding, A. C. (1970): Electron microscopic observations of nasal epithelium after experimental alteration of air flow. Ann. Otol. (St. Louis) 79, 451-460.
10. Hoorn, B. (1966): Organ cultures of ciliated epithelium for the study of respiratory viruses. Acta path. microbiol. scand., suppl. 183.

11. Jahnke, V. (1972): Ultrastruktur der Nasenschleimhaut nach Laryngektomie. Arch. Ohr., Nas., u. Kehlk.-Heilk. 202, 371-379.
12. Jahnke, V. (1972): Ultrastruktur der allergischen Nasenschleimhaut des Menschen. Z. Laryng. Rhinol. 51, 152-162.
13. Lenz, H. (1973): Three-dimensional surface representation of the cilia-free nasal mucosa of man. Acta oto-laryng. (Stockh.) 76, 47-57.
14. Messerklinger, W. (1955): Apokrine Sekretion in Flimmerepithel der Luftwege? Z. Laryng. Rhinol. 34, 661-665.
15. Messerklinger, W. (1970): Physiologische und pathologische Veränderungen des Nasenepithels. Arch. Ohr., Nas., u. Kehlk.-Heilk. 195, 475-480.
16. Mygind, N., Viner, A. S. & Jackman, N.: Histology of nasal mucosa in normals and in patients with perennial rhinitis. In press.
17. Mygind, N. & Bretlau, P. (1973): Scanning electron microscopic studies of the human nasal mucosa in normal persons and in patients with perennial rhinitis. I: Cilia and microvilli. Acta allergol. (Kbh.) 28, 9-27.
18. Mygind, N. & Thomsen, J. (1973): Cytology of the nasal mucosa. A comparative study between a replica-method and a smear-method. Arch. klin. exp. Ohr., Nas., u. Kehlk.-Heilk. 204, 123-129.
19. Terrahe, K. & Backwinkel, K.-P. (1970): Elektronenmikroskopische Untersuchungen über die Herkunft des Nasensekretes bei hyperergischer Rhinopathie. Arch. klin. exp. Ohr., Nas., u. Kehlk.-Heilk. 197, 254-264.

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