IOVS

Investigative Ophthalmology & Visual Science

May 1994 Volume 35, No. 6
ISSN 0146-0404

Fine Structure and Significance of Snakelike Chromatin in Conjunctival Epithelial Cells

Erich Knop and Enrico Reale

Purpose. In patients with several disorders of the ocular surface, in wearers of contact lenses, and occasionally in patients considered "normal," the epithelial cells of the bulbar conjunctiva present in their nucleus peculiar alterations of the heterochromatin arrangement that, because of the shape this assumes, are named "snakelike chromatin," or "snakes." To obtain some information about the yet unknown etiology of these snakes, the authors investigated them by electron microscopy.

Methods. Identical conjunctival epithelial cells, collected by impression cytology from long-time contact lens wearers, were first identified by light microscopy and then observed by scanning and transmission electron microscopy.

Results. In scanning electron microscopy, cytoplasmic and nuclear components of air-dried cells were seen collapsed on the snake, which became prominent at the surface, proving its high degree of compactness and showing its axial position and characteristic shape in the elongated nucleus. In transmission electron microscopy, the marginal heterochromatin of the affected nuclei was detached peripherally, forming thin chromatin strands directed toward the main nuclear axis and accumulating there into the snake structure. An important component of the nuclear skeleton, the fibrous lamina, was altered or lost, whereas the nuclear envelope itself did not move and remained intact. Stages of the snake alteration considered as advanced showed an almost completely reversed eu- and heterochromatin distribution, and nucleoli were usually no longer seen. In cases with increased epithelial alteration, there occurred various stages of segmentation of nuclei, induced by an atypical accumulation of cytoplasmic filaments, rolling up around the nucleus and constricting it like a cuff.

Conclusions. The presence of a mechanical stimulus is shown in the ultrastructural findings, and these strongly suggest that it is altering the nuclear and cytoplasmic skeleton, producing snakes and their segmentation. Therefore, snakelike chromatin is suggested as an indicator of mechanical stress on the ocular surface. Invest Ophthalmol Vis Sci. 1994;35:711–719.

In contact lens wearers, as reported previously,[1,2] epithelial cells of the bulbar conjunctiva undergo squamous metaplasia together with occurrence of various, already light-microscopically detectable, morphologic nuclear alterations, resembling the cytologic aspects of diseases of the ocular surface, for example the dry eye syndrome. The nuclear alterations are pyknosis, heterogeneities of the nuclear chromatin, anisonucleosis, appearance of two or more pyknotic nuclei in one cell, karyolysis or karyorrhexis. However, the most frequent and interesting nuclear alteration is represented by a peculiar condensation of the chromatin into a long snakelike or sticklike structure that is axially arranged in the center of an elongated nucleus. The chromatin with this arrangement, known as "snakelike chromatin" or simply "snakes" for about ten years was first described in keratoconjunctivitis sicca.[3] Since then it has been reported in various other diseases of the ocular surface,[4–9] in contact lens

From the Laboratory of Cell Biology and Electron Microscopy, Medical School Hannover, Germany.
Presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Florida, May 1992.
Supported by the Gesellschaft der Freunde der Medizinischen Hochschule Hannover e.V.
Submitted for publication December 22, 1992; revised September 7, 1993; accepted September 23, 1993.
Proprietary interest category: N.
Reprint requests: Erich Knop, Laboratory of Cell Biology and Electron Microscopy, Medical School Hannover, Konstanty-Gutschow. Str. 8, D-30625, Hannover 61, Germany.

wearers[1,2,6,7] and occasionally in patients considered as "normal."[1-3,30]

At first, snakes were suspected to be artifacts intrinsic to the method of impression cytology even from the first describer[3] because they were not seen in scrapings of the same patients. Later they have been observed in biopsies,[9] proving their independence on the method. On the other hand, apart from the studies on the conjunctiva where snakes have been described only in conditions associated with squamous metaplasia of the normally cuboidal shaped epithelial cells, central chromatin aggregation in general is a rare and nonphysiologic finding in the tissues of the human body.

Probably because of major technical difficulties in performing an electron microscopic analysis on nuclei with snakelike chromatin (as shown below), its fine structure is still unclear although attempts have been made at its resolution.[7] Hence, the purpose of the current study was to investigate the ultrastructure of these characteristic morphologic nuclear alterations and its eventual significance for the pathogenesis of ocular surface diseases.

METHODS

The epithelial cells obtained by the technique of impression cytology[10] according to a slightly modified procedure[2] were fixed either by immersion in glutaraldehyde (GA, 2.5% in 0.1 M cacodylate buffer, pH 7.4) (Figs. 1, 2) or by air-drying (Fig. 3). For light microscopy (LM), staining was performed according to the Tseng's method,[11] with hematoxylin, with basic fuchsin and with the Feulgen nuclear reaction.[12]

Cells, always adhering on the supporting filter material, were first observed in LM. Areas of special interest were then cut out and further prepared for scanning (SEM) and transmission (TEM) electron microscopy of the *identical* cells. For SEM, the specimens were dehydrated in graded alcohols, critical-point–dried, sputter-coated with gold palladium (15 nm) and observed in a Philips SEM 505.[13,14] TEM specimens were postfixed for 1 hour in OsO4 (1% in 0.1 M cacodylate buffer, pH 7.2), dehydrated in graded alcohols and embedded in epoxy resin (Epon^R). Thin sections were cut with a diamond knife, stained with lead citrate and uranyl acetate and observed in a Zeiss EM 10 electron microscope.

To reveal the snakelike structures as known from LM, and to clearly identify them in the sections by TEM, it was necessary to cut the samples, consisting of one cell layer, just a few microns thick, in a plane parallel to the cell itself and passing through the main nuclear axis (Fig. 4). The impression cytologic samples were obtained with informed consent; the tenets of the World Medical Association Declaration of Helsinki were followed; and institutional human experimentation committee approval was granted.

RESULTS

The large cells that had undergone the process of squamous metaplasia had a flat shape and were no longer cuboidal as usually found on the intact bulbar surface.[15] This flat shape was not directly evident in LM (Figs. 1, 3A) but only in SEM (Figs. 2, 3B).

In SEM, after fixation with GA (Fig. 2), the three-dimensional cell shape was well preserved and therefore the nuclei were not seen. In unfixed air-dried cells (Fig. 3B), plasma membrane, cytoplasm, and nuclear envelope (not properly stabilized before the water content of the cell was evaporated) collapsed on the solid nuclear contents, which became prominent at the specimen surface. This procedure allowed to display the shape and size of the nucleus as well as orientation and form of its snakelike chromatin condensations. If present, the snake was detectable as a massive and compact structure even more clearly than in the LM of the same cell (compare Fig. 3A with 3B).

Figure 1 represents one area of special interest showing large cells with altered nuclei in different stages of central chromatin condensation (arrows) suggesting a progressive process finally leading to the snakes.[16] Additionally, in the same photomicrograph,

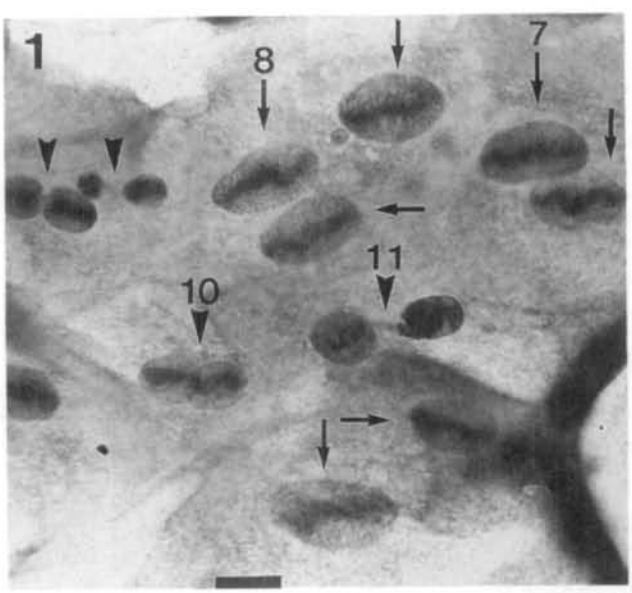

FIGURE 1. Epithelial cells of the human bulbar conjunctiva collected with the technique of impression cytology. The squamous cells show different stages of chromatin condensation ("snake," arrows) as well as different degrees of segmentation (arrowheads) of the altered nuclei. Numbers indicate the figures of the current article showing electron-microscopic aspects of the same nucleus. (Hematoxylin, ×850; bar, 10 μm).

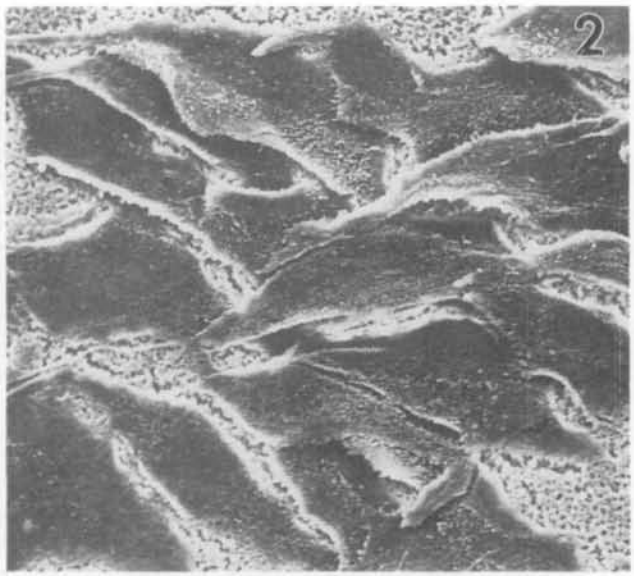

FIGURE 2. Scanning electron microscopy. The nuclei of the squamous cells are not visible because of the GA fixation of the cytoplasm. (Magnification ×850.)

there are aspects of nuclear segmentation (arrowheads). These nuclei seem to be divided but their condensed chromatin with snakelike shape remains evident. The cells present in this area could be successfully cut for TEM.

By TEM, the nucleus of a normal conjunctival cell (Figs. 5, 6A) showed that, as usually described, the heterochromatin predominated as a thick coat in the nuclear periphery in contact, through the fibrous lamina, with the inner membrane of the nuclear envelope. The uncondensed chromatin (euchromatin) prevailed in the central region of the nucleus.

In the nuclei with snake, even if the atypical chromatin arrangement was moderate (Figs. 7A, 7B),the peripheral heterochromatin was represented by a thin, discontinuous, electron dense layer parallel to the nuclear envelope but still attached to this at a few sites (especially at the poles of the elongated nucleus). Most of the heterochromatin was detached from the nuclear envelope and assembled into the axially located snake, remaining connected with the thin marginal layer by numerous thin chromatin strands (Fig. 7B, which shows by TEM the cell marked "7" in the LM image of Fig. 1). The nucleoli usually were no longer visible but the nuclear envelope was found intact.

In the nucleus of normal conjunctival cells the fibrous lamina was clearly detectable as a narrow (40- to 50-nm) homogeneous zone of moderately electron dense, amorphous material (Fig. 6A). In the nuclei with a snake (Figs. 6B, 6C), the space between the inner nuclear membrane and the peripheral heterochromatin was wider (150 to 300 nm, about three- to fivefold normal width) and contained granular or filamentous material. A narrow electron dense coat occasionally marked the inner nuclear membrane (Figs. 6B, 6C) where probably remnants of the fibrous lamina were present. The peripheral heterochromatin was affected by the disintegration of the fibrous lamina: whole areas of it became progressively loose from the nuclear envelope although sometimes it maintained the shape of the nuclear periphery (Fig. 8B, right).

A typical nucleus with snake in a more advanced stage of condensation is seen in Figures 8A and 8B showing LM and TEM of the same cell. All structures faintly visible in the high power LM (Fig. 8A) can be traced in the TEM (Fig. 8B). In contrast to normal nuclei, in the nucleus with snake the distribution of hetero- and euchromatin is almost reversed. The nu-

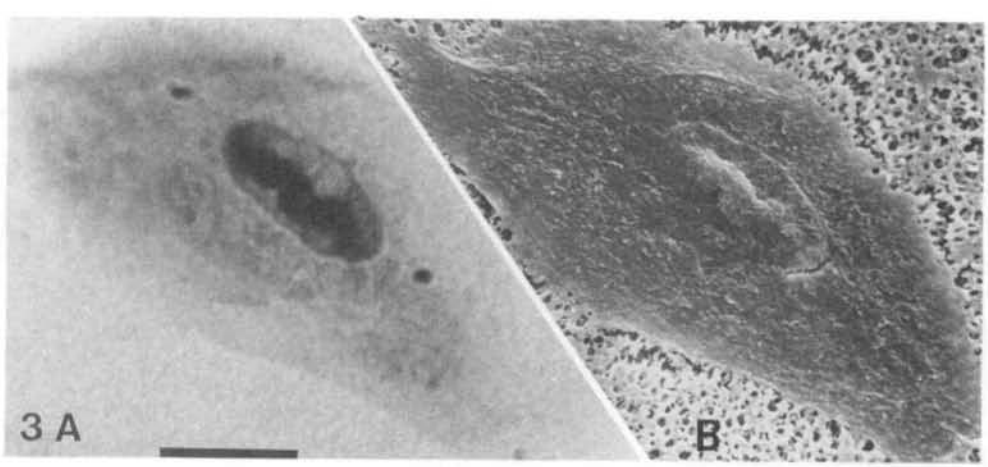

FIGURE 3. The same cell by light (**A**) and scanning electron microscopy (SEM (**B**). (**A**) The squamous cell with axially located, highly condensed chromatin in snakelike shape is stained with hematoxylin. (**B**) Plasma membrane, cytoplasmic, and nuclear components of the air-dried cell are seen collapsed on the snake, which therefore becomes clearly detectable. (bar, 10 μm).

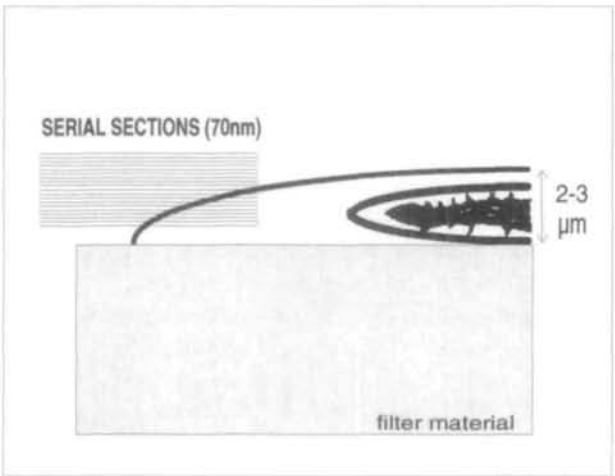

FIGURE 4. The method applied for TEM investigations. Areas of special interest were cut out and serial thin sections were performed through the nuclei and parallel to their main axis. It is evident that the nuclear envelope can be seen in cross section (and hence clearly) only in few serial sections.

clear envelope is mostly free of peripheral heterochromatin and nearly all of this is aggregated in a central axial structure of very high density that remains close to the envelope only at the nuclear poles.

The nuclear segmentation (Fig. 1, arrowheads) was only seen in nuclei with atypical chromatin condensation and its occurrence was increased in cases with advanced cytologic alteration of the epithelium. A comparison of Figures 9A and 9B displays that, what is appearing as a segmentation or division of the nucleus in LM, is indeed rather a deep circular invagination toward the main nuclear axis. The cytoplasm of the squamous cells was filled with filaments of a diameter from about 5 to 10 nm and only few organelles were remaining. These filaments accumulated toward the nuclear envelope (Fig. 9C) as a filamentous belt, lying in an invagination of the nucleus. The nuclear envelope remained intact even in this area. In the advanced stages of segmentation (Figs. 10A to 10C) the belt filaments were even more aggregated than before and formed a very dense ringlike structure (Figs. 10B, 10C, arrowheads).

These segmentations had various shapes, occurred over short or long distances but, although the segments were sometimes appearing as completely loose nuclear fragments in LM (Figs. 1, 11A), they were never seen completely divided in TEM, but still interconnected by a cordlike structure (Fig. 11B). Serial sections showed that it consisted of a small chromatin bridge hidden inside the filament cuff and masses of filaments, dispersed throughout the cytoplasm, were seen to be continuous with and entering into the filament cuff (Fig. 11C, arrows); the nuclear envelope might appear folded under this influence.

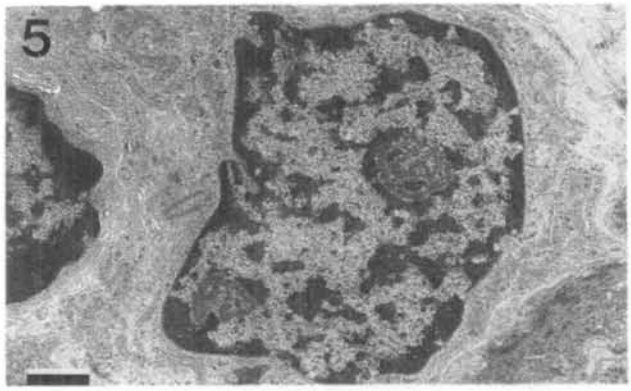

FIGURE 5. Conjunctival epithelial cell with normal nucleus. The heterochromatin prevails in the nuclear periphery, and the euchromatin in the center; two nucleoli can be recognized. (TEM ×8000; bar, 1 µm.)

Not rarely, the chromatin material (eu- and heterochromatin) was seen in a twisted arrangement, like a screw thread, while disappearing inside the filament cuff (Fig. 11C).

DISCUSSION

Snakelike chromatin condensation is an enigmatic morphologic nuclear alteration of the conjunctival epithelium.

At first glance the conditions inducing a snakelike appearance of the chromatin do not necessarily seem to be closely related to each other because they occur in a variety of pathologic and even in apparently "normal" situations. The cytologic picture of the epithelial surface as revealed by impression cytology, on the other hand, is relatively similar in all cases.

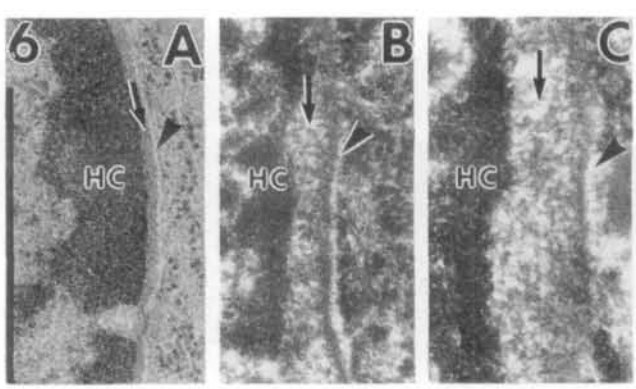

FIGURE 6. Conjunctival epithelial cells, peripheral regions of nuclei with nuclear envelope (arrowhead), fibrous lamina (arrow), and heterochromatin (HC). (**A**) Detail of Figure 5. A normal nucleus. (**B, C**) Details of Figure 8B. Locations with increasing grades of alteration. The fibrous lamina is heterogeneous, shows granular or filamentous appearance, or is almost completely missing. (Magnification ×40,000; bar, 1 µm.)

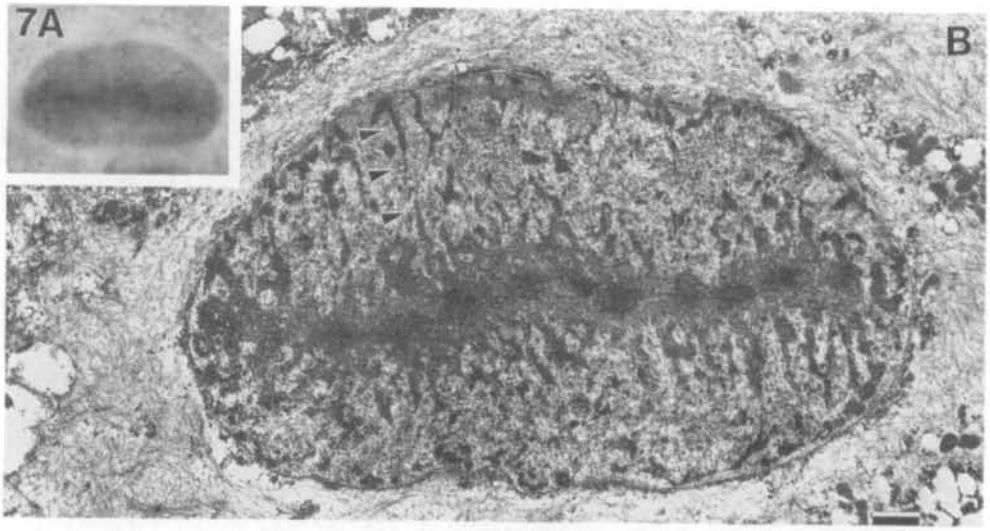

FIGURE 7. A nucleus (labeled "7" in Fig. 1) with a moderate degree of central chromatin condensation on LM (**A**) and TEM (**B**). (**B**) This micrograph displays the alteration of the fibrous lamina, the detachment of the condensed chromatin from the nuclear periphery, and its strands (arrowheads) confluent centrally into the snake. Note the multitude of cytoplasmic filaments in Figures 7 and 8, indicating the squamous metaplasitc nature of the cells. (Bar, 1 μm.)

The common factor of all these conditions is an alteration of the tear film stability by direct influence on the tears,[17,18] by changes of the underlying epithelial morphology, which is known to affect tear film adherence to a large extent,[19–23] or by contact lens wear.[24,25] This decrease of tear film stability may induce an increase of mechanical friction between the eyelid (or a contact lens) and the ocular surface, establishing what, in the pathogenesis of morphologic surface alterations, is known as "chronic mechanical irritation."[26,27] Conceivably, this occurs in contact lens wear as well[1,2] because the eyelid stroke generates a repeating displacement of the contact lens, with consequent friction against the bulbar conjunctival sur-

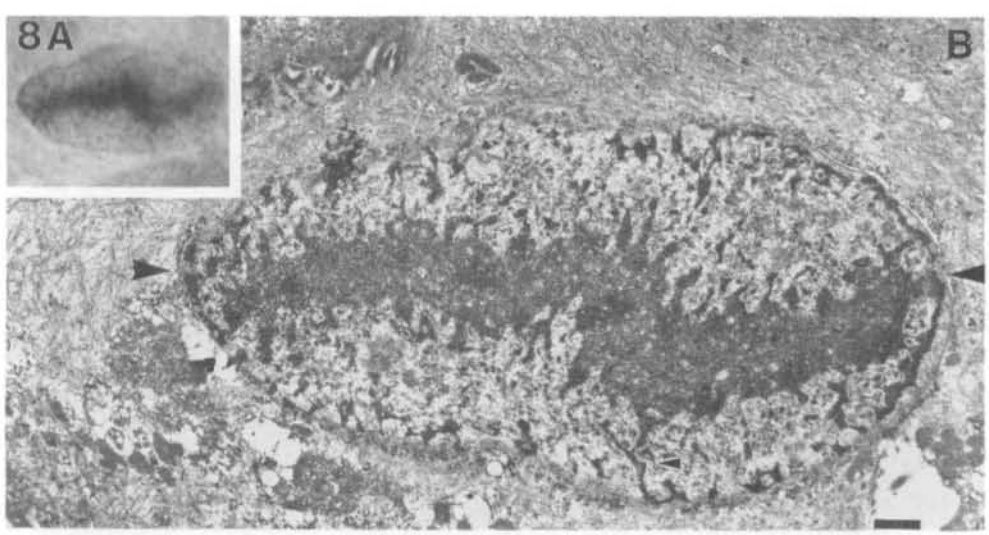

FIGURE 8. More advanced snake stage with nearly complete displacement of chromatin toward the main nuclear axis: LM (**A**) and TEM (**B**). Only few peripheral chromatin strands (small arrowhead) remain, and the inner surface of the nuclear envelope is almost free of condensed chromatin, with the exception of the nuclear poles (large arrowheads). Here the distance between nuclear envelope and snake is small because the displacement of the chromatin is limited. The envelope itself is clearly detectable only in some areas because of the very flat shape of the nucleus (see Fig. 4). (Bar, 1 μm.)

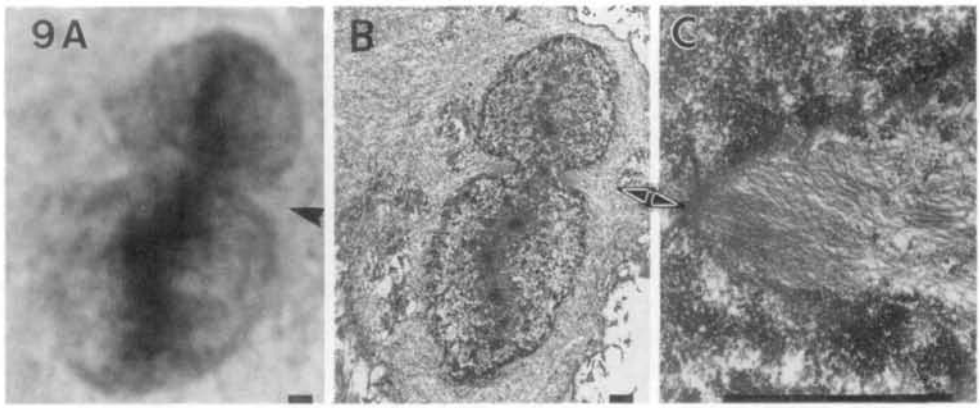

FIGURE 9. Beginning nuclear segmentation in LM (**A**) and TEM (**B, C**). As seen in TEM, a thick bundle of filaments accumulates in a deep indention (arrowhead) on both sides of the nuclear surface. This arrangement suggests that the bundle constricts the nucleus like a ring. A magnification of the right half of the constriction (**B, C**, arrowhead) shows that the filaments are identical and continuous with the other cytoplasmic filaments. The nuclear envelope is intact. (**A**) LM and (**B**) TEM; (**C**) TEM. (Bar, 1 μm.)

face, and because squamous metaplasia (which can be caused by chronic irritation[26,27]) is seen here also.

It could be assumed that similar conditions may also occur in patients that by routine ophthalmologic examination and tear film tests are considered as normal and do not wear contact lenses. In fact, some may already have minor localized disturbances of their preocular conditions in the form of small areas not unlike "dry spots," there resulting in decreased tear film stability and increased mechanical stress without causing any symptoms. This is indicated by the occurrence of some enlarged or squamous cells and snake-like chromatin in a number of normal subjects, as reported previously[3,28–30] so that the diagnosis and definition of a "normal" conjunctiva is difficult[2,31] and probably dependent on the respective applied tests (or criteria).

As seen in the current study by electron-microscopic investigation of the impression cytologic specimens, one of the first manifestations in the development of the snakes is an alteration of the fibrous lamina. This component of the nuclear skeleton forming a thin sheet at the inner surface of the nuclear envelope[32,33] provides the regular adherence of the heterochromatin to the envelope itself.[34–36] The to-and-fro movements on the epithelial surface induced by the eyelid stroke and always occurring in an horizontal direction, transversally to the eyelid movement, could induce the elongation of the nucleus in the same direction, the disintegration of the fibrous lamina with con-

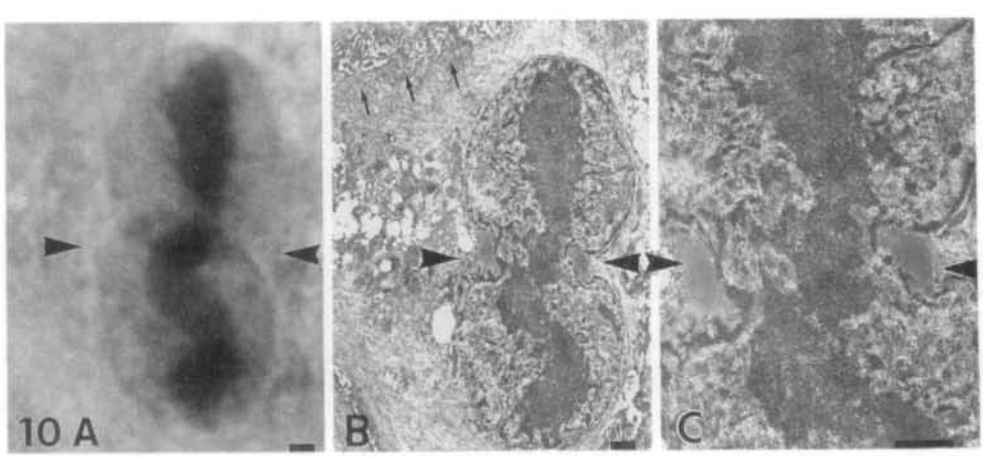

FIGURE 10. Advanced stage of segmentation in LM and TEM. The filament cuff (**A–C**, arrowheads), still not visible by LM (**A**), appears by TEM (**B, C**) as a tightly compact structure. High magnification and cross-section clearly display its ringlike arrangement (**C**). The lateral cell interdigitations (arrows) are well preserved. (**A**) LM and (**B**) TEM; (**C**) TEM. (Bar, 1 μm.)

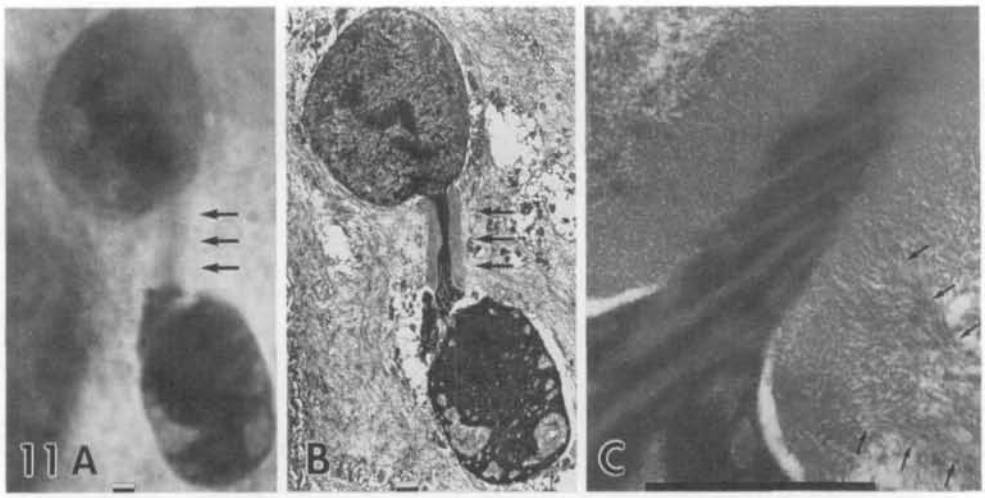

FIGURE 11. A highly segmented nucleus by LM and TEM. Close examination of (**A**) indicates a faint structure in between the two nuclear segments (arrows). TEM of the same nucleus reveals this as a cordlike structure (**B**, arrows) composed of central chromatin core and encircled by a filament cuff, interconnecting the segments. The circular arrangement of the filaments in the cuff is proven as they are observed as electron dense, tightly packed points in this section crossing the cord along its middle (**C**). Long filamentous structures prevail in in the periphery (**C**;120arrows indicate their continuity with other cytoplasmic filaments). Spiral arrangement of hetero- and euchromatin in the bridge suggests that segmentation may occur with a (slightly) different torsion of the segments and hence indicates the presence of a mechanical stimulus. (**A**) LM and (**B**) TEM; (**C**) TEM. (Bar, 1 μm.)

sequent progressively increasing detachment and displacement of the peripheral heterochromatin, and its eventual accumulation along the nucleus major axis. This assumption is supported by the fact that the cells with a snake are on the bulbar surface preferably arranged in longish clusters of horizontal orientation; the individual nuclei share this orientation.[2] A further sign of the alteration of the normal interphase heterochromatin arrangement[37,38] during snake formation is given by the disappearance of the nucleolus, suggesting a disassembly of the chromosomes bearing the nucleolus organizing centers.

The multitude of cytoplasmic filaments in the epithelial cells proves that these are squamous metaplastic, what has before only been assumed by their flattened outer cell shape. For yet unknown reasons, they exhibit an unusual behavior in the cells observed here, and in cases with increased epithelial alteration, they tend to accumulate toward an indentation of the nuclear surface and to form a belt- or cufflike structure around the nucleus. Because intermediate filaments are linked to the nuclear envelope[39,40] and to the plasma membrane[41] they could, through the proposed rolling and to-and-fro movement, gradually be wound around the elongated nucleus, orthogonally to its main axis, constricting it. If this movement is not evenly pronounced for both segments, a relative torsion might result as strongly suggested by the spiral arrangement of the heterochromatin in the cord joining two nuclear segments in Figure 11C.

In general nuclei can show different forms of morphologic alterations in reaction to external stimuli[42] or to intrinsic processes like the programmed cell death (apoptosis, see below). In contact lens wearers there were most frequently characteristics of degeneration, no clear signs of activation as enlargement of nucleoli or mitosis occurred.

The segmentation observed here is remarkably different from the process of regular cell and nuclear division known as mitosis. The segmentation of the snakes is lacking, for example, of dissolution of the nuclear envelope, condensation of the chromatin into chromosomes and formation of a microtubular mitotic spindle. Whether the snakes or their segmentation are related to any atypical kind of mitosis (as amitosis or endomitosis) is unclear but most unlikely, because amitosis would require microtubuli and complete nuclear division[43]; because endomitosis is characterized by the presence of individual chromosomes[44]; and because cryptoendomitosis would not need segmentation of the nucleus.[45] In addition, any kind of mitosis would be induced by an increased metabolic activity that is most unlikely in squamous cells.

Chromatin condensation and disassembly of the nucleolus occur during apoptosis as well (for reviews, see Refs. 46 and 47). However, typically central chro-

matin condensation as in snakes (except of a part of one segment in nucleus 11) is unknown there. At difference from apoptosis, the cells with snake nuclei, as seen by EM, furthermore do not lose their contact with the neighboring ones (Figs. 1, 10B) and have a smooth surface devoid of buddings or convolutions (Fig. 3B). In addition, apoptic bodies (fragments of apoptotic cells) have never been found inside or among the cells of the conjunctival epithelium prepared with the technique of impression cytology. Accumulations of filaments are not known in apoptosis at all.

Central heterochromatin aggregation in general is a rare finding, reported for example in the terminal differentiation process of chick lens epithelium fibers.[48] Furthermore it is found in cells occurring inside of Aschoff granuloma bodies in rheumatic fever (so called Anitschkow cells) as depicted in textbooks.[49] Their origin is debated (either myocytic or fibroblastic[50]) or macrophagic[49,51] but clearly not epithelial. Although their nuclear ultrastructure shows a certain similarity to snakelike chromatin the fibrous lamina seems intact there (see Figs. 7, 12, 23 in Ref. 51) and the cytoplasm is completely different. Closer resemblance is seen in the so-called "bar-shaped" chromatin[52] as found in the human oral epithelium.[53] This shares the central heterochromatin arrangement of the nucleus and, in contrast to its name, it occurs in undulating shape as well (see Figs. 1, 5 in Ref. 53); the squamous appearance of the cytoplasm is also similar. These cells may possibly share, as mucosal surface epithelia at a prominent location (frenulum of the lower lip), mechanical influences that in the conjunctival epithelium we showed to be involved in the production of nuclear alterations. These structures, hence, seem to support our hypothesis of the etiology of snakelike chromatin at the ocular surface.

In previous investigations on the snakes of the human bulbar conjunctival epithelium, we suggested that the alterations are due to chronic mechanical irritation,[1,2] generated by an increased friction. This could produce, as found here, both an alteration of the nuclear skeleton resulting in the formation of snakes and a rearrangement of the cytoskeleton with formation and accumulation of filament bundles that finally may induce a segmentation of the altered nucleus. Hence, our ultrastructural findings strongly suggest that mechanical stimuli play a major role in the etiology of snakes and their segmentation. We would therefore suggest snakelike chromatin as an easy and clearly detectable indicator of mechanical stress on the ocular surface.

Key Words

conjunctiva, epithelium, tear film, dry eye, contact lens, nucleus-ultrastructure, chromatin, cytoskeleton, nuclear skeleton

References

1. Knop E, Brewitt H. Conjunctival cytology in asymptomatic wearers of soft contact lenses. *Graefes Arch Clin Exp Ophthlamol.* 1992;230:340–347.
2. Knop E, Brewitt H. Induction of conjunctival epithelial alterations by start of contact lens wear. A prospective study. *German J Ophthalmol.* 1992;1:125–134.
3. Marner K. 'Snake-like' appearance of nuclear chromatin in conjunctival epithelial cells from patients with keratoconjunctivitis sicca. *Acta Ophthalmol.* 1980;58:849–853.
4. Prause JU, Marner K. Snake-like nuclear chromatin in imprints of conjunctival cells in patients with Sjögrens syndrome. In: Holly FJ ed. Proceedings of the First International Tear Film Symposium. Lubbock, TX: Dry Eye Institute; 1985:157–161.
5. Liotet S, Batellier C, Chaumeil C, Rault J. Interet de l'empreinte conjunctivale en contactologie. *Contactologia.* 1987;9:176–179.
6. Götz ML, Jaeger W, Kruse FE. Die Impressionscytologie der Bindehaut. Eine nichtinvasive Untersuchungsmethode. 2. Lichtmikroskopische Befunde bei normaler Conjunctiva, bei Keratoconjunctivitis sicca und bei Kontaktlinsenträgern. *Fortschr Ophthalmol.* 1986; 83:34–38.
7. Kruse FE, Rohen JW, Götz ML, Schmitz W. Die Impressionscytologie der Bindehaut. Eine nichtinvasive Untersuchungsmethode 3. Der Einsatz von Rasterelektronenmikroskopie und Transmissionselektronenmikroskopie an Impressionscytologischen Präparaten. *Fortschr Ophthalmol.* 1986;83:39–45.
8. Koch JM, Bornfeld N, Waubke TN, Wessing A. Impressionszytologie der Bindehaut nach hochdosierter Brachytherapie maligner Melanome der Uvea mit 106 RU/ 106 RH-Applikatoren. *Fortschr Ophthalmol.* 1988;85:642–645.
9. Koch JM, Waubke TN. Impressionscytologie und ihr licht-und elektronenmikroskopisches Äquivalent bei Patienten mit Keratokonjunctivitis sicca. *Fortschr Ophthalmol.* 1987;84:446–449.
10. Egbert PR, Lauber S, Maurice DM. A simple conjunctival biopsy. *Am J Ophthalmol.* 1977;84:798–801.
11. Tseng SCG. Staging of conjunctival sqamous metaplasia by impression cytology. *Ophthalmology.* 1985; 92:728–733.
12. Böck P. *Romeis-Mikroskopische Technik.* 17th ed. München: Urban und Schwarzenberg Verlag; 1989.
13. Knop E. Employment of a BSE-detector-system for demonstration of morphological nuclear changes. *Beitr Elektronenmikr Direktabb Oberfl.* 1990;23:343–346.
14. Knop E. Air drying of monolayered cell sheets allows SEM observation of morphological nuclear changes in unfractured cells. *Scanning.* 1991;13:116.
15. Steuhl KP. Ultrastructure of the conjunctival epithlium. In: Straub W, ed. *Developments in Ophthalmology.* Vol. 19. Basel: Karger Verlag; 1989.
16. Knop E, Brewitt H. Snakelike condensations of nuclear chromatin. *Cell Biol Int Rep.* 1990;14:221.
17. Dohlman CH. Dry eyes: Aspects of pathophysiology. *Fortschr Ophthalmol.* 1987;84:443–445.

18. Scherz W, Dohlman C. Is the lacrimal gland dispensible? Keratoconjunctivitis sicca after lacrimal gland removal. *Arch Ophthalmol.* 1975;93:281–283.
19. Abdel-Khalek LMR, Williamson J, Lee WR. Morphological changes in the human conjunctival epithelium: I. In the normal elderly population. *Br J Ophthalmol.* 1978;62:792–799.
20. Abdel-Khalek LMR, Williamson J, Lee WR. Morphological changes in the human conjunctival epithelium: II. In keratoconjunctivitis sicca. *Br J Ophthalmol.* 1978;62:800–806.
21. Lemp MA, Dohlman CH. Dry eye secondary to mucus deficiency. *Trans Am Acad Ophthalmol Otolaryngol.* 1971;75:1223–1227.
22. Lemp MA, Holly FJ, Shuzo I, Dohlman CH. The precorneal tear film. 1. Factors in spreading and maintaining a continuous tear film over the corneal surface. *Arch Ophthalmol.* 1983;83:89–94.
23. Nichols B, Dawson CR, Togni B. Surface features of the conjunctiva and cornea. *Invest Ophthalmol Vis Sci.* 1983;24:570–576.
24. Guillon JP. Tear film structure and contact lenses. In: Holly FJ, ed. Proceedings of the First International Tear Film Symposium. Lubbock, TX: Dry Eye Institute; 1985;914–940.
25. Treumer H, Corfield A, Duncker G. Reaktive Veränderungen der Bindehaut und Hornhaut unter Aphakie-Dauerkontaktlinsen: Analysen der Tränenflüssigkeit. *Fortschr Ophthalmol.* 1986;83:403–406.
26. Walter JB, Israel MS. *General Pathology.* Churchill Livingstone; 1974:306.
27. Kissane JM. *Anderson's Pathology.* St. Louis: CV Mosby; 1985:517.
28. Knop E. Impressionscytologische Untersuchung der Conjunctiva bei Trägern weicher Kontaktlinsen unter besonderer Berücksichtigung des 'Snake-like-Chromatin.' Medical School Hannover, Germany: 1988. Dissertation.
29. Knop E, Brewitt H. The morphology of conjunctival epithelium in health and contact lens wear. *Contactologia.* 1992;14:108–120.
30. Knop E, Brewitt H. Zytologische Alterationen des Konjunktivaepithels bei Trägern harter gasdurchlässiger Kontaktlinsen. *German J Ophthalmol.* 1993;2:303.
31. Knop E. How normal is the 'normal' conjunctiva. *Doc Ophthalmologica.* 1993;83:246.
32. Fawcett D. On the occurrence of a fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates. *Am J Anat.* 1966;119:129–146.
33. Aebi U, Cohn J, Buhle L, Gerace L. The nuclear lamina is a meshwork of intermediate-type filaments. *Nature.* 1986;323:560.
34. Franke WW. Structure, biochemistry, and functions of the nuclear envelope. *Int Rev Cytol Suppl.* 1974;4:71–236.
35. Gerace L, Comeau C, Benson MJ. Organization and modulation of nuclear lamina structure. *J Cell Sci.* 1984(suppl 1):137–160.
36. Verheijen R, Venrooij Wvan, Ramaekers F. The nuclear matrix: structure and composition. *J Cell Sci.* 1988;90:11–36.
37. Hancock R, Hughes M. Organization of DNA in the interphase nucleus. *Biol Cell.* 1982;44:201–212.
38. Hubert J, Bourgeois CA. The nuclear skeleton and the spatial arrangement of chromosomes in the interphase nucleus of vertebrate somatic cells. *Hum Genet.* 1986;74:1–15.
39. Carmo-Fonseca M, David-Ferreira JF. Interactions of intermediate filaments with cell structures. *Electron Microsc Rev.* 1990;3:115–141.
40. Staufenbiel M, Deppert W. Intermediate filament systems are collapsed onto the nuclear surface after isolation of nuclei from tissue culture cells. *Exp Cell Res.* 1982;138:207–214.
41. Goldman RD, Goldman AE, Green KY, Jones YCR, Jones SM, Yang HY. Intermediate filaments: Possible functions as cytoskeletal connecting links between the nucleus and the cell surface. *Ann N Y Acad Sci.* 1985;455:1–17.
42. Freudenberg N. *Zytopathologie-Farbatlas und kurzes Lehrbuch.* Stuttgart: Schattauer Verlag; 1988:22–44.
43. Pehlemann FW. Neue Befunde zur Ultrastruktur der Amitose von Interrenalzellen von Rana temporaria L. *Verh Anat Ges.* 1973;67:619.
44. Nagl W. *Endopolyploidy and Polyteny in Differentation and Evolution.* Amsterdam:Elsevier/North Holland; 1978.
45. Krstic RV. *Illustrated Encyclopedia of Human Histology.* Berlin: Springer Verlag; 1984:112.
46. Potten CS. *Perspectives on Mammalian Cell Death.* Oxford: Oxford University Press; 1987.
47. Kerr JFR, Harmon BV. Definition and incidence of Apoptosis: An historical perspective. In: Tomei LD, Cope FO, eds. *Apoptosis: The Molecular Basis of Cell Death. Curr Commun Cell Molec Biol.* Vol. 3. Cold Spring Harbor Laboratory Press; 1991:5–30.
48. Sanwal M, Muel AS, Chaudun E, Courtois Y, Counis MF. Chromatin condensation and terminal differentiation process in embryonic chicken lens in vivo and in vitro. *Exp Cell Res.* 1986;167:429–439.
49. Sandritter W. *Allgemeine Pathologie.* Stuttgart: Schattauer Verlag, 1981:492.
50. Robbins SL. *Basic Pathology.* 3rd ed. Philadelphia: WB Saunders; 1981:301
51. Pienaar JG, Price HM. Ultrastructure and origin of the Anitschkow cell. *Am J Pathol.* 1967;51:1063–1091.
52. Kobayashi TK, Tsubota K, Ugajin Y, Hasegawa T. Presence of bar-shaped nuclear chromatin in cell samples from the conjunctiva. *Acta Cytol.* 1992;32:163–166.
53. Haley RS, Kaplan BJ, Howell R. Ultrastructure of oral cells with bar-shaped nuclear chromatin. *Acta Cytol.* 1979;23:81–84.