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Experimental surgery of the nose, anteroposterior changes of the mucosa on altering the air-flow

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SUMMARY

In 20 rabbits one nostril was surgically closed and the mucous membrane studied 4-90 days after the operation. The density of goblet cells was determined anteriorly and posteriorly on whole mounts, epithelial changes on serial sections from 4 different localities on the septum. Anteriorly on the open side damage to the cilia initiated epithelial processes of repair, viz. hyperplasia of basal cells, transformation of these cells into columnar cells, and differentation into mucous and ciliated cells. On the 16th day the epithelium was again columnar and ciliated. As a consequence of continued trauma new cycles were initiated, but not even after 90 days was there any squamous epithelium. In the middle and posteriorly on the septum no changes were demonstrated, indicating a marked, but gradual decrease in the anteroposterior direction of the influence by the air-flow upon the mucosa. On the closed side there was increased secretory activity and normalization of the epithelium which was changed most anteriorly in normal rabbits.

The nasal mucosa is constantly exposed to trauma by the air-flow. This trauma must be at a maximum anteriorly in the nose and in sites where the air-flow is maximal (Proetz, 1953; Masing, 1967). Owing to individual variations in the shape of the human nasal cavity, caused by irregularities of the septum and conchae, the effect of the air-flow and a possible change of the mucosa may be presumed to exhibit marked topographical variations. In man, where so many other factors, especially recurrent catarrhal conditions and infections in the nose influence the mucosa, it is therefore difficult to establish the magnitude of the changes caused by the air-flow. The presence of squamous epithelium anteriorly on the septum and conchae indicates that the effect of the chronic air-flow is responsible. However, Oppikofer (1907) found squamous epithelium in many normal persons far

posteriorly on the conchae, and it is doubtful whether these changes too are due to the air-flow.

Hilding (1932), in experiments on rabbits, demonstrated squamous epithelium on that side of the nose where the air-flow was doubled and a marked increase of goblet cells on the closed side with no air-flow (Hilding and Hilding, 1970; Hilding et al., 1970). Hilding (1932) concluded that the respiratory tract epithelium is not constant, but changing with external conditions.

We have repeated and extended these experiments, and in examining the mucosa we used blind quantitative histology. First we studied the goblet cell density in 20 operated rabbits and compared it with a normal material (Mogensen and Tos, 1978). On the closed side we found a somewhat higher density than on the open side, but this increase was not statistically significant in relation to the normal material. Also, the density on the open side was not significantly lower than normal. Thus, we did not find changes as pronounced as might be presumed from Hilding's (1932) report, and 90 days after the operation there were no areas devoid of goblet cells to indicate transformation of the epithelium into squamous epithelium. In another study (Tos and Mogensen, 1978) we performed quantitative histology of serial sections from the posterior part of the septum, about 23 mm behind the vestibule, where we found no significant differences between the closed and open side, not even 90 days after the operation. On serial sections 8-10 mm behind the vestibule we found on the open side damage to cilia which had initiated processes of repair in the epithelium, viz. basal cell hyperplasia and differentiation of the cells. At different times there were different forms of reaction in the epithelium, but no squamous epithelium. On these closed side we found increased secretory activity in a tall columnar epithelium which was of a fairly normal appearance 90 days after the operation. In a third study (Tos and Mogensen, 1979) we investigated epithelial changes most anteriorly in the nose, immediately behind the cutaneous border where the action of the air-flow must be at a maximum. At this site we found thickened epithelium with basal cell hyperplasia in normal rabbits. After closure of the nostril this epithelium returned to normal, regular, with ciliated cells and goblet cells extending right to the cutaneous border. On the open side there were similar – but somewhat more marked – changes than about 8-10 mm posteriorly to the cutaneous border. At this site too there was no squamous epithelium, although in some rabbits the basal cell hyperplasia was so pronounced that the epithelium was reminiscent of squamous epithelium.

In the present study we aimed at analysing the anteroposterior decrease of the changes, partly by determining the goblet cell density anteriorly and

posteriorly and partly by comparing the epithelial changes on serial sections taken from different sites on the septum. In the human adult nose we had found an increase of goblet cell density into the anteroposterior direction on the septum (Mogensen and Tos, 1977 a) as well as conchae (Mogensen and Tos, 1977 b), most easily explained by a diminishing influence by the air-flow posteriorad. On the contrary, we had found in the frontal, ethmoidal, and sphenoidal sinuses, where the air-flow has been eliminated, a goblet cell density appreciably lower than in the nose.

MATERIAL AND METHODS

The material comprises 38 adult rabbits of the type Copenhagen White, weighing 3 kg. 11 rabbits were normal, 7 were anaesthetized by intravenous Nembutal and killed at the same time as the operated rabbits. 20 rabbits were operated, the right nostril being completely closed (Mogensen and Tos, 1978a), so that respiration took place exclusively through the left nostril. 4, 6, 10, 16, 20, 30, 60, and 90 days after the operation 2 or 3 rabbits were killed by an overdose of Nembutal. Normal and anaesthetized rabbits living under the same conditions as the operated ones were killed at the same times.

The entire septum was removed after fixation, and the mucous membrane from each side freed, stained by the PAS-alcian blue whole-mount-method, and embedded in a chamber with anise oil-colophonium. On each septum goblet cells were counted in 0.01768 mm² fields. 10 counts were made anteriorly, 8-10 mm behind the cutaneous border, 10 counts posteriorly, 23 mm behind the cutaneous border. The median density anteriorly and posteriorly was calculated.

Haematoxylin-eosin, PAS-alcian blue stained serial sections, taken most anteriorly, immediately behind the cutaneous border, 8-10 mm behind, 15 mm, and posteriorly 23 mm behind the cutaneous border (Figure 1) were compared in each rabbit. The localities are called most anteriorly, anteriorly, in the middle, and posteriorly. In this way it ought to be possible to confirm the gradual anteroposterior decrease of the changes.

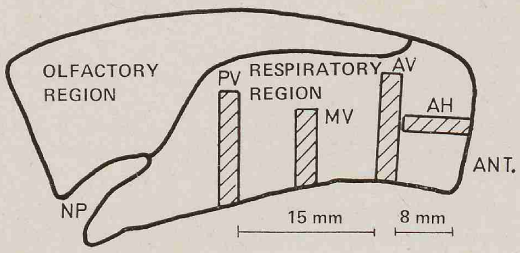

Figure 1. Situation of the mucosal specimens on the rabbit septum: (1) Most anteriorly (AH), (2) anteriorly (AV), (3) in the middle (MV), and (4) posteriorly PV. Anterior border of the nose ANT, nasopharyngeal duct posteriorly (NP).

RESULTS

Goblet cell density

We have previously demonstrated (Mogensen and Tos, 1978) that there were no differences in globlet cell density between the normal and anaesthetized material or any quantitative histological differences in the appearance of the epithelium and mucosa or in the occurence of cilia between the two materials. Therefore, intravenous Nembutal anaesthesia has no influence upon the mucous membrane, so that below the two materials will be designated normal material.

The normal material, consisting of 28 septa (14 left and 14 right) showed anteriorly an interindividual density of 64 cells/field (range 41-130), posteriorly of 74 cells (range 46-111). This difference is not statistically significant (Mann-Whitney test $p > 0.05$), but there was a tendency to a higher density posteriorly. There were marked individual variations in density.

Anteriorly: On the closed (right) side in the operated material there was a median density of 73 cells per field (range 41-105) as compared with 56 cells/field (range 30-110) on the open side. The difference is marked, but not statistically significant ($p > 0.05$). In relation to the normal material the difference is not significant, either on the closed or on the open side.

Posteriorly: On the closed side there was a median density of 73 cells/field (range 47-105), on the open side 70 cells/field (range 35-93), i.e. almost identical.

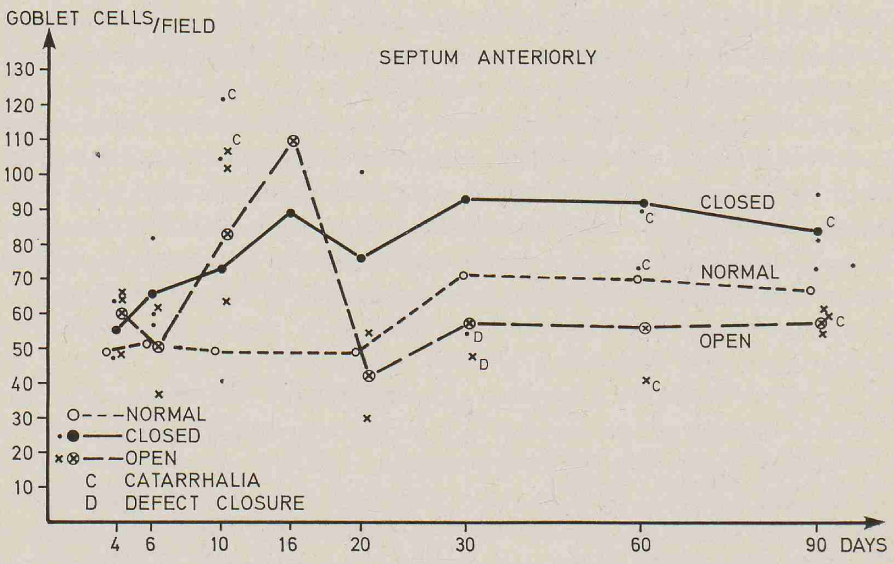

Figure 2. Median density of goblet cells anteriorly in the nose on the open and closed side of the operated material and of the normal material.

Analysis of the density at the various times showed *anteriorly* on the *closed* side (Figure 2) an even increase from the 4th day and a moderately increased density after the 20th day. On the *open* side there was on the 10th and 16th days also an increased goblet cell density, but thereafter a decrease, and from the 20th to the 90th day a reduced density. From the 20th to the 90th day after the operation, then, there is a definite tendency to a higher density on the closed side (92 cells/field) than on the open side (56 cells/field). This difference is significant ($p = 0.04$). However, the differences are not marked enough to be significant as compared with the normal material. As the goblet cell density was determined 8-10 mm behind the cutaneous border, in the immediate vicinity of that part of the mucosa which was serially sectioned, the density found on whole mounts may be compared with the goblet cell count in sections. The high goblet cell density on the open side on the 10th and 16th days is evident also in histological sections. It is due to the first cycle of the process of repair (Tos and Mogensen, 1978), the basal cells having differentiated into mucous cells. Incidentally, the sections after the 16th day showed, in all rabbits, a smaller number of goblet cells on the open than on the closed side.

Posteriorly on the septum (Figure 3) there was a tendency to a higher density on the 20th day, but otherwise no significant difference in density between the closed and open side. Sections also showed no significant differences between the two sides.

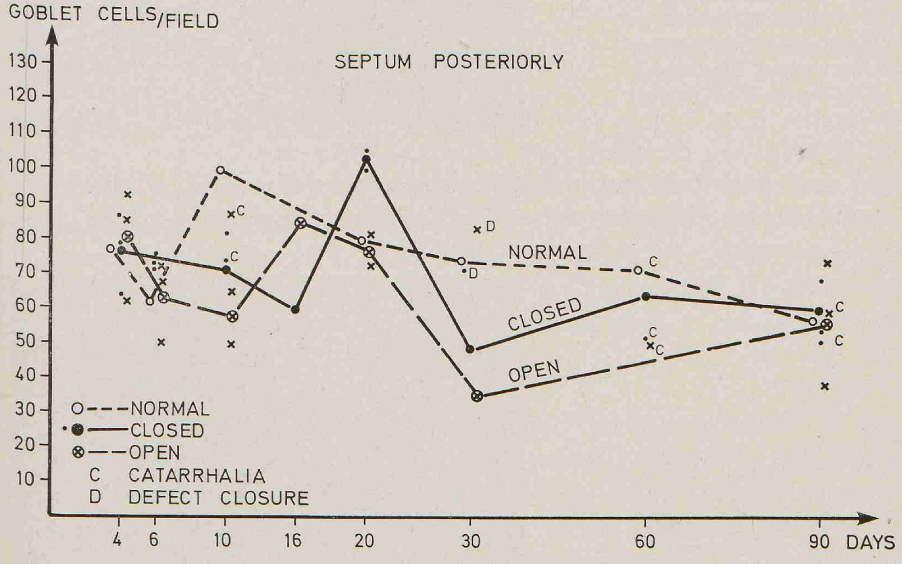

Figure 3. Median density of goblet cells posteriorly in the nose on the open and closed side of the operated material and of the normal material.

In 4 rabbits (one after 10 days', two after 60 days', and one after 90 days' observation) catarrhal conditions occured during the observation period. In one the closure of the nose was defective. These rabbits are not included in the statistical analysis of goblet cell density, although the values are plotted on Figures 1 and 2. In catarrhal rabbits the goblet cell density was higher on the closed than on the open side.

ANTEROPOSTERIOR EPITHELIAL CHANGES

Open side

On the 4th and 6th days the epithelium in the two anterior localities was thickened and frayed, with no or only a few cilia which were damaged. There was appreciable hyperplasia of round basal cells, extending to the surface and pushing the previously columnar ciliated cells and goblet cells towards the surface (Figure 4). The epithelium looked like squamous epithelium, with little or no columnar characteristics. In the middle and posteriorly there were no changes, a nice ciliated epithelium and no differences from the closed side.

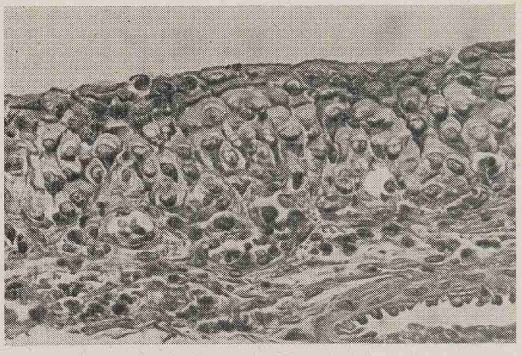

Figure 4.
Open side 8–10 mm behind the cutaneous border 6 days after the operation: Basal cell hyperplasia and transformation of basal cells into columnar. The original columnar cells have been pushed up to the surface. Haematoxylin-eosin, PAS-alcian blue section x 500.

10th day. Anteriorly and most anteriorly the transformation of the round basal cells into columnar cells as well as the differentiation of the latter

Figure 5.
Open side 23 mm behind the cutaneous border 10 days after the operation. Columnar respiratory epithelium with goblet cells and intact cilia.

into mucous cells was in full swing. The epithelium was again more columnar, although it was considerably thickened and somewhat irregular. Luminally there were still some goblet cells by way of being desquamated, basally new columnar goblet cells with mucous granules, and a number of undifferentiated columnar or basal cells. The relatively higher goblet cell density on the open side on the 10th day (Figure 2) is thereby explained. In the middle and posteriorly there were no changes (Figure 5).

16th day. Anteriorly and most anteriorly there was nice, tall columnar epithelium with many goblet cells and ciliated cells. True, the epithelium was somewhat thickened and showed some basal cell hyperplasia, but it contained a number of ciliated cells and many goblet cells (Figure 6). On the whole mounts there was a high goblet cell density (Figure 1 a). In the middle and posteriorly the epithelium was normal.

Figure 6.
Open side 8–10 mm behind the cutaneous border 16 days after the operation. Columnar ciliated epithelium with goblet cells.

20th day. Anteriorly the epithelial changes were similar to those seen on the 6th and 10th days, being again reminiscent of squamous epithelium with hyperplasia of basal cells, transformation of these cells into columnar, or incipient differentiation into mucous cells. The goblet cell density was rather low. Most anteriorly the changes were more pronounced and the epithelium thicker, while in the middle and posteriorly there were no changes and the goblet cell density was high (Figure 3).

30th day. Anteriorly there were changes similar to those on the 16th day, predominated by columnar epithelium with ciliated and goblet cells. However, basal cell hyperplasia was also present. The varying epithelial changes, initiated on the 4th day owing to damage to the cilia, indicate a cyclic process of repair whose first cycle has been completed in 16 days, the second in 30 days. In the middle and posteriorly there were no noteworthy

changes, and if there have been any processes of repair in these sites too they have been of very slight degree.

60th and 90th days. Anteriorly and most anteriorly the appearances were varying: One rabbit showed 90 days after the operation columnar epithelium with cilia and goblet cells and the other rabbit thick, hyperplastic epithelium with basal cell hyperplasia, again reminiscent of squamous epithelium and of the same appearances as on the 6th and 20th days (Figure 7). In the middle and posteriorly the latter rabbit showed no changes and no differences between the open and closed side (Figure 8).

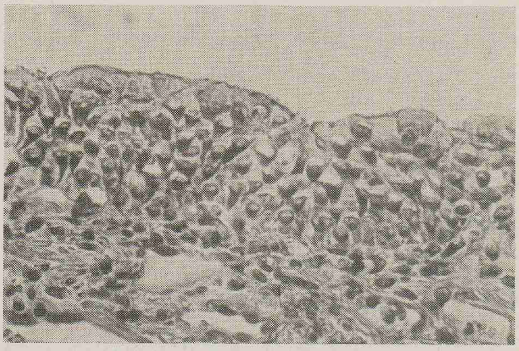

Figure 7.
Open side 8–10 mm behind the cutaneous border 90 days after the operation. Hyperplasia of basal cells and transformation of these cells into columnar cells. The orginal columnar cells are flat and pushed towards the surface.

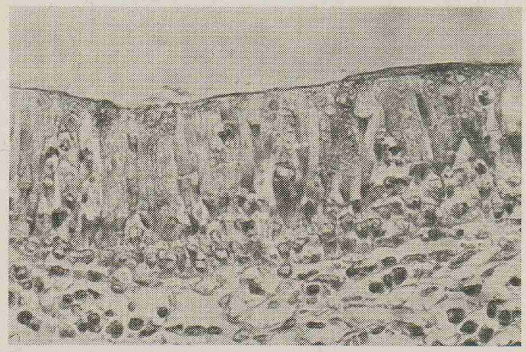

Figure 8.
Open side 23 mm behind the cutaneous border 90 days after the operation. Nice columnar respiratory epithelium with cilia, mucociliary clearance on the surface, and goblet cels. This epithelium is practically normal.

CLOSED SIDE

The changes on the closed side were pronounced, especially most anteriorly, immediately behind the cutaneous border where normal rabbits showed thickened epithelium with basal cell hyperplasia and no or few cilia. This squamous-like appearance presumably represents a phase in the process of repair caused by the influence of the normal air-flow (Tos and Mogensen, 1979). On the closed side this epithelium gradually changed into regular,

ciliated, columnar epithelium reaching to the cutaneous border. In addition to this normalization the changes on the closed side were fairly uniform and have previously been described in detail (Tos and Mogensen, 1978). At all times the epithelium anteriorly was columnar with increased secretory activity. On the 10th and 20th days the cells were fairly tall and amply ciliated. 60 and 90 days after the closure the epithelium was extremely regular, short columnar and ciliated, reaching right to the cutaneous border and containing relatively few basal cells. In the middle and posteriorly the appearances were similar.

DISCUSSION AND CONCLUSION

1. Determination of goblet cell density is the only objective, quantitative measure for assessing the secretory capacity of the mucous membrane, and it also indirectly expresses the transport function by the epithelium. Sections showed that the presence of ciliated cells bears a close relation to the goblet cells. The preparations from the open side with relatively few goblet cells also had a few or no ciliated cells.
2. In a previous analysis (Mogensen and Tos, 1978) the counts were performed all over the septal surface. We found similar tendencies as in the present study, a higher goblet cell density on the closed than on the open side. However, the changes were nowhere as marked as might be presumed from Hilding's study. On the 10th and 16th days we even found an increased goblet cell density on the open side, due to the brisk regenerative process in the epithelium and differentiation of basal into goblet cells.
3. Analysis of sections from various sites on the septum showed on the open side a marked decrease of the air-flow influence upon the mucosa into the anteroposterior direction. Most anteriorly there were, already under normal conditions, the same epithelial changes as found at a doubled air-flow a few mm farther posteriorly. 8-10 mm behind the cutaneous border there were also pronounced changes, whereas 15 and 23 mm behind the cutaneous border there were no changes, and the epithelium was nicely ciliated. Between the open and closed side there were no differences in epithelial appearance on the posterior half of the septum.
4. The air-flow damages cilia, and this initiates a cycle or repair comprising chronologically hyperplasia of basal cells, gradual desquamation of the damaged cells, transformation of basal cells into columnar, and differentiation of goblet cells into ciliated cells. Apparently the first cycle lasts for 16 days, whereupon there are indications of new cycles, but not as characteristic as the first one. As the histological appearance of the epithelium changes rapidly, it depends on the open side highly upon the site and the time of examination. On the 4th, 6th, and 20th days, when basal cell hyper-

plasia predominates, it is reminiscent of squamous epithelium, while on the 10th, 16th, and 30th days it is again predominantly columnar.

5. Even after 90 days' exposure to doubled air-flow we did not anywhere find squamous epithelium. However, longer-lasting experiments are required. There is also a need for re-investigation of normal adult noses to search for squamous epithelium.

6. On the closed side the normal trauma from the air-flow is eliminated, and the normal, mild damage, with subsequent regeneration of the epithelium, does not occur. The existing changes of the epithelium disappear, and the turnover time of the normal epithelium is prolonged. It may be said that the epithelium is resting. It gets somewhat thinner, with a lower basal cell count, and it consists predominantly of slender columnar cells and an increased number of goblet cells and ciliated cells. Similar changes have been reported in the nasal mucosa of laryngectomized patients (Sternberg, 1924; Dixon et al., 1949; Naumann, 1964; Puska et al., 1970; Jahnke, 1972). In atrophic rhinitis and in ozoena one nostril has been surgically closed (Young, 1968, 1971; Shah et al., 1974), with a favourable effect upon the symptoms and histological improvement of the mucous membrane. After long-lasting closure, however, some patients still had squamous epithelial metaplasia, which is pronounced in atrophic rhinitis (Holopainen, 1967), whereas others had allegedly a nice respiratory epithelium. Our studies indicate that long-lasting closure can improve pathological epithelial changes.

ZUSAMMENFASSUNG

Bei 20 Kaninchen wurde ein Nasenloch chirurgisch geschlossen und die Nasenschleimhaut 4–90 Tage nach der Operation untersucht. Die Dichte der Becherzellen wurde vorne und hinten an Ganzpräparaten, die Epithel-veränderungen an Serieschnitten an vier verschiedenen Lokalitäten der Nasenscheidewand bestimmt. Vorne auf der offenen Seite setzt eine Schädigung der Flimmerzellen ein Reparationsprozes ein, der aus Hyperplasie der Bazalzellen, Differentierung von diesem zur Becherzellen und Flimmerzellen besteht. Am 16. Tag ist das Epithel wieder zylindrisch und ciliär. Als Folge der fortgesetzten Traume entstehen neue Schäden, aber das Epithel wurde auch nicht nach 90 Tagen zum Platenepithel verändert. In der Mitte und hinten in der Nase wurden keine Änderungen gefunden, welches auf eine gradweise Abnahme des Einflusses des Luftstroms auf die Nasenschleimhaut deutet.

Auf der geschlossenen Seite wurde zunehmende sekretorische Aktivität und ganz vorne eine Normalisierung des normalt veränderten Epitheles gefunden.

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