Original Article

Morphology of the Conjunctival Epithelium in Spectacle and Contact Lens Wearers – A Light and Electron Microscopic Study

Original title: Morphologie des Konjunktiva-Epithels bei Gesunden und bei Kontaktlinsenträgern. Eine licht- und elektronenmikroskopische Untersuchung

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Abstract

The normal morphology of the conjunctival epithelium and the changes it undergoes in asymptomatic long-term contact lens wearers were studied by impression cytology in 28 subjects. The cell specimens obtained were evaluated by light and electron microscopy. All the lens wearers showed marked cytological changes previously seen only in ocular surface pathology. Clear evidence of squamous metaplasia was observed in the normally stratified columnar epithelium. In addition, numerous nuclear changes occurred, notably a high percentage of "snakes", i.e. long, snakelike chromatin condensations located at the centres of cell nuclei. In a prospective study, all of these changes were found to occur soon after the start of lens wear and to develop very rapidly as a function of the duration of lens wear. After only 6 months of lens wear the changes seen in the group of former spectacle wearers reached the stage seen in long-term wearers. However, in 2 subjects the changes were also found to be reversible.

Key words: Soft hydrogel contact lenses – Conjunctiva – Epithelium – Impression cytology – Squamous metaplasia – Chromatin condensations ("snakes") – Transmission electron microscopy – Scanning electron microscopy

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Introduction

The conjunctival epithelium is crucial to the integrity of the ocular surface as a whole. The outermost part of this surface consists of an epithelium which is highly prismatic in several layers over most of its area (though not in the immediate limbal zone) and whose top layer includes numerous goblet cells (*Lütjen-Drecoll* et al. 1982, *Rohen* 1986, *Steuhl* 1989).

Like the entire ocular surface, the conjunctival epithelium is covered by a three-component tear film (*Wolff* 1954, *Ehlers* 1963, *Rohen* 1986) which, in addition to moistening the epithelium, has an important metabolic role in maintaining the transparency of the cornea. The innermost layer of the tear film, which is in direct contact with the epithelium, consists of the mucus produced by the conjunctival goblet cells. Besides being crucially important to the stability of the tear film (*Norn* 1963, *Proust* 1969, *Kaura* 1985), it is the layer which makes accumulation of the principal, aqueous phase of the tear film possible. A lipid phase constitutes an outer barrier to the aqueous phase.

The ultrastructure of both the conjunctival and corneal epithelium has numerous apical processes (microvilli and microplicae) and is completely covered by a fine glycocalyx layer. The glycocalyx, which some authors regard as the second component of the ocular surface mucus system (or "second" mucus system (*Greiner* et al. 1969), is produced in the uppermost cell layer of the conjunctival epithelium, stored in vesicles, and integrated in the cell membrane by exocytosis (*Dilly* 1985).

There is a close functional interrelationship between the epithelial surface morphology and tear film stability (*Abdel-Khalek* et al. 1978a, 1978b), because the glycocalyx coating of the cells permits the mucus produced by the goblet cells to accumulate, thus forming the innermost layer of the tear film. The mucus in turn enables the epithelial surface, which is in fact hydrophobic, to be wetted by the subsequent aqueous phase (*Holly* and *Lemp* 1971, *Lemp* and *Dohlmann* 1971, *Lemp* et al. 1983, *Nichols* et al. 1983). Tear film stability appears to play

an important role in keeping the epithelial morphology intact (*Levenson* 1973, *Greiner* 1978, *Brewitt* et al. 1979, *Brewitt* and *Joost* 1987), so that the two are interdependent.

In various ocular surface disorders, and especially those associated with "dry eye", the morphology of the conjunctival epithelium displays marked cytological changes (*Marner* et al. 1980, *Nelson* et al. 1983, *Tseng* et al. 1984, *Götz* et al. 1986, *Nelson* and *Wright* 1985, *Liotet* et al. 1987, *Koch* and *Waubke* 1987). However, asymptomatic lens wearers also show changes of the precorneal tear film and signs which may reflect histological changes in the underlying corneal and conjunctival layers (*Greiner* et al. 1978, *Greiner* and *Allansmith* 1981).

It was therefore a logical step to study the morphology of the conjunctival epithelium in long-term contact lens wearers.

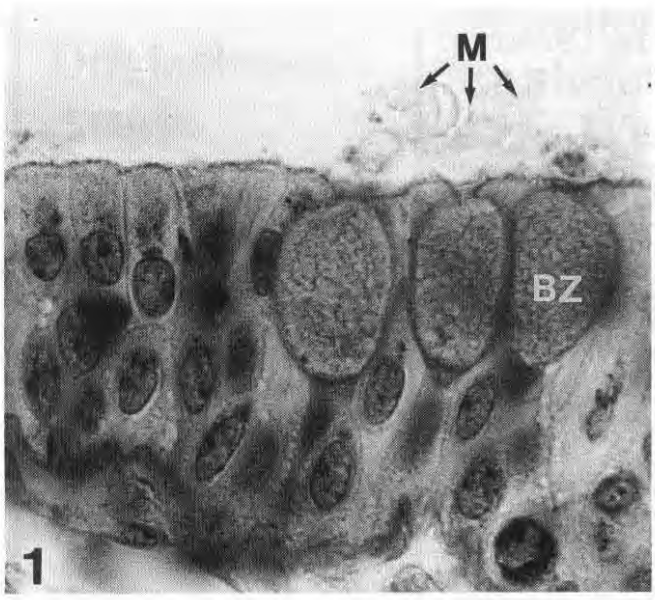
Materials and Methods

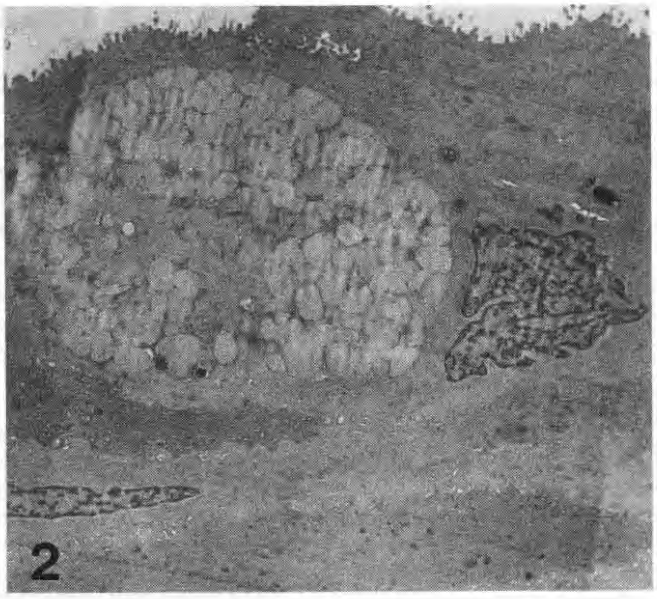
For these studies we used the impression cytology method described by *Egbert* et al. (1977). A small piece of Millipore filter paper (Millipore VSWP 0.025 fm) is applied with light pressure to the surface of the eye and removed a few seconds later. The specimen thus obtained, adhering to the filter paper, is normally a dense cluster from the topmost epithelial cell layer. Since lateral cell cohesion is unaffected, the method also permits qualitative statements concerning the entire epithelium, and not merely limited statements concerning individual cells. In contrast to diagnostic excision, this method is simple to perform and painless, and is therefore also accepted by asymptomatic contact lens and spectacle wearers. Should sampling nevertheless cause discomfort, topical anaesthesia (e.g. Proximetacain) can be administered without affecting the cytological findings.

The specimens thus obtained can be air-dried or chemically fixed in the usual manner and subsequently prepared for light or electron microscopic study. In this study the majority of the specimens were air-dried, stained with an extended PAP stain (*Tseng* 1988) and then studied by light microscopy. The histological specimens were fixed in aldehyde and embedded in paraffin. For transmission electron microscopy (TEM) the specimens were fixed in aldehyde, embedded in synthetic resin, and ultrathin sections were examined using a Zeiss EM 10. Specimens destined for scanning electron microscopy (SEM) were air-dried or chemically fixed, shadowed with gold-palladium and examined using a Philips SEM 505 (*Knop* 1990, 1991).

In all participants the specimens were obtained from the superior bulbar conjunctiva at 12 o'clock, 3D5 mm from the limbus. In some participants, additional specimens were obtained from other regions, e.g. at 3, 6 and 9 o'clock, or 10-15 mm from the limbus.

The prepared specimens were analyzed according to various cytological criteria (*Nelson* 1983, *Tseng* 1985) which are important in the assessment of epithelia and have been applied by other authors. In particular, epithelial cell size, nucleoplasmic ratio (NPR), cell shape, cell cohesion, nuclear morphology, goblet cell count and potential keratinization were studied.





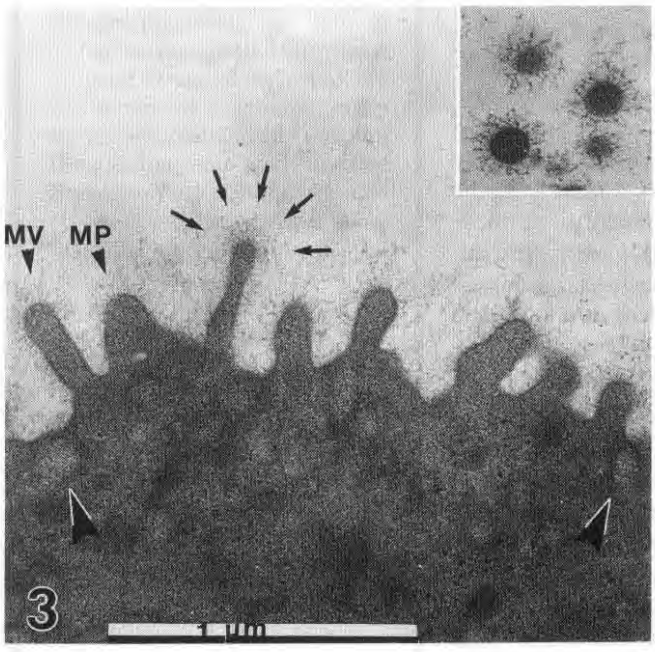


Fig. 1 Normal conjunctiva. The epithelium is highly prismatic in several layers and has numerous goblet cells (BZ) in the top cell layer. Typically, the goblet cells form clusters, with mucus debris (M) visible on their luminal side (LM, 1050 ×)

Fig. 2 Goblet cell (oblique section). The theka is filled with mucus granules (TEM, 4000 ×)

Fig. 3 Surface of conjunctival epithelial cells, with numerous microvilli (MV) and microplicae (MP). The outsides of the cell membranes are covered by the filamentous glyocalyx layer (arrows), radiating into the extracellular spaces. Inset: cross-section through microvilli. The lighter zones (arrows) directly beneath the cell membranes may be the vesicles of the "second mucus secretory system" (TEM, 40,000 ×)

On examining the specimens it was found that the increase in cell size preceding karyopyknosis was principally due toan increase in the diameter of the cytoplasm; the size of the nucleus remained unchanged. The logical conclusion was that up to this stage (Fig. 12, "g" equivalent to approximately twice normal diameter) a certain increase in cell size regularly corresponded to a certain, constant NPR (*Knop* 1988). This NPR was taken as a measure of cell enlargement, since it can be semi-quantitatively assessed simply and reproducibly, in contrast to absolute cell size (in µm) which can only be ascertained morphometrically.

Changes in nuclear morphology were recorded semi-quantitatively as a percentage of the number of cells obtained on the filter strips. The goblet cell counts were based on the number of intact goblet cells on the filter paper or their mucus impressions.

A total of 28 subjects were examined. They were divided into two groups of 14 participants, with the same age and sex distribution. They differed only insofar as one group were spectacle wearers (to establish the normal impression cytological picture of the conjunctival epithelium), while the other comprised long-term contact lens wearers. All the participants were subjectively asymptomatic and appeared to have normal conjunctivae and tear physiology at routine ophthalmological examination (slit lamp, Schirmer test, tear film break-up time).

In the first part of the study the epithelial cell morphology of the long-term lens wearers was compared to that of the control group of spectacle wearers. In the second part, the members of the control group were fitted with contact lenses and the chronological pattern of the cell changes which occurred was observed.

Results

The histological appearance of the normal conjunctiva (Fig. 1) is that of a multilayered, highly prismatic cell cluster, typically with small groups of goblet cells embedded in its uppermost layer. On the epithelial surface, directly at the apices of the goblet cells, one occasionally sees remnants of what is presumably secreted mucus.

Figure 2 is a transmission electron micrograph illustrating the ultrastructure of these goblet cells. The cell nucleus is usually displaced into the cell base, while the theca is filled with numerous small granules which store the mucus products of the cell before they are discharged to the epithelial surface by exocytosis.

The transmission electron micrograph reproduced in Fig. 3 shows the surfaces of the outer conjunctival epithelial cells, which exhibit many variations in the form of microvilli and microplicae. The outer surface of the cell membrane (including all variations) is covered by the fine, hairlike, filamentous glycocalyx layer, which radiates into the extracellular space, forming a dense network between individual microvilli. Directly beneath the cell surface, roundish, lighter zones are seen. These may be the vesicles of the "second mucus secretory system".

Normal conjunctiva: small, roundish cells

The impression cytological specimens obtained from the spectacle wearers, i.e. from normal conjunctivae, (Fig. 4) contained dense, single-layered clusters of small, roundish cells with a narrow cytoplasm border and a NPR of approximately 1:1 (Stage "n", Fig. 12). The scanning electron micrograph (Fig. 6) shows clearly that the cell bodies are not flattened and are roughly isoprismatic in shape. Nuclear morphology was normal, with homogeneous chromatin distribution. Unexpectedly, snakes, i.e. non-homogeneous chromatin distribution, were found in some epithelial cells (less than 10%) obtained from a spectacle wearer. In the majority of these specimens, intact goblet cells were found only at points where the cell clusters comprised more than one layer. In the ordinary, single-layered specimens and where there were no cells it was usual to obtain only impressions of the mucus exuding from the goblet cells (Knop and Brewitt 1991a). Both phenomena were used, with equal weighting, for the goblet cell count, which was found to be 170 $\pm$56 per mm.

Epithelial cells enlarged and flattened in lens wearers

In contrast to this normal finding, extensive changes in the cell picture were found in all contact lens wearers (Figs. 5 and 12). In the majority of cases, clearly enlarged and frequently polygonal epithelial cells were seen, either in loose clusters or even individually on the filter paper. On average, epithelial cells in long-term contact lens wearers were almost twice (1.7 times) as large, with a NPR of approximately 1:5 (Stage "m", Fig. 12). At some points much larger cells were also seen (Fig. 8). The increase in cell size was accompanied by signs of flattening. Under the light microscope (Fig. 5) this was no more than a supposition, based on the curling of the cell borders. In the scanning electron micrograph (Fig. 7), however, the flattened shape of the cells is clearly recognizable. Since both specimens (Figs. 6 and 7) were fixed in glutaraldehyde the cells have their true shape; it cannot be an artefact resulting from air-drying. In cells of more than twice normal diameter a degenerative pyknotic decrease in nucleus size proportional to the increase in cell size was seen (Fig. 8). This decrease continued until cytolysis occurred. At 134 $\pm$95 per mm, the mean goblet cell count in long-term contact lens wearers was approximately a quarter lower than in spectacle wearers.

Goblet cell count reduced

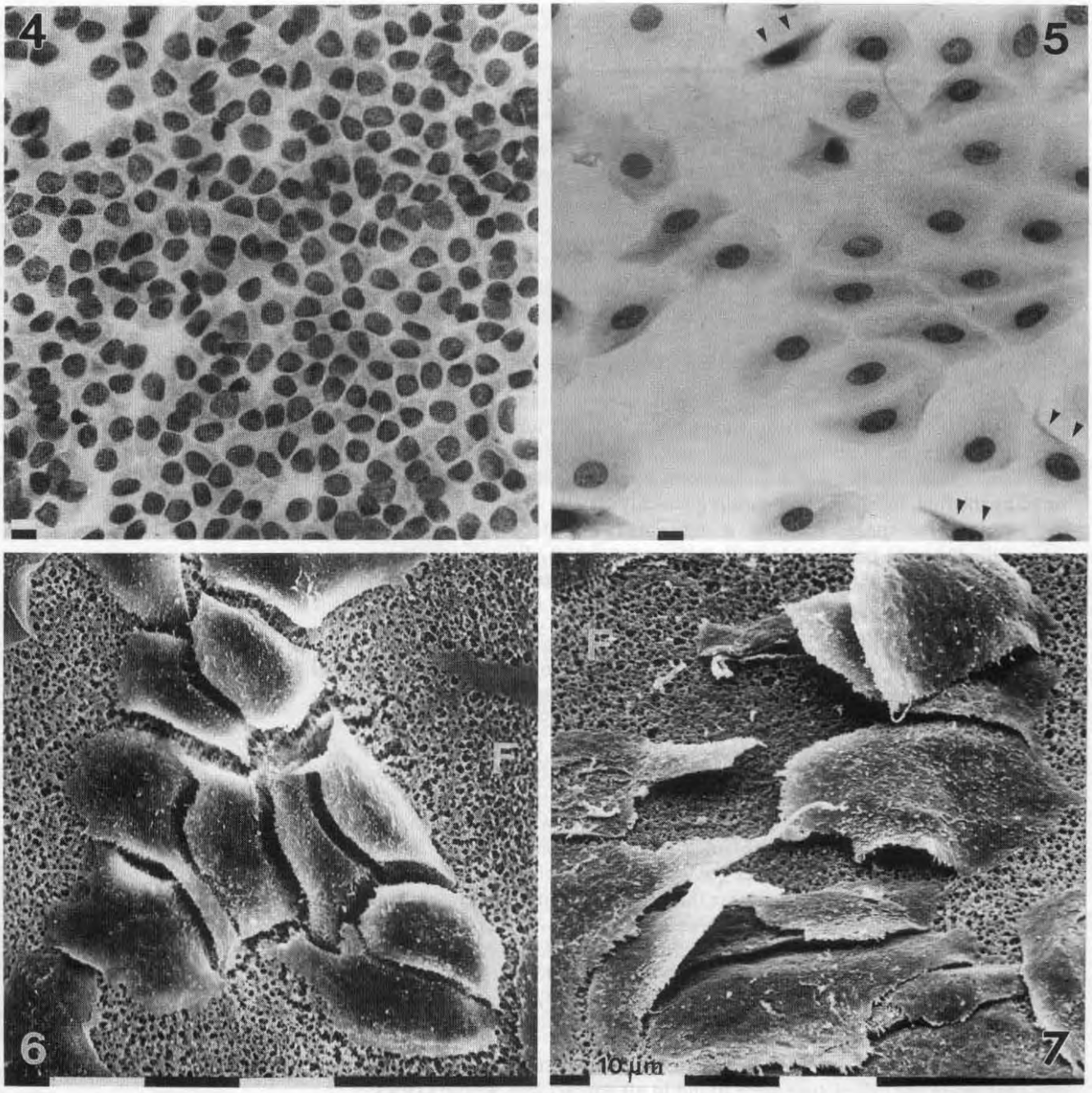


Fig. 4 Impression cytological pattern of normal conjunctival epithelium: homogeneous single-layer cell cluster. The epithelial cells are small and roundish, with a nucleoplasmic ratio of approx. 1:1 (LM, 380 ×)

Fig. 5 In contact lens wearers clearly enlarged, flattened epithelial cells are seen, some with deformed borders (arrows), in loose clusters with an average nucleoplasmic ratio of 1:5 to 1:8 (same scale as micrograph of normal cells; LM, 380 ×)

Fig. 6 Same cell cluster as shown in Fig. 4. The intercellular spaces have been artificially enlarged (the cells are on the filter paper (F) with which they were obtained). (SEM, 1500 ×)

Fig. 7 Same enlarged epithelial cells as shown in Fig. 5. The flattening and spread of the cells in contact lens wearers is clearly apparent (same scale as SEM of normal cells, Fig. 6). (SEM, 1500 ×)

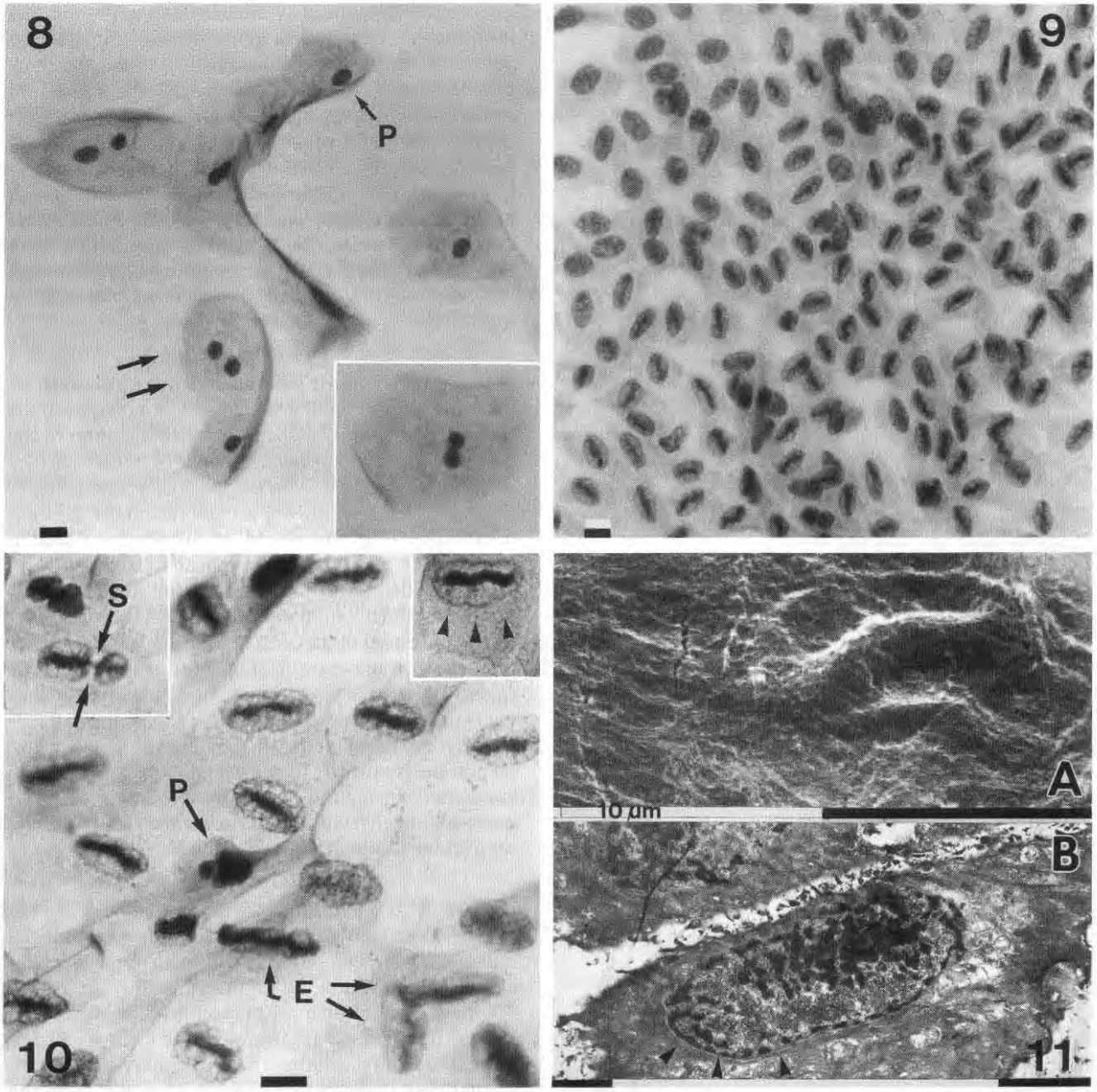


Fig. 8 Pyknotic nuclear compaction (P) was regularly observed in greatly enlarged epithelial cells. Two or more pyknotic nuclei in a single cell (double arrow) were often seen. However, no keratohyalin granules – a sign of incipient keratinization – were seen even in very large cells obtained from contact lens wearers (inset). Same scale as Figs. 4 and 5. (LM 380×)

Fig. 9 "Snakes", i.e. snakelike condensations of the chromatin, were observed frequently, even in only slightly enlarged cells. They were mainly localized in longish zones, though not uniformly distributed within these. This figure shows a snake zone (running from bottom left to top right) in a contact lens wearer in Group IV, in which 51-75% of the specimens studied had "snakes" (LM, 380×)

Fig. 10 Nuclear changes: Besides pyknotic compaction of nuclei (P), numerous "snakes", were observed, i.e. abnormal, evidently progressive snakelike condensations of chromatin at the centres of cell nuclei. Under the light microscope the nuclear membranes appeared intact (arrows). Nuclei were regularly elongated, in some cases extremely so (E). The nuclei of some cells (S) were segmented (LM, 690×)

Fig. 11 a) Snakes as massive, prominent structures beneath the collapsed debris of air-dried cells. The nuclear membrane frequently forms a small border around the condensed chromatin. (SEM, 4200×); b) Nucleus of chemically fixed cell with snake confirms that nuclear membrane (arrows) is intact despite central condensation of chromatin. (TEM, 6150×)

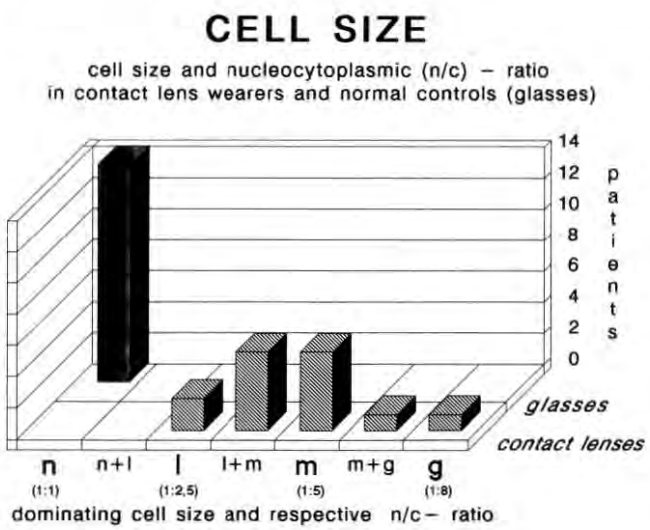


Fig. 12 In comparisons of dominant cell size (at the preferred localization of 12 o'clock on the conjunctiva), markedly enlarged epithelial cells were observed in all long-term contact lens wearers. In the control group of spectacle wearers only normal, small cells were observed.

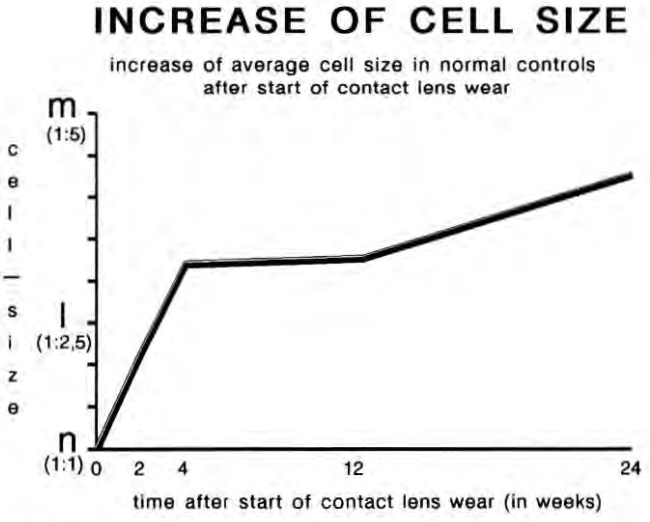


Fig. 13 Within 6 months of starting to wear contact lenses, cell size in these former spectacle wearers also increased rapidly, with an increase in dominant cell size corresponding to squamous metaplasia

In addition to cell flattening (and the resulting increase in cell diameter) and the reduced goblet cell count, many other changes in nuclear morphology were observed (Figs. 8–11). These included pyknosis, as mentioned above, in very large cells, which could subsequently develop into karyorrhexis or karyolysis. Cells with 2 or more nuclei, usually degeneratively diminished in size, compacted and pyknotic, were also seen (Fig. 8). Moreover, some changes were seen which occurred in cells that were only slightly enlarged and some of normal size. Several variations of the normally homogeneous chromatin were seen.

The most striking and frequently seen of these was a long, rodlike or snakelike condensation the chromatin, regularly associated with elongation of the nucleus. These changes are known in ocular surface pathology as "snakes". In the specimens studied here, the nuclear membranes appeared intact under the light microscope (Fig. 10). TEM of an ultrathin section through the nucleus of a chemically fixed cell (Fig. 11b) provided confirmation that the nuclear membrane was intact and that the chromatin was pathologically condensed in the centre of the nucleus. In a scanning electron micrograph (Fig. 11a) of air-dried cells (*Knop* 1991), snakes are apparent as massive, prominent structures beneath the debris of the nuclear membrane, cytoplasm and cell membrane. The nuclear membrane frequently forms a small border around the condensed chromatin. Snakes were found in all specimens obtained from long-term contact lens wearers, on average in about 30% of the cells.

It is interesting to note that none of the cell changes described were distributed uniformly over the bulbar conjunctiva, but only within an approximately circular area around the limbus (Fig. 16), i.e. within the range of movement of the contact lens on the eye. Within this area the frequency of the changes always decreased from the limbus to the periphery of the bulb, so that even if the central changes (3–5 mm from the limbus) were pronounced the epithelium might still be normal at the periphery (10 D 15 mm from the limbus). In addition, the extent of the changes differed in regions equidistant from the limbus. The most marked changes were regularly observed at 12 o'clock. They were less marked at 6 o'clock and least marked laterally.

In order to establish whether contact lenses indeed play a causal role in the development of these cytological changes in lens wearers, and to study the chronological development pattern, the group of spectacle wearers were subsequently fitted with modern hydrogel contact lenses (Knop and Brewitt 1989) with good oxygen permeability and medium water content. The condition of the conjunctival epithelium was examined regularly during a 6-month period, and specimens were obtained for cytological study.

The first cell changes were observed after only one week of lens wear. They were as described above, i.e. an increase in cell size and nuclear changes, and progressed very rapidly. In general, the increase in cell size appeared to take place more rapidly, preceding the nuclear changes (Figs. 12 and 14). After 2 weeks, predominantly normal-sized conjunctival cells at 12 o'clock were observed in only 5 (36%) of the 14 former spectacle wearers. After 4 weeks only one participant (7%) still presented with the normal cell picture. After 12 weeks all the participants had predominantly enlarged cells, and after 6 months the average size of the cells had increased to about 1:5 (Fig. 13).

The rate of change in cell morphology, and in particular the development of snakes, was almost as fast. After 2 weeks 57% of the participants still had no snakelike changes in the nuclear chromatin, but after 12 weeks this figure had shrunk to a good quarter (28%) of the former spectacle wearers. After six months of lens wear, only 2 (14%) of the participants were still free of snakes and the average snake count in this period increased to about one-quarter of the cell specimens obtained (Fig. 15).

Overall, together with a reduction of about one-third in the goblet cell count to 133|47 per mm, these findings correspond to the cell picture observed in the long-term lens wearers.

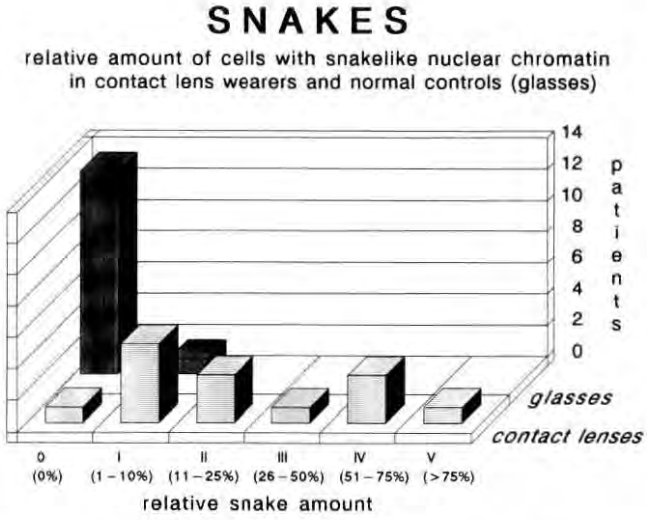


Fig. 14 A comparison of nuclear structure showing that almost all long-term contact lens wearers had substantial numbers of cells with pathologic chromatin condensation (snakes), while with one exception the nuclear structures in the control group of spectacle wearers was normal.

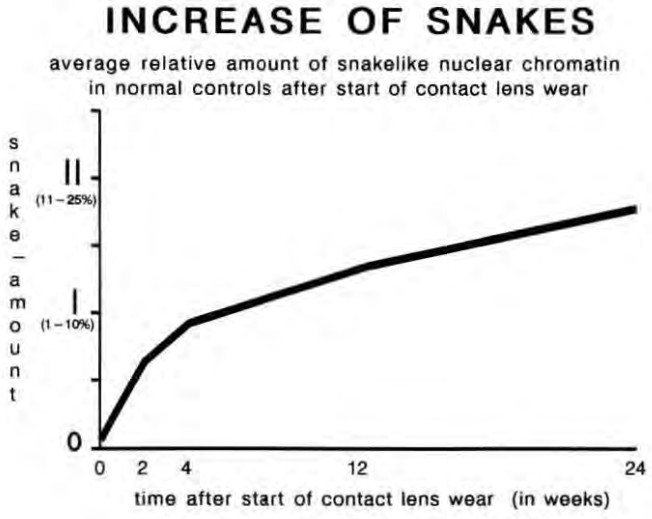


Fig. 15 Numerous "snakes" also developed very rapidly in these former "normal" participants when they started to wear contact lenses

Discussion

In this impression cytological study very clear cytological changes in the conjunctival epithelium were found in all the contact lens wearers examined, as compared to a group of healthy controls matched for age and sex distribution. In the contact lens wearers, marked squamous metaplasia was found, in addition to reduced goblet cell counts and a variety of nuclear changes, in particular snakelike chromatin condensations at the centre of the cell nuclei. These surprising findings show that even asymptomatic lens wearers with apparently normal ocular surfaces exhibit remarkable cytological changes of the conjunctival epithelium.

Apart from the fact that these cytological changes occurred only in the group of contact lens wearers, the assumption that they were caused by lens wear is further supported by the results of the prospective clinical study. In the spectacle wearers who first served as a control group, and in whom conjunctival cytological findings were originally normal, similar cytological changes developed astonishingly fast as soon as they were fitted with contact lenses. After only 6 months the cell picture in these subjects was similar to that previously observed in the long-term lens wearers.

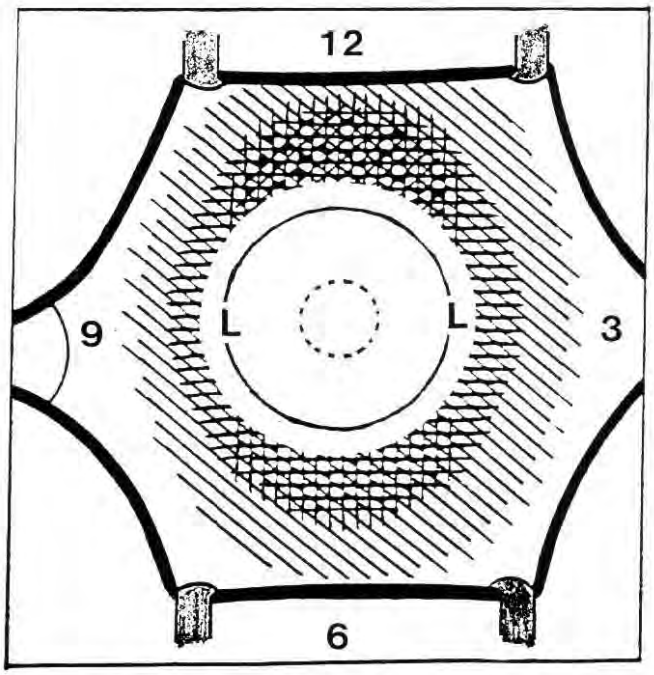


Fig. 16 Rather than being uniformly distributed over the ocular surface, the cell changes described are characteristically localized. Darker hatching denotes a higher degree of change. The changes occur only in the circular zone of lens movement on the conjunctiva bulbi. They decrease towards the fornix and are most pronounced in the principal, vertical direction of movement of the contact lens, in particular at 12 o'clock. The limbus (L) and the cornea were not studied and are therefore shown in white

However, the cell changes proved to be reversible when the supposed causative factor was eliminated. This was observed in 2 subjects who, after wearing soft contact lenses for many years, discontinued wear for about 2 years. The condition of the conjunctival epithelial cells was subsequently found to have reverted to normal (*Knop* and *Brewitt* 1991b).

Besides this chronological coincidence of contact lens wear and cytological changes in the conjunctiva (squamous metaplasia and nuclear changes), the pattern of the topographical distribution of these changes on the conjunctiva suggests a close association with lens wear: the changes described occurred only in an approximately circular area around the cornea, corresponding to the range of movement of the contact lens on the eye. The changes were most pronounced in the vertical direction (12–6 o'clock), corresponding to lens movement caused by blinking. The lateral changes were less marked. Outside this zone, at 10–15 mm from the limbus, the epithelium was usually found to be unchanged.

This topography in particular suggests that the cell changes were directly due to a mechanical cause, i.e. persistent irritation by the edge zone of a contact lens. Other, theoretically possible lensassociated factors (*Roth* 1979, *Ehrich* 1981, *Ruben* 1982, *Ehrich* 1985, *Junker* 1985, *Kok van Alphen* 1987, *Miller* 1987, *Rao* and *Saini* 1987) influencing the development of cell changes, such as insufficient tear quantity or reduced tear film break-up time, were ruled out by examination, which showed all the subjects to have normal tear film status. It is improbable that the changes were due to allergic reactions or infections, since the participants were asymptomatic, slit lamp findings were normal, and leukocytes were absent from almost all the cytological specimens except for some granulocytes seen on one occasion in each of 5 subjects.

Direct effect of contact lens as chronic mechanical irritant

The influence of toxic effects due to lens solutions was minimized by using a preservative-free sodium-dichloroisocyanurate system which is also relatively well tolerated when used incorrectly (*Brewitt* and *Conrads* 1986, *Knop* and *Brewitt* 1989). In addition, however, it would be difficult to explain the different topographical changes, and in particular the difference between the superior and inferior conjunctivae, as being due to a toxic influence. On the other hand it is certainly conceivable that the mechanical load at 12 o'clock, under the combined pressure of the contact lens and the upper lid, is greater than at 6 or 3 o'clock. The hypothesis that the contact lens has a direct effect as a chronic mechanical irritant is further supported by the fact that among the participants with marked cell changes, those with relatively mobile lenses were overrepresented.

No epithelial keratinization observed

Until now, cell changes of this type have been observed only in ocular surface disease, in particular associated with "dry eye syndrome" (*Marner* et al. 1980, *Nelson* et al. 1983, *Tseng* et al. 1984, *Götz* et al. 1986, *Nelson* and *Wright* 1985, *Liotet* et al. 1987, *Koch* and *Waubke* 1987). Squamous metaplasia and large numbers of snakes are also found in these diseases. However, as is clear from published data, keratinization of the epithelium is also regularly observed. No such keratinization (Fig. 8) was seen in the contact lens wearers studied by us.

Thus, while the changes observed in this study are no doubt basically similar to those seen in dry eye syndromes, it appears that they are less severe in contact lens wearers.

Contact lenses alter conjunctival epithelium and lead to morphological changes similar to those seen in many ocular surface disorders

Contact lenses and dry eye disorders evidently alter the integrity of the ocular surface in a similar way. However, considering the results presented here it may be assumed that contact lenses have a direct mechanical influence on the ocular surface and its cell morphology. This may also have a secondary, delayed effect on tear film stability. On the other hand, ocular surface diseases possibly interfere with the complex regulatory system consisting of the surface morphology and mucus physiology at other points, and evidently do so more directly. Ultimately, the tear film stability is – presumably always – reduced as a consequence, causing intensified mechanical stress on the epithelium due to increased friction. This is conceivably due to reduction of the aqueous phase (as in Sjögren's syndrome, or direct cell destruction, e.g. associated with allergies (Stevens-Johnson syndrome, pemphigoid). In any event, the conjunctiva, like the epidermis, reacts to different (pathogenic) stimuli with relatively uniform changes. These include squamous metaplasia and reduction of the goblet cell count. Keratinization may also occur.

Contact lens presumably interferes with mucus system by reducing goblet cell count and changing cell surface

It is still not clear how far contact lenses interfere with the mucus system of the eye. Even though we found reduced goblet cell counts in lens wearers – this has also been observed in dry eye (*Ralph* 1975, *Nelson* 1984, *Tseng* et al. 1984) – signs of an increase in the mucin content of the tear film have also been observed in lens wearers (*Treumer* et al. 1986), possibly due to a greater impact of the "secondary mucus system" (*Greiner* and *Allansmith* 1981, *Rohen* 1986, *Brewitt* 1988). As a direct mechanical infuence, contact lenses may also cause changes in the normal surface morphology (Fig. 3) of the epithelial cells, which then reduce the accumulation of mucus on the cell surface, as a precondition for an intact tear film. However, it proved difficult to count the goblet cells accurately and reproducibly by impression cytology, which also seems to be less reliable than genuine histological methods (*Nelson* and *Wright* 1985, Comments). In most cases it furnished only imprints of goblet cell mucus instead of complete goblet cells (*Knop* and *Brewitt* 1991a). Moreover, goblet cell counts vary widely at different points on the conjunctiva (*Kessing* 1986, *Marquardt* und *Wenz* 1979). This also finds expression in the high standard deviations in goblet cell counts, not only in our study but also in those by other authors (*Nelson* and *Wright* 1984).

"Snakes" also observed in a normal subject

Nor has the reason for the snakelike condensation of chromatin been elucidated. It is evidently a progressive regrouping (*Knop* and *Brewitt* 1990) of the chromatin at the centre of a still intact nuclear membrane (as established by light and electron microscopy) (Figs. 10 and 11b). Snakes were also observed in a few cells obtained from one normal participant. However, since this was clearly a pathologic change, the subject in question may already have had pathologic changes of the ocular surface which were not (yet) detectable at routine ophthalmological examination. This supports the supposition that a variety of factors influencing the ocular surface can cause similar cytological changes. At the same time it may be assumed that snakelike nuclear changes will without doubt be found in a certain percentage of subjects with normal slit lamp findings. This finding also underscores the fact that impression cytology is highly sensitive to pathologic morphological changes of the ocular surface, especially in contact lens wearers, in the fitting of contact lenses (*Liotet* et al. 1987), in wetting disorders and in all so far unexplained disorders associated with lens intolerance.

Wearers asymptomatic despite conjunctival epithelial changes

In view of the similarity of the cytological picture in contact lens wearers and patients with "dry eye" it is all the more surprising that all the contact lens wearers in this study were asymptomatic at the time of examination and that the symptoms observed thus had no obvious pathological significance initially. However, it is not only possible but indeed likely that, at least in the longer term, squamous metaplasia with changes in cell morphology and a reduction in the goblet cell count will have a functional effect on the complex system of production and preparation of an intact precorneal tear film. Therefore, such cell changes must at least be suspected of constituting a possible preliminary stage of dry eye following prolonged contact lens wear.

Cell changes nevertheless a possible preliminary stage of dry eye

Squamous metaplasia a serious morphological change

From a cytological point of view, on the other hand, a metaplasia, i.e. the metaplastic change of a differentiated – in this case highly prismatic – epithelium into a differently differentiated – in this case squamous – epithelium is a change which must always be taken seriously. At the very least it is indicative of increased stress on the epithelium in question (*Walter* and *Israel* 1974, *Freudenberg* 1980). In other medical fields it has been established that metaplastic restructuring (e.g. of the epithelial transition zone between the uterine cervix and portio, and of the bronchial epithelium) is also associated with a risk of neoplastic degeneration (*Robbins* 1974, *Grundmann* 1986). However, since no increase in the incidence of ocular surface neoplasms has been reported in recent decades, it is impossible to gauge the importance of the results of this study as yet. In any event, a further detailed study of these changes appears necessary. We are at present performing such a study. As established with 2 participants in this study, these changes are probably reversible if lens wear is discontinued.

Theoretical risk for neoplastic degeneration

Changes probably reversible if lens wear discontinued

Our results emphasize once more that contact lens wearers should be examined regularly by an ophthalmologist. A more sophisticated diagnostic procedure such as impression cytology may be necessary from time to time to permit qualified assessment of the ocular surface.

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