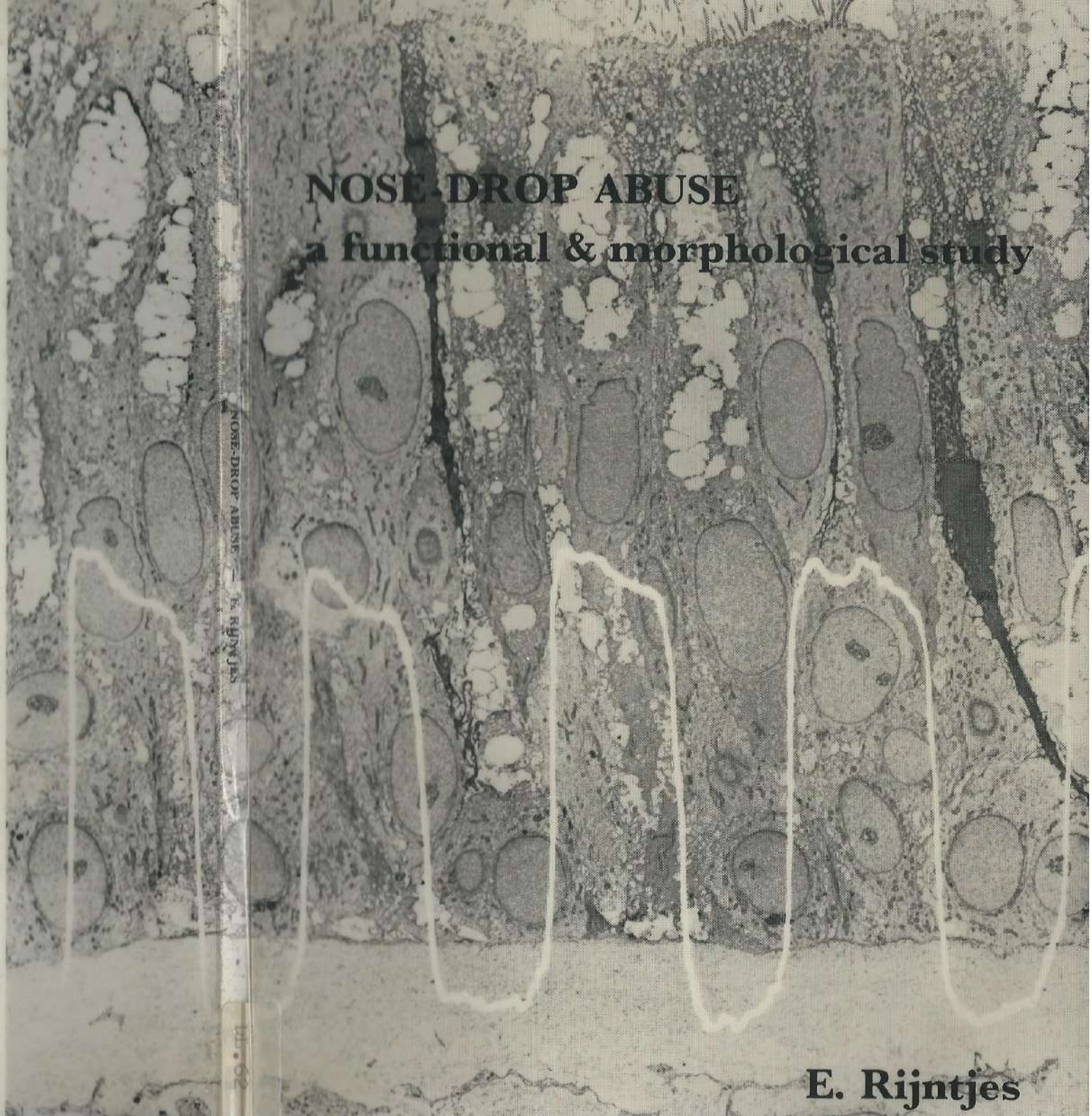
NOSE-DROP ABUSE
a functional & morphological study
E. Rijntjes

NOSE-DROP ABUSE

A FUNCTIONAL AND MORPHOLOGICAL STUDY

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR MAGNIFICUS DR. A. A. H. KASSENAAR, HOOGLERAAR IN DE FACULTEIT DER GENEESKUNDE, VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN TE VERDEDIGEN OP WOENSDAG 6 FEBRUARI 1985 TE KLOKKE 16.15 UUR

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Promotiecommissie:

Promotoren:	Prof. Dr. P.H. Schmidt Prof. Dr. W.Th. Daems
Co-promotor:	Prof. Dr. D.J. Ruiter
Referent:	Prof. Dr. J.P. Scherft
Overige leden:	Prof. Dr. M. van der Ploeg Prof. Dr. E.L. Noach Prof. Dr. J.J. Grote

Dit proefschrift werd bewerkt op de afdeling Keel-, Neus- en Oorheelkunde (Hoofd: Prof. Dr. P.H. Schmidt), in het Laboratorium voor Electronenmicroscopie (Hoofd: Prof. Dr. W.Th. Daems) en in het Pathologisch Laboratorium (Hoofd: Prof. Dr. Ph.H. Hoedemaeker) van het Universitair Medisch Centrum te Leiden.
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Aan mijn ouders

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Introduction

Vasoconstrictive nose drops are used daily by appreciable numbers of people, sometimes for many years in succession, because they think that if they do not so their nose will "close up". Obstructed breathing is the stimulus that leads these patients to the prolonged use of nose drops. Although most of them probably had a real reason to use the drops at the start, for example a head cold, ear infection, or sinusitis, it is not clear why so many continue to use and ultimately abuse nose drops. The most frequently mentioned complaint, nasal obstruction, will disappear in time if the patient stops using the nose drops.

The composition of nose drops has changed many times since such products first appeared on the market. The impression is obtained that the abuse has increased since milder types became available. During the last decade the drops containing xylometazoline (Otrivin®) have become the most commonly used in The Netherlands, and nose-drop abuse is sometimes referred to there as Otrivinism.

The changes induced in the nasal mucosa by prolonged use of nose drops have remained obscure, although it has been assumed by some that the mucosal cilia are 'damaged'. We have been unable to find reports of studies on alterations in the nasal mucosa due to abuse of nose drops over long periods. This gap in the literature led us to undertake the investigations reported in the present thesis. The objective of this work was to determine the functional and morphological changes, if any, in the nose after at least six months of abuse of nose drops.

Chapter 1 gives a review of the subject and a discussion of the pharmacology and mode of administration of nose drops and their effect on the activity of the cilia. The composition of the patient group is described and the regimen used for termination of the use of nose drops is given.

In Chapter 2, the histology and physiology of the nasal mucosa are dealt with and the findings concerning the various types of cell occurring in the respiratory epithelium, obtained by light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM), are reported.

Chapter 3 deals with the light-microscopical results in relation to the glycoprotein composition of the human nasal respiratory epithelium during nose-drop abuse and six months after cessation of the abuse. In view of the interindividual differences in the structure of the nasal mucosa of subjects without nasal complaints, the biopsy specimens of the nasal mucosa of nose-drop abusers during abuse were not compared with similar specimens of non-abusers; instead, the biopsy specimens collected during and after abuse were compared for each patient.

Chapter 4 discusses the results of the comparison of the electron-microscopical (TEM and SEM) findings in the nasal respiratory epithelium during and six months after the abuse of nose drops, and Chapter 5 deals with the nasal patency in the same patient group during and six months after abuse.

Chapter 1

Nose drops then and now

1.1. Historical background

In the course of the centuries many different intranasal medicaments have been used to relieve a stuffed nose. According to Toohill et al. (1981), the oldest advice concerning a stopped-up nose occurs in the Talmud, which refers to "dung of white dog, mixed with myrrh". In ancient times the Hindus used pepper, mustard, oris root and asfoetida to relieve a stuffed nose. Oliver and Schafer (1895) were the first to describe the vasoconstrictive action of adrenaline (epinephrine), a hormone secreted by the adrenal cortex and isolated in 1887. Since then, many substances with vasoconstrictive properties have been investigated. Among the clinical applications of these substances was the use in nose drops to promote patency of the nasal cavity when applied locally. In 1942, Hünermann was the first to point out that long-term use of nose drops could give rise to "*Schnupfen Mittelsucht*", by which he meant a constant impulse to use nose drops. In 1944, Gollum described a group of 30 patients who had become "addicted to the use of Privine". Feinberg and Friedlander (1945) had the impression that Privine® nose drops gave the most effective vasoconstriction. Besides this effect, Privine was thought to have a vasodilative action; according to these authors, the vasodilative effect was weaker than the vasoconstrictive effect but lasted longer than the latter, and they called it the secondary congestive effect. After the initially vasoconstrictive action, prolonged use of Privine nose drops was followed by an increasingly congestive phase. According to Feinberg and Friedlander, none of the sympathomimetic nose drops is free of this effect. Kully (1945) too described the secondary congestive effect of sympathomimetic nose drops and recommended re-evaluation of the indications for the use of what by then had become 240 different kinds of nose drops.

Lake introduced the term rhinitis medicamentosa in 1946 to cover "the condition of the nasal membranes resulting from overuse of nasal vasoconstrictors". In 1947, Ryan published a histopathological study on the nasal mucosa of the rabbit after long-term (up to 10 weeks) contact with nose drops (see section 3.1). In 1949, Fabricant described complications associated with nose-drop abuse no longer encountered today, for example the lipoid pneumonia caused by the use of preparations with mineral oil as vehicle and the argyrosis seen after prolonged use of nose drops containing silver.

Some authors (Feinberg and Friedlander 1945; Kully 1945) considered the secondary congestive effect (also called after-congestion or rebound swelling) to be the stimulus which leads some patients to the repeated use of nose drops. We are now aware that some sympathomimetic nose drops have both an alpha mimetic

effect (giving vasoconstriction) and a beta mimetic effect (giving vasodilatation). After application, the concentration of the drops gradually becomes lower and the beta mimetic effect predominates. The vasoconstriction disappears and the vasodilatation persists.

The search for vasoconstrictive nose drops lacking the secondary congestive effect still continues. According to Miller (1964), Kuhn (1966), and Mayer (1966), secondary congestion does not occur with oxymetazoline (Nasivin®), but according to a conclusion reached by the Council of Drugs of the American Medical Association in 1965: "Like other intranasal medications, oxymetazoline may produce a rebound vasodilatation with prolonged use". The xylometazoline preparation widely used in The Netherlands (Otrivin®) is not free of this secondary congestive effect either.

1.2. Pharmacology of vasoconstrictive nose drops

When nose drops are applied locally to the nasal mucosa, the fluid is absorbed into the lamina propria via the layer of mucus lying on the mucosa and via the epithelial layer. The vasoconstrictive effect of nose drops is exerted on the blood vessels in the lamina propria, and as a result the thickness of the mucosa is reduced.

The blood vessels in the lamina propria are surrounded by adrenergic nerves. The diameter of the vessels is regulated via sympathetic innervation. The transmission of the stimulus to the sympathetic synapse is performed by the neurotransmitter noradrenaline. The adrenaline secreted by the adrenal cortex plays a role as well. The effect of sympathomimetic drugs is based on stimulation of the adrenergic receptors but can also be indirect, for example via the stimulation of the secretion of catecholamines at the nerve ends. On pharmacological grounds, two types of adrenergic receptor are distinguished, i.e., alpha and beta receptors. The alpha receptors are subdivided into alpha-1 and alpha-2 receptors; this subdivision is based on comparisons of the strength of the action of a large number of sympathomimetics, which will not be discussed here. Initially, the alpha-1 receptors were thought to be localized postsynaptically and the alpha-2 recptors presynaptically. Recently, however, it was found that the alpha-2 receptors can also occur postsynaptically. Stimulation of the alpha receptors leads to vasoconstriction and thus to a decrease of the volume of circulating blood in animals (Hall and Jackson 1968; Malm 1974; Änggård and Edwall 1974) as well as in man (Bende 1983).

Locally administerd nasal decongestive agents such as phenylephrine and oxymetazoline are alpha adrenoreceptor agonists, the former acting mainly on the alpha-1 and the latter mainly on the alpha-2 adrenoreceptors (Starke 1981). In man, the presence of beta adrenoreceptors in the blood vessels of the nasal mucosa has not been conclusively demonstrated (Grobler 1966; Bende 1983). It is also not known whether the rebound effect is the result of an active process based on the stimulation of beta receptors or is due to chronic stimulation of alpha receptors leading to exhaustion of the receptors. Bende (1983) showed that the blood flow in the human nasal mucosa is controlled mainly by alpha-2 adrenoreceptors.

There are two ways in which sympathomimetic nose drops can stimulate the alpha receptors of the blood vessels in the nasal mucosa:

1. *Direct stimulation of the alpha receptors:* Noradrenaline and adrenaline are examples of substances that trigger direct stimulation. The effect of these substances is relatively short (about 3 hr), probably because there is considerable accumulation (particularly in presynpatic fibres). Phenylephrine, the derivative of aderenaline used in nose drops, also has a direct stimulatory effect on the alpha receptor but its activity level is much lower.

2. *Indirect stimulation of the alpha receptors:* Ephedrine is an example of a substance that can induce indirect stimulation. The secretion of this neurotransmitter is stimulated by an action on the noradrenaline depots in the presynaptic fibres that gives rise to a sympathomimetic effect. Ephedrine has a moderately vasoconstrictive effect of intermediate duration (4-5 hr). Phenylpropanalolamine has an indirect effect, and its sympathomimetic action is stronger than that of ephedrine.

A special place is occupied by the imidazoline derivates. These substances show an effect on the blood vessels similar to that of the alpha adrenergic agents and act directly on the alpha receptors. Oxymetazoline (Nasivin) and xylometazoline (Otrivin) are examples of imidazoline derivates. All of them act rapidly (within a few seconds) and the duration of the effect is often relatively long (6-8 hr). This might mean that they are not inactivated by binding to tissue cells. Preference is given to imidazoline derivatives on the basis of their pharmacological properties, because they offer the advantage of both rapid and prolonged action.

The recommended dosage of oxymetazoline, i.e., 1 or 2 drops (1%) daily in each nostril, is reported to reduce the blood flow in the nasal mucosa of healthy subjects by about 50 per cent (Bende 1983). In patients who have acute infectious rhinitis, oxymetazoline reduces the blood flow by more than 50 per cent (Bende 1983). If a very intensive and rapid vasoconstriction is desired, e.g. for intranasal inspection or nasal surgery, adrenaline 1% is used by preference.

An ideal nose drop would act rapidly (within a few seconds) and for a long time (about 8 hr) and would not give rise to rebound congestion (see section 1.1). It would also be useful to have the disposal of a kind of nose drop that had no influence on other organ systems, e.g. for patients with hypertension. Modern nose drops have fewer side effects than the older types, but cannot be called ideal.

1.3. Dependence on sympathomimetic nose drops

The reason why some individuals continue to use nose drops, even for many years, is unknown. These abusers say frequently that "My nose doesn't function properly because it is stuffed up, so I use nose drops". This dependence on nose drops is a very different phenomenon to the addiction seen in, for example, heroin

addicts, where a central role is played by the physical dependence expressed by withdrawal reactions. There is an impression that for rather many users the nose drops serve as a fetish, the small bottle being given its own special place on the bedside table or in a pocket.

Since nose-drop abuse does not seem to lead to a tendency to increase the concentration of the drops repeatedly, there is no reason to speak of addiction, which involves not only an uncontrolled urge to use the agent in question, sometimes in increasing dosage, but also distress under deprivation, as in, for example, cocaine addiction. In cases where an urge develops and discomfort occurs when the agent is not available, reference is made to habituation. Nose-drop abuse is closer to habituation than to addiction.

1.4. Administration of nose drops

The way in which nose drops are administered is extremely important for the desired effect. In the treatment of, for example, sinusitis or otitis media, the drops must be introduced into the nose in a particular way. When the standing patient bends his head backward and introduces the drops with a pipette, most of the fluid runs along the bottom of the nasal cavity to the hypopharynx in the direction of the oesophagus and stomach. The ostea of the nasal activity and the Eustachian tubes are not reached. To deliver the fluid to these areas, the patient should lie down, first on one side and then on the other (possibly with the head hanging down) before applying the drops in the respective nostrils.

The pharmaceutic industry supplies nose drops in two forms at present: a drop bottle (with dropper) and a nebulizer with or without fixed dosage. Both types of nebulizer deliver the fluid to the mucosa as an aerosol, the latter in an amount determined by the force with which the plastic container is squeezed. If a nebulizer is used when the patient is lying down, the plastic container is inverted and thus loses its ability to create an aerosol, which means that an excessive amount of fluid is applied. This explains why it is inadvisable to use a nebulizer for a sick child in the supine position: a well-meaning parent can easily administer an excessive amount of a sympathomimetic fluid, which can lead to severe intoxication (Mygind 1979). For all modes of administration it is important to keep in mind the difference in concentration for children (0.5‰) and adults (1‰).

1.5. Nose drops and ciliary activity

One of the aims of the present study was to find out whether long-term use of nose drops has morphological or functional effects on the nasal mucosa. To this end, the cilia were investigated to see whether they undergo morphological changes during prolonged abuse of nose drops (section 4.3.1). Other investigators have studied the influence of medicaments on the activity of cilia on ciliated mucosa, and have designed experiments to measure this influence (Proetz 1939; Hutcheon and

Cullen 1955; Bos 1964; Dudley and Cherry 1978; van der Donk et al. 1980), but all of these designs have the disadvantage that they cannot be used for *in vivo* measurements in human subjects. Extrapolation of such results to patients is inadmissible in the absence of confirmation by *in vivo* experiments.

Van Ree and van Dishoeck (1962) described an *in vivo* test in man. They applied a dye (Edicol® orange) to the septum and used rhinoscopy to time the arrival of the dye in the nasopharynx. The duration of this interval is a measure of the quality of the mucus transport.

The intranasal saccharine test (Ginzel et al. 1980) too can be used to study mucus transport in the nose *in vivo*, but this test has the drawback of being subjective, because the patient must indicate when the saccharine placed on the nasal mucosa by the investigator gives rise to a sweet taste in the mouth. The more exact but also more complicated method developed by Simon et al. (1977) (determination of the distribution of labelled particles over the nasal mucosa) permits a more objective estimation of the influence of a drug on the ciliary activity of the human nasal mucosa. With this method, retardation of mucus transport under influence of xylometazoline was established (Mygind et al. 1978). The work done by van der Donk et al. (1981) provided evidence indicating that the nature of the preservative used in nose-drop solutions is more determinative for cilia activity than the pharmacologically active substance. Mercury compounds are thought to have a particularly deleterious effect.

1.6. Objectives of the present study

This study was undertaken in an attempt to answer the following questions:

1. Is it correctly asserted that the ciliated epithelium of the human nasal mucosa changes under the influence of prolonged use of nose drops?
2. If so, are such changes reversible when the patient stops using the drops?
3. What is the relationship between complaints about the nasal passages made by abusers of nose drops and the rhinoscopic findings at examination by a physician?
4. Does this relationship change after the nose drops are withdrawn?

With respect to the first two questions it may be said that to the best of our knowledge, no studies have been done on changes in human nasal mucosa under the influence of prolonged abuse of nose drops (at least once a day for more than six months). Animal studies have, however, shown changes in the structure of the nasal mucosa during long-term abuse of nose drops (e.g. Ryan 1947). Concerning the last two questions it may be said that the physician examining a nose-drop abuser by anterior rhinoscopy often sees a picture that is difficult to reconcile with complaints of obstruction of the nasal passages. It seems to him that at that moment the patient must be able to breath through the nose without difficulty. Six months after the withdrawal of the nose drops he hears from the patient that the complaints about

the nasal passage have disappeared and anterior rhinoscopy shows a picture indicating ease of breathing.

1.7. Composition of the patient group

All patients who come as out-patients to the Ear, Nose, and Throat Department of the Leiden University Hospital with complaints about their nasal passages are asked whether they have used nose drops. Over a period of two years (1981-1983) those who had done so for more than six months (and at least once a day) were invited to participate in the study, and the drawbacks of prolonged use of nose drops were explained to them. They were also given a schedule to help them terminate the use of the drops (see section 1.8).

Because we wished to obtain an impression of the response of human nasal mucosa to a chronic stimulus, patients in whom a role was played by other exogenous or endogenous influences on the mucosa were excluded from the study as much as possible. This was the case for the following groups of abusers:

1. patients with benign "tumours" in the nasal cavities (e.g. polyps);
2. patients with a malignant tumour in the nasal cavities;
3. patients accustomed to inhale and/or exhale tobacco smoke through the nose;
4. patients with demonstrated atopy;
5. patients applying more than one drug to the nose at the same time; and
6. pregnant patients.

On this basis, 21 patients were selected as suitable subjects. In this group, the patency of the nose was measured (rhinorheomanometry, see Chapter 5) during and six months after the termination of the abuse of nose drops. Of these 21 patients, 11 were willing to contribute a biopsy specimen of the mucosa of the middle third of the inferior turbinate (first on the left and then on the right side) during and six months after abuse (see Chapters 3 and 4).

Table 1.1. Age and sex composition of the series.

Age groups (in yr)	Number	
	men	women
11-20	3	3
21-30	3	2
31-40	—	5
41-50	1	1
51-60	1	1
61-70	—	—
71-80	1	—

The group of 21 patients included 12 women and 9 men. The youngest was 16 years of age, the oldest 75 years, and 16 of the latter group were aged between 16 and 38 years; the mean age was 32 years. The age distribution is shown in Table 1.1, where the group is divided into seven age classes, each class representing a ten-year interval.

1.8. Treatment

Patients who had used nose drops excessively for at least six months with at least one application a day, were told that this abuse was mainly responsible for their complaints of obstruction of the nasal passages, and they were urged to stop this practice. To relieve their complaints after withdrawal of the drops, the following drug regimen was advised:

a. 2 mg dexchlorpheniramine maleate and 0.25 mg dexamethasone in one tablet, 3 times a day during the first week, twice a day during the second week, and once a day during the third week; and
b. 50 μg beclomethasone dipropionate nose spray, one puff on each side 3 times a day during the first week, twice a day during the second week, and once a day during the third week.

The patients were seen again three weeks after the start of this regimen. Most of them were surprised at the improvement of the patency of their nasal passages. They were urgently advised once again not to resort to the nose drops. Five months later, the second biopsy was performed (Chapters 3 and 4) and the rhinorheomanometric investigation was repeated (Chapter 5).

Chapter 2

Structure and function of the nasal mucosa

2.1. Anatomy of the nasal cavity

The nasal cavity is divided into a left half and a right half by the nasal septum. Each half ends anteriorly in a nostril and posteriorly communicates with the rhinopharynx via a choana, one of the paired openings between the nasal cavity and the nasopharynx. The combined surface area of the two cavities is about 150 cm^2 and the total volume about 15 ml. Both halves are covered with nasal mucosa composed of a superficial epithelium lying over the lamina propria. Between these two layers a basal membrane is present. Each half of the nose can be divided into two parts, the vestibule and the cavity (Fig. 2.1). Cranially and dorsally the vestibule is bordered by the internal orifice (limen nasi). An arched elevation situated laterally from the orifice corresponds with the lower margin of the upper lateral cartilage, which can be felt under the finger as a sharp edge, about 1.5 cm from the nostril. The orifice (ostium internum) has an important function in the nose, because it forms the narrowest part of the respiratory tract. This area is only 0.3 cm wide on each side (Masing 1967; Procter et al. 1967). As the cross-section in Fig. 2.1 shows, due to the shape of the turbinates the nasal cavity is a laterally flattened tube ranging in width from 1 mm anteriorly to 5 mm posteriorly. The olfactory epithelium covers a surface measuring about 10 cm^2 on the superior turbinate. The remainder of the nasal cavity is covered with respiratory epithelium. For good vision within the nose, the mucosa should be shrunk by the application of e.g. adrenaline 1%. After that, the large inferior turbinate can be clearly seen on the lateral wall at anterior rhinoscopy. The middle turbinate can be seen deeper in the cavity (about halfway down). The superior turbinate, which is situated high and far back in the nose, is seldom visible. These three turbinates divide the nasal cavity into a lower, a middle, and an upper nasal passage.

Among the structures which are not visible at anterior rhinoscopy are the lowermost nasal passage with the orifice of the nasolacrimal duct, the orifices of the frontal and maxillary sinuses, and anterior ethmoidal cells in the middle nasal passage. These orifices are roughly 2-6 mm in diameter. The nasal septum rarely lies along the median line.

2.2. Histology of the respiratory epithelium

The histological picture of the respiratory epithelium has been described by e.g. Schaeffer (1932), Jahnke (1972, 1978), Lenz (1973), Okuda and Kauda (1973),

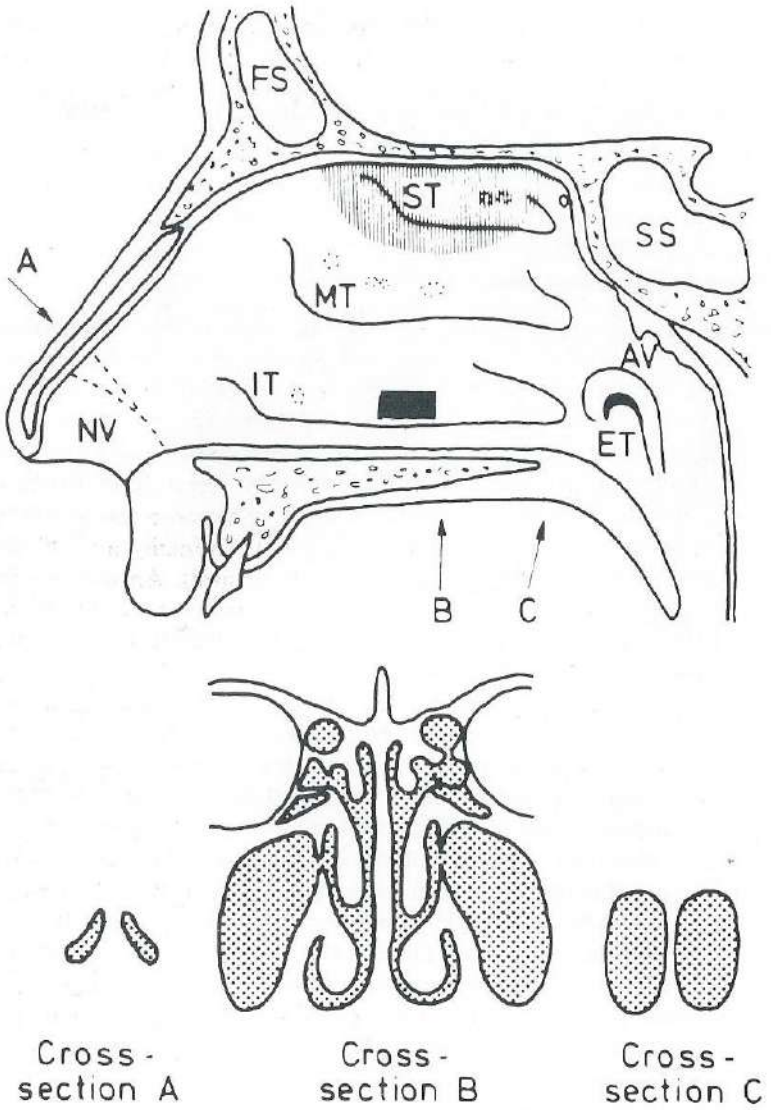


Fig. 2.1. Schematic representation of the lateral wall of the nasal cavity (above) and cross-sections through the ostium internum (A), the cavum nasi (B), and the choana (C) (below). The dotted area in the lateral wall represents olfactory epithelium, and the black rectangle indicates the site at which a mucosa sample was taken (see Chapter 3); the arrows show the location of the cross-sections in the lower part of the Figure.
NV: vestibulum nasi; IT: inferior turbinate and orifice of the nasolacrimal duct; MT: from left to right, middle turbinate and orifice of frontal sinus, anterior ethmoidal cells, and maxillary sinus; ST: superior turbinate and orifice of posterior ethmoid cells, and above the superior turbinate the orifice of the sphenoidal sinus; FS: frontal sinus; SS: sphenoidal sinus; AV: adenoidal vegetation; ET: orifice of the Eustachian tube. After Mygind, 1979.

Mygind (1975), and Busutill et al. (1977). These publications give detailed descriptions of particularly the ciliated cylindrical epithelium (composed of cylindrical cells with and without cilia, mucus-containing goblet cells, and basal cells). Boysen (1982) studied biopsy specimens taken from the anterior tip of the middle turbinate and found that four transitional forms could be distinguished in the respiratory nasal epithelium in varying ratios, their occurrence depending on the age of the subject and specific external stimuli (see Fig. 2.2), i.e.:

I. ciliated cylindrical epithelium,
II. stratified cuboidal epithelium,
III. mixed stratified cuboidal-stratified squamous epithelium, and
IV. stratified squamous epithelium.

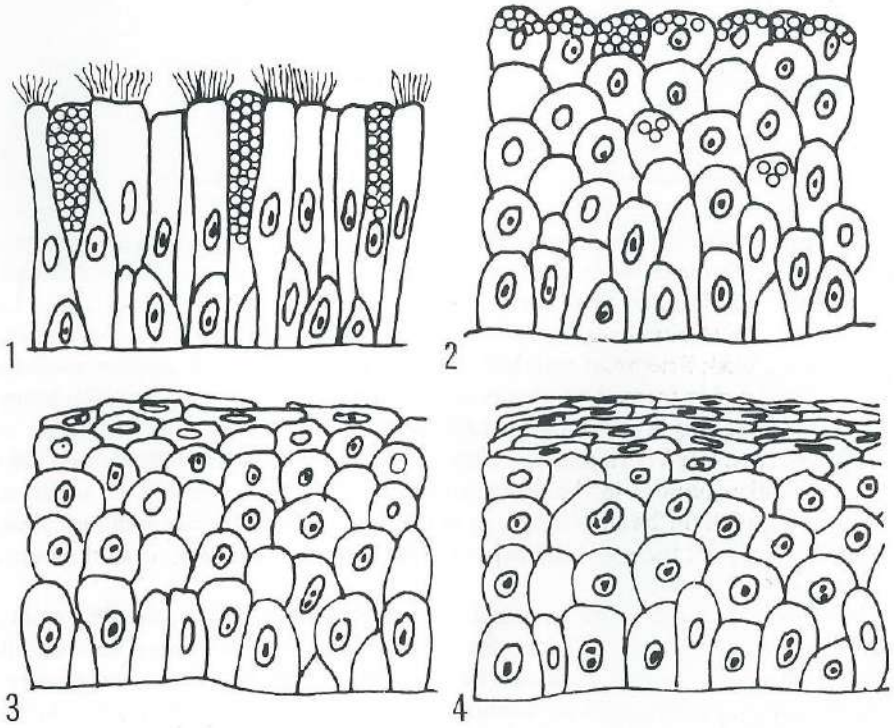


Fig. 2.2. Schematic representation of the four types of epithelium occurring in the nasal mucosa. 1. Ciliated cylindrical epithelium. 2. Stratified cuboidal epithelium. 3. Mixed stratified cuboidal-stratified squamous epithelium. 4. Stratified squamous epithelium.

Each of these four types of epithelium is invariably found in association with the one preceding it or following it on this list, or with both. Thus, transitions occur between these types. For example, Boysen (1982) found a higher proportion of squamous epithelium in older individuals than in young subjects. The same picture was also seen in workers in nickel mines.

In view of these differences in the structure of "normal" human nasal epithelium, it will be useful here to discuss the proportional relationship between the various types of epithelium at a given site in the nasal cavity. Ciliated cylindrical epithelium is found particularly in the posterior two-thirds of the nasal cavity, and mainly stratified cuboidal and stratified squamous epithelium in the anterior third. The term metaplasia may only be used when changes in the type of epithelium are seen at a given site. Because the age of the patients in our series ranged from 16 to 75 years and the structure of the nasal mucosa differed widely between individuals in each group (due e.g. to anatomical differences in the nasal cavity), the present study was based on comparison, in each subject, of the nasal epithelium in the middle third of the inferior turbinate during the abuse of nose drops with the same epithelium six months after the cessation of the nose-drop abuse.

2.2.1. *Cilia*

Cilia are minute hair-like processes occurring on the free surface of a cell. They range in length between 5 and 10 μm and in width from 0.1 to 0.3 μm. The number of cilia per cell varies according to the localization of the ciliated cell in the respiratory tract. In the human trachea the individual cell has about 250 cilia (Rhodin 1974), the cells in the anterior part of the human nose have 50-100 (Mygind and Bretlau 1974), and those in the human middle ear 40-50 (Shimada and Lim 1972). The TEM ultrastructure of the cilia shows a characteristic pattern (Fawcett and Porter 1954; Friedman and Bird 1971; Rhodin 1974). In a typical cross-section of a cilium, a ring formed by nine pairs of microtubules and two central tubules is seen (Fig. 2.3); this is called the 9+2 pattern, but atypical patterns also occur, i.e., 9+4, 8+2, and 10+2 (Friedman and Bird 1971; Takasaka et al. 1980; Herzon 1981). The two microtubules in the centre end in a basal plate, whereas the others are continuous with the basal body. Each of the nine pairs has two projections, always on the same side; these are the dynein arms, which contain the protein molecule with ATP-ase activity.

Dynein is thought to be responsible for the motility of the cilia. The pairs of microtubules are connected by the nexin links, and the pair of central microtubules is surrounded by the central sheath. Between this sheath and the peripheral pairs of microtubules, radial spokes are found. The complex composed of the microtubules with their dynein arms, the central sheath, the nexin links, and the radial spokes, is called an axonema.

Movement of the microtubules is thought to lead to movement of the cilia (Satir 1974). This motility theory is supported by the absence of dynein arms in patients suffering from Kartagener's syndrome, a congenital disease characterized by immobility of the cilia (Afzelius 1979). However, the mobility of the cilia does not seem to depend solely on the presence of dynein arms, because immotile cilia with morphologically normal dynein arms have been described (Herzon and Murphy 1980). Within a restricted area, all of the cilia move in the same direction but not at the same time. This produces a metachronal wave (Proetz 1953).

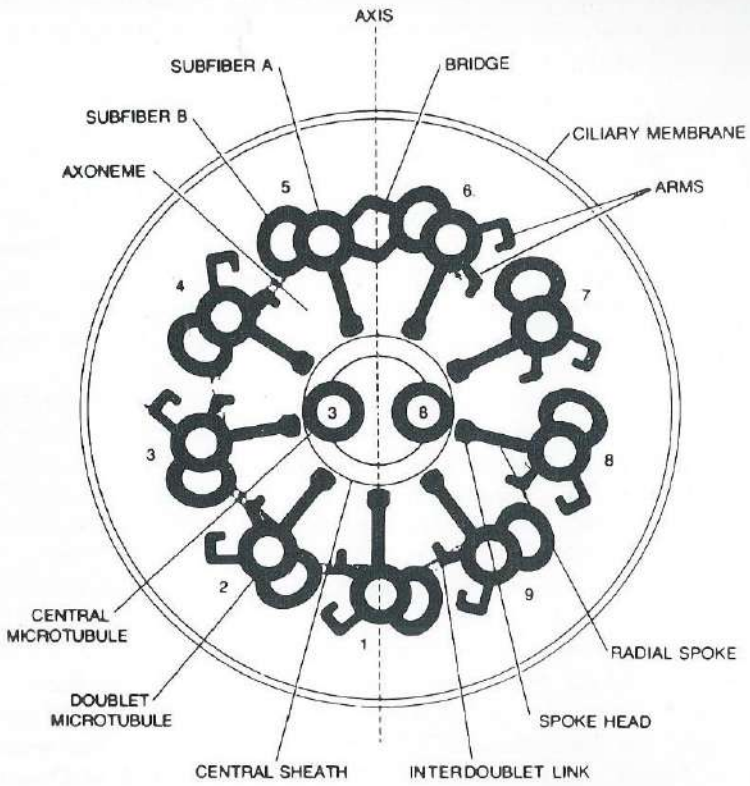


Fig. 2.3. Cross-section of a cilium. Each pair of microtubules has two subfibres (A and B), each carrying a dynein arm. Interdoublet (nexin) links connect adjacent microtubules. The two microbutules in the centre are surrounded by a central sheath, and radial spokes run between the central sheath and the peripheral pairs of microtubules (after Satir, 1974).

Indications for a neural control of cilia movement have not been obtained. Roth (1958) suggested that the basal bodies play an important role in the coordination of cilia movements, but nothing is known about the mechanism involved.

Function of the cilia

The respiratory epithelium is covered with two layers of mucus. The cilia move in the lower layer, and the upper layer is transported by the cilia. These layers are each about 5 μm thick. The lower layer has a low viscosity (Yoneda 1976); the upper layer is viscous and can capture inhaled particles. In the nose the upper layer is shifted inward by the movement of the cilia. Each cilium completes about 20 beats per second and continues to move as long as the cell lives (Hubermann et al. 1977), the tip reaching a maximum speed of about 30 mm a minute. The velocity of the

mucus transport in the nose is 4-6 mm/min (Hilding 1932). Because one of the functions of the nasal mucosa is the capture and removal of foreign particles, this combined action of the mucus layers and the cilia is sometimes called clearance.

Acquired characteristics of ciliated cells

It has frequently been reported that ciliated cells are exceptionally sensitive to external influences (Gould 1971; Stenback 1973; Becci et al. 1978). The responsive changes in ciliated cells are sometimes restricted to the cilia themselves. Proetz (1933), Hutcheon and Cullen (1955), Gallay (1960), Simon et al. (1977), Dudley and Cherry (1978), and van der Donk et al. (1981) investigated the influence of various kinds of nose drops on the beat frequency of the cilia. Among others ephedrine, adrenaline, Tyzine®, Privine®, Afrin®, and Otrivin® have been found to reduce this rate.

Morphological changes in the cilia can also be induced by external stimuli. For example, Herzon (1981) described ultrastructural abnormalities of cilia on the nasal mucosa of abusers of nose drops, and Ailsby (1973), McDowell et al. (1976), and Tasaka et al. (1980) found compound cilia in bronchial mucosa of heavy smokers, but compound cilia have also been described in control subjects without respiratory-tract complaints. Frasca et al. (1967) exposed dogs to tobacco smoke for 44 days and found that after this exposure almost all of the ciliated cells in the bronchial mucosa had been replaced by secretory cells. Jeffery et al. (1976) demonstrated loss of cilia of the respiratory epithelium of rats chronically exposed to SO_2 fumes. Temperature, humidity, chemicals, and dust also probably play an important role in changes occurring in the nasal mucosa (Malaty 1970; Lenz 1973; Mogensen and Tos 1977; Busutill 1978). Regeneration of cilia can occur after elimination of an unfavourable external influence (Andrews 1974; McDowell et al. 1979).

2.2.2 *Lamina propria*

The lamina propria is the part of the nasal mucosa situated between the basal membrane and the supportive tissue (cartilage or bone) on which the mucosa rests. It is composed of a loose mesh of fibro-elastic connective tissue containing many blood vessels, nerves, and glands. Between these structures, cells of various types occur, i.e., lymphocytes, plasma cells, mast cells, and eosinophilic granulocytes.

Blood supply

The blood reaching the nasal mucosa is supplied by the external carotid artery via the sphenopalatine artery to the turbinates, the lateral wall of the nasal cavity, and the lower parts of the septum. From the internal carotid artery the blood flows to the upper parts of the nose via the anterior and posterior ethmoidal arteries.

In the lamina propria itself there are small arteries, arteriolae, capillaries, cavernous sinusoids, and small veins. Arteriovenous anastomoses are also encountered. The arteriolae play an important role in the effect exerted by nose

drops. The cavernous sinusoids are situated mainly in the turbinates, i.e., in the basal part of the lamina propria. The endothelial coat is continuous and is supported on the upper surface by a network of basal membranes combined with collagen and elastic fibres which in turn are covered by smooth muscle cells. Normally, the sinusoids are found to be in a contracted state and are considered to be specialized capillaries that satisfy the functional demands of the air passages, such as protection against inhaled air containing a large amount of pollen.

Adrenoreceptors

The blood vessels in the lamina propria of the nasal mucosa are surrounded by adrenergic nerves. The vascular bed contains both alpha adrenoreceptors (which respond to stimuli by causing vasoconstriction) and beta adrenoreceptors (which respond by inducing vasodilatation; McGrath 1981). Functionally, there is a predominance of alpha adrenoreceptors. Stimulation of these receptors leads to a reduction of the blood volume and of the blood flow in the nasal mucosa of animals (Hall and Jackson 1968; Malm 1974a; Änggård and Edwall 1974) and man (Richardson and Seebohm 1968; Bende 1983).

Locally applied nasal decongestive agents such as phenylephrine and oxymetazoline are alpha adrenoreceptor agonists, the former acting mainly on the alpha-1 adrenoreceptors and the latter mainly on the alpha-2 type (Starke 1981). The presence of beta adrenoreceptors in the blood vessels of the human nasal mucosa is not certain (Grobler 1966; Bende 1983). Bende (1983) showed that the blood flow in the human nasal mucosa is controlled mainly by alpha-2 adrenoreceptors.

Innervation

The lamina propria of the nasal mucosa is liberally supplied with a higly ramified and complex system of nerves belonging to the central and autonomous nervous systems. The sensory fibres of the nasal mucosa run together with the branches of the trigeminus nerve (Christensen 1934; Malcolmson 1959; Donek 1974), and the sympathetic fibres arise from the superior cervical ganglion (Christensen 1934). The parasympathetic fibres arise from the superior secretory nucleus in the brain stem. The sensory fibres transmit sensation, for example cold, pressure, heat, and pain, which can lead to sneezing, increased mucus secretion in the nose, or blood-flow changes. The parasympathetic system innervates both glands and blood vessels (Ishii and Toriyama 1972; Grote 1974), and the autonomous innervation plays an important role in swelling and shrinkage of the nasal mucosa and thus in the patency of the nose.

Glands

The lamina propria in the region of the vestibule of the nose is characterized by the presence of both sebaceous and sweat glands. Such glands do not occur in the nasal cavity itself. Both serous and mucus glands are present in the lamina propria of the respiratory epithelium. On the basis of localization, these glands can be classified as anterior nasal glands which drain into crypts in the region of the ostium

internum and as small tubulo-alveolar glands dispersed over the entire respiratory mucosa (Bojsen-Møller 1965).

The serous acini of the anterior nasal glands lying in the deeper part of the lamina propria drain via long ducts in the region of the ostium internum where the vestibule borders on the respiratory epithelium (see section 2.1). Bojsen-Møller (1965) concluded from the number of orifices anterior to the septum that there must be 50-80 glands, mainly in the cranial part of the ostium internum. The smaller tubulo-alveolar glands and their orifices were found throughout the entire respiratory epithelium (Bojsen-Møller 1965; Terrahe 1970; Tos 1976) and these glands drain via short ducts, many of which have a funnel-shaped orifice (Bojsen-Møller 1965; Tos 1976).

The epithelial lining of the ducts is composed of cylindrical cells. Close to the orifice, this epithelium merges with that of the mucosa with its goblet and ciliated cells (Schiefferdecker 1900).

2.3. Physiology of the nose

Nasal breathing is a vital function for most mammals. This includes the newborn, for whom a good development of this function in the first few weeks is essential. Later in life oral breathing can suffice, but blocked nasal respiration is unpleasant and in principle also undesirable, because of the loss of the conditioning of the inspired air, which takes place mainly in the nose.

As mentioned in section 2.1, the nasal cavity is narrowest in the region of the internal orifice and becomes wider at the back (see Fig. 2.1). When the subject is at rest, the entering air-stream is partially laminar and partially turbulent. The turbulence arises just behind the narrow ostium internum (Ingelstedt and Toremalm 1960, 1961), and increases with increasing velocity of the inhaled air (e.g. under stress) when affected by anatomical abnormalities (e.g. deviated septum) or an abnormally wide nasal cavity, e.g. in atrophic rhinitis (ozaena) and after a partial maxillectomy.

Because the entire nasal passage is relatively narrow, resting respiration involves close contact between the inhaled air and the nasal mucosa. This contact insures efficient exchange of warmth and moisture (Proetz 1953; Ingelstedt and Toremalm 1960, 1961). An abnormal nasal respiration sometimes results in reduced contact between the inhaled air and the nasal mucosa. Under respiration at rest, 5-10 per cent of the inhaled air reaches the olfactory region. Stronger inhalation can raise this percentage to 20 (Donek 1974).

Warming and moistening

In medieval times the main purpose of respiration was thought to be cooling of the blood. In that period the respiratory passages were seen as nothing more than a passive system of tubes serving for transportation of air to and from the lungs. In 1829, Magendie postulated that the nose had an active function, being responsible for warming and moistening the incoming air. In the twentieth century, many investigators have shown that the nose prepares the inhaled air for the lungs. Ingelstedt (1956) showed that when respiration occurs at say 23°C and 40% relative humidity, the inhaled air is brought to 32°C and 98% relative humidity. Oral inhalation also provides conditioning of the air but less efficiently than the nasal route.

Filtration

On average, an individual inhales 10,000 litres of air a day. This air contains a large number of particles of different kinds (bacteria, viruses, dust, pollen, scales, etc.), all of which can have a deleterious effect if they reach the pulmonary alveoli in excessive quantities. Most of them are trapped by the layer of mucus lying on the respiratory epithelium and are transported together with this mucus by the movement created by the cilia (see section 2.2.1). Thus, a good mucociliary transport system is essential for a good filter function of the nose. The average rate at which the mucus layer moves in the nose is about 5 mm/min (van Ree and van Dishoeck 1962; Andersen et al. 1971).

2.3.1. *Histological changes in the respiratory epithelium under the influence of unconditioned air*

In a patient who has not been able to breathe through the nose (e.g. tracheotomy) for a considerable time, the number of ciliated cells in the respiratory epithelium of the nose increases (Ewert 1965; Jahnke 1972; Mygind et al. 1974a), but the first 5 cm of the tracheal mucosa below the tracheostoma shows changes in the direction of squamous epithelium. The anterior part of a nasal polyp, which has the greatest exposure to the incoming air, is often covered with squamous epithelium as well, whereas the posterior surface is covered with ciliated epithelium (Mygind et al. 1974b). When intranasal surgery results in excessive enlargement of the nasal cavity (turbinate surgery, ethmoidectomy), the considerable supply of poorly conditioned air to the respiratory epithelium leads to a transformation into squamous epithelium accompanied by crust formation in the nose. When the nostrils are closed surgically in such cases, the squamous epithelium reverts to ciliated epithelium (Shah et al. 1974). Drettner (1979) attempted to find correlation between such changes and the nasal cycle. The nasal cycle is a physiological mechanism which leads to alternating reduction of the patency of the two sides of the nose due to an increase of the blood volume in the mucosa of the turbinates, the total nasal patency remaining constant. Under normal conditions this nasal cycle is not noticed by the breather; but if a cold in the head develops, the alternation of patency is sometimes experienced consciously. According to Drettner, this nasal cycle might contribute to the recovery of the respiratory epithelium, i.e., occurring after the epithelium has been in close contact with the inhaled air for about six hours and has had about six hours to recover while the mucosa on the other side of the nose is mainly occupied with condition of the air. The neural mechanism underlying the nasal cycle is unknown.

Chapter 3

Histological structure and glycoprotein composition of human nasal respiratory epithelium during and after abuse of nose drops

3.1. Introduction

In animals exposed to external stimuli (ephedrin, formalin, tobacco smoke, SO_2 fumes) the columnar epithelium in the nose can change into squamous epithelium (Ryan 1947; Burian 1958; Frasca et al. 1968; Jeffrey et al. 1976). The same changes have been found in the human nasal mucosa under the influence of ammonia fumes or nickel particles (Bablik and Burian 1961; Boysen 1982). The squamous epithelium in the nose of factory workers exposed to ammonia fumes usually reverts to columnar epithelium when the exposure to the irritant ceases (Bablik and Burian 1961).

Transformation of one type of tissue into another type was called metaplasia by Virchow (1884). Under this term he understood a direct conversion of the differentiated cells forming a given type of tissue into differently differentiated cells. The type of tissue changes because of alterations in the shape of some of the cells composing the original tissue.

Brenkman (1970) defined metaplasia as a dynamic process by which the original tissue at a given site is replaced by tissue of another type but one that does not occur under normal conditions and shows a permanent tendency to revert to the original situation. Metaplastic tissue originates via a transitional stage in which the mother cells give rise to daughter cells with a divergent differentiation pattern.

Besides the histological changes seen in response to external stimuli, alterations in the glycoprotein composition of the mucus-containing cells of the nasal mucosa have also been described. The various kinds of glycoprotein can be identified by the use of histochemical techniques (McCarthy and Reid 1964; Lamb and Reid 1969, 1970). For example, in rat mucosa Jones (1977) showed a shift from neutral to acid glycoproteins under the influence of tobacco smoke.

We were unable to find any data in the literature on morphological changes or changes in the glycoprotein composition of the human nasal respiratory epithelium under prolonged exposure (more than six months) to xyolmetazoline nose drops. In this chapter the histological and histochemical changes induced by nose-drop abuse will be discussed on the basis of the light-microscopical findings.

3.2. Methods and materials

Composition of the patient group and sampling of tissue

Of the 21 patients who participated in the rhinorheomanometric study, 11 were willing to contribute a biopsy specimen of the middle third of the inferior turbinate during nose-drop abuse and six months after withdrawal of the drops. This group comprised six men and five women aged between 16 and 75 years (mean age: 32 years) (see Table 1.1). All of these patients had applied xylometazoline 1‰ nose drops (Otrivin, Ciba-Geigy, Basel, Switzerland) to both nostrils at least once a day for at least six months.

In the present study, the mucosa of each patient during abuse was compared with the contralateral mucosa sampled six months after termination of the abuse. All biopsy specimens were taken from the middle third of the inferior turbinate (on the left side during abuse and on the right six months later; Fig. 2.1). All tissue samples were collected under the same conditions by the same investigator, except that in three cases the biopsy was performed under general anaesthesia, the others having been done under local anaesthesia. However, in the three cases with general anaesthesia the local anaesthetic agent was also applied, and therefore the only difference between the two groups was the administration of the general anaesthetic agent via an oral tube. For local anaesthesia use was made of induction with crystalline cocaine hydrochloride with adrenaline 1% applied on a cottonwool applicator (Hinderer 1971). Three of these applicators were introduced as follows:

1. one high in the anterior part of the nose to block the anterior ethmoidal nerve;
2. one posteriorly in the middle passage under the middle turbinate and against the lateral wall blocking the sensory fibres coming from the foramen sphenopalatinum, and
3. one posteriorly on the floor of the nasal cavity to block the branches of the greater palatine nerve.

After the mucosa had been completely anaesthesized in this way, two vertical incisions were made with a hooked Beaver scalpel (no. 66), the mucosa was lifted, and a straight Beaver scalpel (no. 64) was used to detach the specimen, which was removed with an anatomical forceps. The tissue thus obtained was divided into four approximately equal fragments, and the part held by the forceps was discarded. The other three pieces were reserved for light microscopy and scanning and transmission electron microscopy. The biopsy was performed in this way to reduce the chance of mechanical damage as much as possible.

Histology

The specimen reserved for light microscopy was fixed in buffered 10% formalin and embedded in paraffin, after which it was cut into sections of about 6 μ thick and stained with haematoxylin eosin (HE), periodic acid Schiff (PAS), or alcian blue (AB) (pH 2.6). Despite all the precautions taken, parts of the epithelium sometimes showed mechanical damage. In each biopt the total length of undamaged epithelium was measured and the percentages of the following types of epithelium were determined: ciliated columnar epithelium, stratified cuboidal epithelium,

mixed stratified cuboidal-stratified squamous epithelium, and stratified squamous epithelium.

The first criterion selected for the identification of columnar or stratified cuboidal epithelium was the shape and direction of the mucus-containing compartments. When these compartments of the secretory cells in the respiratory epithelium were orientated perpendicular to the basal membrane and elongated (goblet cells), the epithelium was called columnar. When mucus-containing cells were round or almost round, the epithelium was called cuboidal. Thus, mucus-containing cells can be found in columnar epithelium (goblet cells) as well as in cuboidal epithelium. In dubious cases the composition of the surrounding epithelium was taken into account and particularly the shape and orientation of the nuclei. The term mixed stratified cuboidal-stratified squamous (type III) was reserved for epithelium with a distinctly layered structure and the cells of the topmost layer showing flattening; the epithelium was called stratified squamous (type IV) when the top layer of flattened cells lay on a number of horizontally orientated polygonal cells.

In 10 of the 11 biopsied patients the epithelium sampled during and after abuse of nose drops could be compared; in the eleventh case the tissue had suffered too much damage to permit light-microscopical analysis. For the morphological study use was made of a ×40 oil immersion objective (magnification: 400), each field having a diameter of 0.18 mm. The number of fields in which the various types of epithelium were found was used to calculate the percentage of each type in each mucosal biopt.

Changes in the number of mucus-containing cells during and after abuse of nose drops were assessed by counting all mucus-containing cells on the surface of each biopt and dividing the total by the number of fields to obtain the number of mucus-containing cells per field. These counts were performed in the individual epithelia and compared within patients. The lowest count for mucus-containing cells in contact with the surface amounted to 72, and the highest count for an individual patient was 827. The mean number of mucus-containing cells for the patients in this group was 486.

Morphometry

For the morphometric studies use was made of a microscope (Leitz Orthoplan®) equipped with a camera lucida drawing attachment in combination with a light-emitting diode (LED) directly visible in the microscope, and an X-Y tablet connected to a graphic digitizer (MOP-AMO3, Kontron-Messgerate, Munich, West Germany). The thickness of the epithelium was measured every 35 μm over the total length of undamaged epithelium. The smallest number of measurements in a mucosa biopt was six, the highest 88, with a mean of 61 for the series. For each biopt the mean thickness of the epithelium was calculated and the findings during and after the abuse of nose drops were compared.

For the statistical analysis of the results shown in Tables 3.2 and 3.3, use was made of Student's t-test, with significance at $p < 0.05$.

Histochemistry

Modifications of the AB-PAS method (AB (pH 2.6) and PAS; sialidase, AB (pH 2.6), and PAS; AB (pH 1.0) and PAS) were used to determine whether the mucus-containing cells had neutral and/or acid glycoproteins, and could also show whether the acid glycoproteins were composed of sialomucins (sensitive or insensitive to sialidase) or sulphomucins (Jones and Reid 1973a,b). Since this classification of the acid glycoproteins is based on a subjective colour evaluation, we decided to limit the distinction to neutral and acid glycoproteins for the present study. No other biochemical technique to distinguish the various types of glycoproteins further is available. The mucosa studied histochemically derived from the patients described in section 4.3 in connection with the TEM study of the mucus-containing granules.

The sections were examined at a magnification of 400 under oil immersion. Comparison of the biopt of the patients provided an impression of the changes in the glycoprotein composition during and after the abuse of nose drops.

Inflammatory infiltrate

The inflammatory infiltrate carrying cells with round nuclei and found in the lamina propria and epithelium was sampled and scored semi-quantitatively for comparison of the pictures during and after nose-drop abuse. The occurrence of neutrophil granulocytes was assessed at the same times.

3.3. Results

3.3.1. *Histology*

The respiratory epithelium in the mucosa biopts was easily recognized light microscopically, but at a magnification of 400 (under oil immersion) the margins of the cells could not always be clearly distinguished. The same holds for the presence or absence of cilia on a cell. In most cases, however, the staining methods mentioned in section 3.2 allowed recognition of the position of mucus-containing secretory cells and this made it possible to distinguish between columnar and cuboidal epithelium. The shape of the nuclei was usually easy to determine.

Of the four types of epithelium described by Boysen (1982), we saw mainly type I (columnar epithelium) and type II (stratified cuboidal epithelium). The difference in the shape of the mucus-containing cells in these types of epithelium can be clearly seen in Figs. 3.1 and 3.2. The mixed stratified cuboidal-stratified squamous epithelium (type III) was only seen once and even then the amount was not sufficient to fill one microscopical field (∅ 0.18 mm). Stratified squamous epithelium (type IV) did not occur in our material.

The ratio of columnar to cuboidal epithelium during and after nose-drop abuse was recorded for each patient, and the results were compared. As Table 3.1 shows, more cuboidal and less columnar epithelium occurred during than six months after abuse in all cases. In other words, the nasal respiratory epithelium of nose-drop

abusers showed a shift from cuboidal to columnar when the drops were withdrawn. Table 3.1 also shows that no columnar epithelium was found in three patients and no cuboidal epithelium was found six months after cessation of the abuse in seven patients. The thickness of each type of epithelium was measured to find out whether any change occurred after the cessation of abuse (Table 3.2). In seven of the ten patients the thickness of the columnar epithelium during and after abuse was compared. A decrease was found in all cases, and was significant in five ($p < 0.05$). In the other three patients the thickness of the cuboidal epithelium was measured during and after abuse and compared; two patients showed a significant decrease and one a non-significant decrease of the thickness ($p < 0.05$). Thus, the thickness of both the columnar and the cuboidal epithelia decreased in nine of the ten patients after termination of the abuse.

Table 3.1. Composition of the nasal respiratory epithelium (columnar and/or cuboidal) during and six months after cessation of nose-drop abuse. The patients are listed in order of declining duration of abuse. In all cases the proportion of columnar epithelium increased and that of cuboidal epithelium decreased.

Age (yr)	Duration abuse (yr)	Columnar/cuboidal	
		during abuse	six months after cessation
35	24	50/50	100/0
49	17	70/30	100/0
75	7	85/15	100/0
22	6	25/75	65/35
20	5	0/100	60/40
42	3	25/75	100/0
72	3	75/25	100/0
34	2	85/15	100/0
19	1	0/100	30/70
16	0.5	0/100	85/15

Changes in the number of mucus-containing cells during and after nose-drop abuse were also investigated for these patients. Table 3.3 shows the mean numbers (and standard deviation) of cells of each of the types of epithelium seen in one field (∅ 0.18) during and after nose-drop abuse in these patients. Comparison of the number of mucus-containing cells in the columnar epithelium during and after abuse was possible in seven of the ten patients. All seven showed an increase, which was significant in six of the seven cases ($p < 0.05$). In four patients the comparison could be made for the cuboidal epithelium: two showed an increase and two a decrease of the number of mucus-containing cells.

The mononuclear cellular infiltrate seen in the lamina propria and epithelium showed no change after withdrawal of the nose drops in six of the ten patients, three

Table 3.2. Mean thickness of respiratory epithelium (columnar and cuboidal) during and six months after cessation of abuse of nose drops (in μm, with standard deviation). The patients are listed in order of decreasing age. ↓ = decrease of mean thickness; ↑ = increase of mean thickness; s = significant; ns = not significant at $p < 0.05$.

Age (yr)	Duration abuse (yr)	Thickness of columnar epithelium			Thickness of cuboidal epithelium		
		During abuse	Six months after cessation	Change	During abuse	Six months after cessation	Change
75	7	153.4 ± 11.2	94.9 ± 11.5	38.1%↓(s)	86.3 ± 7.3	—	—
72	3	102.6 ± 14.7	100.3 ± 11.2	2.3%↓(ns)	91.3 ± 16.2	—	—
49	17	82.7 ± 19.7	65.0 ± 10.9	17.7%↓(s)	58.4 ± 12.9	—	—
42	3	88.5 ± 16.2	63.9 ± 7.5	27.9%↓(s)	75.0 ± 9.3	—	—
35	24	98.3 ± 2.3	72.8 ± 5.6	25.9%↓(s)	65.8 ± 13.9	—	—
34	2	87.4 ± 6.4	84.8 ± 13.3	2.9%↓(ns)	93.1 ± 11.3	—	—
22	6	118.4 ± 13.7	88.8 ± 8.6	25.1%↓(s)	75.2 ± 9.1	—	—
20	5	—	75.3 ± 6.3	—	87.5 ± 5.4	81.3 ± 9.1	7.1%↓(s)
19	1	—	86.4 ± 24.0	—	84.3 ± 9.4	59.8 ± 7.0	29.1%↓(s)
16	0.5	—	99.1 ± 11.4	—	65.9 ± 6.4	72.1 ± 3.0	9.4%↑(ns)

others showed a decrease, and one an increase of the infiltrate. No changes were seen with respect to the neutrophil granulocytes. In sum, withdrawal of the nose drops did not lead to any distinct changes in the composition of the inflammatory infiltrate.

Table 3.3. Mean number of mucus-containing cells in columnar and cuboidal epithelium during and six months after cessation of nose-drop abuse. The patients are listed according to decreasing age. ↓ = decrease of mean number; ↑ = increase of mean number; s = significant; ns = not significant at $p < 0.05$.

Age (yr)	Duration abuse (yr)	Number of mucus-containing cells in columnar epithelium			Number of mucus-containing cells in cuboidal epithelium		
		During abuse	Six months after cessation	Change	During abuse	Six months after cessation	Change
75	7	37.9 ± 4.2	39.4 ± 3.8	3.9%↑(ns)	18.4 ± 2.1	—	—
72	3	29.5 ± 3.7	37.3 ± 3.1	26.4%↑(s)	16.0 ± 2.3	—	—
49	17	21.1 ± 2.8	32.9 ± 2.8	55.9%↑(s)	14.0 ± 1.8	—	—
42	3	9.9 ± 2.3	26.2 ± 2.9	136.4%↑(s)	7.6 ± 2.0	—	—
35	24	22.2 ± 3.2	34.3 ± 4.5	54.5%↑(s)	13.7 ± 2.2	—	—
34	2	12.8 ± 2.8	25.0 ± 3.7	92.3%↑(s)	8.0 ± 1.2	—	—
22	6	24.4 ± 3.0	39.3 ± 3.6	61.1%↑(s)	22.4 ± 3.6	27.5 ± 2.8	22.8%↑(s)
20	5	—	18.7 ± 2.1	—	8.0 ± 1.1	14.4 ± 6.3	75.0%↑(s)
19	1	—	21.0 ± 2.8	—	15.6 ± 3.0	10.1 ± 4.0	26.7%↓(s)
16	0.5	—	18.7 ± 1.7	—	16.1 ± 1.9	16.0 ± 1.0	0.6%↓(ns)

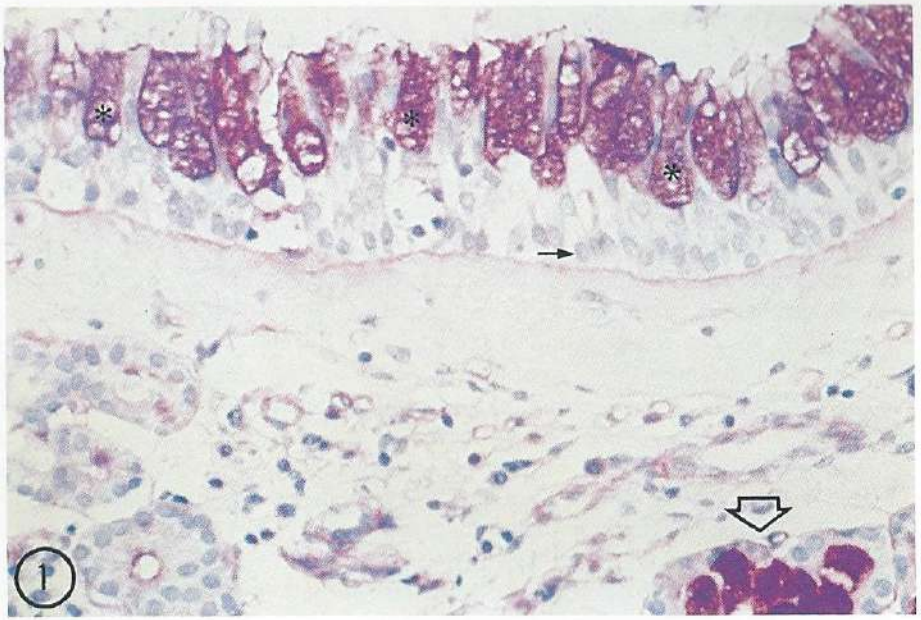


Fig. 3.1. Light-micrograph of nasal mucosa of a patient who had used nose drops for three years, made six months after termination of the abuse. Columnar epithelium containing columnar mucus-containing cells (*), basal cells (small arrow) (BC), and a seromucous gland in the lamina propria (large arrow). Formalin fixation. Staining: AB-(pH 1.0)-PAS. ×400.

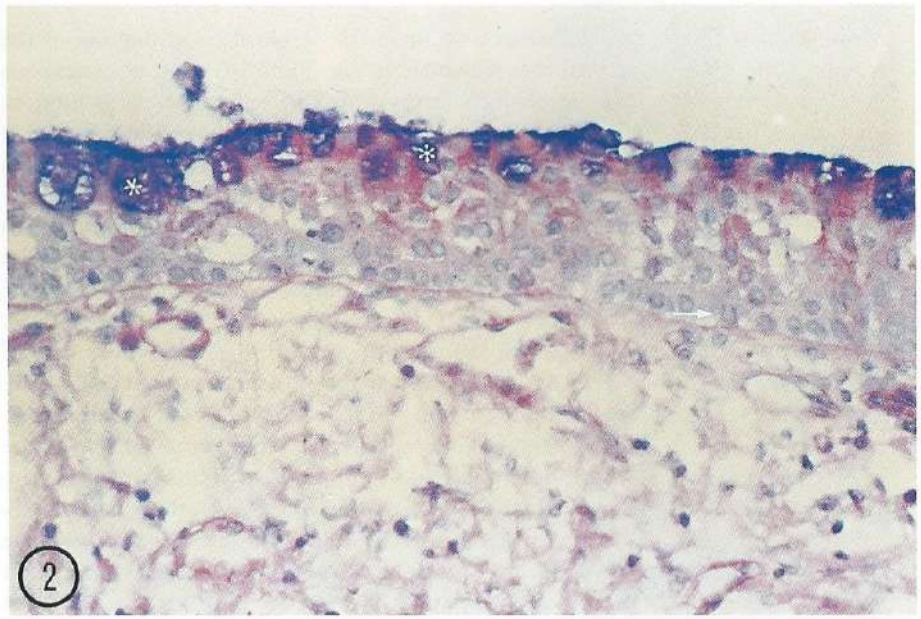


Fig. 3.2. Light-micrograph of nasal mucosa of a patient who had used nose drops for 24 years, made during abuse. Cuboidal epithelium including round or almost round mucus-containing cells (*) and

3.3.2. *Histochemistry*

For five patients the glycoprotein composition of the mucus-containing cells in the nasal mucosa of the middle third of the inferior turbinate during and after nose-drop abuse could be compared. The TEM study of the secretory granules of these patients is reported in section 4.3.1. The comparison could not be made for the other six patients because too little representative tissue was available. Three patients showed no change in the glycoprotein composition, two patients showed a change in the glycoprotein composition of the cuboidal epithelium, i.e., from neutral and acid during abuse to exclusively acid six months later. Thus, no change in the glycoprotein composition was found in three cases. In the other two patients the changes were in the direction of the acid glycoproteins.

The biopsy specimens of one patient showed both PAS- and AB-positive goblet cells, and also AB-positive cells in the layer of intermediate cells. In all probability these last cells are the same as the cells described in section 4.3.1 (see Fig. 4.3), and are thought to be precursors of the secretory cells on the surface of the epithelium. Apparently, some "candidate" secretory cells contain only acid glycoproteins but after reaching the surface contain neutral glycoproteins. In our material we did not find the mucus-containing cells with a PAS-positive and an AB-positive vacuole within the same cell, as described by Jones (1978) for a case where granules with acid glycoproteins were situated apically and those with neutral glycoproteins lay more basally.

3.4. Discussion

Changes due to external influences occur in the respiratory epithelium of the nose. It is known from animal experiments that the administration of exogenous noxae can induce metaplastic changes from stratified columnar epithelium to squamous epithelium (Ryan 1947; Burian 1958; Bablik and Burian 1961; Frasca et al. 1968; Malaty et al. 1970; Lenz 1973; Jefferey et al. 1976; Mogensen and Toss 1977; Busutill 1978).

Because the effect of prolonged use of xylometazoline 1‰ nose drops (Otrivin) on the nasal mucosa was not known, we compared the histological picture and histochemical composition of nasal mucosa during this abuse with the findings six months after termination of the abuse. This could have been done best by comparing the picture of this tissue before, during, and after the abuse, but of course specimens from the period before nose drops were used were not available. Furthermore, because of the marked interindividual differences described by among others Boysen (1982), comparison could not be reliably made between the mucosa of abusers and that of individuals without nasal complaints. We therefore compared the mucosa of individual patients during abuse with their mucosa six months after withdrawal of the nose drops. This approach had the disadvantage that we could not exclude the effect of time on the structure of the epithelium, but it seems likely that the chosen six-month period between the biopsies was too short

for the occurrence of a major structural change. Other external factors can also exert an influence on the structure of the epithelium, including infection by a virus or bacterium, warm air, dusty surroundings, nicotine, and many others.

In all cases, more stratified cuboidal epithelium was present during abuse than six months after its cessation, and correspondingly, less columnar epithelium during than six months after abuse. Thus, when xylometazoline nose drops were withdrawn, the nasal epithelium showed a shift from stratified cuboidal to columnar epithelium. This stage is probably characterized by increased mitotic activity in the epithelium, which showed a disturbed architecture histologically. The reversibility of these changes observed when the noxious agent was eliminated is consistent with metaplasia. Our findings also showed a decrease of the thickness of the epithelium after the withdrawal of the nose drops, which is indicative of normalization of the epithelium after removal of the stimulus. An increase of the number of mucus-containing cells was also seen six months after withdrawal of the nose drops, and this too must be taken as an expression of differentiation after recovery. Half of the patients showed no change in the glycoprotein composition, and in the other half the proportion of acid glycoproteins increased after the cessation of abuse.

Fewer ciliated epithelial cells were found in mucosa of the middle third of the inferior turbinate during abuse than six months after withdrawal of the nose drops. It seems probable that this decrease has an unfavourable influence on the mucus transport and therefore on the filtration function of the nose.

The exact mechanism underlying the xylometazoline effect on the nasal mucosa is not entirely clear from the present findings. In the first place, it is not certain which of the components of the nose drops is responsible for the effect. In the second place, a role can be played by both direct and indirect effects on the epithelium due to the changes in the nasal passage caused by the prolonged vaso-constriction induced by xylometazoline. It is conceivable that prolonged constriction of the blood vessels in the lamina propria leads to undesirable enlargement of the nasal cavity, giving a physiologically unfavourable situation accompanied by a change from laminar to turbulent air flow. This could give rise to metaplastic changes in the epithelium (Shah et al. 1974). No clear-cut relationship was found between the duration of the nose-drop abuse and the degree of epithelial abnormality, but more of the columnar type was found in older patients than in the younger group six months after withdrawal of the drops. Since in general more columnar epithelium was also found during abuse by older patients, the metaplastic effect of xylometazoline nose drops seems to be less pronounced in older than in younger individuals.

Boysen (1982) investigated changes in the nasal epithelium under the influence of nickel dust and the associated successive stages of changes leading to metaplasia in the direction of squamous epithelium. The longer the individual was exposed to the nickel dust, the more often squamous epithelium was found. Boysen described two intermediate stages, one characterized by stratified cuboidal epithelium and the other by mixed stratified cuboidal-stratified squamous epithelium.

The fact that in the present study only changes toward cuboidal epithelium were seen might mean that the stimulus associated with xylometazoline abuse is relatively slight by the absence of squamous epithelium after prolonged abuse. It is important, however, that in Boysen's study the biopsy specimens were collected from the anterior tip of the middle turbinate, a region in which metaplasia in the direction of squamous epithelium is likely to occur sooner or more often because this head can be expected to undergo more turbulence of inspired air than the middle part of the inferior turbinate, where we took our biopsy material.

Most of the patients showed no differences between the amounts of inflammatory infiltrate found during and after nose-drop abuse. This makes an effect of nose drops on the inflammatory inflitrate unlikely. We therefore think the term rhinitis medicamentosa (Lake 1946; Blue 1968; Feinberg and Feinberg 1971) should be discarded and replaced by the term rhinopathia medicamentosa. It must be kept in mind here, however, that the former term was not restricted to the clinical picture caused by nose-drop abuse but included the nasal complaints due to the side effects of, for instance, oral hypertensive agents (such as *Rauwolfia* preparations).

Chapter 4

Electronmicroscopy (TEM + SEM) of the human nasal respiratory epithelium during and after the abuse of nose drops

4.1. Introduction

The histology of the human nasal respiratory epithelium has been described in section 2.2, and the light-microscopical evidence indicating changes in the respiratory epithelium under nose-drop abuse has been discussed in Chapter 3. This chapter will deal with the transmission and scanning electron microscopy of various types of cell during and after abuse of nose drops.

To the best of our knowledge, there are no data in the literature dealing with the effects of the nasal respiratory epithelium in patients who have made excessive use of nose drops (i.e., use for more than six months). Petruson and Hansson (1982) reported a study done with transmission and scanning electron microscopy (TEM and SEM) on the nasal mucosa (lower edge in the inferior turbinate, about 4 cm from the tip of the nose) of subjects without nasal complaints who had used xylometazoline nose drops for six consecutive weeks. No changes were observed.

In the present study special attention was given to the various types of mucus-producing cell. Jefferey (1973) and Baskerville (1975) distinguished between two types, i.e., the F (full) type and the S (small) type. Jones (1978) called these cells L and S (large and small), respectively.

F and L cells: The cytoplasm of the cells is filled with secretory granules. The nucleus of the cells is situated basally. It is not clear whether the description refers solely to the classic goblet cell or to a round or virtually round cell found among cuboidal epithelial cells.
S cells: Mucus-secreting cells with secretory granules in the apical area.

Thus, in principle four types of mucus-producing cells can be found:

1. Clindrical cell filled with secretory granules (goblet cell)
2. Cylindrical cell with secretory granules in the apical area only
3. Cuboidal cell filled with secretory granules
4. Cuboidal cell with secretory granules in the apical part only

In our material a TEM study was performed to find out which of these types of cell was or were present during and after nose-drops abuse, and the granules in these cells were compared at the same time. To obtain information about the histochemical characteristics of the granules containing mucus, silver proteinate

staining was applied to ultrathin sections. The other types of cell found in the respiratory epithelium during and after abuse of nose drops were also studied. In addition, a comparative SEM study was performed to investigate the surface of the nasal mucosa during and after nose-drop abuse.

4.2. Materials and methods

4.2.1. *Biopsy material*

To obtain information about the various types of epithelium during and after nose-drop abuse as well as an impression of the occurrence of the various types of epithelium in the nasal cavity in subjects without nasal complaints, biopsy specimens of mucosa collected at different sites in the nose of healthy volunteers and patients were studied by TEM.

The composition of the total patient group is described in section 1.6. Of the 21 patients who participated in the rhinorheometric study, 11 were willing to contribute biopsy specimens, to be taken during and six months after the cessation of excessive use of nose drops. Each biopsy specimen was investigated light microscopically (2.3.1) as well as by TEM and SEM. For six patients, the TEM results during and after abuse could be compared. The TEM survey provided micrographs in which 15 to 30 adjacent cells between the surface and the basal membrane could be studied. For the other five patients this was not possible, because the relevant areas in the preparations were too small for an electron-microscopical survey. For seven patients the SEM results during and after abuse could be compared. This was not possible for the other four patients because too much of the area in the sections was covered with mucus (preventing adequate examination of the epithelium) or the tissue was found to have suffered too much mechanical damage.

4.2.2. *Transmission electron microscopy*

The biopsy material reserved for TEM was cut into five to ten pieces. Two methods of fixation were applied. In one, direct osmium fixation was performed with 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) 323 mOsm for 4 hr at 4°C. The fixative was then replaced by 0.9% NaCl at 4°C, and the material was dehydrated in an ascending ethanol series (70-80-90-100% C_2H_5OH), orientated, and embedded in Epon (Luft 1961). Polymerization was performed for 48 hr at 60°C. For the other method, use was made of a modified McDowell-Trump fixative. This fixative is composed of 1% gluteraldehyde and 4% paraformaldehyde in 0.14 M cacodylate buffer (pH 7.4), to which the tissue was exposed for at least 1 hr at room temperature. Next, it was rinsed in tap-water, dehydrated in a graded acetone series up to 100%, orientated, and embedded in Epon. Sections 1 μm thick were then cut with a glass knife and stained with Richardson's stain or toluidine blue. These sections were examined light microscopically and the selected preparations were trimmed and then cut ultrathin with a diamond knife on an ultramicrotome (L.K.B. or Reichert). The ultrathin sections were stained for 10 min in a saturated aqueous solution of uranyl acetate followed by 10 min exposure to lead citrate (Reynolds 1963).

To obtain information about the histochemical characteristics of the granules with a diameter of 0.5-1.5 μm found in the cells, a silver proteinate stain was applied to ultrathin sections (Thiéry 1967) which were then studied and photographed in a Philips EM 200, EM 201, or EM 410 electron microscope. When both cylindrical and cuboidal epithelial cells were found in the same specimen light microscopically, an attempt was made to section as much Epon-embedded material as possible for TEM studies.

4.2.3. *Scanning electron microscopy*

The biopsy specimens reserved for SEM were first shaken in 0.9% NaCl for 15 min at 37°C; this was intended to remove the layer of mucus on the mucosa to permit clear observation of the surface of the epithelium. Next, the material was fixed for on average 2 days in 1.5% glutraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4, 354 mOsm). The fixative was then replaced by a 0.9% NaCl solution at 4°C, after which dehydration was performed in an ethanol series (70-80-90-100%) followed by drying under CO_2 in a Polaron critical point apparatus. The preparations were mounted with conducting adhesive on stubs, with the surface of the mucosa facing up. After coating with a layer of gold about 20 nm thick in a Polaron sputter coater, the specimens were studied and photographed in a Cambridge, Stereoscan model S180 at 15kV.

4.3. Results

4.3.1. *Transmission electron microscopy*

In healthy volunteers and nose-drop abusers (during and after the abuse), ciliated cylindrical epithelium was found not only in the anterior but also in the posterior third of the nasal cavity, in the former area fewer ciliated and more cuboidal. Although small fields of mixed stratified cuboidal-stratified squamous, and stratified squamous cell epithelium were seen light microscopically in a few preparations of tissue from the middle third of the inferior turbinate, these types of epithelium were not found in the TEM studies. The TEM surveys permitted excellent observation of the cells and the contents of cells occurring in the cylindrical and cuboidal epithelium.

Of the four types of epithelium described by Boysen (1982; section 3.1) for the mucosa on the head of the middle turbinate, two were seen with TEM in the middle third of the inferior turbinate during and after abuse of nose drops, i.e., ciliated cylindrical epithelium and stratified cuboidal epithelium. Cylindrical and stratified cuboidal epithelium were found in both abusers of nose drops (during as well as after abuse) and individuals without nasal complaints.

In these two kinds of epithelium TEM showed a number of cell types which will be discussed here in detail.

A. *Ciliated cylindrical epithelium* (Fig. 4.1)

1. Cylindrical cells without cilia
2. Goblet cells
3. Intermediate cells
4. Basal cells

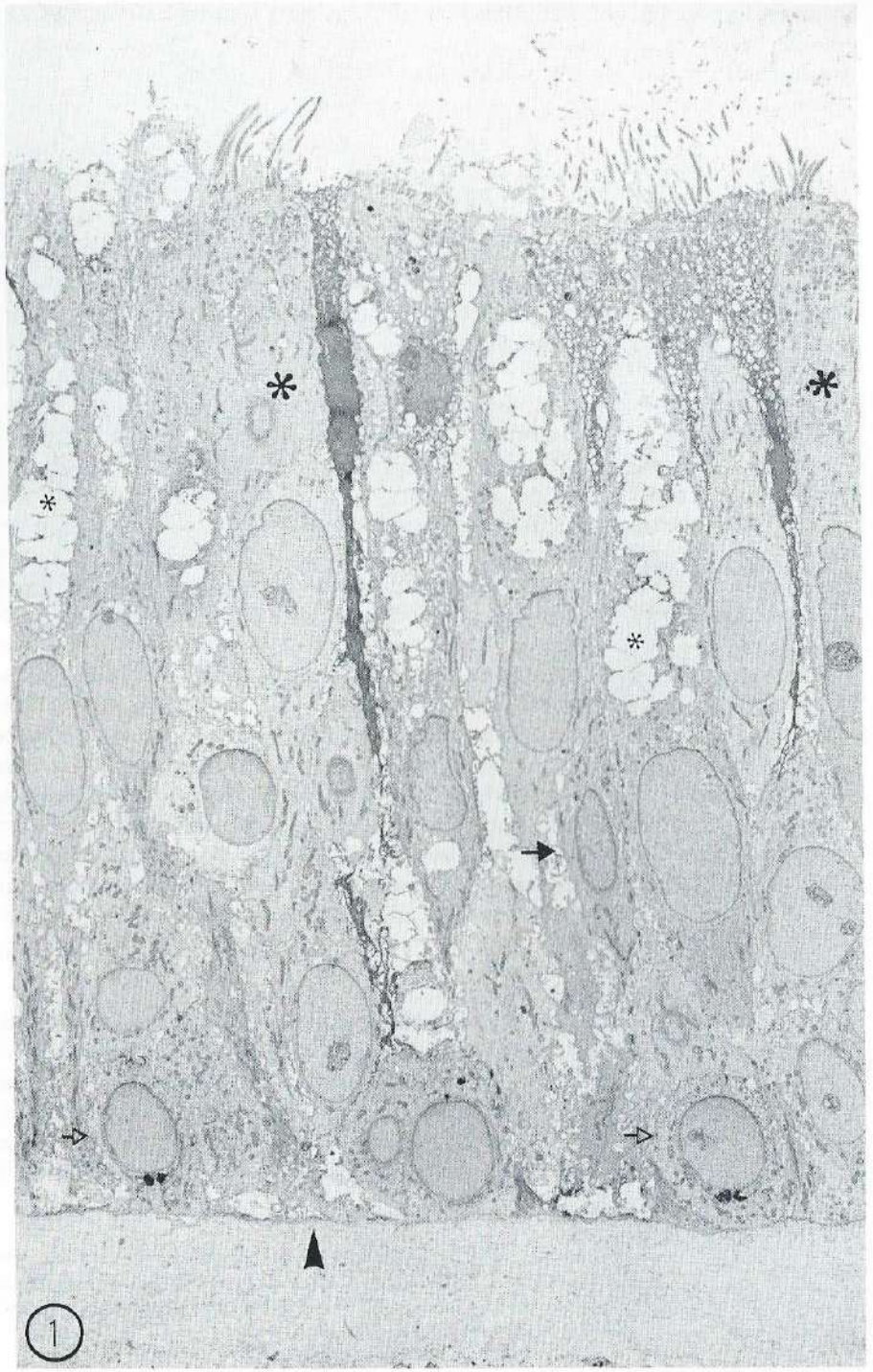
These types of cell all lie on the basal membrane, and the first two also have contact with the surface. Both TEM (Figs. 4.1 and 4.9) and SEM (Figs. 4.10, 4.13, and 4.15) showed that the epithelial cells on the surface are contiguous. On the apical side of the cells the cell membrane forms finger-shaped projections which are tightly interlaced. The individual cell membranes have a thickness of 70-80 Å. In this region the intercellular space is about 100 Å, the cell membrane complex between two cells thus measuring about 250 Å. Desmosomes are easily recognized in these regions. Although the cells lie close together on the lumen side, basally in the epithelium the intercellular spaces become increasingly large, as can be seen in Fig. 4.9.

1. Cylindrical cells with and without cilia

The cylindrical cells with and without cilia (Fig. 4.1) are the most frequently occurring cells in the respiratory epithelium of the nasal mucosa. They have a round to oval nucleus lying at different levels, which sometimes gives the misleading effect of stratification of the cells. Therefore, this epithelium is more properly called pseudostratified cylindrical epithelium. The surface of the lumen side of both types of cell bears microvilli which increase the surface area. These finger-like projections have a diameter ranging from 0.03 to 0.1 μm and a maximal length of 2 μm. The ciliated cells have many mitochondria distributed throughout the cytoplasm but in greater numbers in the apical than the basal part of the cell. The mean diameter of the mitochondria is 0.3-0.5 μm, and the length can be several times the width. The cristae in the mitochondria show ramification. On the apical side of the nucleus there are cisternae, vesicles, and vacuoles forming the Golgi apparatus. Endoplasmic reticulum is scarce and is composed predominantly of vesicles containing ribosomes.

The cytoplasm of the cells without cilia show the same organelles but strikingly fewer mitochondria. The apical part of both types of cell shows round to oval inclusion bodies with a diameter of about 0.5 μm, which at higher magnification appear to be surrounded by a single membrane. These inclusions, called here lysosome-like bodies (see under *Discussion*), can be divided into three groups:

Fig. 4.1. Transmission electron micrograph of the epithelial layer of the nasal mucosa of a subject without nasal complaints (middle third of the inferior turbinate). Cylindrical epithelium showing ciliated cells (*), goblet cells (*) with fusing electron-lucent granules, intermediate cells (→), basal cells (arrows), and the basal membrane (arrowhead). Fixed in OsO_4. ×2,100.



lysosome-like bodies with a lamellar structure (Fig. 4.9), lysosome-like bodies with granules (Fig. 4.6), and a third kind with a number of equally large electron-lucent vesicles, called multivesicular bodies (Fig. 4.4.).

The ultrastructure of the cilia is discussed elsewhere (2.2.1.).

2. The goblet cells

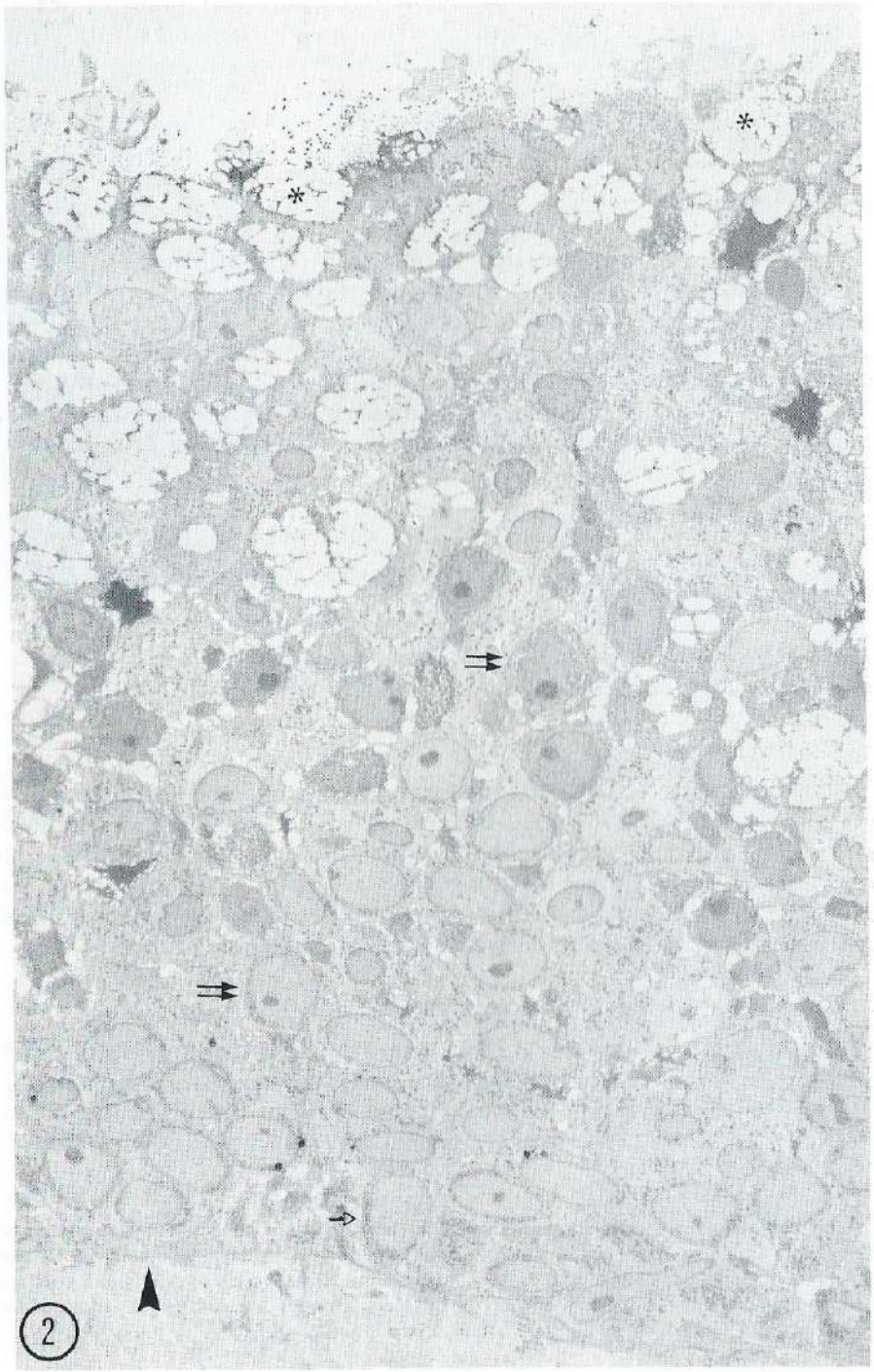
In the normal nasal mucosa of adults without nasal complaints the number of goblet cells ranges from 4,000 to 7,000 per mm^2 (Mogensen and Tos 1977), with a slightly higher number in the posterior than the anterior part of the nose (Tos 1976). The goblet cells are characterized by numerous apically located secretory granules. The secretory granules in the goblet cells of the human nasal mucosa are electron-lucent granules with a diameter of 0.5-1.5 μm and have a tendency to fuse and form larger granules. The lumen side of the cell bears microvilli which disappear when the goblet cell becomes filled with secretory granules. The mature goblet cell then expells its contents into the lumen, as can be clearly observed with SEM (Fig. 4.16). The apex of the mature goblet cell is often slightly wider than the base of the cell. Fusion between the membrane of a secretory granule with the surface membrane of the cell leads to the release of part of the secretion into the extracellular space. When this has occurred, the cell starts to synthesize secretory granules again. It is now known how many secretory cycles a goblet cell can undergo.

In the totally filled goblet cell the organelles are pushed toward the base of the cell. The nucleus is round to oval. Between the nucleus and the secretory granules, there are closely packed cisternae, vesicles, and vacuoles in the Golgi region. The vesicles are usually extremely dilated. On the apical side of the Golgi region there are secretory granules which show a stronger tendency to fuse the closer they lie to the surface of the cell. There are many ribosomes and an abundance of granular endoplasmic reticulum. Mitochondria are found not only basally in the cell but also between the mucus-containing compartment and the cell membrane. The goblet cells have fewer mitochondria than the ciliated cells and show no lysosome-like bodies. The silver proteinate staining shows a finely granular reaction product in the various cell organelles, and also makes the cell coat visible.

3. Intermediate cells

The intermediate cells (Fig. 4.1) represent intermediate stages of development (differentiation) from basal cells to mature ciliated or goblet cells. These cells also occupy an intermediate position in the epithelium in that they rest on the basal membrane but do not reach the surface of the epithelium. These oval to elongated cells represent a heterogeneous group of cells representing intermediate stages between basal cells and the ciliated and goblet cells. Granules are sometimes seen in

Fig. 4.2. Transmission electron micrograph of the epithelial layer of the nasal mucosa of a patient who had used nose drops excessively for five years (middle third of the inferior turbinate). Stratified cuboidal epithelium showing type F secretory cells (*), intermediate cells (paired arrows), basal cells (arrow), and basal membrane (arrowhead). Fixed in OsO_4. ×2,400.



the cytoplasm of these cells. The nucleus of the intermediate cells lies at a higher level than the nucleus of the basal cells. The volume of the nucleus is relatively large compared with the volume of the cell. The occurrence in the cytoplasm of mitochondria, ribosomes, endoplasmic reticulum, and the like is dependent on the degree of differentiation of the cell, i.e., the more differentiated the more ribosomes and mitochondria. No lysosome-like bodies are found in the intermediated cells.

4. Basal cells

The basal cells are stem cells that give rise to cylindrical and the goblet cells. Of the four cell types (Fig. 4.1), the basal cell is the smallest. These cells lie on the basal membrane and do not reach the surface. The nucleus, which is round to oval, accounts for a relatively large part of the cell volume. Usually, one or more nucleoli are visible in the nucleus. Reflecting the limited degree of differentiation of these cells, the cytoplasm shows hardly any endoplasmic reticulum, few mitochondria, and only an occasional Golgi region. Free ribosomes occur in large numbers.

B. *Stratified cuboidal epithelium*

The following types of cell are found in stratified cuboidal epithelium (Fig. 4.2):

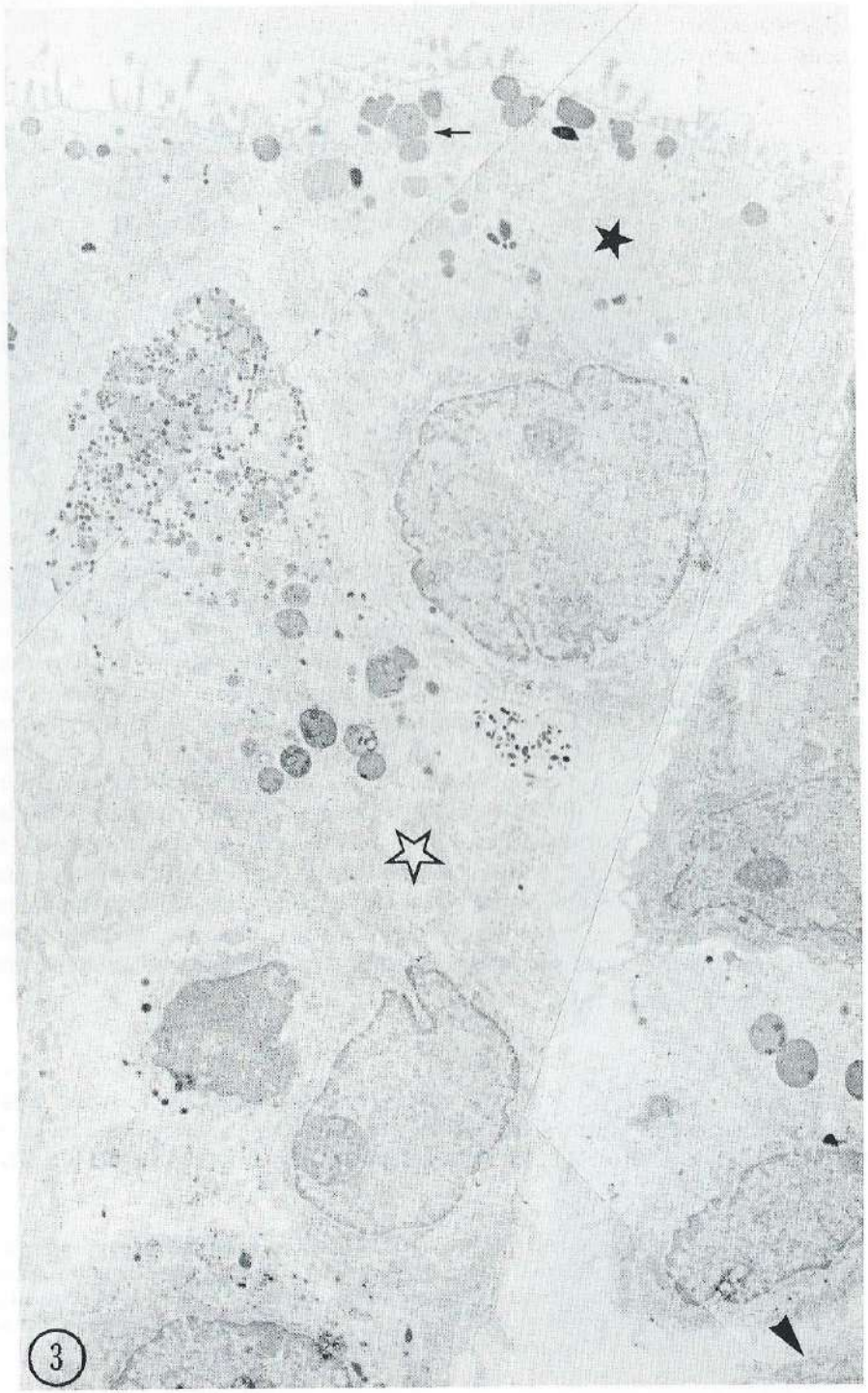
1. Cells without cilia (and without secretory granules)
2. Cells containing mucus, type F according to Jeffery (1973)
3. Cells containing mucus, type S according to Jefferey (1973)
4. Intermediate cells
5. Basal cells

Of these types of cell, only the basal cells and some of the intermediate cells are in contact with the basal membrane, and most of the intermediate cells have contact with neither the basal membrane nor the surface. Here, too, the intermediate cells represent a transitional stage between on the one hand the basal cells and on the other the type F and S secretory cells and non-ciliated cells situated on the surface of the epithelium. Thus, the various types of cell lie in successive layers.

1. Cells without cilia (and without secretory granules)

The surface of these cells (Fig. 4.2) shows microvilli which are less numerous and shorter than those found in cells without cilia in the cylindrical epithelium. The number and diameter of mitochondria are both smaller in the non-ciliated cells in the cylindrical epithelium. Lysosome-like bodies occur in the apical part of the cell,

Fig. 4.3. Transmission electron micrograph showing a detail of the epithelial layer of the nasal mucosa of a patient who had used nose drops for ten years. The epithelium contains a type S secretory cell (★) and an intermediate cell (☆) with two kinds of granule, both occurring mainly in the apical part of the cytoplasm. The granules in the secretory cell (arrow) show few signs of fusion. The arrowhead in the lower right corner points to the basal membrane. Fixed in OsO_4. ×8,600.



isolated ribosomes are found throughout the cytoplasm, and there is little rough endoplasmic reticulum. Only a few cisternae and vesicles are seen in the Golgi region.

2. Secretory cells, type F according to Jefferey (1973)

These cuboidal cells whose cytoplasm is almost completely filled with secretory granules (Fig. 4.2) are found in the stratified cuboidal epithelium on the surface of the mucosa. The cells filled with granules show few microvilli. The secretory granules have a strong tendency to fuse. Besides the electron-lucent secretory granules there are similar granules with a dark centre; the organelles are concentrated in the basal area of the cell. The nucleus is round, and between it and the secretory granules there are many cisternae, vesicles, and vacuoles pressed together in the Golgi region. Many free ribosomes are present and rough endoplasmic reticulum is abundant. The number of mitochondria is approximately the same as in the goblet cells. No lysosome-like bodies were seen.

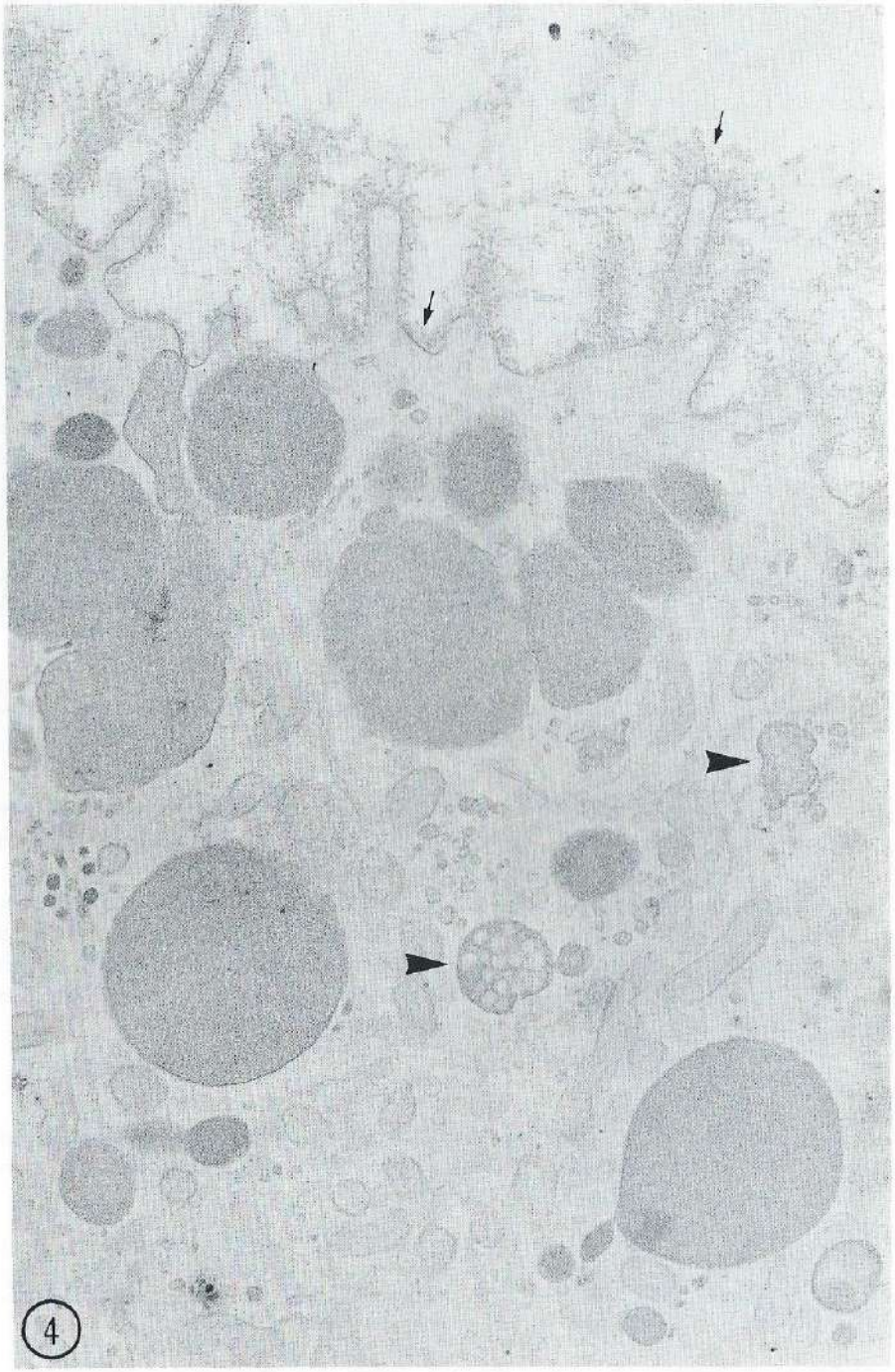
3. Secretory cells, type S according to Jefferey (1973)

The number of microvilli visible on the cell surface is related to the number of secretory granules in the cytoplasm. The more apically situated granules there are, the fewer microvilli. As a rule, the secretory granules in these cells (Fig. 4.3) seem to be smaller and more electron dense than those in the goblet cells of the cylindrical epithelium, and also seem to have less tendency to fuse. The granules are surrounded by a single membrane. Staining with silver proteinate showed positive secretory granules and also made the cell coat easy to observe (Fig. 4.4). Light microscopically, the same material showed PAS-positive staining in the apical part of the cell. These two findings taken together justify the conclusion that the granules contain glycoproteins. The nucleus of these cells is round to oval and is situated between the middle and slightly basal regions of the cell. Lysosome-like bodies occur in the apical cytoplasm, and there are fewer mitochondria than in the goblet cells. The Golgi region shows few cisternae, vesicles, and vacuoles, ribosomes are dispersed in the cytoplasm, and there is little rough endoplasmic reticulum.

4. Intermediate cells

The intermediate cells (Figs. 4.2 and 4.3) have no contact with the basal membrane except for a few whose nucleus lies just above the level of the nuclei of the basal cells, and also have no contact with the surface of the epithelium. The

Fig. 4.4. Transmission electron micrograph of a superficial cell (cuboidal epithelium) of a patient who had been using nose drops for ten years. In the apical cytoplasm of the mucus-containing cell, mucus granules are seen. Some of them are fusing, and show fine granular contents, and the same material is seen in the multivesicular bodies (arrowheads). At the surface a finely granular reaction product can be seen in the cell coat (arrows). Silver proteinate staining. Fixed in OsO_4. ×27,000.



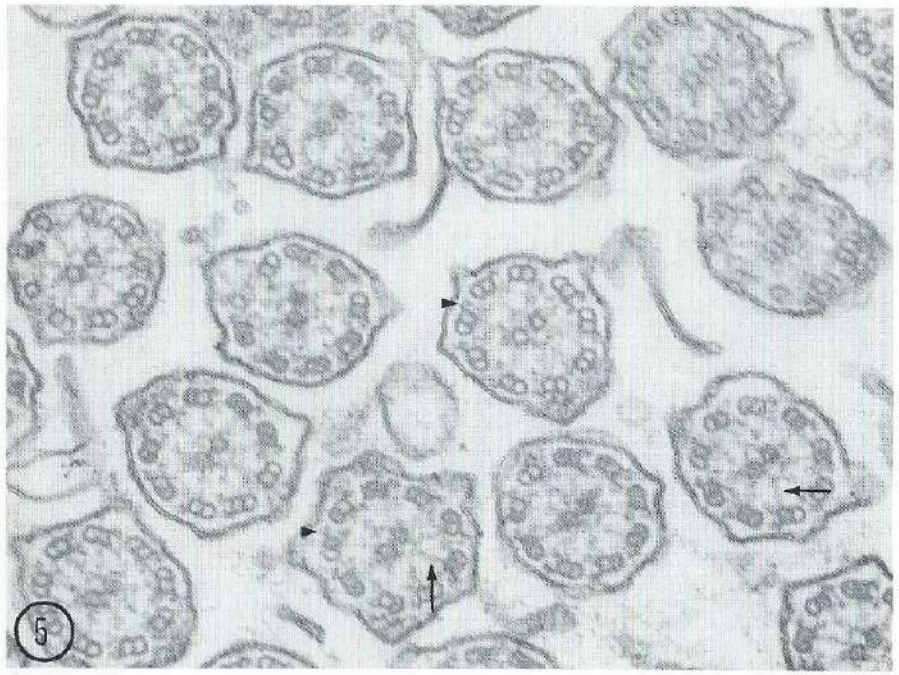


Fig. 4.5. Transmission electron micrograph of transversely sectioned cilia from a subject without nasal complaints. The 9+2 pattern is recognizable, as are the dynein arms (arrowheads) and radial spokes (arrows). Fixed in Trump's solution. ×98,000.

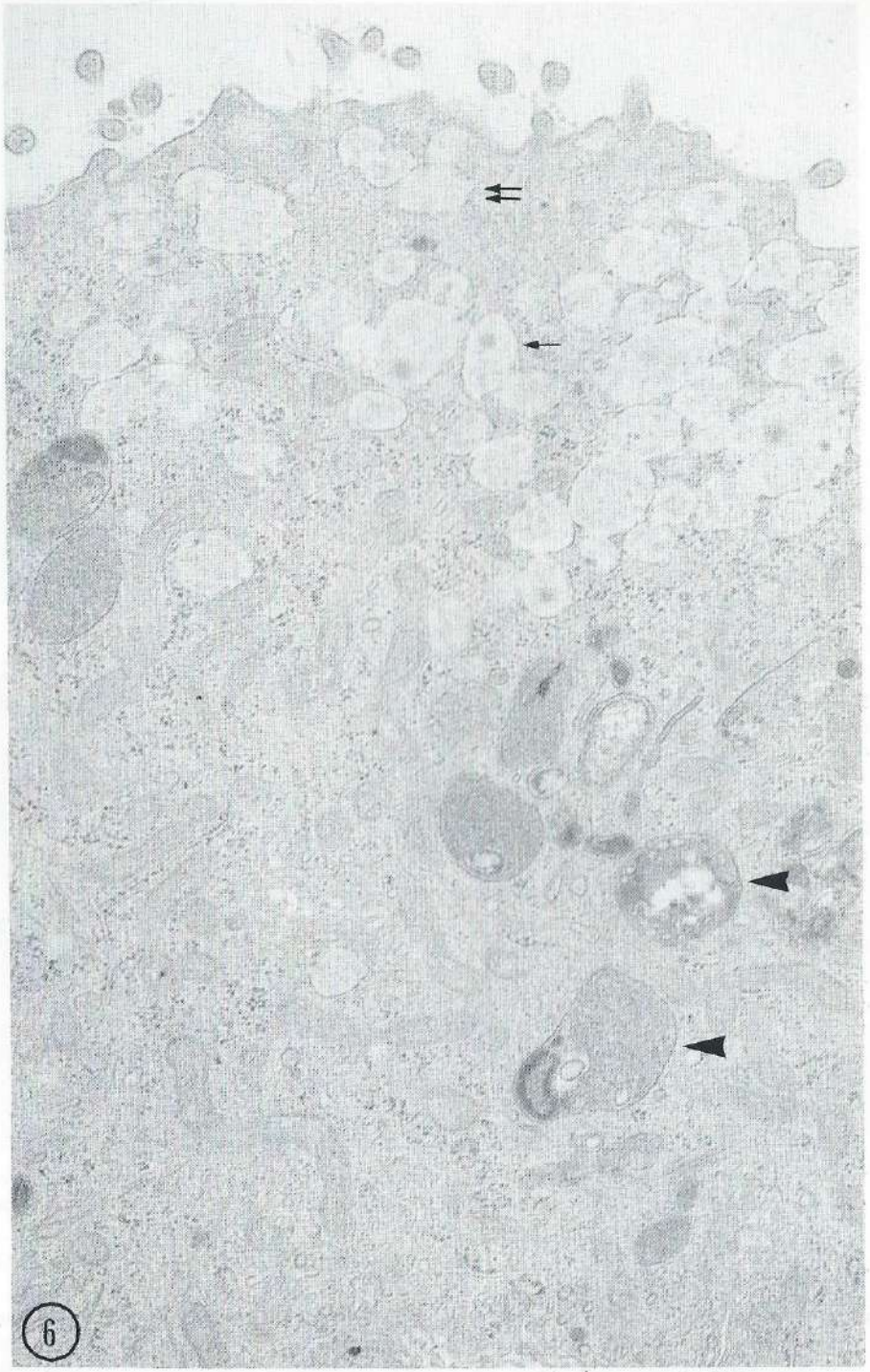
more layers of these cells, the thicker the epithelium. Secretory granules can occur in intermediate cells (Fig. 4.2), probably those which are precursors of the superficially situated secretory cells (Type S and/or type F).

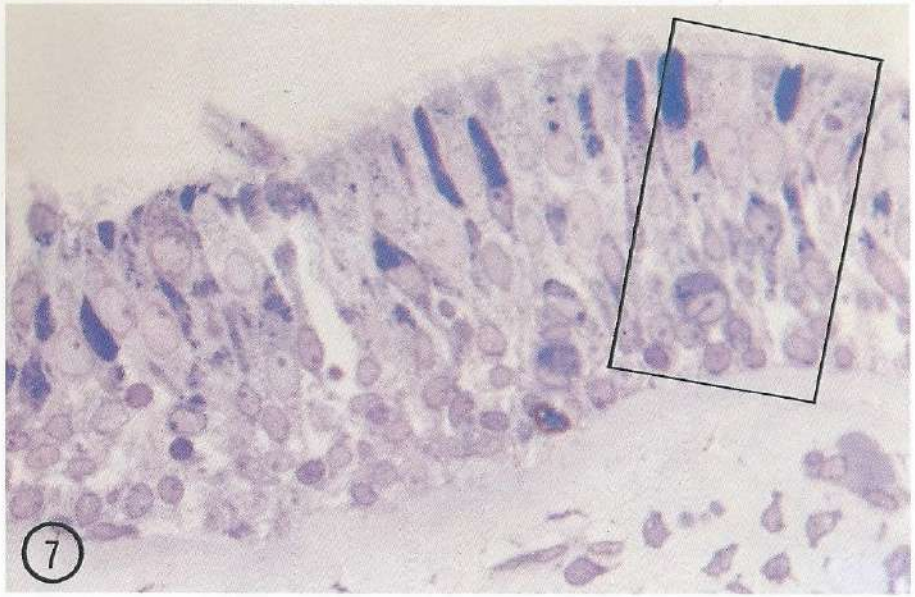
The nucleus of the cuboidal cell is round, often centrally situated, and occupies a relatively large part of the cell. The occurrence of mitochondria, ribosomes, endoplasmic reticulum, etc., is dependent on the degree of differentiation of the cell. The more differentiated the cell, the more ribosomes and mitochondria are found. No lysosome-like bodies are found in the intermediate cells.

5. Basal cells

The basal cells are the stem cells from which not only cells without microvilli (and without secretory granules) but also the type S and F secretory cells develop. These

Fig. 4.6. Transmission electron micrograph of a detail of a type S secretory cell (stratified cuboidal epithelium) of a patient who had been using nose drops for 24 years. The cell contains electron-lucent granules (paired arrows) and electron-lucent granules with an electron-dense core (arrow). Lysosome-like bodies are also present (arrowheads). Fixed in Trump's solution. ×27,000.





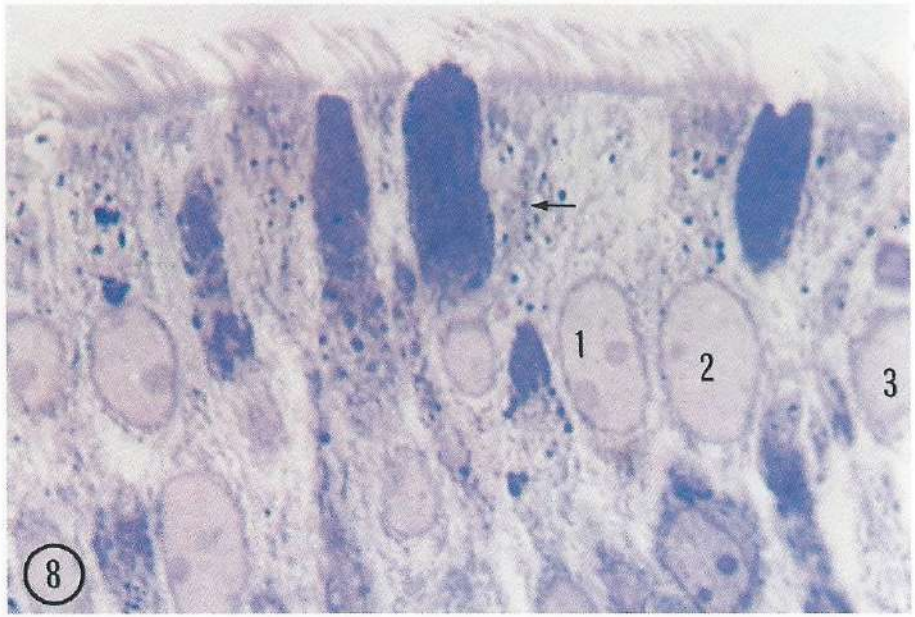
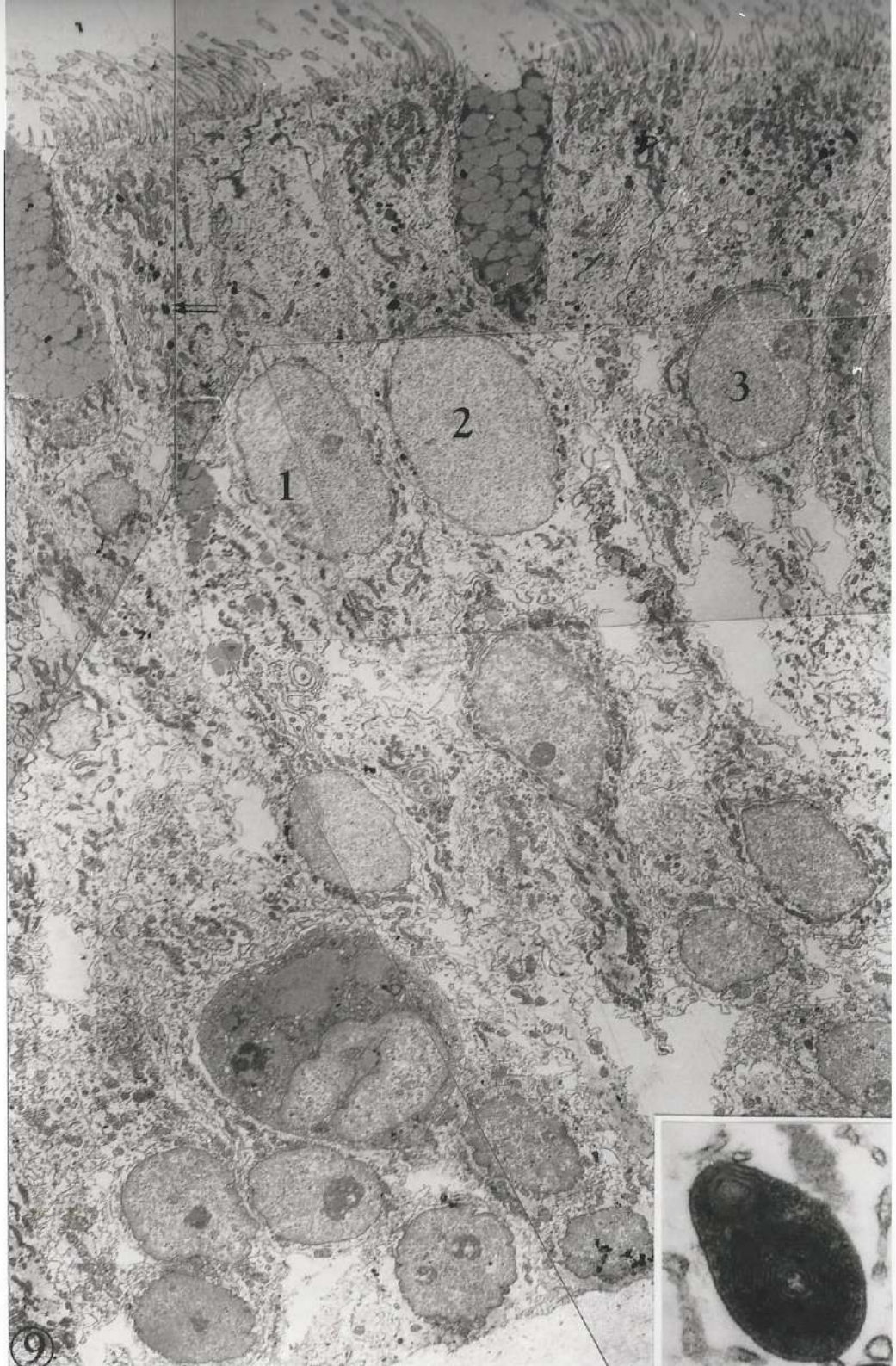


Fig. 4.7. Light-microscopical appearance of nasal mucosa showing ciliated cylindrical epithelium, six months after withdrawal of nose drops, abused for six months. Fixed in OsO_4, embedded in Epon, stained with toluidine blue. Area corresponding with TEM view indicated in Fig. 4.9. ×1,100.

Fig. 4.8. Detail of Fig. 4.7. The single arrow indicates the same organelle as the paired arrows in Fig. 4.9. ×3,000.

Fig. 4.9. Transmission electron micrograph of the same area as shown in Figs. 4.7 and 4.8. Cytoplasm of the surface cells shows electron-dense structures (e.g., paired arrows) corresponding with blue-stained granules in surface cells in those figures. The paired arrows indicate the location of the detail in the



round to oval cells (Fig. 4.2) lie on the basal membrane. The nucleus, which is round to oval, is large relative to the size of the cell. The nucleus usually shows one or more nucleoli. The cytoplasm contains little endoplasmic reticulum, few mitochondria, and only rarely a Golgi region. Free ribosomes are numerous.

Secretory cells bearing microvilli were not seen in either cylindrical or cuboidal epithelium, and cylindrical cells with only secretory granules in the apical part of the cell were not found in our material. In one case intermediate cells containing two kinds of secretory granules were found (Fig. 4.3), i.e., large electron-lucent and smaller electron-dense granules.

The ultrastructure of cilia, secretory granules, and lysosome-like bodies during and after nose-drop abuse

Cilia

In transverse sections the ultrastructure of cilia in the nasal mucosa of individuals without nasal complaints shows the characteristic 9 + 2 pattern with radial spokes running between the centre and the doublets, which normally bear dynein arms (Fig. 4.5), as already described. The corresponding findings in abusers of nose drops showed the same ultrastructure, and this picture persisted six months after the termination of the abuse.

Secretory granules

During and after nose-drop abuse, two kinds of secretory granules were found with TEM (Figs. 4.1, 4.2, and 4.6), i.e., electron-lucent granules and electron-lucent granules with an electron-dense core. Both types are surrounded by a single membrane. Fusion of granules is frequent (Fig. 4.4), and is brought about by merging of these membranes. Table 4.1 shows the occurrence of the various types of granule during and after nose-drop abuse.

It is clear from Table 4.1 that the nasal mucosa of the ten patients in question showed predominantly electron-lucent fusing granules both during and six months after nose-drop abuse. The electron-lucent granules with an electron-dense core found during abuse in five of the ten patients, disappeared after withdrawal. No electron-dense granules were found. There seems to be no relation between the duration of abuse and the presence or absence of a particular type of granule (see Table 4.1).

Lysosome-like bodies

In the description of the ultrastructure of cylindrical cells with and without cilia, three kinds of lysosome occurring in human nasal mucosa are mentioned. Lysosomes occur not only in the cells with and without cilia in the cylindrical epithelium but also in the cuboidal epithelium. The latter was found to show lysosomes in the cells without cilia (or secretory granules) and in type S secretory cells. No marked difference was found between the number of lysosomes during

Table 4.1. Types of secretory granules present during and after nose-drop abuse.

	During abuse		Six months after termination of abuse	
Duration of abuse (yr)	electron-lucent fusing granules	electron-lucent granules with elec.-dense core	electron-lucent fusing granules	electron-lucent granules with elec.-dense core
24	+	+	+	—
17	+	—	+	—
7	+	+	+	—
6	+	+	+	—
5	+	+	+	—
3	+	—	+	—
3	+	+	+	—
2	+	—	+	—
1	+	—	+	—
0.5	+	—	+	—

and after abuse of nose drops, but the impression was obtained that the multivesicular bodies were seen less frequently during and after abuse.

When human nasal mucosa was fixed in osmium, embedded in Epon, and stained with 1% toluidine blue (3.3.3), light microscopy showed dark-blue granules in the cytoplasm of the superficially situated cells (Figs. 4.7 and 4.8). To assess correspondence with the lysosomes in the apical part of these cells, the following sections of ciliated cylindrical epithelium were studied: three sections, 600-900 Å, on a grid, one "thick" section, 0.2 μm thick, on a coverslip, and the next three 600-900 Å thick sections on another grid. The TEM picture of the first three of these sections showed cylindrical epithelium (Fig. 4.9). Lysosome-like bodies were frequently found in the apical part of ciliated cells. The TEM picture of the last three sections was very similar to that of the first three. Light microscopy of the "thick" section showed the same cells as had been visible electron microscopically (Figs. 4.7 and 4.8), and the blue-stained granules seen light microscopically appeared as lysosome-like bodies in the electron microscope.

4.3.2. *Scanning electron microscopy*

To obtain information about the various kinds of epithelium during and after nose-drop abuse, as well as an impression of the incidence of the various kinds of epithelium in the nasal cavity in individuals without nasal complaints, biopsy specimens of mucosa were taken from several sites in the nose of healthy subjects and abusers of nose drops for an SEM study. It proved possible to predict the composition of the underlying epithelium from the appearance of the surface of the mucosa. In healthy subjects, ciliated epithelium (Fig. 4.10) was found predominantly in the middle and posterior thirds of the nasal cavity. Funnel-shaped

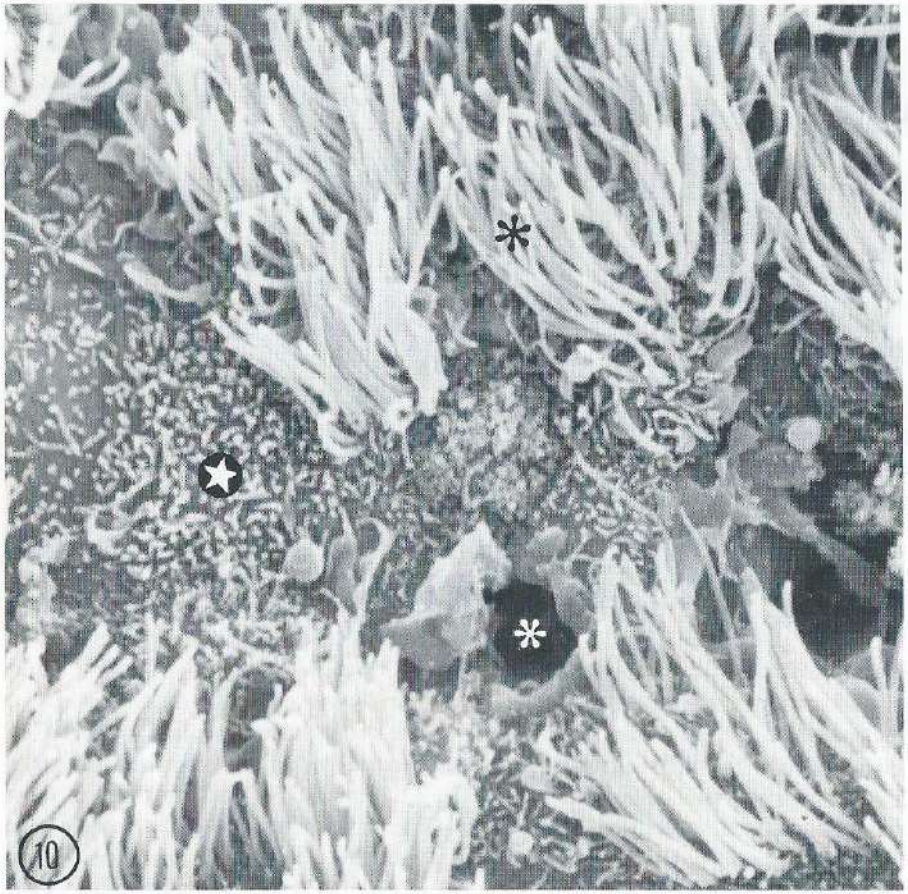


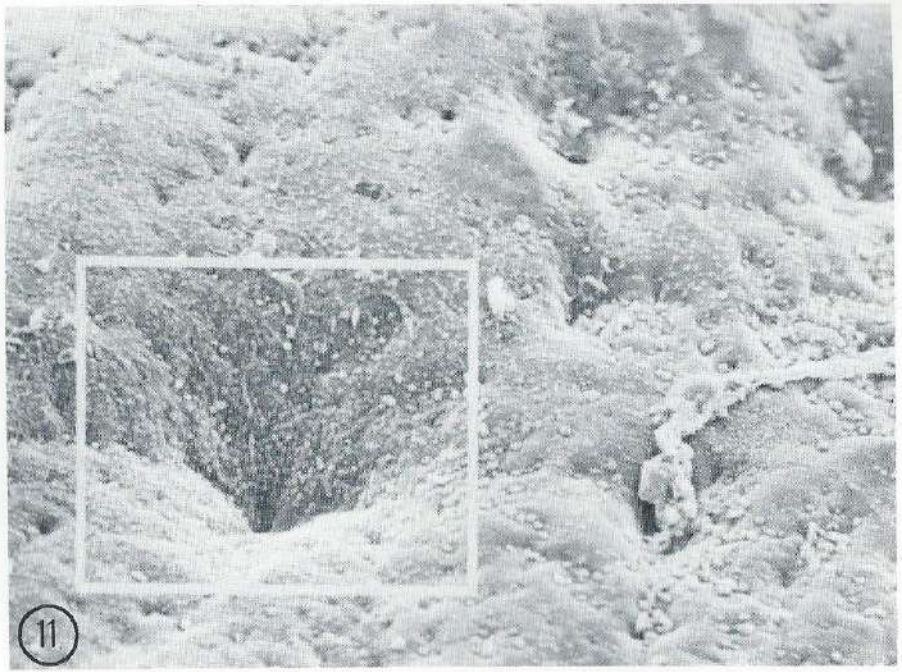
Fig. 4.10. Scanning electron micrograph of ciliated epithelium of a subject without nasal complaints (middle third of the inferior turbinate). Ciliated cells (*), non-ciliated cells with microvilli (☆), and open goblet cells (★) are indicated. Fixation in glutaraldehyde 2%. ×4,300.

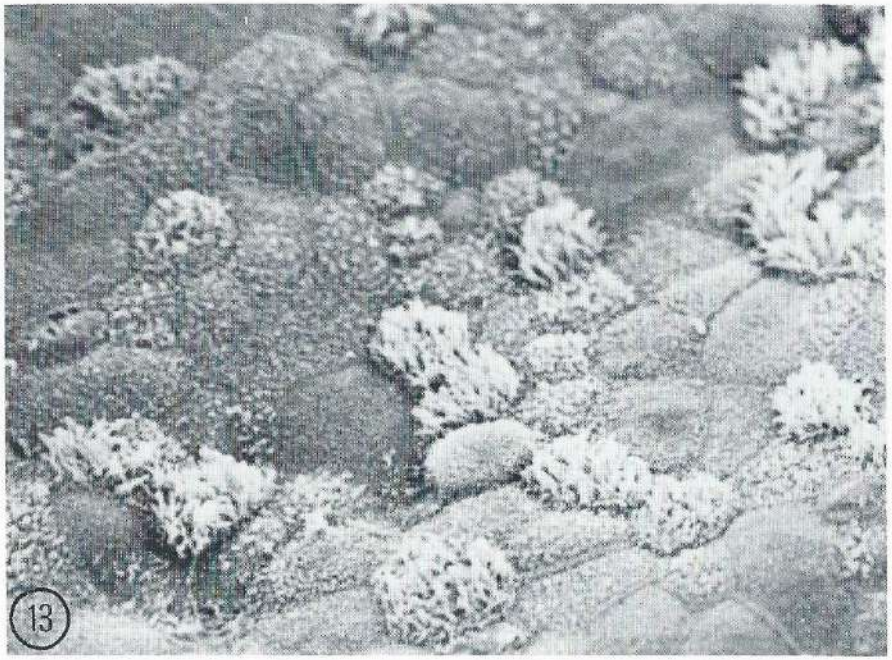
endings of tubulo-alveolar glands were found in this epithelium (Figs. 4.11 and 4.12).

The anterior third of the nasal cavity showed less ciliated and more cuboidal (Fig. 4.13) and squamous-cell (Fig. 4.18) epithelium. Outlets of the anterior nasal glands were found in the latter (Fig. 4.14).

Fig. 4.11. Scanning electron micrograph of area with ciliated epithelium of a subject without nasal complaints (middle third of inferior turbinate), showing funnel-shaped opening of a tubulo-alveolar gland. Fixed in glutaraldehyde. ×160.

Fig. 4.12. Enlarged detail of indicated area in Fig. 4.11. ×370.





Of the four types of epithelium (3.1) found by Boysen in the mucosa over the head of the middle turbinate, SEM showed three in our material (middle third of the inferior turbinate), i.e.:

Type I. Ciliated cylindrical epithelium:

The surface of this epithelium is formed by ciliated and non-ciliated cells and goblet cells. The cilia on the ciliated cells (Fig. 4.15) have a maximal length of about 2 μm and a diameter ranging from 0.03 to 0.1 μm. The distinction between non-ciliated cells and goblet cells is not always simple to make on the basis of SEM.

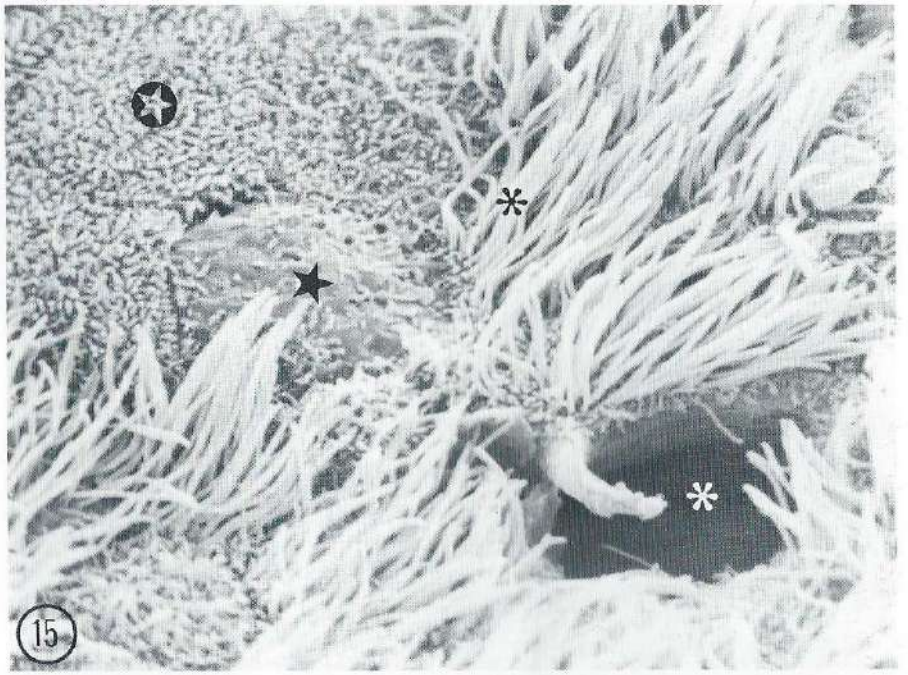


Fig. 4.15. Scanning electron micrograph of ciliated epithelium six months after withdrawal of nose drops in a patient who had used the drops for 7 years. The surface shows a ciliated cell (*), non-ciliated cells with many microvilli (☆), a goblet cell with few microvilli (★), and an open goblet cell (✯). Fixed in glutaraldehyde. ×4,300.

Fig. 4.13. Scanning electron micrograph of epithelium of anterior third of the nasal cavity of a subject without nasal complaints. There are fewer ciliated cells than in Fig. 4.14, and more non-ciliated cells with slightly raised borders, as seen in stratified cuboidal epithelium. Fixed in glutaraldehyde. ×1,700.

Fig. 4.14. Scanning electron micrograph of squamous epithelium from the anterior third of the nasal cavity of a subject without nasal complaints, showing the orifice of an anterior nasal gland. This opening is about 7 times smaller than that of the tubulo-alveolar gland in Figs. 4.15 and 4.16. Fixed in glutaraldehyde. ×1,400.

Goblet cells carry microvilli varying strongly in length and number independent of the secretory stage of the cell. The more secretory granules in the apical part of the cell, the shorter and fewer the microvilli. When the cell is ready to secrete, the surface of the goblet cell is rounded and shows few microvilli (Fig. 4.16). The surface of the non-ciliated cells is always flat and has a larger number of microvilli than a secretory cell has (Fig. 4.15).

Type II. Stratified cuboidal epithelium:

The surface of the stratified cuboidal epithelium is formed by cuboidal cells (without microvilli) with or without secretory granules in the cytoplasm. The more granules in a cell, the rounder its shape. When the surface of the epithelium is formed solely by secretory cells, SEM shows a mucosal surface with a cobblestone appearance (Fig. 4.17). The size and number of the microvilli on the secretory cell of the cuboidal epithelium are dependent, just as for the goblet cells in the cylindrical epithelium, on the amount of mucus in the cell. With increasing amounts of mucus the size and number of microvilli decrease.

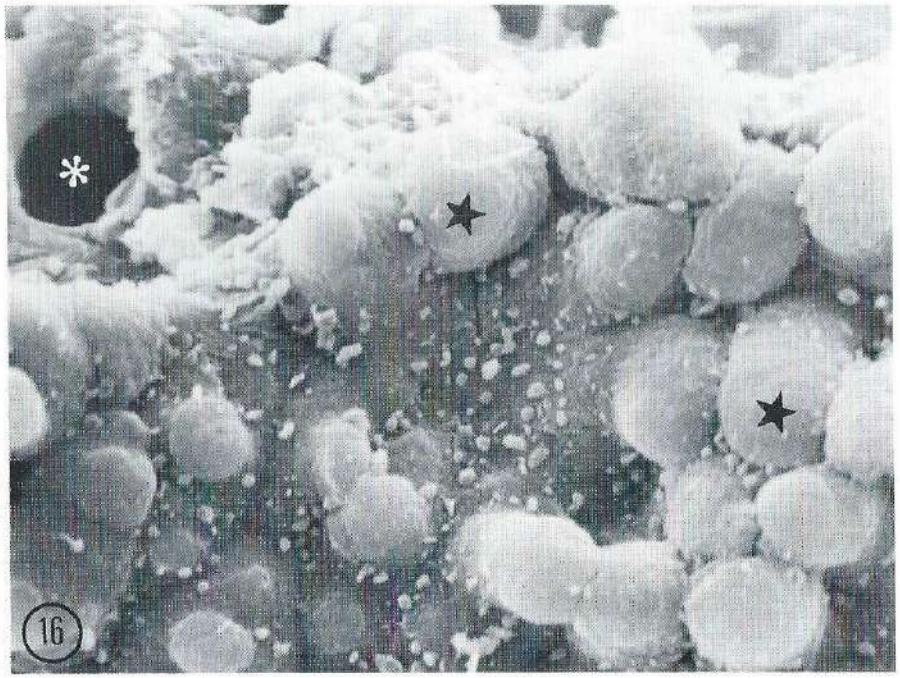


Fig. 4.16. Scanning electron micrograph showing epithelium with a surface formed predominantly of secretory cells, six months after withdrawal of nose drops used for 7 years. There are several distended goblet cells with a smooth surface (★), one of which is still open after secretion (☆). Fixed in glutaraldehyde. ×4,300.

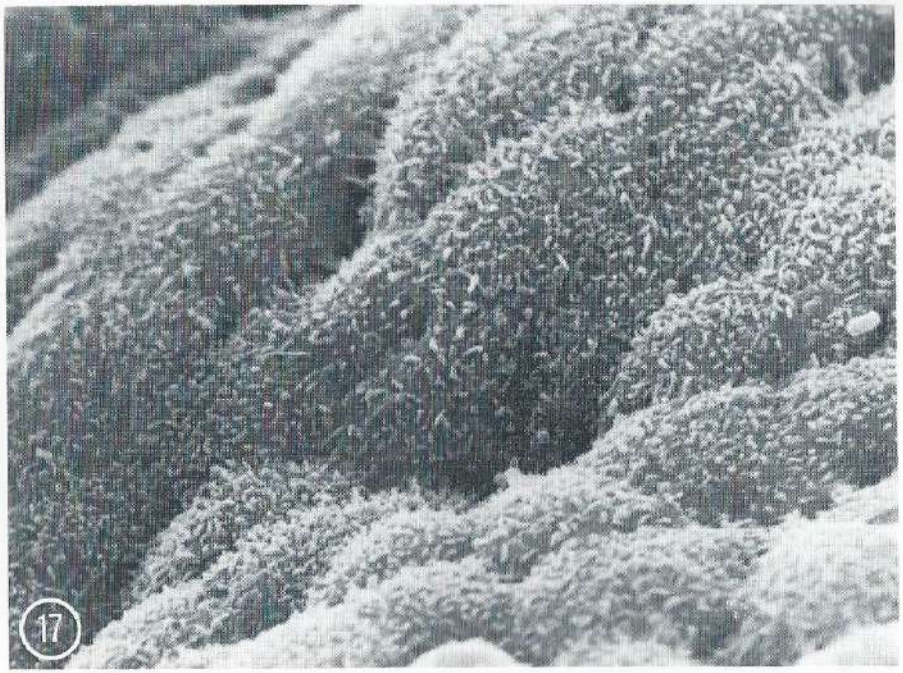


Fig. 4.17. Scanning electron micrograph of nasal mucosa during abuse of nose drops in a patient who had been using the drops for two years. Stratified cuboidal epithelium with the cobblestone appearance. Glutaraldehyde fixation. ×4,000.

Type III. Mixed stratified cuboidal and stratified squamous-cell epithelium:

The surface of this epithelium is formed by flat polygonal cells. The cell boundaries are easily recognizable by their slight elevation relative to the cell membrane. Some of the flat cells overlap each other (Fig. 4.18). In our material the stratified squamous epithelium was not found in the SEM pictures.

The SEM pictures could be analysed for seven of the ten patients whose nasal mucosa (middle third of the inferior turbinate) was investigated histologically during and six months after nose-drop abuse. The various types of epithelium could be recognized from the appearance of the surface, and no morphological changes in the various types of epithelium occurred under the influence of nose-drop abuse.

Because the biopsy specimens reserved for SEM proved rather frequently to be damaged and the epithelium was sometimes obscured by a layer of mucus, it was not possible to quantify the various types of epithelium in each biopt. It was found, however, that each type of epithelium always lies adjacent to one with the preceding or following grade of metaplasia.

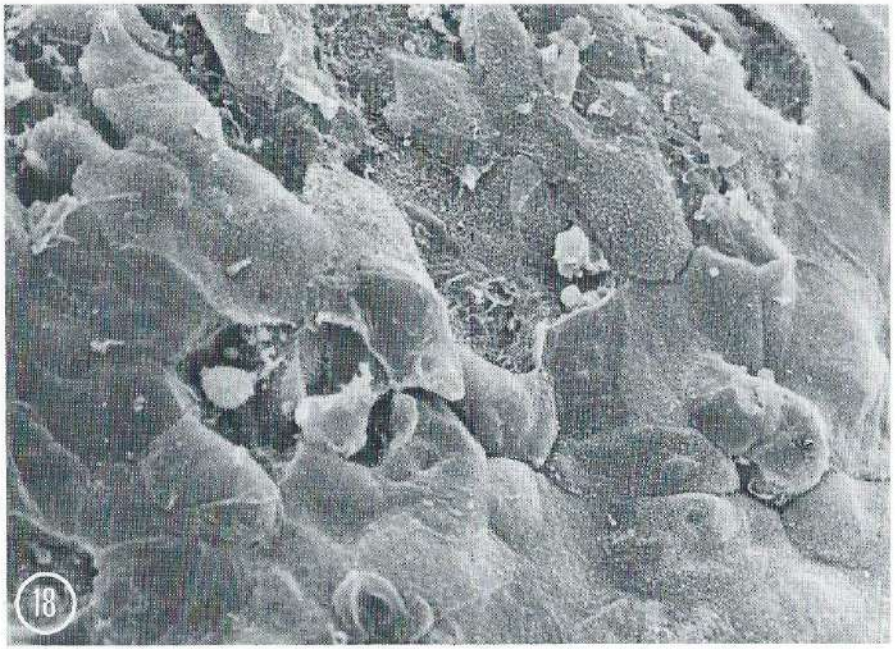


Fig. 4.18. Scanning electron micrograph of nasal mucosa of a patient during abuse of nose drops for 17 years. Mixed stratified cuboidal stratified squamous epithelial cells. On the surface, partially overlapping flat polyglonal cells can be seen. Glutaraldehyde fixation. ×1,300.

4.4. Discussion

In the present study it proved possible to recognize different types of epithelium in the nasal mucosa of the middle third of the human inferior turbinate in the electron-microscopical picture (TEM or SEM). Of the four types described by Boysen (1982) in the anterior part of the middle turbinate, two were found in our material (middle third of the inferior turbinate) by TEM and three by SEM.

With TEM, the ciliated cylindrical and stratified cuboidal epithelia were recognized, and with SEM, ciliated, stratified cuboidal, and mixed stratified cuboidal and squamous cell epithelia were recognized on the surface of the tissue. Classification according to type appeared to be difficult, because transitional stages between the various types of epithelium are also present (2.2). With TEM, these transitional forms were found among the ciliated cylindrical epithelium (all cells in contact with the basal membrane) and the stratified cuboidal epithelium (only the basal and some intermediate cells in contact with the basal membrane). SEM showed that each type of epithelium also lies adjacent to the preceding or following type in terms of degree of metaplasia. These findings support the hypothesis that transition between types of epithelium occurs.

TEM surveys (4.2) provided a good impression of the structure of the various types of epithelium. Ciliated cylindrical epithelium and stratified cuboidal epithelium were found, both during and six months after nose-drop abuse, in the mucosa of the middle third of the inferior turbinate (3.3). Structural changes during and after nose-drop abuse could not be demonstrated electron microscopically, as in the work done by Petruson and Hansson (1982). The different types of cell and their organelles were studied with TEM. Striking aspects were shown by the intermediate cell, whose place, as to both localization and differentiation, falls between the basal and superficial cells. The intermediate cell is found in both the cylindrical and the cuboidal epithelia and sometimes contains secretory granules; this suggests that it is the precursor of the secretory cells which come to lie on the surface of the mucosa. In one patient intermediate cells showing two kinds of secretory granule were found, i.e., large electron-lucent and small electron-dense granules, possibly representing two kinds of glycoprotein (Jefferey 1973).

Secretory cells

The secretory cells which come to lie on the surface of the mucosa include not only the classic goblet cell (cylindrical cell filled with secretory granules) but also the cuboidal cell which has secretory granules either everywhere or only in the apical part of the cell. Jefferey (1973) and Baskerville (1975) distinguished between type F (full) and type S (small) secretory cells, and Jones (1978) called them type L (large) and type S (small). These subdivisions represent an attempt to distinguish between cells whose cytoplasm is virtually stuffed with secretory granules (F and L) and cells with secretory granules only in the apical part of the cytoplasm (S). It is not clear from the literature whether F and L refer solely to classic goblet cells or also to cuboidal cells whose cytoplasm is virtually filled with secretory granules. In principle, four types of secretory cell can be found:

1. Cylindrical cell filled with secretory granules (= goblet cell)
2. Cylindrical cell with secretory granules in the apical region
3. Cuboidal cell filled with secretory granules
4. Cuboidal cell with secretory granules in the apical region

The second of these types did not occur in our material. This division into types might be related to the maturation phase of a mucus-containing cell, but it is not always possible to determine the phase a cell represents on the basis of the microscopical picture. It may be asked, for instance, whether a cuboidal cell with secretory granules in the apical region is a cell which is in the process of filling with secretion and is therefore not yet "ripe" or a cell which has just released its secretion and is therefore "over-ripe". In view of the foregoing considerations, the value to be assigned to the subdivision of secretory cells into F, S, and L types seems limited.

The conclusion reached by such authors as Hilding and Hilding (1970) that secretory cells with cilia do not occur in the respiratory epithelium, is confirmed by the findings in our material. Our results indicate, furthermore, that the degree to which a secretory cell is filled with secretory granules can be assessed from the

number of microvilli on its surface membrane. The more secretory granules in the apical area of the cytoplasm, the fewer microvilli are found on the cell surface.

Glycoprotein composition

In a TEM study, Jefferey (1973) found changes in the glycoprotein composition of the secretory granules in the respiratory epithelium of the lowermost air passages of the rat under the influence of tobacco smoke. We used TEM to compare the secretory granules during and after nose-drop abuse. In half of our cases electron-lucent granules were found during and after abuse, and the other half showed a shift from electron-lucent granules with an electron-dense core during abuse to exclusively electron-lucent granules six months after withdrawal of the nose drops. After cessation of abuse there is a shift from neutral to acid glycoproteins. These findings are in agreement with the results of the histochemical study (3.3.2). The above-mentioned shift is not characteristic of nose-drop abuse. Nevertheless, it can be seen as a reflection of mucous membranes stimulated by the misuse of nose drops.

The electron-lucent granules in cells occurred either alone or in combination with electron-lucent granules having an electron-dense core. It is conceivable, however, that the former granules had a core which was not sectioned.

With respect to the size of the secretory granules, our findings confirm Boyson's (1982) conclusion that in general, secretory granules in metaplastically changed respiratory epithelium are smaller and less likely to fuse than are those of the goblet cells in cylindrical epithelium.

Lysosome-like bodies

In addition to the secretory granules found in the cytoplasm of the superficially situated cells, smaller granules were also encountered. Light microscopically, the latter showed strong staining after exposure to toluidine blue. Comparison of the light-microscopical and TEM pictures of serial sections showed that the toluidine blue staining granules correspond with round to oval inclusions with a diameter of about 0.5 μm and surrounded by a single membrane. Three types of inclusions were found in our material, i.e., electron-dense material with a lamellar structure, electron-dense material with granules, and electron-dense material containing a number of uniformly sized electron-lucent vesicles (multivesicular bodies). Morphologically, these inclusions resemble the lysosome-like bodies described in the literature (Daems et al. 1969; Ginsel et al. 1973; Holtzman 1976). In our material the lysosome-like bodies were found in the apical part of the cells with and without cilia in the cylindrical epithelium and in the apical part of the cells without cilia (and without secretory granules); in the mucus-containing cells (type S) they occurred in the cuboidal epithelium. No marked difference was found between the numbers of lysosomes during and after nose-drop abuse, but we obtained the impression that both during and after nose-drop abuse the multivesicular bodies were found less frequently than the electron-dense material with lamellar structure and the electron-dense material with granules.

Ultrastructure of the cilia

Herzon (1981) has described the ultrastructure of the cilia in patients with various rhinological diseases including nasal polyps, Kartagener's syndrome, mucoviscoidosis, and also what he calls rhinitis medicosa. In connection with the last of these conditions he described cilia with an anomalous pattern (9+4 and 9+3 instead of 9+2) or lacking radial spokes. Talaat et al. (1981) described abnormal cilia in rabbits given 1% ephedrine nose drops three times a day for two or three weeks. After two weeks of this treatment the cilia showed clumping into large groups. Because these authors did not observe the 9+2 patterns, they concluded that the ultrastructure of the cilia is lost after two weeks of the above-mentioned nose-drop treatment.

According to our findings (3.3), the percentage of ciliated epithelial cells is lower during than after abuse. Because cilia were found at both times, we were able to compare the structure during and after abuse. Neither TEM nor SEM showed structural changes between the two time-points, and the same was found in healthy subjects. Thus, our results are not in agreement with those of Herzon and of Talaat et al. but do confirm those of Petruson and Hanssen (1982), who studied human respiratory epithelium (of the middle third of the inferior turbinate) and found no SEM changes in the cilia after six weeks of nose-drop use (Otrivin) by healthy subjects without nasal complaints. These last authors did not describe any TEM changes, but saw no difference in the mucociliary function after six weeks of administration of nose drops.

Chapter 5

Influence of nose-drop abuse on nasal patency and the nasal cycle

5.1. Introduction

5.1.1. *Nasal patency*

Poor nasal patency is the most prominent complaint of patients who have used nose drops excessively for a long time. It is this complaint which leads the patient to continue using the drops and eventually makes him an abuser. When these patients are examined, anterior rhinoscopy often gives the impression that the patency of the nasal passages is adequate, but the patients claim that these passages feel obstructed. This discrepancy led us to assess the nasal patency of patients who had made excessive use of nose drops.

5.1.2. *Determination of nasal patency*

Several methods for the measurement of nasal patency are described in the literature. Good historical reviews can be found in Uddströmer (1940), Guillerm et al. (1961), Keuning (1968), Williams (1968), and Graamans (1980). The simplest method was described by Zwaardemaker in 1889 in a paper entitled "Adembeslag als diagnosticum der nasale stenose". This method is based on the relationship between the patency of the nasal cavity and the size of the condensation patches produced on a cold mirror by the air expired through the nose. Since then, many investigators have attempted to quantify the patency of the nasal passages. The principle underlying these measurements concerns the relationship between the transnasal pressure drop and the resulting airflow in the nose. The measurement of this pressure drop is called rhinomanometry, and the measurement of the flow induced by this drop is called rhinorheometry. When the airflow and pressure drop are measured simultaneously, the procedure is called rhinorheomanometry.

The pressure and flow measurements can be performed both actively and passively. For passive rhinometry the subject holds his breath while air under a known pressure or flow is led through the nasal passage and the resulting flow or pressure drop is measured. For active rhinometry the subject breathes through his nose and the pressure drop and flow are determined simultaneously. For measurement of the pressure in the posterior part of the nasal passage, i.e., in the nasopharynx or oropharynx, a tube connected to the manometer is introduced into the oral cavity under airtight conditions (Fig. 5.1). Alternatively, the manometer

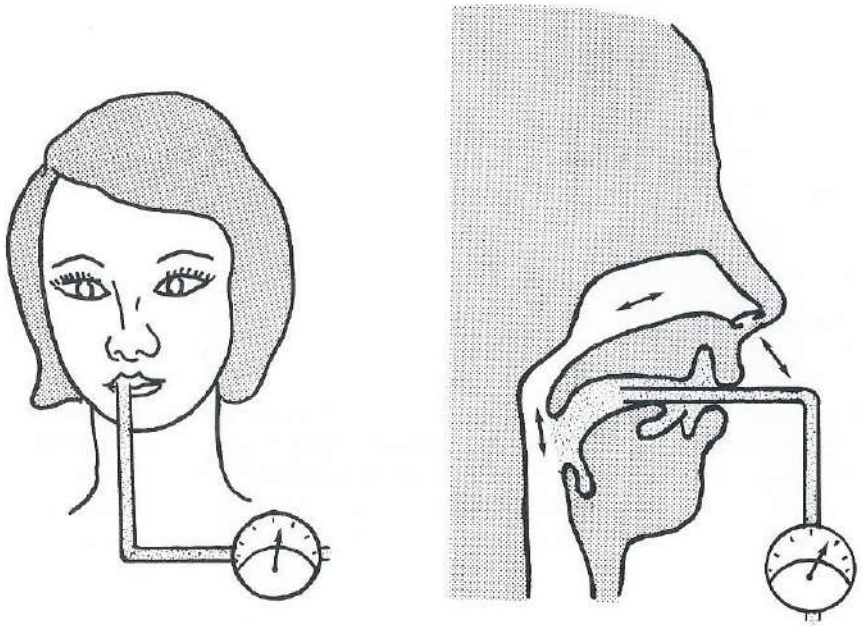


Fig. 5.1. Posterior rhinomanometry. The nasopharyngeal pressure is measured after the manometer tube has been placed in the mouth under airtight conditions, the subject breathing through the nose. Since there is no airflow in the oral cavity, this cavity can be seen as a continuation of the manometer tube up to the oropharynx. The manometer thus registers the pressure in the oropharynx, which is roughly the same as that in the nasopharynx.
⟷: air current in nasal cavity, nasopharynx, oropharynx, and hypopharynx.
▒: oral cavity without air current

tube can be placed in the oral cavity or in one side of the nose, which therefore cannot develop an air flow or a pressure difference. Under such conditions the oral cavity or that side of the nose can be considered as a continuation of the tube attached to the manometer. The manometer will then register the pressure in the oropharynx or nasopharynx, which together with the pressure anterior to the nose determines the drop in presure within the nose (Fig. 5.2).

With both methods the difference between the pressure in the nasopharynx and anterior to the nose is measured. During anterior rhinometry the patient breathes through the contralateral nostril, and for posterior rhinometry he breathes through both nostrils. Combining of these principles has led to the development of the following methods to assess nasal patency.

1. *Passive anterior rhinometry,* which can be subdivided into two groups:

   a. *Passive anterior rhinorheometry.* Air under known pressure is led through the nose and the flow is measured. The introduced air can leave the body either via

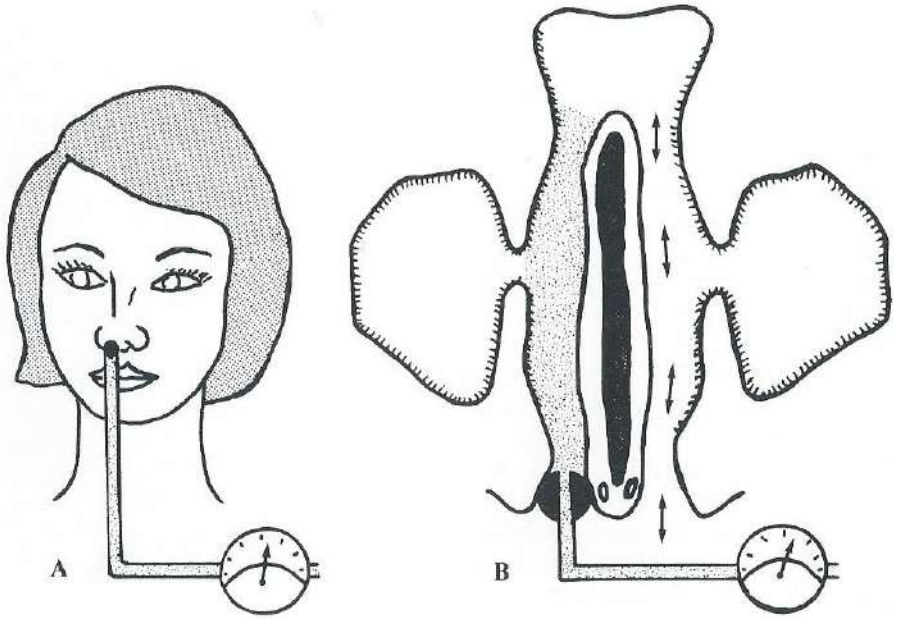


Fig. 5.2. Anterior rhinomanometry. The nasopharyngeal pressure is measured with the tube of the manometer in one vestibule under airtight conditions, the subject breathing through the other side of the nose. Since there is no airflow in the nasal cavity connected to the manometer, this cavity can be seen as a continuation of the manometer tube up to the nasopharynx. The manometer thus registers the nasopharyngeal pressure.
⟷: air current in one side of nasal cavity
▒: opposite nasal cavity without air current

the opened mouth or the other nostril with the mouth closed. This method has been described by e.g. Kayser (1895), Courtade (1903), and Benesi (1911).

b. *Passive anterior rhinomanometry.* Air is led through the nasal passage at a known flow and the pressure needed to maintain that flow is measured. This method has been discussed by van Dishoeck (1935), Malcolmson (1959), McLaurin et al. (1960), Ingelstedt and Runderantz (1964), Cottle (1968), Knothe and Aschoff (1969), Bentley and Jackson (1970), and van Dishoeck and van Dishoeck (1970).

2. *Active anterior rhinorheomanometry:* The flow is measured during inhalation through one nostril. The pressure in the other side of the nose is measured at the same time and is considered to represent the pressure in the nasopharynx. This method has been discussed by e.g. von Sémerák (1958), Cottle (1960), Soubeyrand (1963), von Arentschild (1966), Guillerm et al. (1966), Keuning (1968), Akyildiz (1969), Kortekangas (1971), Jenssen (1973), and Masing (1974).

3. *Passive posterior rhinomanometry:* Air is led through the nose at a known flow and is released via the mouth. The pressure required to achieve this flow is measured in the mouth or in the pharynx. This method has been discussed by e.g. Scheideler (1939), Seebohm and Hamilton (1958), Perks and Rozner (1964), Richardson and Seebohm (1958), and van Dishoeck and van Dishoeck (1970).

4. *Active posterior rhinomanometry:* While the patient continues to breathe regularly the pressure in the pharynx or oral cavity is registered and at the same time the flow in the nasal passages is measured. This method has been described by e.g. Sternstein (1937, 1941), Aschan et al. (1958), Guillerm et al. (1961), Klajman and Sitowski (1961), Pattle (1961), Craig et al. (1965), Masing (1965, 1967), Solomon et al. (1965), Solomon and Stohrer (1965), Spoor (1967), Cohen (1969), Ingelstedt et al. (1969), Taylor and Shivalkar (1971), Feenstra (1972), Kern (1973), Barrée and Feenstra (1974), and Warren et al. (1974).

In passive rhinometry the airflow in the nasal passages is usually non-physiological, and for this reason active rhinometry is to be preferred. It must, however, be kept in mind that during active rhinomanometry, with one side of the nose closed off, the volume of the air on the other side will be greater than when both sides are open. Anterior rhinometry cannot be performed where one side of the nose is blocked (for example by severe deviation of the septum making it impossible to measure the pressure in the nasopharynx) or where the septum is perforated. Furthermore, in anterior rhinometry the introduction of the measuring instruments into the nose can easily lead to deformation of the vestibule and the valve. Because with this method the subject breathes through one side of the nose, non-physiological air currents can arise more easily under the non-physiological conditions and the resistance can only be measured in each side of the nose separately, not the total resistance.

On these grounds we chose for the present study active posterior rhinorheomanometry performed with the conductivity meter designed by Spoor (1967). This instrument measures simultaneously the air flow ($\dot{V}$) through the nose and the associated pressure drop (P), and yields the conductivity (C) automatically. $\dot{V}$, P, and C are registered continuously. The conductivity meter can be used to measure not only the conductivity of the total nose but also that of the two sides of the nose separately. For the latter, a small wad of cottonwool is carefully introduced into one of the vestibules, so that an air flow can only occur on the other side. However, two conditions must be satisfied: after insertion of the cottonwool, there must be no

contact between it and the mucosa of the nasal cavity and no deformation of the valve area on the other side. The changes in the conductivity of both sides of the nose provides an impression of what is called the nasal cycle.

5.1.3. *Nasal cycle*

The nasal cycle was first described in 1889 by Kayser, who found alternating changes in the conductivity of the passage of both sides of the nose and called this pattern the nasal cycle. He ascribed this physiological phenomenon to changes in the volume of the blood in the nasal mucosa. According to his view, the nasal cycle had no influence on the conductivity of the nasal passages. Lilly (1923) described patients who complained frequently that first one side of the nose and then the other felt obstructed, and he too called this a physiological process, one in which the mucosa on one side swelled to the point of causing obstruction while the other side opened and "released its secretions". Heetderks (1927), who performed a rhinoscopic study on the effect of various climatological conditions on the nasal mucosa, found a nasal cycle in 80 per cent of his subjects, most prominent in adolescents and much less distinct in older individuals. Stoksted (1952) too found a nasal cycle in 80 per cent of his series. He was the first to investigate the cycle rhinomanometrically and his findings confirmed Kayser's (1895) conclusion that the total resistance in the nose remains roughly the same.

Flottes et al. (1961) attempted to determine the mean duration of the nasal cycle. Of their subjects, 80 per cent showed a cycle lasting between 2 and 5 hours, with a mean of 3.5 hours, and 20 per cent one of between 5 and 8 hours. In individuals the duration of the cycle proved to be constant. These authors also investigated the effect of vasoconstrictive nose drops, and found that the cycle disappeared after the administration of such drops and returned after the effect of the drops had ceased. In addition, these authors established the duration of the effect of various types of nose drops.

Keuning (1968) found a nasal cycle in 80 per cent of a group of 17 volunteers aged between 20 and 30 years. In this series the shortest cycle lasted 2 hours, the longest 7 hours, and the mean duration was 4 hours and 20 minutes. In children aged between 3 and 6 years, van Cauwenberge and Deleye (1984) found not alternating but parallel changes in the two sides of the nose, and therefore concluded that the conductivity changed simultaneously on both sides.

The mechanism underlying the regulation of the cyclic swelling and shrinking of the nasal mucosa is not known.

5.2. Methods and materials

The composition of the group of 21 patients who used nose drops excessively is described in section 1.7. Under abusers of nose drops we understand here patients who have used xylometazoline nose drops at least once a day for a period longer

than six months. The following groups of patients were excluded from the study because of affections that can lead to changes in the nasal mucosa:

a. patients with a tumour of the nose or sinuses (e.g., polyposis nasi);
b. patients accustomed to inhaling or exhaling tobacco smoke through the nose;
c. patients with demonstrated atopy;
d. patients applying more than one drug to the nose at the same time; and
e. pregnant patients.

The group of 21 patients selected in this way, comprised 12 woman and 9 men. The youngest was 16 years old, the oldest was 75. Sixteen of the 21 were aged between 16 and 32 years.

To exclude a direct effect of xylometazoline at the time of the measurements, the patients were asked to use nose drops for the last time on the evening before the conductivity assessment (i.e., at least 12 hours after the last application of drops). Anterior rhinoscopy was performed before the measurements, and the physician's subjective impression of the patency of the nose was recorded. The measurements were repeated six months after termination of the abuse of the nose drops (see under *Treatment schedule* §1.8). The nasal conductivity measurements were performed under the same climatological conditions in all patients.

5.2.1. *Spoor's (1967) conductivity meter*

In the original design for this meter, the flow ($\dot{V}$) was measured via a tube with an inner diameter of 10.5 mm. The difference between the pressure on each side of the diaphragm was measured with a differential pressure transducer of the strain-gauge type. The output of the transducer proved to be proportional to the square of the flow. In the rhinomanometer used in the present study the tube and diaphragm have been replaced by a Fleisch tube, i.e., a tube containing a large number of very small tubes, making the pressure difference proportional to the flow, which also holds for the output of the flow transducer. The Fleisch tube is attached to a mask which covers the entire nose (Fig. 5.3). The pressure difference between the nasopharynx (measured via an oral tube) and the mask enclosing the nose is recorded by a differential pressure transducer.

The electrical output of the transducer used to measure the pressure in the nose is proportional to that pressure. Both electrical outputs (flow and pressure) are fed into an electronic divider. When a diaphragm is present in the flow tube, the results of the measurements are proportional to $\dot{V}^2/P$. If we call this quotient C^2, we have $C^2 = c.\dot{V}^2/P$, in which c is a constant. By calibrating the instrument appropriately, we can have $c = 1$ and $C^2 = \dot{V}^2/P$. This means that C will represent the square of the flow at a pressure of 1 cm water. With the use of the flow tube provided with a diaphragm, the measured values are registered on a quadratic scale. The curve plotted on log paper then shows the flow at a pressure of 1 cm water. Spoor (1965) called the value of C the conductivity, and showed that during normal respiration C is constant and independent of the depth of the respiration. With the Fleisch tube,

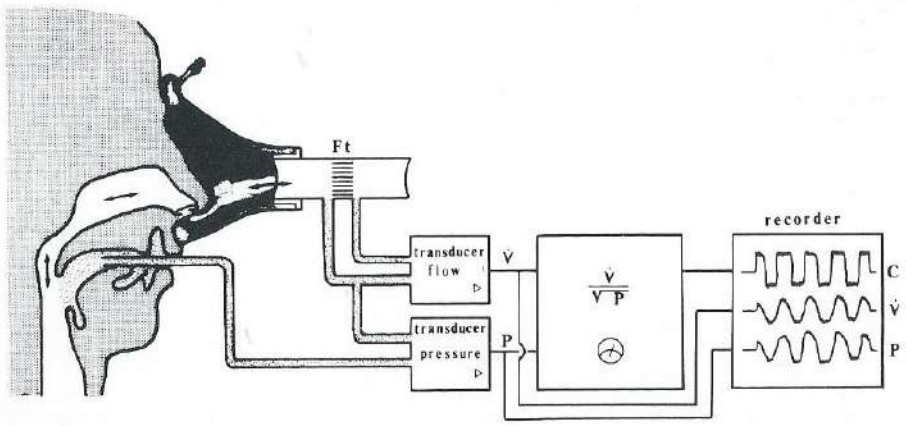


Fig. 5.3. Schematic representation of Spoor's conductivity meter in the experimental set up. The air flow in the nose is determined via a nose mask attached to a Fleisch tube (Ft). A transducer converts the flow V into an electrical voltage output. The pressure drop in the nose represents the difference between the pressure anterior to the nose (in the mask) and that in the posterior part of the nose (in the nasopharynx), which is measured via a tube placed in the mouth. The pressure drop P is also converted into voltage output by a transducer. The conductivity C is calculated electronically according to the formula $C^2 = \dot{V}^2/P$, thus $C = \dot{V}/\sqrt{P}$. The conductivity, flow, and pressure are registered simultaneously by a recorder during a number of inhalations and exhalations.

the pressure drop in the tube is proportional to the flow, and the scale on the meter of the instrument and the tracing registered on the paper are linear. For the calculation of the conductivity, the value of the flow is automatically divided by the square root of the pressure.

Exhalation gives an ascending line (positive values) and inhalation a descending line (negative values), and this holds for not only the conductivity but also for the flow and the pressure (Fig. 5.4).

In all patients the conductivity was measured in both sides of the nose separately and simultaneously at 15-minute intervals. Because the data in the literature (e.g., Flottes et al. 1961; Keuning 1968) and a preliminary study in subjects without nasal complaints indicated that the mean duration of a nasal cycle is approximately four hours, the measurements were performed over a period of at least that duration. If conclusive evidence of the existence of a nasal cycle was not obtained within this period, supplementary measurements were performed to register a cycle of longer duration, if any. For all measurements the pressure and flow were measured simultaneously first. After that, one nostril was blocked by careful insertion of a wad of cottonwool into the vestibule such that there was no contact between the wad and the mucosa of the nasal cavity and no deformation of the vestibule and valve on the other side of the nose. Next, the pressure, the flow, and the resulting conductivity were measured on the unblocked side. The same procedure was then performed for measurements on the first side, the meter providing the values for the

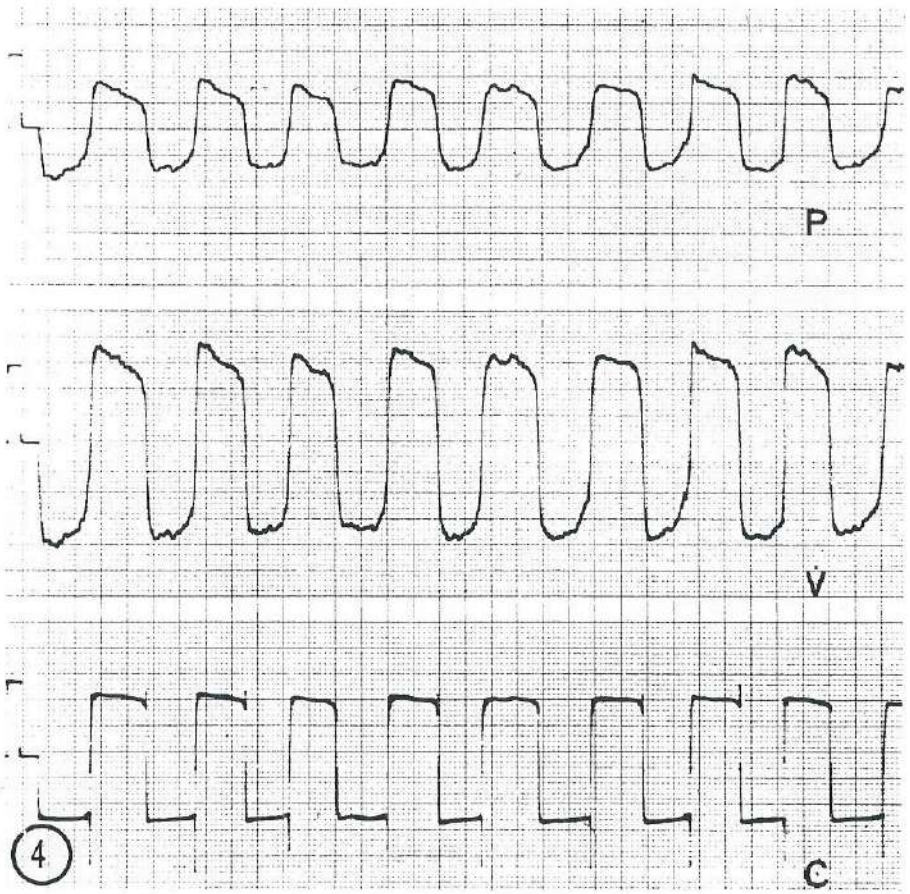


Fig. 5.4. Tracing showing simultaneous registration of the pressure (P), flow ($\dot{V}$), and conductivity (C) in both sides of the nose in a healthy subject.

conductivity of the total nose as well as of each side separately. The latter values gave an impression of the nose cycle where present. In the literature (see section 5.1.3) the nasal cycle is usually only described qualitatively, i.e., as opposite changes in the nasal patency alternating between the two sides of the nose.

5.2.2. *Definition of the nasal cycle*

The nasal cycle has been described qualitatively in section 5.1.3. The literature does not offer a quantitative definition, and we know of only one attempt to arrive at a qualitative definition (Hasegawa and Kern 1978), but the result is incomprehensible, at least to us. We define the nasal cycle qualitatively as an alternating

change in the conductivity of the two sides of the nose. On this basis, the following quantitative condition can be added: the difference between the maximum and minimum values for the difference in conductivity between the two sides of the nose must amount to at least a certain percentage (χ) of the average of the conductivity on both sides during the same period. This can be written:

$$(C_r - C_l)_{max} - (C_r - C_l)_{min} = \frac{\chi(C_r + C_l)_{mean}}{100} \quad .$$

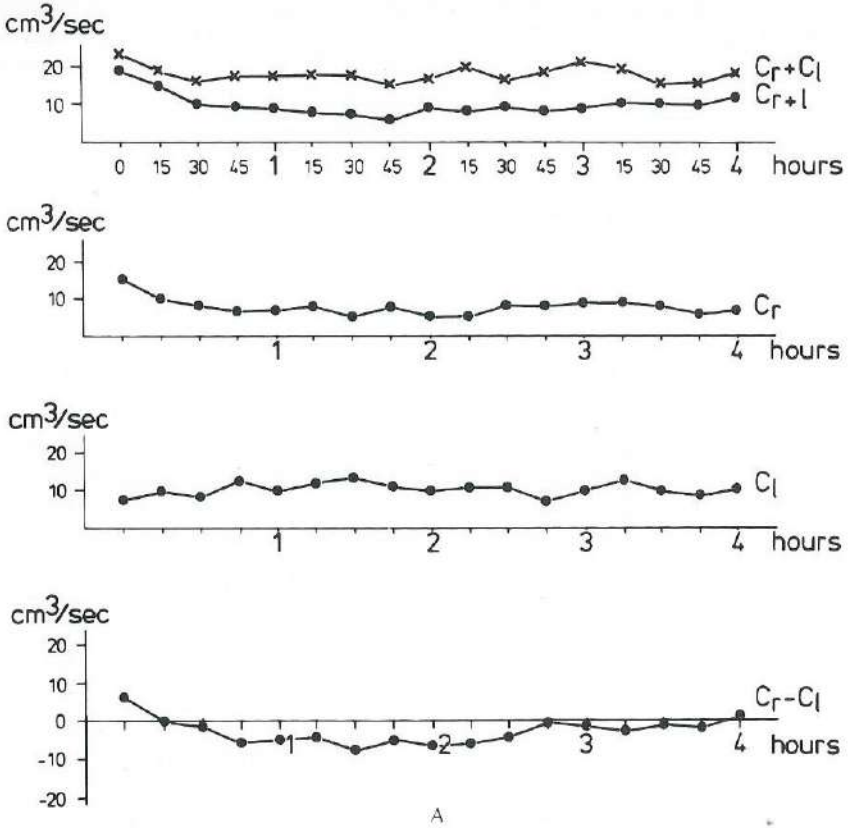
5.3. Results

Fig. 5.5a shows the results of conductivity determinations in a patient who had used nose drops excessively for five years. According to our definition (see 5.2.2) this patient showed a nasal cycle.

Alternating conductivity between the sides of the nose did not occur and was not apparent after subtraction of the conductivity of one side of that of the other ($C_r - C_l$). According to our definition (see 5.2), this patient did not have a nasal cycle. Fig. 5.5b shows the results of corresponding measurements in the same patient six months after withdrawl of the nose drops. The conductivity of both sides of the nose has increased relative to that during abuse, and an alternating pattern is evident. Subtraction of the conductivity of the left side from that of the right ($C_r - C_l$) shows the presence of a nasal cycle. Calculation of χ (see 5.2) shows that this percentage amounts to 63.5. This means that according to our definition a nasal cycle was present.

When anterior rhinoscopy was performed just before the first measurement (i.e., when nose drops had not been used for 12 hours) we invariably obtained the impression that nasal patency was worse than at the first visit. Questioning revealed in all cases that the patient had used nose drops less than 8 hours before the first examination. The rhinomanometric results are shown in Table 5.1.

In Table 5.1 the patients are listed in order of increasing duration of abuse of nose drops. Twenty of the 21 patients showed an increase (ranging from 19 to 383 per cent) in the mean conductivity values of the entire nose measured at 15-minute intervals. The mean increase amounted to 109 per cent. One patient (no. 18) showed neither an increase nor a distinct decrease of the conductivity. During abuse of nose drops, none of the patients showed a nasal cycle. Six months after the withdrawal of the drops, a nasal cycle was found in 6 of the 21 patients; the mean age of this group was 21 years. When the presence of a nasal cycle could be demonstrated qualitatively the change in conductivity proved to be substantial. Thus, the criterion in our definition that χ must be greater than 10 per cent was more than satisfied, the value ranging between 64 and 136 per cent.



A

Fig. 5.5, a and b. Conductivity measurements during (a) and 6 months after termination of (b) abuse of nose drops lasting 5 years.

C_{r+l} : conductivity of right and left sides together
C_r : conductivity of the right side
C_l : conductivity of the left side
$C_r - C_l$: conductivity of the right side minus conductivity of the left side
$C_r + C_l$: conductivity of the right side plus conductivity of the left side

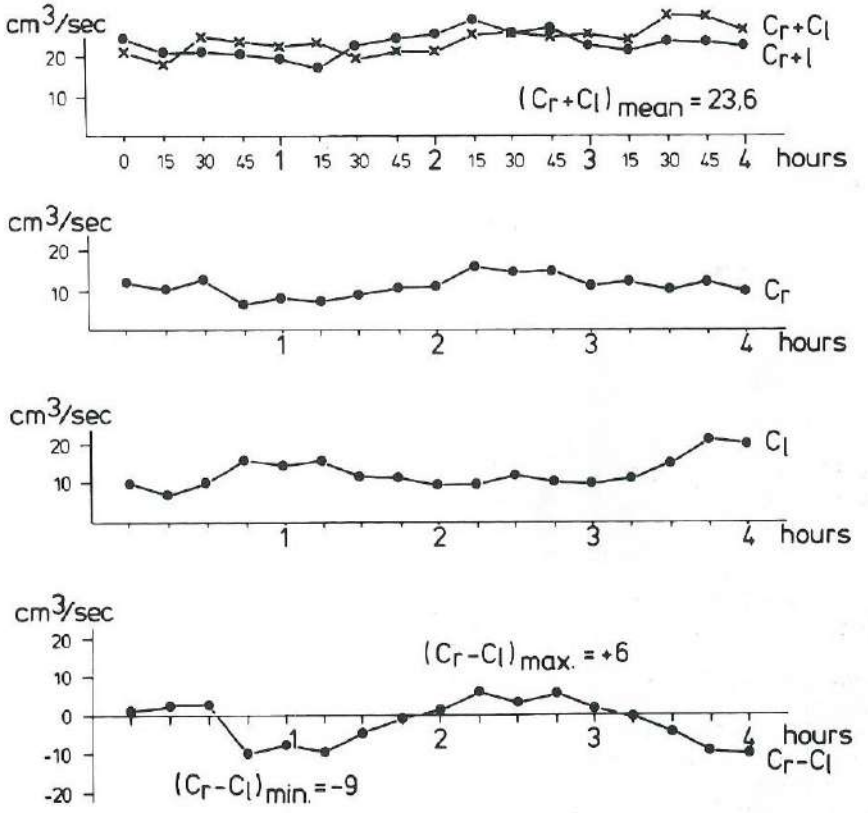
a: No alternating changes can be seen for either side of the nose, and the same holds when the conductivity on the left side is subtracted from that on the right ($C_r - C_l$). When the conductivity values for the two sides of the nose are added together ($C_r + C_l$), the resulting curve lies above that of the curve obtained by simultaneous measurement of the conductivity on both sides (C_{r+l}). This difference is explained by the fact that when one side of the nose is closed off, the volume of air transported via the other half is greater than its share under normal conditions. (For this compensatory pattern, see section 5.4).

b: The combined conductivity (C_{r+l}) has increased as compared with the corresponding value during abuse, and the changes in conductivity on the right and left sides now show alternation. Subtraction of the conductivity on the left side from that on the right side ($C_r - C_l$) shows this change even more clearly.

$(C_r - C_L)_{max} = +6$
$(C_r - C_l)_{min} = -9$
$(C_r + C_l)_{mean} = 23.6$

$$\chi = \frac{(C_r - C_l)_{max} - (C_r - C_l)_{min}}{(C_r + C_l)_{mean}} \times 100\%$$

$$\chi = \frac{(6) - (-9)}{23.6} \times 100\% = 63.5\%$$



B

Table 5.1. Conductivity (measured simultaneously on both sides of the nose, C_{r+l}), increase of the mean conductivity, and presence of a nasal cycle during and 6 months after cessation of abuse of nose drops in 21 patients. Changes in conductivity are expressed as per cent (conductivity during abuse = 100%). Statistical analysis performed with Student's t-test (significant at $p < 0.05$). Qualitatively, a nasal cycle could not be detected during abuse, but a cycle was clearly visible in 6 patients, 6 months after cessation of abuse.

			both sides together (C_{r+l})$_{mean}$				after cessation of abuse		
pat. no.	age (yr)	duration abuse (yr)	(C_{r+l})$_{mean}$ during abuse	(C_{r+l})$_{mean}$ 6 months after cessation abuse	Increase of (C_{r+l})$_{mean}$ (%)	Alternating changes in the conductivity of the two sides of the nose ($C_r - C_l$)	χ (in %)	Cycle
1	35	24	9.8 ± 2.5	27.6 ± 4.8	182 (s)			
2	49	23	13.9 ± 6.6	25.3 ± 3.7	82 (s)			
3	49	17	14.1 ± 4.0	24.3 ± 1.7	74 (s)			
4	23	10	10.3 ± 4.7	27.4 ± 4.2	166 (s)			
5	75	7	21.2 ± 1.3	29.2 ± 9.2	38 (s)			
6	22	6	16.4 ± 3.5	25.1 ± 5.7	53 (s)			
7	27	5	29.5 ± 12.6	40.3 ± 6.3	37 (s)			
8	30	5	19.7 ± 1.5	40.6 ± 3.6	106 (s)			
9	20	5	10.5 ± 3.0	23.8 ± 3.3	127 (s)	yes	64	+
10	29	4	7.1 ± 3.8	34.3 ± 12.3	383 (s)			
11	35	4	3.6 ± 2.7	8.9 ± 3.6	147 (s)			
12	38	4	22.2 ± 5.2	34.4 ± 2.6	55 (s)			
13	16	3	20.6 ± 3.7	35.4 ± 5.6	72 (s)	yes	64	+
14	19	3	11.7 ± 6.8	22.9 ± 7.6	96 (s)	yes	104	+
15	42	3	5.3 ± 1.3	9.8 ± 2.1	85 (s)			
16	34	3	7.4 ± 1.5	36.3 ± 6.6	391 (s)	yes	80	+
17	20	2	10.5 ± 5.3	13.2 ± 3.5	26 (ns)	yes	136	+
18	29	2	12.7 ± 2.6	11.7 ± 4.0	−7.9(ns)			
19	19	1	14.1 ± 5.1	26.8 ± 5.0	90 (s)	yes	82	+
20	51	0.5	7.3 ± 5.6	24.1 ± 4.3	230 (s)			
21	16	0.5	15.3 ± 6.8	18.1 ± 4.5	29 (ns)			

s: significant; ns: not significant; +: nasal cycle present.

5.4. Discussion

Patients who abuse nose drops complain that their nasal passages feel obstructed. When such patients are examined, the physician has the impression that they ought to be able to breath fairly well through the nose. If such patients are then asked not to use nose drops for at least 12 hours before the next examination, the same physician receives the impression that the condition of the nasal passages is poor. Closer questioning reveals that the patient used nose drops less than about eight hours before the first examination. Since the mean duration of the effect of xylometazoline nose drops is about eight hours, the discrepancy between the complaints about obstruction and the impression of adequate passages at the first examination can be explained by the use of nose drops during the eight hours preceding the first examination.

Patients who cease to abuse nose drops note improvement of the nasal passages after some time. In the present study, Spoor's conductivity meter was used to obtain a quantitative picture of this improvement. The improvement of the conductivity of the nasal passages may in all probability be ascribed to the decrease in the thickness of the lamina propria, which has become swollen during the abuse of the nose drops. This decrease may in turn be ascribed to the calibre of the blood vessels in the lamina propria (Bende 1983). The conductivity meter also permits assessment of the conductivity of each side of the nose separately. When the conductivity is measured separately on the two sides of the nose and the values are added together, the sum is higher than the value obtained when the conductivity is measured simultaneously on both sides. This phenomenon was described but not explained by Kern (1981). In our opinion, the explanation lies in compensation, more air being taken in on the open side of the nose when the other side has been closed for the conductivity measurements.

To obtain a reliable impression of the changes in the volume of the nasal mucosa, the conductivity of the passages was determined at 15-minute intervals over a period of four hours, and for both passages together as well as on each side separately. When the changes in the two sides occur alternately, this is usually accepted as a nasal cycle (Kayser 1895; Heetderks 1927; Kern 1981). Knowing that rhinomanometry provides qualitative data, we defined the nasal cycle qualitatively. None of the patients in our series showed a nasal cycle during nose-drop abuse, whereas six months after withdrawal of the nose drops a cycle was found in six of the 21 patients. A review of the literature, where the nasal cycle is only defined qualitatively, showed that a nasal cycle had been found in about 80 per cent of the subjects (Kayser 1895; Lillie 1923; Heetderks 1927; Kern 1981). Keuning (1968) also found a cycle in 80 per cent of his subjects, but he had selected individuals in the age group of 20-30 years. In our series the mean age of the six patients who showed a nasal cycle six months after withdrawal of the drops was 21 years and the mean age of the entire group without a cycle six months after cessation of abuse was 35 years. This is in agreement with reports of other authors (e.g., Stoksted 1952), i.e., that a nasal cycle is found more often in younger persons than in individuals belonging to

older age groups. In our series, 12 of the patients were aged 30 years or younger, and in five of these 12 a nasal cycle was found six months after termination of the abuse of nose drops.

Whether these patients had had a nasal cycle before they abused nose drops is not known. It is also not known whether the nasal cycle can be lost under the influence of nose-drop abuse, but this seems probable. To answer these questions, abusers would have to be studied before they started to abuse nose drops, which is obviously not feasible.

Chapter 6

General discussion

The study reported in this thesis had two objectives. In the first place an attempt was made to determine whether abuse of xylometazoline nose drops leads to morphological and/or functional changes in the epithelium of the nasal mucosa in man and, if so, whether this process can be reversed by withdrawal of the drops. In the second place, an attempt was made to explain the discrepancy between poor nasal patency about which nose-drop abusers often complain and the fairly good nasal patency which is frequently found at rhinoscopy.

Histological investigation

According to Boysen (1982), four types of epithelium are found in the nasal mucosa, i.e., ciliated-pseudostratified, stratified-cuboidal, mixed stratified-cuboidal-stratified-squamous, and stratified-squamous epithelium. The relative proportions in which these types of epithelium occur is dependent on the localization in the nose. In the posterior two-thirds of the nasal cavity ciliated columnar epithelium predominates, whereas in the anterior third, mixed stratified-cuboidal and stratified-squamous epithelium predominates.

However, the distribution of the various types of epithelium can be influenced by a number of exogenous stimuli. As early as 1932, Hilding commented: "I think no structure in the body is so delicate and so severely exposed to the environment as the nasal mucosa". In miners continually exposed to nickel-containing dust and aerosols, Boysen (1982) found predominantly cuboidal and squamous epithelium in the nose. When the exposure of these workers to nickel diminished, the epithelium in the nose changed to the columnar type. Transitions from one type of epithelium to another have been shown to occur in response to a number of stimuli, and these changes proved to be reversible when the stimulus was eliminated. In the present paper these transitions were studied electron-microscopically. Transitional forms between ciliated columnar epithelium and stratified cuboidal epithelium were found. Scanning electron microscopy (SEM) showed that each type of epithelium was invariably related to the preceding or succeeding stage of metaplasia.

The appearance of the various types of epithelium in nasal mucosa of patients who used nose drops excessively was compared with the appearence of the types of epithelium in the nasal mucosa of the same patients six months after withdrawal of the nose drops. In our material deriving from the middle third of the inferior turbinate, all of the above-mentioned kinds of epithelium were present except the

stratified squamous type. Neither light nor electron microscopy showed any changes in the various types of epithelium during and after nose-drop abuse. However, the relative proportion of cuboidal epithelium was greater during abuse than six months after cessation of exposure. This corresponds with a smaller proportion of columnar epithelium during abuse as compared with the situation six months later.

Since ciliated pseudostratified epithelium is normally found over the middle third of the inferior turbinate, our findings indicate that the excessive use of xylometazoline nose drops induces a change from columnar to stratified cuboidal epithelium in the mucosa of this region. This kind of change has been described as representing a transitional phase of metaplasia in the direction of squamous epithelium (Boysen 1982). The transitional phase is probably characterized by increased mitotic activity of the epithelial cells, as reflected by the histological appearance. The reversibility after withdrawal of the noxa is consistent with metaplasia. Furthermore, we found that after withdrawal of the nose drops the thickness of the epithelium decreased, which is compatible with normalization of the mucosa after elimination of the stimulus. It was also found that the number of secretory cells had increased six months after withdrawal of the drops, and this too should be seen as an expression of normal differentiation after withdrawal of the noxe.

Since we only found changes in the ratio of columnar to cuboidal cells, it may be concluded that the stimulus associated with xylometazoline abuse is relatively limited. This is supported by the absence of squamous epithelium after long-term abuse. It should, however, be kept in mind that our biopsy specimens were taken from the middle third of the inferior turbinate, whereas those of Boysen (1982) originated from the anterior tip of the middle turbinate. Because the latter region can be expected to be exposed to more turbulence of inhaled air than the former, metaplasia toward squamous epithelium can be expected to occur sooner or more frequently in the latter than the former region.

During the misuse of nose drops, electron-lucent granules with and without an electron-dense core were found. Six months after withdrawal of the drops, these granules could no longer be found. This change probably reflects a shift in the glycoprotein composition of the mucus-containing cells from neutral to acid. The same shift was found in our histochemical light-microscopical studies. In the presence of a chronic stimulus, Jones (1977) found a shift in the opposite direction in mucus-containing cells of the bronchial epithelium. However, his results and ours cannot be compared, because of the physiological differences between the mucosa of the upper and lower parts of the respiratory tract.

The present study has not provided indications as to the identity of the stimulus responsible for the metaplasia. We did not attempt to determine which of the components of the nose drops is the active agent in this respect. Furthermore, an indirect effect could be involved as well, i.e., a change in the volume of the nasal passage due to long-term vasoconstriction caused by xylometazoline. It is conceivable that persitent constriction of the blood vessels in the lamina propria

leads to long-term shrinkage making the nasal cavity wider than is favourable for the physiological status, and that this is accompanied by a shift from laminar to more turbulent air currents. A nasal cavity which is too wide could give rise to metaplastic changes in the epithelium. Since the degree of inflammatory infiltration of mononuclear cells was the same during abuse and six months after withdrawal of the nose drops and the composition of the infiltrate did not change either, we may conclude that nose-drop abuse does not lead to inflammation of the nasal mucosa, as is suggested by the term rhinitis medicamentosa (Lake 1946; Blue 1968; Feinberg and Feinberg 1971).

One of the changes induced in the mucosa by excessive and prolonged use of nose drops is a reduction of the number of ciliated cells in the epithelium, which in turn has an unfavourable effect on mucus transport and thus on the clearing of the inhaled air. We studied the ultrastructure of the various types of cell in the epithelium of the nasal mucosa during and six months after the misuse, and found no marked changes in the three types of lysosome-like bodies seen in our sections (electron-dense with a lamellar structure, electron-dense with granules, and multivesicular bodies) or in the ultrastructure of the cilia.

Functional investigation

Because patients who use nose drops excessively often complain of a feeling of obstruction of the nasal passage and because at rhinoscopy the passage seems to be adequate, we evaluated the patency of the nasal passages of our patients by rhinorheomanometry. Many methods have been described for such measurements. A review of these techniques is given and the reason for our choice of active posterior rhinorheomanometry are discussed. Patency was determined with a slightly modified version of Spoor's (1967) conductivity meter. With this instrument measurements can be made in both nasal cavities simultaneously or separately. Repeated measurements in the two sides separately over a period of several hours gives an impression of the nasal cycle. This cycle is qualitatively defined as an alternating increase and decrease of the conductivity of one side of the nose while the reverse occurs on the other side. The quantitative changes in nasal conductivity underlying the nasal cycle were studied. The patients were asked to stop using nose drops 12 hours before the first rhinomanometric determinations. During the measurements, anterior rhinoscopy often gave the impression that the patency of the nasal passage was worse than it had been at the first examination, and when questioned, the patients admitted to having used the nose drops shortly before that first examination. This finding might explain the seeming discrepancy between the patient's complaint of obstructed breathing and the physician's subjective impression of adequate nasal passages.

In all but one case, conductivity measurements showed improvement of the nasal passage after the withdrawal of the nose drops. During abuse, a nasal cycle was not observed in any of the patients. Six months after cessation of abuse, the presence of a cycle was established according to our criteria in six of the 21 patients. Five of

these six patients were younger than 30 years. These findings are in agreement with the reports in the literature that a nasal cycle is found more frequently in young than in older individuals. In our series, only five of the 11 patients younger than 30 years of age were found to have a nasal cycle. This finding could mean that the nasal cycle can be lost under the influence of nose-drop abuse.

General conclusions:

1. Abuse of xylometazoline nose drops is accompanied by changes in the nasal respiratory epithelium accompanied by a slight disturbance of cell differentiation, as a result of which fewer ciliated cells are present on the surface of the mucosa. This can have an unfavourable effect on mucus transport and thus on the filtration function of the nose.
2. The slight disturbance of differentiation of the nasal respiratory epithelium induced by nose-drop abuse is reversible, disappearing when the abuse ceases.
3. The seeming discrepancy between the nose-drop abusers' complaints of nasal obstruction and the adequate passages found at rhinoscopy can be explained by the use of nose drops by these patients shortly before this examination. If these patients have not used nose drops for at least 12 hours, the nasal patency is found to be inadequate.
4. When abusers of nose drops stop using these drops, the patency of their nasal passages improves and this leads to adequate nose-breathing.
5. When abusers of nose drops stop using the drops, the epithelium of the nasal mucosa becomes some μm thinner. This small change cannot explain the spectacular improvement in nasal patency. The increased nasal patency can, however, be ascribed to decreased thickness of the lamina propria. In all probability, this decrease is caused by a decrease in the volume of the vascular components of the lamina propria.

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Summary

Vasoconstrictive nose drops are used daily by many, sometimes for years in succession. Poor nasal patency is the stimulus responsible for the repeated use of nose drops by these patients. This thesis reports a study on the functional and morphological changes in the nasal mucosa of abusers of nose drops.

Chapter 1: Nose drops then and now

This chapter gives a review of the literature on nose-drop abuse, and describes the pharmacology and forms of administration of nose drops. The influence of these drops on the motility of the nasal cilia is discussed as well as the composition of the patient group and recommendations for the treatment of abusers during withdrawal of the nose drops.

Chapter 2: Structure and function of the nasal mucosa

In many text-books the respiratory epithelium in the nose is described as ciliated cylindrical epithelium, but recent studies have shown that a number of types of epithelium can occur, depending not only on the site in the nose but also on the amount and quality of the air passing through the nasal passages. Four types of nasal epithelium are described as well as the various functions of the nasal structures.

Chapter 3: Histological structure and glycoprotein composition of human nasal respiratory epithelium during and after abuse of nose drops

In all patients more cylindrical epithelium was found after the cessation of nose-drop abuse, and more cuboidal epithelium during abuse than six months after the withdrawal of the drops. After the cessation of abuse the respiratory epithelium was thinner than during abuse. More mucus-containing cells were found after the cessation of abuse. These changes point to metaplasia caused by nose-drop abuse.

In half of the patients the glycoprotein composition of the mucus-containing cells in the respiratory epithelium of the nasal mucosa showed no changes. The other half showed a shift from neutral to acid glycoproteins.

Chapter 4: Electronmicroscopy (TEM and SEM) of the human nasal respiratory epithelium during and after the abuse of nose drops

With electron-microscopical techniques (transmission (TEM) and scanning (SEM) electron microscopy), several types of epithelium were recognized. No differences were found between these types during and after abuse. Transition forms between types of epithelium were seen. No changes were found in the number and types of

lyosome-like bodies in the cytoplasm of the cells on the surface of the mucosa during and after nose-drop abuse, and the same holds for the ultrastructure of the cilia.

The changes found with TEM in the mucus-containing granules of epithelial cells of half of the patients corresponded with the changes in the glycoprotein composition described in Chapter 3.

Chapter 5: Influence of nose-drop abuse on nasal patency and the nasal cycle

Patients who abuse nose drops complain of poor patency of the nasal passages. When they are advised to stop this abuse and do so, they experience improvement of the nasal patenty after some time. For all but one of the patients included in the study, this improvement could be demonstrated objectively. During abuse, a nasal cycle was not found in any of the patients, but six months after termination of the use of the nose drops a cycle was found in six of the 21 patients.

Chapter 6: General discussion

The composition of the respiratory epithelium of the nasal mucosa is influenced by a number of exogenous stimuli. The present study has shown that prolonged use of nose drops (at least six months) can lead to metaplastic changes in this mucosa. The ciliated cylindrical cells can then be replaced by cuboidal cells without cilia. This change probably has an unfavourable effect on mucus transport and therefore on the filtration function of the nose.

The mild disturbance of the differentiation of the nasal respiratory epithelium under the influence of nose-drop abuse is reversible and disappears when the abuse is terminated. When the abuser stops using nose drops, the nasal patency improves. After the cessation of abuse the epithelial layer of the mucosa becomes thinner, but this small change cannot explain the spectacular improvement of the nasal patency. The improved patency of the nasal passages can, however, be ascribed to the decrease in thickness of the lamina propria. In all probability this decrease is due to the decreased volume of the vascular components of the lamina propria.

Samenvatting

Vasoconstrictieve neusdruppels worden dagelijks door velen, soms jaren achtereen gebruikt. Een slechte neuspassage is de prikkel die deze patienten ertoe brengt de druppels steeds weer opnieuw te gebruiken. In dit proefschrift worden de functionele en morfologische veranderingen in het neusslijmvlies van neusdruppelmisbruikers beschreven.

Hoofdstuk 1: Inleiding

In dit hoofdstuk wordt een litteratuuroverzicht betreffende neusdruppelmisbruik gegeven. Tevens wordt de farmacologie en toedieningsvormen van neusdruppels beschreven. De invloed van neusdruppels op de trilhaarbeweging wordt besproken, evenals de samenstelling van de patientengroep en een advies om neusdruppelmisbruikers van hun gewenning af te helpen.

Hoofdstuk 2: Bouw en functie van het neusslijmvlies

In het algemeen wordt het respiratoire epitheel in de neus als cylindrisch trilhaardragend epitheel beschreven. Recente studies hebben echter aangetoond dat meerdere typen epitheel gevonden kunnen worden, afhankelijk van de plaats in de neus waar het weefsel vandaan komt en van de hoeveelheid en de kwaliteit van de door de neus getransporteerde lucht. In dit hoofdstuk worden vier verschillende typen epitheel en de verschillende functies van de neus beschreven.

Hoofdstuk 3: Histologie en histochemie van het menselijk neusslijmvlies tijdens en na neusdruppelmisbruik

Tijdens het misbruik van neusdruppels wordt er meer cubisch epitheel gevonden dan een half jaar na het misbruik. Na het stoppen wordt bij alle patienten in het neusslijmvlies meer cylindrisch epitheel gevonden dan tijdens het misbruik. Het epitheel wordt na het neusdruppelmisbruik dunner, vergeleken met de dikte van het epitheel tijdens het misbruik. Tevens worden er ook na het misbruik meer slijmbevattende cellen gevonden. Deze veranderingen wijzen op metaplasie ten gevolge van neusdruppelmisbruik. In de glycoproteïne samenstelling van de slijmbevattende cellen in het respiratoire epitheel van het neusslijmvlies kon bij de helft van de patienten geen verandering gevonden worden. In de andere helft werd er een verschuiving van neutrale naar zure glycoproteïnen gevonden.

Hoofdstuk 4: Electronenmicroscopie (TEM en SEM) van het menselijk nasale respiratoire epitheel tijdens en na neusdruppelmisbruik

Met electronenmicroscopische technieken werd het voorkomen van verschillende soorten epitheel bevestigd. Electronenmicroscopisch kon geen onderscheid gemaakt worden tussen het voorkomen van de verschillende celtypen tijdens en na neusdruppelmisbruik. Overgangsvormen van het ene epitheeltype naar het andere epitheeltype werden wel gevonden. In de aantallen en typen lysosoomachtige insluitsels, die tijdens en na neusdruppelmisbruik in het cytoplasma van de oppervlakkig gelegen cellen gevonden worden, konden geen veranderingen aangetoond worden. Hetzelfde geldt voor de ultrastructuur van de trilharen. De bij de helft van de patienten gevonden electronenmicroscopische veranderingen in de slijmbevattende korrels komen overeen met de in hoofdstuk 3 gevonden veranderingen in glycoproteïne samenstelling.

Hoofdstuk 5: Invloed van neusdruppelmisbruik op de neusdoorgankelijkheid en neuscyclus

Patienten die neusdruppels misbruiken klagen over een slechte neusdoorgankelijkheid. Wanneer hen geadviseerd wordt het misbruik op te geven, ervaren zij na verloop van tijd een verbeterde neusdoorgankelijkheid. Voor alle onderzochte patienten (op één na) kon deze ervaring worden geobjectiveerd. Tijdens het misbruik is bij geen van hen een neuscyclus gevonden. Een half jaar na het staken van de druppels kon er bij zes van de 21 patienten een neuscyclus worden aangetoond.

Hoofdstuk 6: Discussie

De bouw van het respiratoire epitheel van het neusslijmvlies kan door meerdere exogene prikkels worden bepaald. In dit onderzoek is aangetoond dat langdurig neusdruppelgebruik (langer dan een ½ jaar) metaplastische veranderingen in het neusslijmvlies kan bewerkstelligen. Als gevolg hiervan worden de cylindrische trilhaardragende cellen, door cubische cellen zonder trilharen vervangen. Aangenomen mag worden dat deze verandering een ongunstig effect heeft op het slijmtransport en dus op de filtreerfunctie van de neus. Deze verstoring van uitrijping van het nasale respiratoire epitheel onder invloed van neusdruppelmisbruik is reversibel en verdwijnt wanneer het misbruik opgegeven wordt. Lichtmicroscpisch en electronenmicroscopisch kon bij de helft van de patienten geen verandering aangetoond worden in de glycoproteïne samenstelling van de slijmbevattende cellen in het respiratoire epitheel van het neusslijmvlies. In de andere helft werd er een verschuiving van neutrale naar zure glycoproteïnen gevonden.

Wanneer neusdruppelmisbruikers stoppen de druppels te gebruiken, dan krijgen de patienten een betere neusdoorgankelijkheid. Na het stoppen van het misbruik wordt de epitheellaag van het slijmvlies dunner. Deze kleine verandering kan echter de gemeten spectaculaire verbetering in neusdoorgankelijkheid niet verklaren. De toegenomen neusdoorgankelijkheid kan wel toegeschreven worden aan de afname van de dikte van de lamina propria, die waarschijnlijk wordt veroorzaakt door de afname van het volume van de vasculaire componenten in de lamina propria.

Curriculum vitae

De schrijver van dit proefschrift werd geboren op 11 maart 1949 te Rotterdam. Het basis- en middelbaar onderwijs volgde hij in Rotterdam. In 1967 haalde hij het diploma H.B.S.-B aan het "Rotterdamsch Lyceum" en in datzelfde jaar maakte hij aanvang met de studie Geneeskunde aan de Rijksuniversiteit Leiden.
Na het behalen van het artsexamen in september 1975 werd op 1 oktober 1975 de opleiding tot Keel-, Neus- en Oorarts aangevangen in het Academisch Ziekenhuis Leiden (opleider Prof. Dr. P.H. Schmidt).
Na het beëindigen van deze opleiding bleef hij als Chef de Clinique en later als Chef de Policlinique aan de afdeling Keel-, Neus- en Oorheelkunde van het Academisch Ziekenhuis verbonden.
Op 1 juli 1984 vestigde hij zich als Keel-, Neus- en Oorarts in het Ziekenhuis Leyenburg te Den Haag.