Band keratopathy in a rabbit colony

Joanne W. Economom,* Arthur M. Silverstein, and Lorenz E. Zimmerman

A high incidence of band keratopathy was observed in a rabbit colony being utilized in uveitis experiments. Unrelated to the uveitis, the animals had developed, in addition to the corneal lesions, deposits of calcium in the kidneys, arterial tree, trachea, and smooth muscle of the intestine suggestive of hypercalcemia due to an accidental hypervitaminosis D. The clinical and histopathologic characteristics of band keratopathy in these rabbits proved to be quite similar to those observed in man. Band keratopathy has never been observed in rabbits with uveitis alone. Even in the presence of a disease characterized by widespread metastatic calcification in the visceral organs, band keratopathy was virtually restricted to the eyes in which uveitis had been produced. There even appeared to be some positive correlation between the incidence of band keratopathy and the severity of the uveal inflammation. Thus, it would appear that the pathogenesis of band keratopathy in the rabbit may be dependent on the concomitant contributions of both hypercalcemia and uveitis.

Band keratopathy is a clinical entity that is easily recognized by slit-lamp examination. In the human being it is characterized by a hazy grayish opacification of the cornea. The earliest lesions are observed at the termination of Bowman's membrane on the nasal and temporal sides, within the interpalpebral space. Opacification spreads centrally in the plane of Bowman's membrane, the lesions often uniting to form a band-shaped opacity. Fenerations may appear within the opacity. It is generally recognized that this superficial corneal opacity is the result of the deposition of calcium salts. Band keratopathy may be observed in eyes that are otherwise normal (primary form), or it may be secondary to some other ocular disease such as uveitis (secondary form). The primary form occurs in association with hypercalcemia due to such conditions as hyperparathyroidism, hypervitaminosis D, and multiple myeloma. The secondary form is observed in conditions which exhibit both hypercalcemia and intraocular disease (e.g., Still's disease and sarcoidosis). It may also accompany degenerative changes such as phthisis bulbi.

During the course of an extended study of experimental immunogenic uveitis in rabbits, there developed in the rabbit colony an epizootic disease characterized by widespread metastatic calcification. This happy coincidence provided a unique opportunity to observe the development of band keratopathy in a large number of these animals in association with abnormal calcification of the kidney, gastrointestinal
tract, arterial tree, and trachea. Corneal deposits of calcium salts, varying from minimal limbal involvement to a massive band-shaped opacification, were observed in 19 rabbit eyes.

The clinical and histologic picture presented by these animals was comparable to that seen in human band keratopathy. Since, to our knowledge, the literature contains no other reports of observations of band keratopathy in laboratory animals, it appeared desirable to present these observations as a possible basis for further experimental study.

Materials and methods

A group of adult albino rabbits of the Giant White New Zealand strain that were being followed in connection with a long-term uveitis experiment formed the animal colony described in this report. Immunogenic uveitis had been induced in all animals by an intravitreal injection of crystalline egg albumin into the right eye. (The techniques employed and observations made in the immunologic study have been described elsewhere.) Four groups of rabbits were involved in the uveitis studies at the time the band keratopathy appeared in the colony. These were:

I. A group of 13 animals that had been given an intravitreal injection of egg albumin into the right eye in May, 1962. All injected eyes developed clinical manifestations of uveitis within 9 days after the injection.

II. A group of 6 animals that had received the same intravitreal injection in the first week of June, 1962, 24 hours after the administration of 450 r of x-rays to the head with the rest of the body shielded; again, all injected eyes developed clinical uveitis within 9 days.

III. A group of 6 animals that had received the intravitreal injections in May and June, 1962, 24 hours after the administration of 450 r of whole-body irradiation with the head shielded. None of the injected eyes in this group displayed clinical evidence of uveitis over a 3½ week period.

IV. A group of 6 animals that had been given an intravitreal injection of 2 mg. of egg albumin into the right eye in late May, 1962, and, as expected, had developed uveitis within 9 days. Seven weeks after the injection, these animals received an intravitreal injection of bovine gamma globulin into the left eye. These globulin-injected eyes uniformly developed uveitis within 10 days.

By the end of July, 1962, the uveitis in these eyes had subsided clinically. However, histopathologic examination of other eyes treated in a similar fashion has consistently shown the persistence of a mild, subclinical infiltration of round cells in the uveal tract for some months following the resolution of the clinical uveitis. We may thus assume that during the critical period of

Fig. 1. Band-shaped opacification of the cornea of the right eye of rabbit IT. The opacity is fairly well confined to the interpalpebral fissure. (AFIP Neg. No. 63-1521.)

Fig. 2. Right eye of rabbit 548. There are punctate and lacelike opacifications present below the corneal epithelium. (AFIP Neg. No. 63-1522.)
Band keratopathy in rabbit colony

Fig. 3. Right eye of rabbit 54S. Much of the retina is detached, and there are areas of chorioretinal scarring (arrows). (Hematoxylin and eosin. x6.) (AFIP Neg. No. 63-1242.)

development of the postulated hypercalcemia in these rabbits, a very mild, subclinical uveitis was present in the previously involved eyes.

The eyes of all rabbits involved in this study were examined clinically with the slit lamp. For histopathologic examination, the eyes from 3 rabbits were selected as representing the different degrees of clinical involvement typical of those found in the colony. The animals were killed, and all 6 eyes were fixed in formalin. Rabbit 54S from Group I and rabbit 1T from Group II have shown clinical evidence of band keratopathy only in the right eye; rabbit 54S from Group IV had bilateral involvement. The eyes were embedded in paraffin and sectioned at 7 μ. Hematoxylin and eosin, Von Kossa's method, and alizarin red stains were used in studying sections from all 6 eyes.

Observations

During early August, 1962, there developed within the entire colony of 65 rabbits (including animals not used in our uveitis experiments) a clinical syndrome which was diagnosed as "mucoid enteritis," consisting of diarrhea, lassitude, convulsions, and paralysis. At approximately the same time, it was observed that one or both eyes of some animals had a "white film spreading over the cornea" which was not associated with a red eye. In spite of extensive antibiotic treatment, 20 animals (30 per cent) died with symptoms of this syndrome. Of these, 13 died within a 3 week period.

Only after the termination of the acute phase of the "mucoid enteritis" did careful ophthalmologic observation suggest the similarity of the corneal lesions to band keratopathy, and did the autopsy data show the presence of abnormal calcification of the glomeruli and tubules of the kidneys, smooth musculature of the gastrointestinal tract, trachea, and the endothelium and smooth muscle of aorta and other great vessels, indicative of metastatic calcification. At the same time, a check of the records of the Animal Care Branch revealed that the onset of the disease in the rabbit colony followed soon after the introduction of a new batch of feed to the animals. Moreover, the acute phase of the disease and the death rate in the colony dropped precipitously upon the exhaustion of this batch of feed and the change to a new supply.

When all of these findings were as-
Fig. 4. Band keratopathy, same eye shown in Fig. 3. The atrophic epithelium has become separated from the stroma in several places. There is dense basophilic stippling of the stroma immediately beneath the epithelium (arrows*), and a much less densely basophilic layer is present at a slightly deeper level (arrows*). (A, Hematoxylin and eosin. ×50. B, Hematoxylin and eosin. ×265.) (AFIP Neg. No. 63-1238.)

sembled, the full significance of this curious concatenation of events became apparent. Unfortunately, it was then too late to obtain meaningful serum calcium assays on the affected animals, or analysis of the suspect batch of feed. It was therefore on retrospective evidence that a working hypothesis of the causal factors of this syndrome could be formulated. Nutritional hypervitaminosis D with associated hypercalcemia was the most likely pathogenetic factor which could satisfactorily explain all the aspects of the clinical and pathologic picture presented by these animals.

In all, 31 rabbits were examined clinically in this retrospective study. A total of 30 eyes from these 31 animals had shown clinical uveitis. Of these 30 eyes, 19 showed the corneal band-shaped lesions. In Group I there were 13 animals observed; all had experienced uveitis in the right eye. Six of these eyes, 6 of which demonstrated the subepithelial corneal opacity. No corneal lesions were observed in the left eyes, which had not experienced uveitis. Group II consisted of 6 head-irradiated animals, all of which had had unilateral uveitis. Five of these showed the typical band-shaped lesions. Of the 6 body-irradiated animals in Group III, none showed clinical signs of uveitis, but one of these animals did show a marked opacification of the right cornea in the subepithelial region. Group IV was composed of 6 animals that had had bilateral uveitis with the right eyes showing maximal clinical involvement in May and the left eyes in July. Eight of the 12 eyes exhibited band keratopathy. Five of these 8 were the left eyes that had had the clinical uveitis in July during the episode of "mucoid enteritis." In 2 of the 3 animals of this group showing bilateral corneal opacities, the left cornea was more markedly involved than the right.
In summary, band keratopathy was observed in 19 of 30 eyes that had experienced immunogenic uveitis, but in only 1 of 32 control (nonuveitis) eyes in the same series of rabbits.

The keratopathy was seen by slit-lamp biomicroscopy as a subepithelial opacification situated along the interpalpebral space. It was manifested either as a partial band with segments at the medial and lateral limbus or as a complete band form. In a few cases, fenestrations were observed within the opacity, while in a few others punctate opacities were distributed in a band-like arrangement (Figs. 1 and 2). The juxtalimbal area remained clear in all rabbits, and no conjunctival lesions were noted. In one rabbit in which vascularization of the cornea had taken place, clearing of the opacification around the vessel had occurred.

In an attempt to remove the corneal opacities clinically, 2 rabbits were treated with ethylenediaminetetra-acetic acid. Eight instillations of a 1.85 per cent solution of EDTA were administered into the conjunctival sac every 5 minutes, after denuding of the epithelium. In both instances, the opacities were removed, as evidenced by the slit-lamp observation.

The eyes of 3 rabbits were examined histopathologically. In 2 of these (54S and IT) only the right eyes showed microscopic evidence of band keratopathy. In the third rabbit (84S) both corneas were involved, although the changes were minimal in the

Fig. 5. Same cornea shown in Figs. 3 and 4 but stained by Von Kossa’s indirect method for calcium salts. The subepithelial infiltrate is a conglomerate mass of minute granules of calcium salts. Individual granules can be identified just beneath the dense band (arrows). (x264.) (AFIP Neg. No. 63-1237.)

Fig. 6. Same cornea shown in Figs. 3 to 5, stained with alizarin red after microincineration. The dark bands at levels 1 and 2 are stained red, indicating presence of calcium salts. (x265.) (AFIP Neg. No. 63-1238.)
left eye while marked in the right. In all 3 rabbits the right eye with the well-developed band keratopathy showed extensive retinal detachment (Fig. 3). The left eye showed no effects of a previous endophthalmitis or uveitis in rabbits 54S and IT, and very minimal retinal disturbance, without detachment, in rabbit 84S. The affected corneas of rabbits 54S and IT showed fine basophilic stippling of the superficial stroma, usually immediately beneath the epithelium (Fig. 4), but sometimes involving more deeply situated lamellae. The granular deposits were stained black by Von Kossa's indirect method for demonstration of calcium salts (Fig. 5), and after microincineration they were stained red by alizarin red (Fig. 6). In rabbit 84S the right cornea showed much more advanced degeneration than was observed in the other two animals, with marked scarring and moderate leukocytic infiltration of the outer corneal lamellae. The left cornea, however, showed just a few foci of light calcification.

In addition to the corneal deposits, intraocular calcification was observed in all 3 right eyes. In rabbits 54S and 84S, considerable calcification of the ciliary epithelium and lens cortex was observed (Fig. 7), while in rabbit IT there was only minimal involvement of the ciliary epithelium. The lesions of the ciliary epithelium were particularly interesting for they were confined to the ciliary processes where the deposits were concentrated along the innermost aspect of the ciliary epithelium (basement membrane surface of the pars ciliaris retinae) (Fig. 8).

Discussion
Band keratopathy as a clinical entity representing a degenerative process secondary to intraocular inflammation has been documented for some time. Band keratopathy as an expression of hypercalcemia was first reported in the literature by Meesman. Since that time, several observers have reported the related occurrence of band keratopathy with hyper-
calcemia of any cause,\textsuperscript{4, 6, 10} definitely establishing the relationship of the two. Hypercalcemia is present in many conditions, including hyperparathyroidism, vitamin D intoxication, sarcoidosis, milk-alkali syndrome, multiple myeloma, and renal insufficiencies. Abnormal deposition of calcium in tissues previously normal but with a tendency toward alkalinity\textsuperscript{10} is the pathologic change present and is known as metastatic calcification.

The present report concerns the observation of a large number of cases of band keratopathy in a rabbit colony. The animals employed in a series of uveitis experiments developed a syndrome interpreted in retrospect to have been hypercalcemia due to accidental nutritional hypervitaminosis D. In addition to widespread metastatic calcification in the visceral organs, a number of these animals were found to have concomitant opacifications of the cornea. The corneal opacities were grayish white, localized in the subepithelial stromal layers in the typical band shape, and dissolved upon application of EDTA. Microscopically, these deposits of calcium salts in the subepithelial layers of the corneal stroma proved to be similar to those observed in human patients with band keratopathy.

There have been extensive experiments\textsuperscript{11, 12} on the experimental reproduction of metastatic calcification. The fact that experimental band keratopathy has not, to our knowledge, been reported hertofore is not surprising, since it occurred in the rabbit colony described herein in association with concomitant or resolving uveitis, and was virtually absent in the eyes that had not experienced prior uveitis. Of the 30 eyes with uveitis, 19 showed band keratopathy. It is interesting to note that about one half of those animals whose peak of clinical uveitis occurred 2 months prior to the clinical syndrome of hypercalcemia showed corneal lesions. Where the clinically apparent uveitis predated the hypercalcemic episode by only a very short
interval, the incidence of band keratopathy was greater. This was especially significant in the group of rabbits with uveitis induced in both eyes at different times. In the more recently involved eyes, the incidence of band keratopathy was greater and the lesions more severe than that in the contralateral eyes. Of the 31 animals, only one eye that had not experienced uveitis showed the corneal opacity. Of the several hundred rabbit eyes previously observed during uveitis experiments in this laboratory, this type of corneal lesion has never been observed.

It is evident from these data that, with rare exceptions, neither hypercalcemia nor uveitis alone is sufficient to produce band keratopathy in the rabbit, but rather the two would appear to be required as temporally associated events.

We wish to acknowledge the competent technical assistance rendered by Miss Shirley Johnson and Mrs. Ann Eastham.

REFERENCES

8. Meesman, A.: Quoted by Cogan, Albright, and Barter.4