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Induction of conjunctival epithelial alterations by contact lens wearing *,**

A prospective study

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Abstract. In a prospective clinical study, contact lenses were applied to 14 wearers of glasses with clinically and cytologically normal conjunctiva. The alterations of conjunctival cytology after the start of contact lens wearing were recorded with the technique of impression cytology over a period of 6 months. Within the first few weeks after application of the contact lenses, the patients developed a rapidly increasing alteration of cell size and nuclear morphology. Enlargement of the cell diameter with flattening of the cell body was seen together with numerous different nuclear alterations, in particular snakes (condensations of the nuclear chromatin into a sticklike or snakelike shape, centrally arranged in the nucleus). After this very rapid onset, the alterations increased more slowly towards a possible saturation point (and it seemed that the ultimate changes might possibly not be reached within the investigated period). At about 3–6 months, the patients reached a degree of cytological alteration which was seen in long-time contact lens wearers in our previous study and before this, except for the lack of keratinization, only observed in diseases of the ocular surface. There was a distinct squamous metaplasia of major parts of the conjunctival epithelium which normally is columnar and a decrease of goblet cell density. Squamous metaplasia and nuclear alterations increased with the length of time which had elapsed since the application of contact lenses and with extending the daily wearing time but were always restricted to the contact lens excursion zone on the eye and disappeared after contact lens omission, as seen before. Even patients with major cytological alterations remained free of symptoms, and the conjunctiva was still clinically normal.

* Dedicated to Professor Dr. med. Enrico Reale for his 65th birthday

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Introduction

In our preceding study [23], the conjunctival epithelium of long-time contact lens wearers was systematically investigated with the technique of impression cytology (IC). Surprisingly, the picture of distinct alterations of the epithelial cytology was seen in the form of a squamous metaplasia of the normally stratified columnar [27, 39, 43] epithelium together with numerous nuclear alterations. This is, with the exception of keratinization, an epithelial status only reported before in diseases of the ocular surface, mainly associated with dry eye syndromes; but in contrast to these, in the year-long contact lens wearers, it was not accompanied by any symptoms.

The fact that these alterations were found in all contact lens wearers but not in a comparable group of wearers of glasses gave clear indications of the possible causal importance of the contact lens for the induction of these changes. Hence, this study was performed in order to investigate the question of whether the observed cellular alterations would also arise in a primarily normal conjunctival epithelium after the start of contact lens wearing, and if so, which dynamics this process would show.

For evaluation of the cytological status, as in the preceeding study, IC was employed because it is noninvasive and painless, is tolerated by asymptomatic contact lens wearers and even wearers of glasses, and does not harm the conjunctival epithelium as would be unavoidable with biopsies. Nevertheless, it revealed sufficient information about the conjunctival epithelium because this kind of exfoliative cytology, developed by Egbert et al. [6] and modified by Adams [3], Marner [28], Nelson and Wright [31], and Tseng [44] alows us to obtain a relatively homogeneous, mostly one-layered sheet of the superficial conjunctival cells on a small piece of Millipore filter paper, preserving the lateral cell adhesion. Hence, it gives qualitative as well as quantitative information on the status and alterations of the epithelium, which usually is histologically classified by the morphology of its outermost cell layer [7].

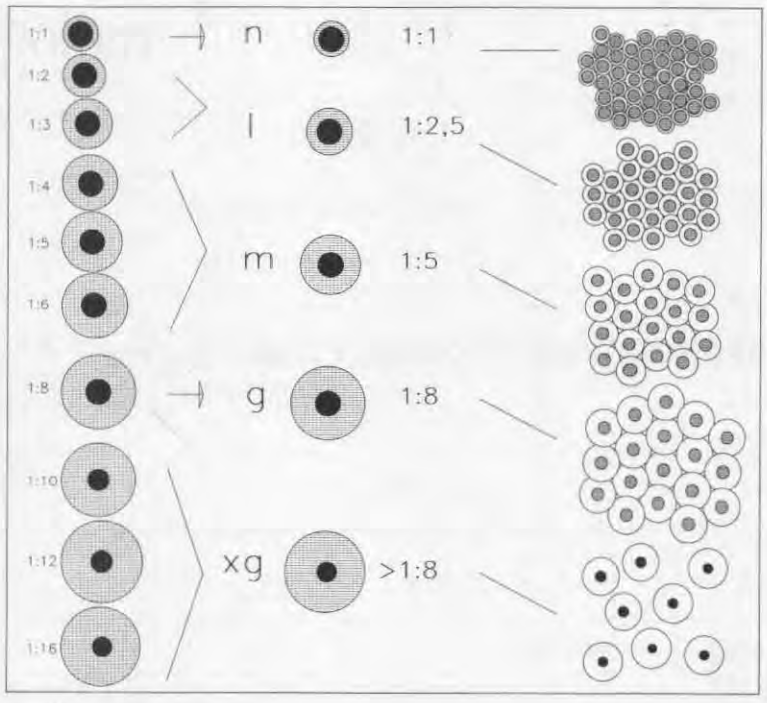


Fig. 1. Computer simulation of the observed cell sizes (*left*); 5 of these were used for classification (*middle*). With unaltered nuclei, every increase of cell diameter is correlated with a respective change of the nucleocytoplasmic (n/c) ratio until nuclear pyknosis (indicated by dense nuclear structure, *right*) occurs beyond double normal diameter ($\approx$n/c-ratio $>1:8$). Simulations of homogeneous cell clusters of just one respective size was helpful for recognition in the actual specimen (*right*)

Material and methods

For this study only patients were admitted who were free of symptoms, showed a normal ocular surface as detected by macroscopic inspection and slit-lamp microscopy, had normal tear film parameters in respect to tear volume and quality as demonstrated by Schirmer's test and tear film break-up time test, and presented a normal impression cytology. The group consisted of 14 wearers of glasses, volunteering for a contact lens study [18]. They had never worn a contact lens before, and the only abnormality consisted in minor refractive disorders.

After verifying the normal status of the conjunctival epithelium, a modern daily wear soft contact lens (polyalcylmethacrylate-NVP-copolymer) with a water content of about 55% in the hydrolyzed state and a oxygen transmission coefficient of 17×10^{-11} [$cm^2 \cdot ml\ O_2 \cdot s^{-1} \cdot ml^{-1} \cdot hPa^{-1}$] was applied. The lens was regularly treated with a preservative-free contact lens hygiene system on an oxidative basis.

The lenses were worn daily for a time of about 11 h and showed good objective wearing results and subjective wearing comfort. They were 13.70 mm or 14.30 mm in diameter (mostly 14.30 mm) and from 8.10 to 9.30 mm in radius (preferably 8.70 mm) to give good lens fitting with sufficient mobility, according to the manufacturer's advice (the lens base curve was about 0.90 mm larger than the corneal radius in average). The mean refraction was in the moderate minus area at about -3.00 D [18].

The cytological and clinical status of the conjunctiva after the start of contact lens wearing was recorded over a period of 6 months, first at weekly and later at monthly intervals. The examinations were carried out in order to guarantee an optimal fit of the lens, to detect contact lens associated problems, and to correlate these with the eventual alterations of epithelial cytology. These examinations included:

- Routine ophthalmological investigation including slit-lamp examination of the ocular surface, test of visual acuity, and anamnesis according to a standardized protocol
- Slit-lamp examination of the contact lens
- IC of the conjunctival epithelium with photorecording

The ICs were obtained by gently applying small pieces of Millipore filter paper (VSWP 0.025 µm) to the conjunctival surface. For light microscopy (LM) they were processed by air drying, stained according to the procedure of Tseng [44], and treated with xylol to make them transparent. The employment of a tensiometer [4, 8, 28, 36] to obtain cells under controlled pressure proved to be unnecessary and carried the risk of later spoiling the whole specimen with dissolved glue-mud during the xylol treatment. In contrast, our technique of mounting the forceps-held specimens with double adhesive tape only on one end to a glass slide avoided this and allowed us to process even large amounts of specimens without employing any special equipment [44]. For transmission and scanning electron microscopy, the specimens were either air-dried or fixed by glutaraldehyde immersion and then processed conventionally [19, 20].

In all patients, the specimens were taken from the upper bulbar conjunctiva at 12.00 o'clock in the 3–5 mm limbal distance. This is

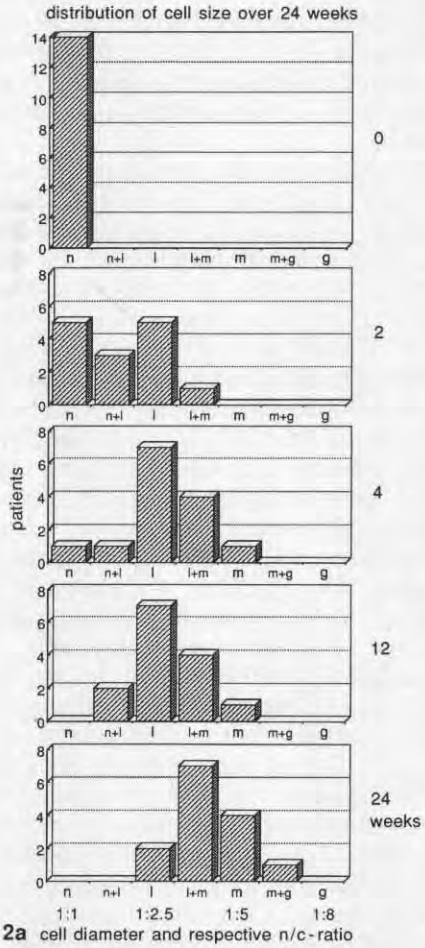
Fig. 2a–c. After the start of contact lens wearing, the dominant cell size changed progressively from the initial normal size (*left*) towards the more enlarged stages (*right*, **a**). The number of patients with a still predominately normal cell size decreased dramatically within the first weeks of contact lens wearing (**b**). The average cell diameter and the respective n/c ratio increased rapidly after the application of lenses, indicating squamous metaplasia of the epithelium (**c**)

Fig. 3. Early stage of cell size alteration with small zone of cell enlargement (*central*) and a surrounding area of normal cell size (*upper and lower margin*); in part (*upper left corner*) the specimen shows a double cell sheet ($\times 221$)

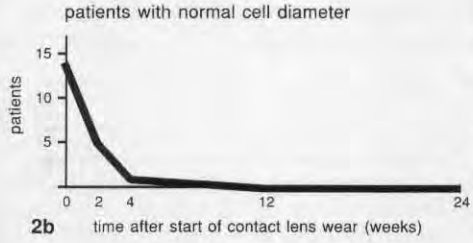
Fig. 4. Advanced stage of cell enlargement with a broad longitudinal zone of enlarged cells in the middle of the picture (preferably in horizontal direction at the upper bulbar conjunctiva) which is limited by neighboring zones of less alteration ($\times 56$)

Fig. 5. With continuing contact lens wearing the affected zones of cell enlargement are confluent. Centrally, in the limbus direction (*middle and lower part*), there are predominately very large, flat, and deformed cells, while towards the bulbar periphery (*upper corners*), the cells are less altered ($\times 56$)

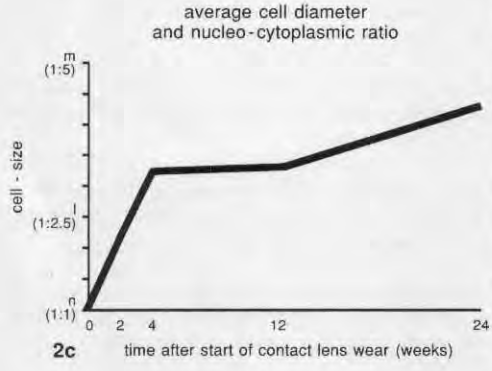
Cell size



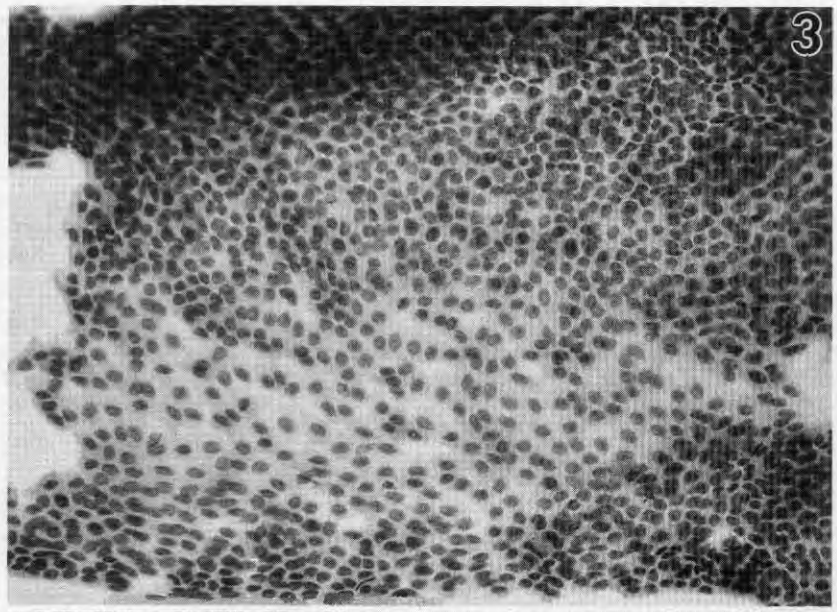
2a cell diameter and respective n/c-ratio

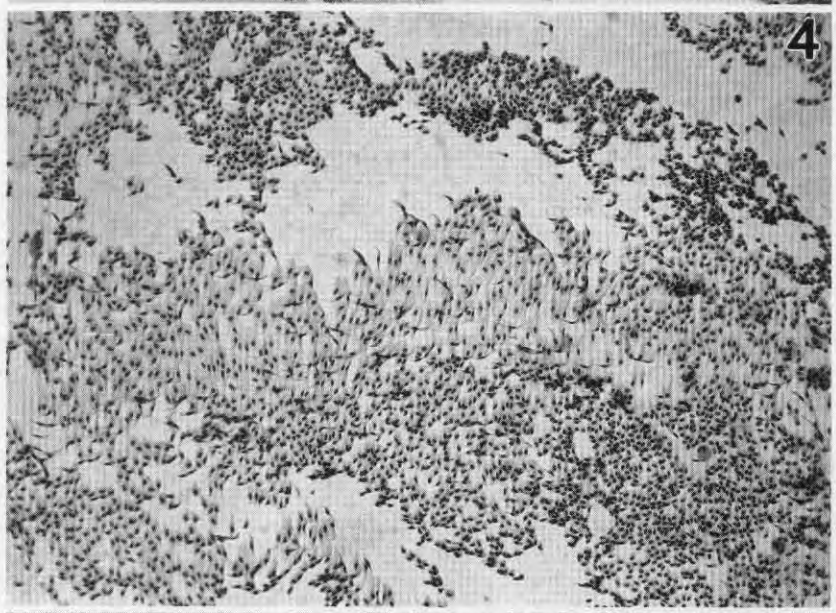


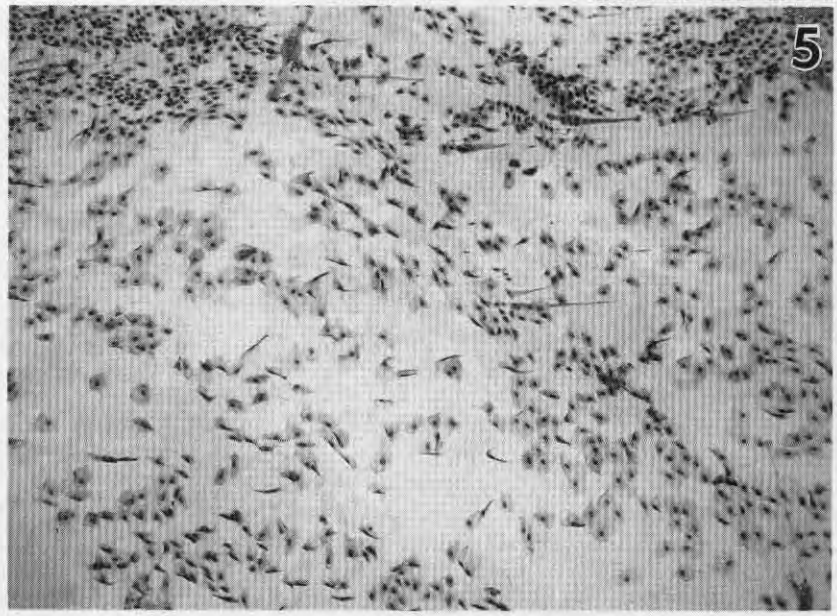
2b



2c







a region which already shows (as proven by the initial ICs) at least isoprismatic cells and has been chosen by most of the other authors in studies on various functional disorders of the ocular surface. Our findings of epithelial cytology are therefore directly comparable with other data in the literature. Additional specimens were taken at other locations around the limbus (3.00, 6.00, and 9.00 o'clock positions) to gain information about the topographical distribution of the alterations. Cell assessment was performed according to the cell size and nucleocytoplasmic ratio (n/c ratio), alterations of nuclear morphology (especially snakes), goblet cell density, and possible incidence of keratinization.

In order to obtain a qualified assessment of the cell size without direct measurement of the actual cell, which would have caused major practical problems because of the multitude of cells obtained (and hence reduced clinical practicability), we chose a semiquantitative method [17] which gave differentiated and reproducible results. The cell sizes present were observed, schematical drawings of all steps of cell enlargement (Fig. 1, left) performed, and 5 of these used for classification (Fig. 1, middle). Until the onset of nuclear pyknosis beyond double the normal cell diameter, obviously only the cell diameter increased, while the size of the nucleus usually remained unchanged. Hence, a certain grade of cell diameter enlargement was correlated to a certain n/c ratio, which was easily detectable by cell assessment. For better recognition of these different stages in the specimen, a computer simulation of ideal cell clusters composed of just one respective size was performed (Fig. 1, right), which proved to be very helpful in the practical work at the microscope.

The 5 different steps of cell size ranged from n/c ratio 1:1 (=*n*, normal) to beyond 1:8 (=*xg*, very large cells) (Figs. 1, 10). The specimens were rated according to the dominant size in the sample or two equally dominant neighboring sizes (Fig. 2a). The occurrence of snakes was classified into 6 steps (0–5) according to the relative number (0% to more than 75%; Fig. 6a) of cells with snakelike chromatin to all cells obtained in the specimen. Goblet cells and their mucous impressions on the filter were counted for the determination of the goblet cell density.

Results

The majority of the specimens had a relatively homogeneous, one-layered cell sheet, and all showed the typical IC morphology of a normal conjunctival epithelium with densely adjacent cells of equal size, roundish shape, and a small cytoplasmic rim resulting in a n/c ratio of 1:1 (Fig. 3). Usually there were few or no complete goblet cells in these specimens, but only imprints of their mucous products on the filter without nucleus and cell membrane. Complete goblet cells were only seen in occasionally obtained multilayered cell sheets. One of the wearers of glasses surprisingly already had some snakes in less than 10% of his cells.

After the application of contact lenses, the cell diameter increased rapidly (Fig. 2c), and the number of patients with a still predominantly normal cell size decreased in the same dynamics (Fig. 2b). Already after 1 week, there were areas of cell enlargement, and after 2 weeks, only ⅓ of the patients still had a predominately normal cell size, while in ⅔ of them epithelial cells of more than the n/c ratio 1:1 dominated the specimens (Fig. 2a).

Another 2 weeks later, the picture had changed completely and only 1 of the patients still had predominately cells of normal size, while in more than 90% the average cell size had increased considerably to a n/c ratio value of about 1:3. After 3 months, none of the former wearers of glasses showed a predominately normal cell size. Till the end of the study (6 months), the average cell size was still rising up to a level of about n/c ratio 1:5. With this increase of cell diameter there was a progressive flattening of the cell (Fig. 11a) combined with a higher incidence of single cells (Fig. 4) on the filter, so that pyknotic cells (with more than double the normal diameter) were mostly seen isolated (Fig. 5). Keratinization was not observed in these cells.

Changes of the nuclear morphology were seen as the reported pyknosis, furthermore karyorrhexis, nuclear doubling resulting in two roundish, pyknotic nuclei, various forms of nuclear inhomogeneities, and, most frequently, snakelike aspects of the chromatin. These obviously progressive [21] condensations of the chromatin into an elongated sticklike or snakelike structure were centrally arranged in a nucleus with an intact nulear envelope. With scanning electron microscopy (SEM), it was possible to demonstrate that the chromatin condensations were of very massive structure (Fig. 12b), which became prominent upon air drying under the collapsing rest of the cell body and the nuclear envelope. The shape of the chromatin structure was exactly like that in the LM picture of the same cell (Fig. 12a). Transmission EM showed the atypical central position of the chromatin condensations (Fig. 12c). Snakes were again seen in the less enlarged epithelial cells rather than in the very large ones (which had pyknotic nuclei), and the affected cells were regularly arranged as clusters interspersed in the layer of normal cells. Within these clusters, the snakes were preferably arranged in the same orientation, with the longer axis of the nucleus corresponding to the longer axis of the cluster (Figs. 7, 8): They were most frequent at the upper bulbar conjunctiva in the 12.00 o'clock region, less so in the other regions of the bulbar conjunctiva.

The development of snakelike chromatin after the start of contact lens wearing (Figs. 6) was, at least in the first 4 weeks, not quite as rapid as the incidence of squamous metaplasia. But nevertheless, after only 2 weeks of contact lens wearing, about 40% of the patients

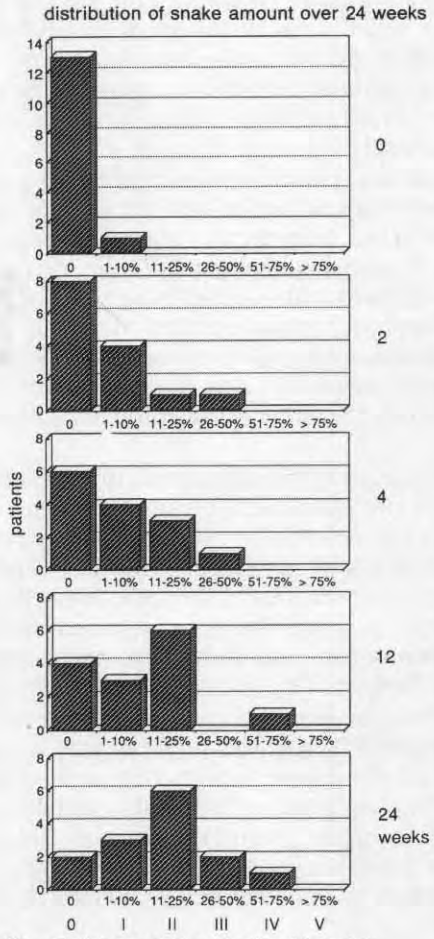
Fig. 6a–c. After the start of contact lens wearing and depending on the elapsed wearing time, the number of cells with snakelike chromatin in their nuclei increased (indicated as a move to the right side of the graph, **a**). The number of patients which were still free of snakelike nuclear condensations decreased progressively during the wearing period (**b**). The average proportion of cells with snakes increased not quite as rapidly as the cell size, but the curve is still more than linear (**c**)

Fig. 7. Early stage of cell alteration with a few snakes, typically arranged in a cluster, interspersed in an area of mild cell enlargement (×250)

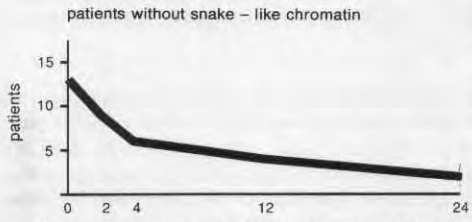
Fig. 8. Large clusters of snakes, mostly longish and horizontally arranged (as in the upper middle and lower margin), frequently accompanying zones of major cell enlargement (×175)

Fig. 9. Nearly all the enlarged, loosened, and partly deformed cells of this specimen show snakelike chromatin condensations in their nuclei, representing stage V of the snake classification (>75% snakes, ×357)

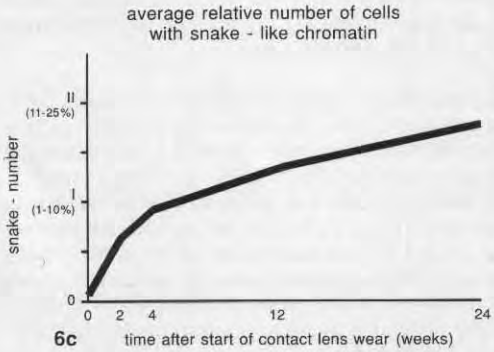
Snakes



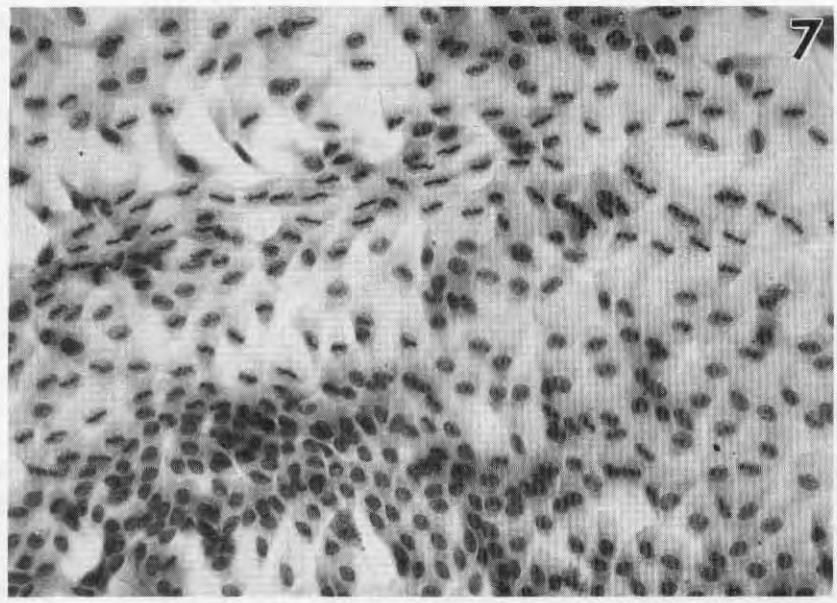
6a relative percentage of cells with snakes

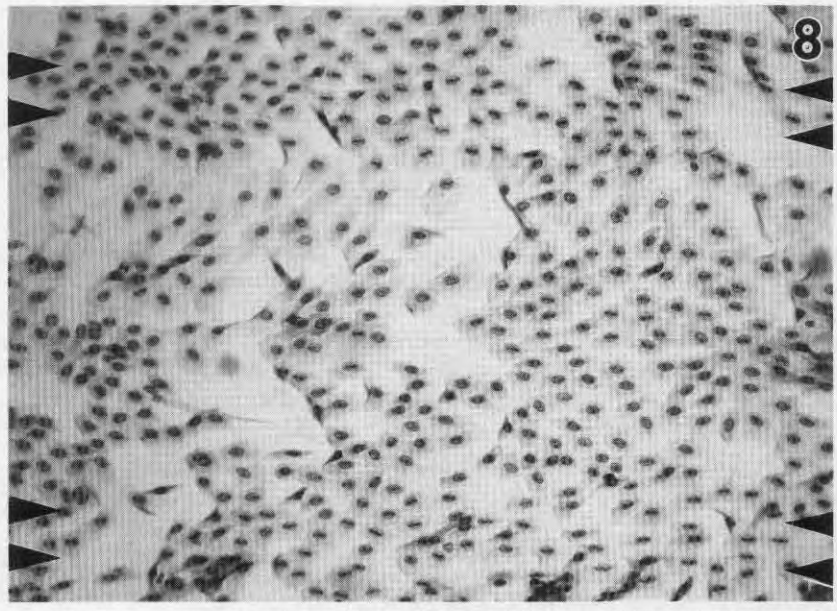


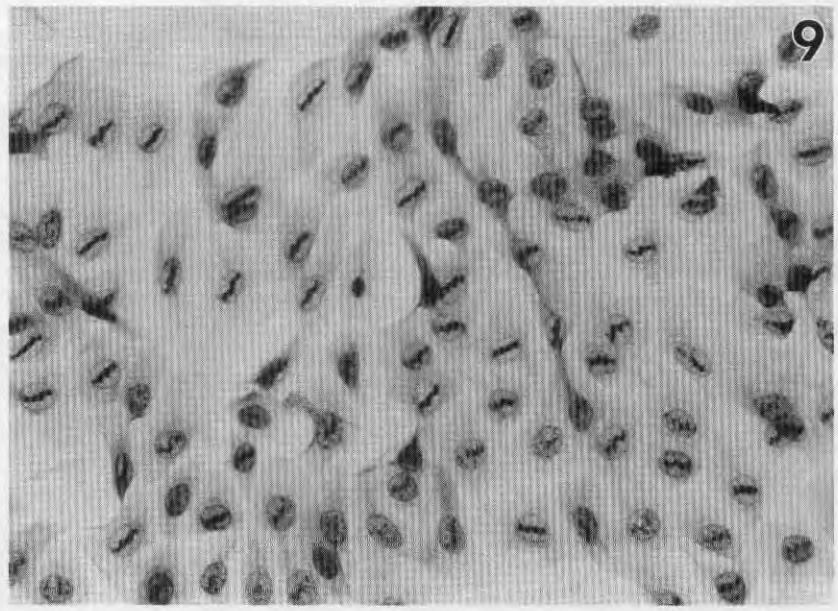
6b time after start of contact lens wear (weeks)



6c time after start of contact lens wear (weeks)







showed snakes, and after 4 weeks more than 50% had them in an average of about 10% of their obtained cells. Within 3 months of contact lenses wearing, more than ⅔ of the patients had developed snakes, and the relative percentage of this phenomenon had further risen to 11%–25% of their cells (Fig. 9).

By 6 months after the use of contact lenses, only 2 patients were still free of snakes, while about 80% of the formerly snake-free patients had developed this nuclear alteration. Both of them did not wear the lenses consequently and, if so, had a relatively low daily wearing time (less than 8 h compared with the average of more than 11 h), but even 1 of these 2 had a few snakes once (at the investigation 4 weeks after application). The average relative percentage of snakes was at this time about 25% of the cells obtained at the upper bulbar conjunctiva.

The observed changes in epithelial cytology first occurred in localized areas of small extension and roundish or ovoidal elongated shape (Fig. 3, lower center). Later, these areas increased and usually had an elongated shape (Figs. 4, 8], with a preferably horizontal orientation at the upper bulbar conjunctiva. Clusters of snakes were often found colocated with the areas of cell enlargement and in the same orientation (Fig. 8). With continuing contact lens wearing over more than 3 months, these areas of cell alteration increased and were confluent in all patients (Fig. 5).

The characteristic topographical distribution of the cell alterations on the bulbar conjunctiva, as observed in long-time contact lens wearers [23], with an expression of alterations only in a ring-like zone of about 1 cm in diameter around the limbus and normalizing epithelium in the bulbar periphery (Fig. 5, upper corners) combined with the most prominent alteration at 12.00 o'clock, less at 6.00 o'clock, and even more reduced at the sides, was confirmed in this study.

The goblet cells did not show a morphological alteration detectable by light microscopy due to contact lens wear. Before contact lens application, the patients had an average density of 194.75 ($\pm$120.63) goblet cells/mm^2 at the preferred location (12.00 o'clock, 3–5 mm limbal distance). At the end of this study, the average goblet cell density had decreased to a value of 133.54 ($\pm$46.97). The starting value is already relatively low compared with histologic [15] goblet cell counts and pathologically low compared with other IC-based counts [32, 33] at this location.

This study showed a reduction of goblet cell density of about ¼ within 6 months of contact lens wearing. The goblet cell values found in this study are to be considered in respect of some technical difficulties (see Discussion), which might reduce their validity. This is a result of the IC generally and of the position on the conjunctiva that was chosen to allow comparability with other authors.

Discussion

The IC method proved to be a very beneficial tool for the evaluation of conjunctival epithelial morphology by obtaining relatively intact superficial cell layers without harming the epithelium or causing any irreparable loss of epithelial substance. These advantages are not known in any other cytological or bioptical technique.

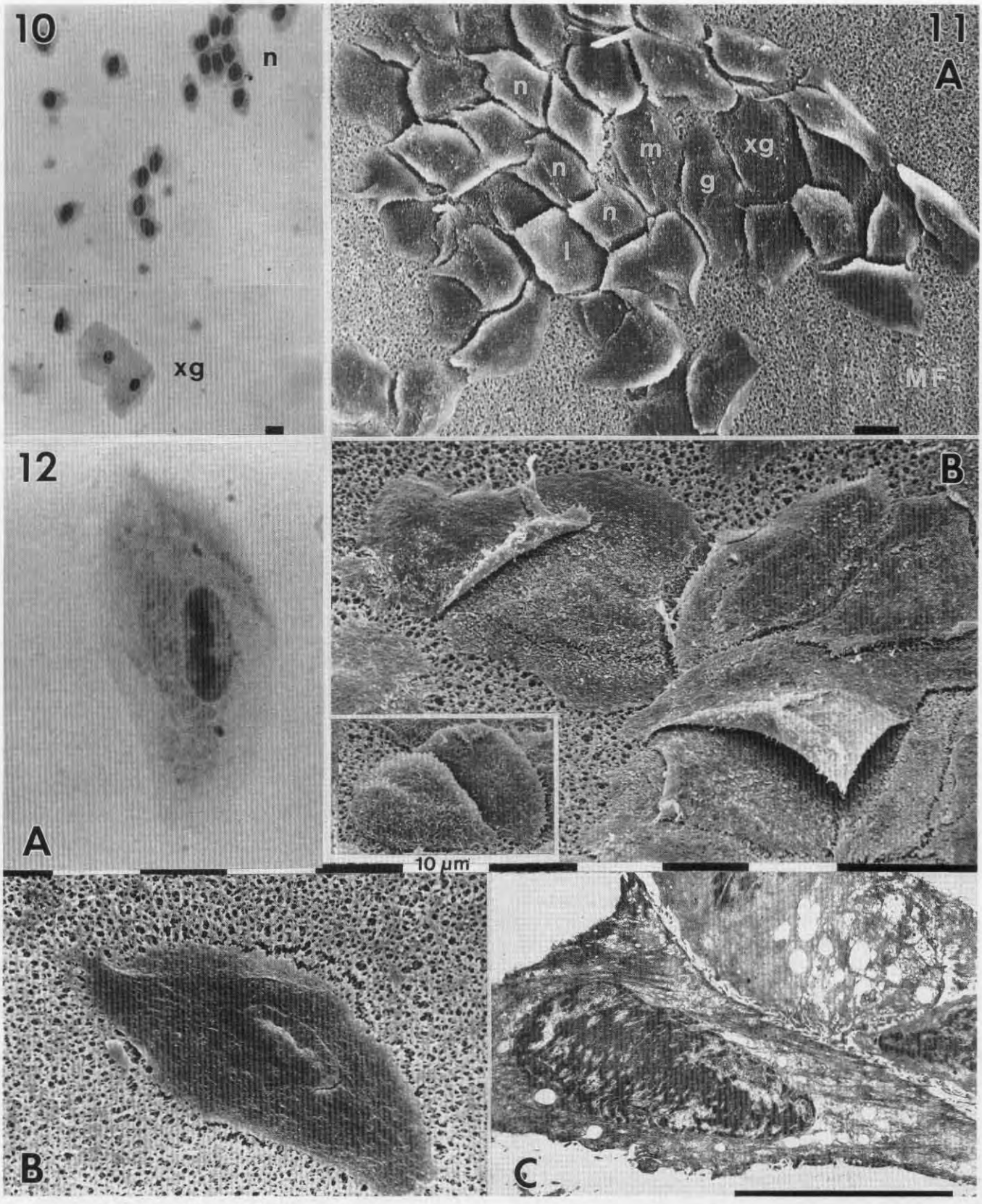
If goblet cell information is needed, the advantage of obtaining mere impressions turns into a disadvantage. Surprisingly, the IC did not obtain complete goblet cells in the usual one-layered cell sheets, although they are situated in this epithelial layer. They were preferably found in occasionally removed sheets of more than one cell layer in thickness. Instead of intact goblet cells, there were mostly only impressions of the mucus protruding out of their apical cell faces on the filter which was used for the counts. The employment of a different filter material (Millipore GSWP) with a larger pore size (0.22 µm) did routinely remove multilayered cell sheets, but this procedure proved in a self-trial to be painful, only tolerable under topical anesthesia, and could, at least in repeated performance, possibly cause epithelial alteration itself.

For principal reasons, the assessment of mere mucus imprints seems to be insufficient because missing mucus impressions do not necessarily exclude the presence of goblet cells, while numerous mucus stains could be the result of repeated impressions of the same few cells. Besides that, mucus imprints are often smeared and not clearly detectable in this case. Hence, the intra- and interindividual differences in goblet cell density as well as the standard deviation were relatively high in our studies and in the literature [32, 33]. This might in part be influenced by the high physiological differences in this region depending on the exact position, with goblet cell densities possibly doubling within 2 mm [15]. Keeping an exact position within 1 mm is obviously easier in biopsies [29] or flat preparations [15] of conjunctival material than in IC.

Fig. 10. Normal-sized conjuntival epithelial cells (*n*, n/c-ratio 1:1) together with 2 cells of highly increased cell diameter and pyknotic nucleus (*xg*, ×275)

Fig. 11 A, B. Scanning electron microscopy of aldehyde-fixed material in a cell enlargement zone shows that normal cells (*n*) have a small size and a prominent cell body (indicated here by deep clefts between the cells) while progressive steps of cell diameter enlargement (*l-xg*) due to epithelial alteration are accompanied by increasing flattening of the cell shape into the typical form of squamous epithelial cells. The cells are still on the Millipore filter paper (*MF*; **A**, ×734). Areas of highly enlarged cell diameters express the typical picture of squamous metaplasia with flat cells, showing alterations of the regular shape as rolling cell margins and separation into thin sheets (**B**, ×1489; *bar* = 10 µm)

Fig. 12 A–C. Enlarged cell with typical snakelike chromatin (condensed and undulated structure) in light microscopical picture (**A**, ×1440). Scanning electron micrograph of the very same cell shows that the snakes are massive structures becoming prominent on the surface if the rest of the cell collapses due to air drying (**B** ×1440). The shape of the snake remains unchanged independent of the technique applied and is recognized as identical in both pictures. Transmission electron micrograph of a similar cell shows the development of the uncommon central arrangement of chromatin leading to the ondulated snake appearance in the right part of the nucleus (**C**, ×4000; *bar* = 10 µm)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In contrast to this, our reported semiquantitative method of the assessment of cellular and nuclear changes proved to be easy to perform and to give valid and reproducible data, as seen when repeated assessment of identical specimens was performed from time to time. A certain amount of practice is necessary, anyway, both in obtaining the specimens from the patient and in performing the actual cell assessment. For these reasons, the results seem generally more homogeneous if IC is performed by the same person every time.

Although our preceeding study with long-time contact lens wearers had shown a remarkable amount of cellular alterations (resembling, except for keratinization, those in ocular surface diseases), in the present study the exceptionally rapid dynamics surprised us, as these alterations arose after the application of a soft contact lens in prior wearers of glasses with normal conjunctival epithelium. Recent results of our latest investigations on hard, gas-permeable lenses indicate similar changes to those with the soft ones. Within the first 4 weeks, the development of morphological changes (Figs. 2, 6) reached the picture of localized squamous metaplasia with nuclear changes. After a rapid onset, in the second part of the study, they increased more slowly and, after about 3–6 months of contact lens wearing, showed a picture as observed in long-time contact lens wearers, with alterations in major areas of the bulbar conjunctiva. The incidence of cytological alterations appeared in all patients as a characteristic pattern, with an early increase of cell diameter and subsequent snakelike nuclear condensations.

The causal importance of the contact lenses for the onset of these changes seems indisputable since the patients had a normal conjunctival epithelium before they started wearing contact lenses, and the symptoms increased with length of elapsed time. In addition, the epithelial changes were only seen with in the excursion zone of the contact lens on the ocular surface. Observations under the same controlled circumstances in a group of long-time contact lens wearers [17], to whom the same lens had been fitted, showed that the duration of daily contact lens wear is also of major importance: Within half a year, these patients developed further, increasing cellular alterations as the daily wearing time was extended. The importance of the daily wearing time is furthermore indicated by the fact that patients who were less affected (such as the 2 patients without snakes) wore the lenses less and had a reduced daily wearing time.

Luckily, this cytological picture of the conjunctival epithelium seems not directly correlated with its functional status because there were no findings detectable by routine ophthalmological techniques and no complaints from the patients. Hence, these alterations obviously do not carry any direct pathological significance. The exact pathomechanism leading to these epithelial changes is still unclear, but the majority of reasons usually cited for problems in contact lens wearing [25, 30, 37, 40] are most likely not involved in this case. Inflammation or allergic reactions are less likely, since they would not show the topographical restrictions, and additionally they are excluded by the normal slit-lamp findings. A possible influence of the contact lens solution was reduced by use of a preservative-free solution [18]. Relative anoxia under the contact lens on the other hand is unlikely to produce the observed topographical differences in the grade of cytological change.

A direct mechanical induction of the squamous metaplasia and snakes via chronic mechanical irritation is indicated by the restriction of alterations to the contact lens excursion zone around the limbus with normalization to the bulbar periphery and their prominent expression in the preferred vertical (12.00 to 6.00 o'clock) direction of contact lens movement, especially at 12.00 o'clock. The longish zones of cell changes (Figs. 4, 8) seen in the first weeks after the start of contact lens wearing could in this sense very well correspond to impression of the contact lens margin. The homogeneous orientation of the snakes in the direction of the longer axis of the clusters (Figs. 7, 8) possibly gives an indication of a direct mechanical etiology.

The further importance of the squamous metaplasia and nuclear alterations is still unclear, but it is possibly related to contact lens problems not rarely observed in very long-time contact lens wearers, resembling mild dry eye symptoms. It seems unlikely that distinct morphological changes of the ocular surface, such as squamous metaplasia of major parts of the conjunctiva normally showing a columnar epithelium with frequent goblet cells and playing an important role in the maintenance of the integrity of the ocular surface [1, 2, 10, 14, 22, 35], can be without consequences in the long run.

The prognosis of squamous metaplasia in general differs to a certain extent in the pathology literature. Unquestionable at least is that squamous metaplasia represents the change of one type of differentiated epithelium into another type of similar differentiation grade which is usually caused by chronic irritation [12, 16, 41, 45] and is of a principally reversible nature [16, 41, 45]. This process of alternating cell differentiation obviously increases the risk of possible neoplastic dedifferentiation, and some carcinoma are known to arise via squamous metaplasia [12, 38] but still it is usually not regarded as an obligate precancerosis [16, 41, 42], although the prognosis is dependent on the actual organ affected [11, 45].

Anyway, there are more direct results of squamous metaplasia. At first, a decrease of goblet cell density could lead to a lack of mucus [26] on the ocular surface, and secondly, this alteration of the epithelial surface morphology might result in a decreased adhesion of the remaining mucus [2] on the conjunctiva. Both will most likely result in a reduction of tear film stability [4, 5, 13, 26, 29, 34], with increased friction and the possible development of dry eye symptoms. The functional integrity of the ocular surface seems to be relatively resistant to changes of its morphology, as seen by others [10], but it is unclear how long a presumed alteration of the function can be compensated.

Surprisingly, one patient already showed some snakes in his conjunctival cells while he was still wearing glasses. With the exception of one other study [28] showing snakes in a control person, normal subjects are supposed to be completely free of such alterations [33, Comments and responses]. This finding, together with the fact that

even otherwise normal conjunctival epithelium sometimes showed small areas of slightly enlarged cells or even single squamous cells, indicates that probably a certain percentage of persons considered as normal indeed already express a few morphological changes like snakes. The problem seems to be to decide what is to be considered as a normal conjunctiva and how it is to be recognized as such. Since snakes have already been seen in a variety of ocular disorders, especially those associated with dry eye symptoms, it might very well be that mild, yet undetectable ocular disorders have already caused cellular morphological alterations in some persons. Therefore, it is questionable whether single snakes in 'normal' persons are to be regarded either as only a variant of the normal status or as an early indication of an already existing but still asymptomatic ocular disorder. With an increasing number of snakes, the latter obviously becomes more likely.

The authenticity of the snake phenomenon on the other hand is not in doubt. After first having been theoretically suspected as an artifact [8, 9, 28, 36], possibly of the IC method, they have now also been shown in scrapings [46] and histology specimens [24]. We found snakes after different fixation and staining techniques, identical in appearance under electron and light microscopy, and in our present study (just like in the preceding one) the occurrence of snakes was clearly related to an external factor, which is the contact lens.

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