Clinical and electron microscopic observations of the conjunctiva of adult patients with Bitot's spots

James P. Ganley and Claire M. Payne*

Three adult patients with Bitot's spots of the conjunctiva were observed in the American Southwest; all were in good health without obvious cause for vitamin A deficiency. Serum vitamin A levels were low normal in two; the third patient was on replacement vitamin A therapy with resultant high serum levels. Serum β-carotene levels were low in two and normal in one. Schirmer test without anesthesia was low normal in two and with topical anesthesia was abnormal in all. After 8 weeks of oral vitamin A therapy, the Schirmer test and Bitot's spots showed little response. Electron microscopy of Bitot's spots showed changes characteristic of keratinizing squamous epithelium: absence of goblet cells, increased tonofibrils, flattening of intermediate cells, loss of superficial cell nuclei, and a keratin layer. Light microscopy of the inferior cul-de-sac conjunctiva showed increased surface goblet cells in two and absence of such cells in the third; by electron microscopy the substructure of the majority of the goblet cell mucin granules had a reticulated appearance in which an electron-dense fibrillar network was present within the granule matrix. The non-mucin-containing epithelial cells of the inferior cul-de-sac for the most part appeared normal.

Key words: Bitot's spots, adults, vitamin A deficiency, conjunctiva, electron microscopy

Bitot in 1863 described foamy, greasy excrescences of the superficial conjunctiva in the intrapalpebral space at the 3 and 9 o'clock position and their association with hemeralopia. These spots often appear triangular in configuration, with their base located adjacent to the corneal limbus. Bitot's spots typically occur in children and are associated with vitamin A deficiency and xerophthalmia. In countries where malnutrition is common, up to 3% of the young may demonstrate these lesions.2

In Western countries the finding of xerophthalmia in healthy adults is uncommon.3 They have been observed during periods of war under conditions of severe starvation,4 in cachectic hospitalized patients,5 and in conditions of extreme debilitation with alcoholic cirrhosis.6

Over the past 3 years we have had the opportunity to observe three adult patients, in otherwise good health, who were referred to the University of Arizona eye clinic for evaluation of Bitot's spots. The purpose of this paper is to describe the clinical findings of these patients and the histopathologic changes of the conjunctival biopsies taken from the Bitot's spot and inferior bulbar cul-de-sac. To our knowledge the ultrastructure
of these lesions and of the clinically uninvolved conjunctiva have not been previously described in humans.

Materials and methods
The patients were referred to the ophthalmology clinic of the University of Arizona Health Sciences Center for evaluation of Bitot's spots. The ophthalmic examination included best-corrected visual acuity, slit-lamp biomicroscopy of the anterior segment, a 5 min Schirmer test with and without topical proparacaine (Ophthaine; E. R. Squibb, Princeton, N. J.) anesthesia, and blood drawn for serum vitamin A and β-carotene levels.

A 1 mm Holt corneal-scleral punch was used to take conjunctival biopsies from the Bitot's spot and inferior bulbar cul-de-sac at the 6 o'clock position after topical Ophthaine anesthesia. A small piece of superficial conjunctiva was obtained by this method which measured approximately 0.5 by 1.0 by 0.4 mm.

For light and electron microscopy, the biopsy specimens were placed immediately in cold 3% glutaraldehyde buffered with 0.1M phosphate buffer, pH 7.2, for 2 hr. The specimens were postfixed for 1.5 hr in cold phosphate-buffered osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Sport's low-viscosity epoxy. Plastic sections (1 μm) were cut and stained with toluidine blue. Thin sections of selected blocks were stained with uranyl acetate and lead citrate and examined with a Hitachi HU-12 electron microscope.

Goblet cells were quantitated by counting the total number of goblet cells present in a representative 1 μm plastic section of the biopsy. All cross-sections examined measured approximately 0.5 by 1.0 mm.

Case reports

Patient 1. A 44-year-old Mexican-American male presented to the eye clinic with a pterygium OD of several years' duration and a Bitot's spot of the left nasal and temporal conjunctiva of unknown duration. The patient related no difficulty with night vision; he worked as a truck driver on the night shift at a local mine. His diet was prepared by his wife and appeared adequate in vitamin A and β-carotene levels.

Examination revealed a corrected visual acuity of 20/20 OU. In the conjunctiva of both eyes foamy, mucoid lesions with a triangular distribution could be removed with some difficulty, and the underlying conjunctiva was slightly thickened and leathery but without pigmentation. The corneas

mately 1 mm onto the cornea. On the left nasal and temporal conjunctiva a small, foamy Bitot's spot was observed measuring approximately 2 mm at the corneal base and 2 mm to the tip. The cornea did not stain with fluorescein, and no signs of conjunctival or corneal xerosis were present. The appplanation tonometry result was 15 mm in both eyes, and the remainder of the ocular examination was normal. Serum vitamin A level was 2 μg/100 ml, and that of β-carotene was 50 μg/100 ml.

The patient was examined approximately 4 weeks later with the Bitot's spot still present. Schirmer test without anesthesia measured over 35 mm of wetting in both eyes in less than 3 min. A Schirmer test after anesthesia with topical 0.1% Ophthaine measured only 5 mm of wetting in both eyes after a 5 min period. Conjunctival biopsies were taken from the temporal Bitot's spot and the inferior cul-de-sac of the left eye and submitted for electron microscopy. Bacterial culture grew diphtheroids.

The patient was started on 10,000 U of vitamin A orally per day. He returned approximately 6 weeks later with the Bitot's spot present temporarily as well as nasally in the left eye. A Schirmer test performed at this time after topical anesthesia measured 7 mm of wetting in both eyes after 5 min. The vitamin A level in serum drawn at this visit was 0 μg/100 ml, and the β-carotene level was 290 μg/100 ml.

Patient 2. The second patient was a 29-year-old white female television producer who, 4 months prior to our examination, first noted lesions of the bulbar conjunctiva that have remained essentially unchanged over this period. She worked long hours, and her diet frequently came from fast-food stores but was not otherwise abnormal. She has worn contact lenses without complications for the past 10 years. She has been in essentially good health, taking no medications, and without symptoms related to vitamin A abnormalities. She did not notice any particular difficulty with night vision and did not spend much time outdoors in the sunlight or dry wind. She did not believe that these lesions had increased in size over this 4-month period of time, nor had she noted her eyes to be particularly injected. She did tear with emotional upsets.

Examination revealed a corrected visual acuity of 20/20 OU. In the conjunctiva of both eyes foamy, mucoid lesions with a triangular distribution were noted medially and laterally. The foam could be removed with some difficulty, and the underlying conjunctiva was slightly thickened and leathery but without pigmentation. The corneas
Fig. 1. Photograph of foamy type of Bitot's spot present on temporal conjunctiva of Patient 3. The lesion measured approximately 3 by 3 mm. A central defect corresponding to the biopsied area can be observed.

were clear, and the anterior chambers were deep. A Schirmer test after topical anesthesia measured 1 to 2 mm of wetting in both eyes after 5 min.

A conjunctival smear showed no inflammatory cells, 3+ epithelial cells, and 4+ bacteria. After 2 days no growth was observed on bacterial culture media.

Serum level of vitamin A was 20 μg/100 ml, and that of β-carotene was 215 μg/100 ml. She was seen again approximately 1 week later, with the foamy discharge on the bulbar conjunctiva unchanged as before. A Schirmer test without anesthesia showed 9.5 mm of wetting in the right eye and 4 mm of wetting in the left. A Schirmer test after topical anesthesia measured 1.5 mm in both eyes after 5 min. No evidence of corneal or conjunctival dryness was observed.

A conjunctival biopsy was taken from the inferior cul-de-sac and from the medial bulbar conjunctiva in the left eye. She was then started on 10,000 U of oral vitamin A per day. She did not return for the follow-up examination. Phone contact 1 year later revealed persistence of the Bitot's spots, which had remained unchanged.

Patient 3. A 42-year-old Mexican male was referred for evaluation of bilateral Bitot's spots of approximately 8 months' duration. The patient was an airline pilot, divorced, who usually ate out in restaurants and fast-food chains. Otherwise, his diet was essentially normal; he related no symptoms referable to possible abnormalities in vitamin A intake, storage, or transfer. He has noted difficulty in night vision, being unable to either drive or fly after dark. Three months previously he
Microscopic observation of Bitot’s spots

Table I. Biographic and clinical manifestations of three patients with Bitot’s spots

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitot’s spots:</td>
<td>Truck driver</td>
<td>Television producer</td>
<td>Airline pilot</td>
</tr>
<tr>
<td>Location</td>
<td>OS: medial/lateral*</td>
<td>OU: medial/lateral*</td>
<td>OU: medial/lateral*</td>
</tr>
<tr>
<td>Duration</td>
<td>Unknown</td>
<td>4 months</td>
<td>8 months</td>
</tr>
<tr>
<td>Conjunctival xerosis</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Nyctalopia</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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</tbody>
</table>

*Unchanged after at least 6 weeks of oral 10,000 IU vitamin A daily.

Table II. Schirmer test, vitamin A, and β-carotene levels of three adult patients with Bitot’s spots

<table>
<thead>
<tr>
<th></th>
<th>Normal subject</th>
<th>Patient 1 Before treatment</th>
<th>After treatment*</th>
<th>Patient 2 Before treatment</th>
<th>After treatment*</th>
<th>Patient 3 Before treatment</th>
<th>After treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schirmer test (5 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Without anesthesia</td>
<td>&gt;15 mm</td>
<td>&gt;35 mm</td>
<td>4/9 mm†</td>
<td>—</td>
<td>—</td>
<td>5/10 mm</td>
<td></td>
</tr>
<tr>
<td>With anesthesia</td>
<td>&gt;15 mm</td>
<td>5 mm</td>
<td>7 mm</td>
<td>1/2 mm†</td>
<td>—</td>
<td>2/3 mm</td>
<td></td>
</tr>
<tr>
<td>Vitamin A level</td>
<td>20-80 µg/100 ml</td>
<td>2 IU</td>
<td>0 IU</td>
<td>20 µg</td>
<td>—</td>
<td>—</td>
<td>410 µg</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>30-65 IU</td>
<td>50 µg</td>
<td>250 µg</td>
<td>215 µg</td>
<td>—</td>
<td>50 µg</td>
<td></td>
</tr>
<tr>
<td>Conjunctival biopsy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitot’s spot</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cul-de-sac</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

*At least 6 weeks of 10,000 IU oral vitamin A daily.

was told he had a vitamin deficiency by an ophthalmologist, and vitamin supplementation was started. He noted no improvement of night blindness nor Bitot’s spots over this period.

Examination revealed bilateral foamy secretions of the nasal and temporal bulbar conjunctiva of both eyes (Fig. 1). Visual acuity was 20/15 OU. The conjunctiva did not appear to be particularly dry, and the cornea had no evidence of xerosis. Serum vitamin A level was 118 µg/100 ml, and that of β-carotene was 65 µg/100 ml. An additional 10,000 U of vitamin A per day was prescribed.

He was re-examined 2 months later without improvement in night vision or Bitot’s spot. Examination revealed a 1+ injection of the bulbar conjunctiva. The Bitot’s spots were slightly more prominent temporally than nasally. A Schirmer test without anesthesia after 5 min showed 5 mm of wetting in the right eye and 10 mm in the left; with topical anesthesia it measured 2 mm in the right and 5 mm in the left after 5 min. On this second visit the vitamin A level was 410 µg/100 ml, and the β-carotene level was 65 µg/100 ml. A conjunctival biopsy was taken from the right Bitot’s spot and from the inferior temporal bulbar conjunctiva.

Salient features of these three patients are summarized in Tables I and II.

Results

Light microscopy

Bitot’s spot. Light microscopic examination of 1 µm sections made from epoxy-embedded tissue revealed that the Bitot’s spots of Patient 1 had the typical appearance of keratinizing squamous epithelium (Fig. 2, A). No goblet cells were observed in the biopsies of the Bitot’s spots from all three patients.

Conjunctiva. Biopsy specimens taken with a 1 mm punch from the inferior cul-de-sac conjunctiva of all patients and from a normal 40-year-old male were approximately the same size. The conjunctival epithelia of two of the patients with Bitot’s spots were histologically similar to normal bulbar conjunctiva in having the appearance of stratified squamous epithelium with no evidence of keratinization or keratohyalin granule formation and the presence of numerous goblet cells (Patient 1,
Fig. 2. Light micrographs of Bitot's spot and inferior cul-de-sac biopsies. A, Representative biopsy of a Bitot's spot has the typical appearance of keratinizing squamous epithelium. No goblet cells are present (Patient 1). B, Bulbar conjunctiva from Patient 1 with Bitot's spots. The epithelium is similar to that of normal bulbar conjunctiva. Ten goblet cells can be identified in the field (arrow).

Electron microscopy

The ultrastructural features of the Bitot's spots from all three patients were consistent with that of keratinizing squamous epithelium (Fig. 3). The basal epithelial cells appeared to be of normal size and configuration. As the cells approached the stratum corneum, the cells and their nuclei became flattened and gradually elongated and lost their nuclei. The more superficial cells showed keratohyalin granules, and in the most superficial layers keratinization was apparent. Amorphous debris was interspersed between the most superficial layers of keratin. Tonoofilaments organized to form tonofibrils could be observed in all layers of the epithelium but became more numerous as the cells approached the surface. No goblet cells were observed in the biopsies of the Bitot's spots from all three patients. In the substantia propria of Patient 1, the collagen fibrils appeared disorganized, and calcium deposition was apparent.

Conjunctiva.

Biopsies taken from the inferior cul-de-sac of all three patients with Bitot's spots were not strikingly abnormal (Figs. 4 and 5). The intercellular spaces appeared widened in some areas and in the basal layer appeared filled with some amorphous material (Fig. 4). In these same areas the surface of the basal cells bordering the substantia propria exhibited a scalloped appearance. The projections from the surface epithelial cells also showed more variation in distribution and length (Fig. 5) than did normal conjunctiva taken from an age-matched control. The surface projections appeared clustered in some areas where they formed "tufts." The increased length of some of the projections gave the latter a more "microvillous" appearance.

Table III. Total number of goblet cells in cul-de-sac conjunctival biopsy and percentage at epithelial surface in a normal subject and three patients with Bitot's spots

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total number of goblet cells in cul-de-sac biopsy</th>
<th>Percentage of goblet cells at epithelial surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>75</td>
<td>17</td>
</tr>
<tr>
<td>Patient 1</td>
<td>193</td>
<td>39</td>
</tr>
<tr>
<td>Patient 2</td>
<td>162</td>
<td>66</td>
</tr>
<tr>
<td>Patient 3</td>
<td>0</td>
<td>—</td>
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Fig. 3. Low-power electron micrograph of Bitot's spot from Patient 1. The epithelium has the typical appearance of keratinizing squamous epithelium: flattening of intermediate cells and their nuclei, loss of superficial cell structure and nuclei, presence of keratohyalin granules, and a keratin layer. The most superficial keratin plates progressively separate, and the intercellular spaces are filled by amorphous cellular debris. Scattered melanosomes are present in the epithelial cells. The basement membrane is somewhat thickened in appearance and contains concentric electron-dense crystalline deposits most likely representing calcium. No goblet cells are present. (X3600; bar = 1 μm.)
Fig. 4. Low-power electron micrograph showing basal layers of inferior cul-de-sac conjunctival epithelium from Patient 1 before vitamin A therapy. Many of the cells contain electron-dense bands that course through the cytoplasm and represent aggregates of tonofilaments. The intercellular spaces are widened in some areas (arrow) and in the basal layer appear filled with some amorphous material. One goblet cell and part of three others are pictured here. The goblet cells contain numerous mucin granules which exhibit great variation in size, shape, and density. Even at this low magnification, the granule contents can be seen to have a reticulated appearance. (x5700.)
Fig. 5. Low-power electron micrograph showing surface layers of inferior cul-de-sac conjunctival epithelium from Patient 1 before vitamin A therapy. Many of the cells contain electrondense bands that course through the cytoplasm (arrow) and represent aggregates of tonofilaments. The upper two to three layers of cells have retained their nuclei and do not form keratohyalin granules. The surface projections from the uppermost layer of epithelial cells are clustered to form “tufts” in some areas. Some projections are quite long and have a “microvillus” appearance. (×5500.)
High-powered magnification of the majority of the goblet cell mucin granules from normal conjunctiva showed a fine homogeneous substructure (Fig. 6, A). This was in contrast to the reticulated appearance of the majority of the mucin granules from the patient’s conjunctiva in which an electron-dense network was present within the granule matrix (Fig. 6, B). As with normal conjunctiva, the mucin granules exhibited variation in size and shape; however, they did show a greater variation in density than did the control.

Discussion

In Western countries it is rare to find Bitot’s spots of adult onset in an otherwise healthy individual. Our three patients appeared to have reasonable dietary vitamin A intake by history and had no intestinal disease affecting vitamin A absorption, no obvious gall bladder, pancreatic, or liver dysfunction, and no reason to suspect an abnormality of the vitamin A transport system. Our findings of decreased basal lacrimal secretion, a similar light microscopic appearance of the Bitot’s spots in our patients to those seen in children with avitaminosis A,8,9 electron microscopic changes similar to those seen in the conjunctiva of rats deficient in vitamin A,10 and the low serum vitamin A levels in two patients would suggest that the Bitot’s spots in our patients are associated with vitamin A deficiency, although the location and source of the abnormality could not be determined from this study.

We could find no previous report of electron microscopic changes in patients with Bitot’s spots. By light microscopy conjunctival biopsies from children with xerophthalmia have shown absence of goblet cells, flattening of the nuclei, and keratinization of the superficial conjunctival layers8,9; similar findings have been demonstrated in adults with acute xerophthalmia.3,6 Light and electron microscopic observations from the Bitot’s spots of our patients also revealed that the conjunctival epithelium had undergone metaplastic change similar to that of keratinizing squamous epithelium with an absence of goblet cells. The conjunctiva away from the Bitot’s spot in two of the patients did not show any evidence of squamous metaplasia. These two patients had ample goblet cells present, whereas the third patient showed none. At the ultrastructural level there was no evidence of keratinization or keratohyalin formation in the epithelial cells. The epithelial cells in the basal layer showed some surface irregularities and widened intercellular spaces, and the surface epithelial projections were noted to be longer in some areas compared with normal conjunctiva. This latter finding may represent a transition from microvilli to microvilli which would be abnormal for this particular epithelium. Scanning electron microscopy is needed however to determine the true nature of these surface projections.

We have shown that Bitot’s spots are indeed focal areas of keratinizing epithelium occurring in the mucosa of the bulbar conjunctiva. They have clinically been subdivided into the dry and the foamy types. Our patients all had the foamy type of Bitot’s spot. The source of this foamy material remains controversial. Some have suggested that the foam might be formed from the keratolytic effect of the diphtheroids frequently present in xerotic conjunctiva.1 In this study the biopsy of Patient 2 showed, by electron microscopy, bacterial cells trapped between plates of keratin. These bacteria appeared as diphtheroids on Gram stain. The conjunctiva of Patient 1 grew numerous diphtheroids on culture. However, by electron microscopy no abnormal swelling or dissolution of the keratin layers was evident as would be expected from a keratolytic effect.

Electron microscopic observations of the surface of the Bitot spot showed a separation of the more superficial layers of keratin with intervening spaces filled with amorphous material and cellular debris. It is possible that this amorphous material may represent cellular remnants and secretions perhaps present in tears, which become trapped between hydrated keratin plates, giving the appearance of foam.

Although there is variation in the substructure of normal human mucin granules,11,12
Fig. 6. Comparison of mucin granules from a normal conjunctival epithelium and from a patient with Bitot's spots. A, Electron micrograph of normal mucin granules at high magnification. All the granule contents have a homogeneously granular appearance. (×58,600.) B, Electron micrograph of mucin granules from Patient 1 before vitamin A therapy. The mucin granules have an electron dense network present within the granule matrix. (×58,600.)
the mucin granules from all goblet cells from the conjunctiva of two patients away from the Bitot's spots had a reticulated appearance, whereas the mucin granules from all goblet cells examined from normal conjunctiva prepared in the same manner had a homogeneous appearance. Since neutral and four types of acidic glycoproteins are secreted by conjunctival mucous cells, it is possible that the reticulated granules represent a distinct subgroup of mucin granules which are biochemically distinct from the homogeneous granules. It has been shown that low vitamin A levels can affect mucopolysaccharide synthesis and may account for the greater preponderance of reticulated granules as observed in this study of patients with Bitot's spots. The finding of large numbers of goblet cells in the conjunctival fornix in these two patients is contrary to previous findings reporting absent goblet cells in children with Bitot's spots. We are unable to explain the greater number of goblet cells at the conjunctival surface in two of our patients than in the control; these findings could represent compensatory hyperplasia and/or hyperactivity of the goblet cells, or the goblet cells might be unable to adequately discharge their contents.

These goblet cell changes, however, do not adequately explain the deficient Schirmer tests found in our patients. The Schirmer test without anesthesia demonstrates decreased lacrimal gland function in two patients, and the test with anesthesia demonstrates deficiency of basal conjunctival secretion in all three patients. In humans the aqueous portion of basal conjunctival secretion is derived from the accessory glands of Krause and Wolfring; it is presumed that a deficiency of these glands accounts for the decrease in basal secretion. In the acute vitamin A−deficient rat model, in addition to loss of goblet cells abnormalities of the meibomian, lacrimal, and harderian glands of the conjunctiva can be found. In none of our three patients were the observed functional changes consistent with keratoconjunctivitis sicca, i.e., deficient tear film, rapid break-up time, punctate keratitis, mucus debris in the tear film, or filamentous keratitis. On transmission electron microscopy similarities can be demonstrated between conjunctival manifestations of keratoconjunctivitis sicca and xerophthalmia. In both conditions loss of goblet cells and stratification of the more superficial layers are characteristic. The differences, however, are more apparent; a keratin layer and submicroscopic changes in goblet cells have not been documented to occur in keratoconjunctivitis sicca. In keratoconjunctivitis sicca there appears to be a change in cell orientation but no change within the cell structure itself, whereas in our patients with Bitot's spots there appears to be a change in both cell orientation and structure. The change in cell orientation in keratoconjunctivitis sicca most likely occurs in response to decreased tear secretion, whereas the structural and orientation changes in our patients probably reflect the biochemical effects on cell metabolism and function of the absence of vitamin A.

There is some suggestion that vitamin A may have a regulatory role on some cells similar to the action of hormones. Vitamin A appears to control differentiation of epithelial tissues: skin, trachea, salivary glands, testes, gut, and conjunctiva. Low vitamin A appears to favor formation of squamous epithelium clinically and experimentally, whereas high vitamin A appears, at least in embryonic chick ectoderm, to favor mucous membrane development. Different epithelial tissues may react to varying levels of vitamin A, so that a certain range of circulating vitamin A might be necessary for the various subtypes of epithelial cells to maintain their respective states; that is, the mucosal epithelium of the conjunctiva in the presence of low vitamin A may undergo metaplasia to squamous epithelium before similar changes occur in nasal mucosa. The opposite phenomenon, i.e., metaplasia of adult squamous epithelium to mucosal epithelium in hypervitaminosis A, has not been observed. Bitot's spots typically occur on the exposed bulbar conjunctiva at the 3 and 9 o'clock position adjacent to the limbus. This area nor-
mally has fewer goblet cells than the more peripheral bulbar conjunctiva. It is possible that this area adjacent to the limbus might by its nature be less mucosal in character and more likely to respond to low vitamin A levels by metaplasia to squamous epithelium. In contrast, the conjunctiva of the fornix, having more mucous membrane characteristics, may be less likely to develop squamous differentiation in the presence of low vitamin A levels.

Return of goblet cells and tear function in a patient with acute vitamin A deficiency but without a Bitot’s spot has been documented histopathologically. In this patient epithelial keratinization was present, and no goblet cells found. After 3 weeks of vitamin A therapy, a few goblet cells had returned, and keratinization decreased. By 8 weeks, the epithelium had assumed a normal cuboidal appearance, and the number and size of cells had increased. In our study the lack of response of the conjunctiva to long-term vitamin A replacement therapy, particularly in Patient 3, is somewhat puzzling. It is possible that at some point the metaplastic change may become irreversible with persistence of the Bitot’s spots.

REFERENCES

10. Mori S: Primary changes in eyes of rats which result from deficiency of fat soluble A in diet. JAMA 79:197, 1922.