Spontaneous Development of Corneal Crystalline Deposits in MRL/Mp Mice

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Purpose. The presence of corneal opacities associated with dacryoadenitis and lacrimal gland destruction has led investigators to consider MRL/Mp mice as models for band keratopathy and Sjögren syndrome. In this study, the authors examined the time course of the corneal opacification and investigated whether the opacities were associated with altered serum levels of parathyroid hormone, calcium, and phosphorus, as well as quantitative and qualitative differences in tear production.

Methods. Corneas were analyzed microscopically and tear fluid production was measured by a modified Schirmer test.

Results. Corneal lesions were observed as early as the fifth week after birth. The lesions consisted of calcium phosphate and appeared as punctate, crystalline opacities located subepithelially. Lesions were present in 72% (56 of 78) of the MRL/Mp mice, with no significant difference in incidence between MRL/Mp +/+ and MRL/Mp lpr/lpr mice. Corneal calcification was occasionally associated with a self-limiting keratitis and neovascularization. In control mice, corneal opacities were not observed before the animals were 6 months of age. Levels of circulating parathyroid hormone decreased significantly during the first 16 weeks of age in MRL/Mp mice. In addition, MRL/Mp mice of both sexes had a significantly lower tear fluid production as compared to BALB/c mice of the same age.

Conclusion. Because corneal lesions start to develop in 5-week-old MRL/Mp mice, thereby preceding the clinical signs of systemic autoimmune disease, and may develop in 6-month-old nonautoimmune-prone mice, it is suggested that calcification develops independent of the systemic autoimmune disease and might be restricted to the cornea. Invest Ophthalmol Vis Sci. 1995;36:454-461.

Autoimmune disease-prone mice are generally considered as useful models for human systemic autoimmune disorders. Investigations into the genetic defects and the immunologic aberrations in these mice have significantly increased our understanding of the mechanisms that may underlie the induction and progression of autoimmune diseases. Mice of the MRL/Mp strain develop a systemic vasculitis with features of systemic lupus erythematosus such as high levels of serum immunoglobulin G, circulating immune complex antibodies and antinuclear antibodies. The congenic MRL/Mp lpr/lpr mice, which carry the recessive lymphoproliferative (lpr) gene, develop an accelerated autoimmune disease that is associated with vasculitis, massive lymphadenopathy, splenomegaly, and glomerulonephritis. These mice are unique in that they develop polyarthritis and are positive for rheumatoid factors.

In addition to the systemic autoimmune disease, both MRL/Mp +/+ and MRL/Mp lpr/lpr mice may develop ocular abnormalities. Choroiditis, scleritis, corneal calcification, and a conjunctivitis that is characterized by mononuclear infiltrates in the bulbar and palpebral conjunctiva have been reported. Associated with the ocular lesions are pronounced perivascular and perilactal infiltrates in the lacrimal glands. A time course study on the histopathology of the lacrimal glands demonstrated the presence of inflammatory lesions in mice at 16 weeks of age, with differences between the MRL/Mp +/+...
Inbred male and female MRL/Mp +/+ + , MRL/Mp mice

MATERIALS AND METHODS

lands. The mice were bred for two generations and

Spontaneous Corneal Calcification in

Mice

Breeding of Laboratory Animals, Zeist, the Nether-

Harlan Sprague-Dawley, Central Institute for the

development of pathologic changes of the anterior eye

Ipr/lpr mice of

closely resembles Sjogren syndrome in humans.2'8'12

In addition, lacrimal gland function was assessed quali-

and phosphorus and parathyroid hormone (PTH) levels.

Serum Calcium and Phosphorus Levels

The anterior segment of the eye was examined regu-

larly in unanesthetized mice using a slit-lamp biomicro-

scope by an experienced ophthalmologist who was

unaware of the mouse strain being studied. In total, 78 male and female MRL/ Mp mice, including 18

MRL/Mp lpr/lpr mice, aged 1 to 20 weeks were stud-

ied. Twenty BALB/c mice, ten female and ten male, were used as negative controls. In addition, several

mouse strains at two other animal care facilities in

Amsterdam were examined by slit-lamp biomicro-

scopy.

Tissue Preparation

Ten MRL/Mp mice (five male and five female, 12–16 weeks of age) with corneal crystalline deposits and ten sex-and age-matched BALB/c mice were killed by an intraperitoneal injection of 30 mg pentobarbitone sodium (150 µl Euthesate; Aphenno, Arnhem, The Netherlands). The eyes were fixed in 0.1 M cacodylate buffer, pH 7.4, containing 1% formaldehyde and 1.25%

% glutaraldehyde and embedded in Technovit 7100

(Kulzer; Wehrheim, Germany), a methylmethacrylate-

based resin. Eye sections were also stained according to von Kossa’s silver nitrate method to detect deposits of phosphate minerals. In addition, corneal tissue was subjected to energy dispersive x-ray microanalysis. Corneas that showed profound central opacification were dissected, snap-frozen in liquid nitrogen and ly-

ophilized. Corneal tissue was scanned at the site of

opacification and at the clinically healthy periphery,

with a Philips (Eindhoven, The Netherlands) scan-

ning electron microscope 505 equipped with an

EDAX-PV9800 and a windowless detector.

To determine the health status of each mouse used, a histopathologic analysis of kidneys was per-

formed. The tissue specimens were snap-frozen in liq-

uid nitrogen and stored until further use. Ten 8 µm

frozen sections of each kidney were stained with hema-

toxin and eosin. All tissue sections were analyzed by an

experienced pathologist who was unaware of the

mouse strain being examined.

Serum Calcium and Phosphorus Levels

Blood was obtained from 10 MRL/Mp mice (5 male and 5 female, 12–16 weeks of age) with corneal crystal-

and lpr/lpr strain.4,10 The intensity of lacrimal gland

inflammation was significantly more severe in female as compared to male mice. The predominance of T

lymphocytes and plasma cells in the lacrimal gland

lesions and the enlargement of the salivary glands closely resembles Sjogren syndrome in humans.2'8'12

The corneal calcification is regarded as a model for human band keratopathy.4,5,9,11-15 Corneal calcifi-

cation in MRL/Mp mice has been reported by Hoff-

man and colleagues, and was described as a typical

bilateral band keratopathy characterized by deposits of calcium in Bowman’s layer.9 Results indicated that

hormonal aberrations resulting in hypercalcemia and

elevated levels of parathyroid hormone might have contributed to development of these corneal lesions. A similar band keratopathy develops spontaneously in diabetic KK mice but without signs of hypercalcemia.16

Moreover, corneal calcification has been observed in nonautoimmune-prone BALB/c mice fed a cholesta-

nol-enriched diet.17 Fine and associates showed that

rabbits develop corneal calcareous deposits 14 days after carbon dioxide laser irradiation.18 The authors

contended that the extracellular localization of the
calcium deposits opposes hyperparathyroidism-associ-

ated calcification that is located intracellularly.

These conflicting results and the ambiguity about the underlying pathologic mechanism prompted our reassessment of the applicability of MRL/Mp mice as models for Sjogren syndrome and band keratopathy. We analyzed in MRL/Mp +/+ and lpr/lpr mice of both sexes and at different ages the spontaneous development of pathologic changes of the anterior eye segment. The kinetics of the corneal calcification were compared to serologic parameters including calcium, phosphorus and parathyroid hormone (PTH) levels. In addition, lacrimal gland function was assessed qualitatively and quantitatively. We demonstrated that corneal calcification precedes systemic autoimmune disease by several weeks and that the initial site of calcification is in the corneal center without affecting the periphery. In addition, while impaired tear fluid production was noted in both strains, this did not correlate with corneal lesions. Therefore, it is concluded that results obtained in MRL/Mp mice as models for Sjogren syndrome and, in particular, band keratopa-

thy in humans should be received with caution.

MATERIALS AND METHODS

Mice

Inbred male and female MRL/Mp +/+ , MRL/Mp lpr/lpr and BALB/c mice were obtained initially from

Harlan Sprague-Dawley, Central Institute for the

Breeding of Laboratory Animals, Zeist, the Nether-

lands. The mice were bred for two generations and

reared under specific pathogen-free conditions at the animal care facilities of the Netherlands Ophthalmic Research Institute. All animal experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were carried out under the responsibility of the ethical committee of the Royal Netherlands Academy of Arts and Sciences acting in accordance with the European Communities Council Directive (86/609/EEC).

Biomicroscopy

The anterior segment of the eye was examined regu-

larly in unanesthetized mice using a slit-lamp biomicro-

scope by an experienced ophthalmologist who was

unaware of the mouse strain being studied. In total, 78 male and female MRL/Mp mice, including 18

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Tissue Preparation

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toxin and eosin. All tissue sections were analyzed by an

experienced pathologist who was unaware of the

mouse strain being examined.

Serum Calcium and Phosphorus Levels

Blood was obtained from 10 MRL/Mp mice (5 male and 5 female, 12–16 weeks of age) with corneal crystal-
line deposits and 10 sex- and age-matched BALB/c mice. Mice were anesthetized with 15 mg fluanison and 3 mg phentanyl (15 μl Hypnorm; Janssen Pharmaceutica, Tilburg, The Netherlands) injected intramuscularly and 50 mg valium (Diazepam; Hoffmann-La Roche, Basel, Switzerland) injected intraperitoneally. Blood was allowed to clot for 2 hours at room temperature. After separating the serum from the clot by centrifugation (800g), the serum was collected and stored at −20°C until further use. Calcium levels were determined by colorimetric assay according to Baginski et al.18 Phosphorus levels were measured by the phosphomolybdate method as described by Goldenberg and Fernandez.19 The nonparametric Kruskal-Wallis test was used to test for statistical differences.

**Serum Parathyroid Hormone Levels**

PTH levels were determined by a radioimmunoassay (Inc Star, Stillwater, MN). In addition to the aforementioned samples, sera from an additional group of MRL/Mp lpr/lpr mice (4 male, 3 female) were analyzed. These mice were bled at 4 weeks of age when there were no corneal lesions, and at 12 weeks of age when the mice had developed profound corneal opacities. Statistical analysis of altered PTH levels in time by MRL/Mp lpr/lpr mice was performed using the Wilcoxon matched pairs test based on ranks.

**Assessment of Tear Fluid Production**

Quantitative and qualitative differences in tear fluid production were assessed by a modified Schirmer test, according to Kurihashi,20 and sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE), respectively. Before the Schirmer test and the collection of tear fluid, the mice were examined with a slit-lamp biomicroscope to determine the presence of corneal lesions. The mice were anesthetized by an intramuscular injection of 15 mg fluanison and 3 mg phentanyl (15 μl Hypnorm; Janssen Pharmaceutica, Tilburg, The Netherlands). Tear fluid production was measured using a cotton thread, which at the tip had been prestained for 2 mm with fluorescein.20 Differences in tear fluid production were analyzed by the nonparametric Kruskal-Wallis test.

To analyze the protein contents of the tears, fluid was collected from the surface of the eye using a 5-μl glass capillary. A total volume of 0.3 to 1 μl of milky fluid was obtained for each mouse. The tear fluid was diluted 1:40 in phosphate-buffered saline (pH 7.5) and mixed with an equal volume of sample buffer containing 20 mM Tris/HCl (pH 8.0), 2 mM EDTA, 5% (wt/vol) SDS, 0.02% (wt/vol) bromophenol blue and 0.01% (wt/vol) sodium azide. Samples were applied to 10% to 15% gradient polyacrylamide gels and separated using the Phast System (Pharmacia LKB Bio-

**RESULTS**

**Time Course of Corneal Lesions in MRL/Mp Mice**

All ophthalmologic changes of the anterior segment of the eye in MRL/Mp and BALB/c mice are listed in Table 1. Mice were examined as early as 8 to 12 days after birth and regularly thereafter, until the animals were 16 to 20 weeks of age. Slit lamp biomicroscopy revealed that at 5 weeks of age both MRL/Mp lpr/lpr and lpr/lpr mice started to develop opacities in the superficial stroma of the central portion of the cornea. These opacities consisted of many grey dots that, as the process advanced, coalesced to form a round or oval fenestrated zone directly beneath the basal layer of the corneal epithelium. The course of the corneal opacification was slowly progressive up to week 12 and usually affected both eyes. End-stage opacities had a granular or crystalline appearance and were situated at the level of the central part of the cornea, while the corneal periphery remained lucid and free of deposits (Fig. 1). In seven mice, the corneal opacities were associated with keratitis and corneal neovascularization, and in three mice, there were signs of corneal ulceration affecting the epithelium and superficial corneal stroma. The onset of the keratitis varied between weeks 6 and 8, and the clinical signs of corneal inflammation persisted for approximately 4 weeks. The keratitis was self-limiting, but the newly formed blood vessels remained visible for several weeks. Occasionally, the epithelium appeared dehydrated. By weeks 8 to 9, three mice with corneal opacities and keratitis developed scleritis. Moreover, from the age of 9 weeks on, 72% (56/78) of the MRL/Mp mice developed subconjunctival hemorrhages. Similar hemorrhages were observed in 20% (2 of 10) of the BALB/c mice. Gender-related differences or differences in clinical signs between MRL/Mp and BALB/c mice were not significant (data not shown).

| TABLE 1. Incidence of Spontaneous Ocular Lesions in 16- to 20-Week-Old MRL/Mp and BALB/c Mice |
|-----------------------------------------------|-----------|-----------|
| MRL/Mp (%) | BALB/c (%) |
| Corneal deposits | 56/78 (72) | 0/10 (0) |
| Keratitis | 7/78 (9) | 0/10 (0) |
| Corneal melting | 3/78 (4) | 0/10 (0) |
| Scleritis | 3/78 (4) | 0/10 (0) |
| Subconjunctival bleeding | 56/78 (72) | 2/10 (20) |

Corneal crystalline deposits were not observed in technology, Woerden, The Netherlands), followed by silver staining.
Spontaneous Corneal Calcification in Mice

FIGURE 1. Dark-field photomicrograph of the cornea of an 18-week-old MRL/Mp mouse. An oval area of crystalline deposits surrounded by a zone of grey dots is present in the center of the cornea.

age- and sex-matched control BALB/c mice. However, slit-lamp examination of several mouse strains housed at two independent research institutes in Amsterdam revealed that nonautoimmune-prone mice may develop similar lesions (Table 2). These mice were at least 6 months old. Interestingly, corneal calcification was not restricted to albino mice because C57BL/6 also had symptoms (2 of 3). When comparing the incidence at both institutes, it is striking that corneal lesions were observed only at one institute. This suggests that the housing condition may partly contribute to development of the corneal lesions.

TABLE 2. Corneal Calcification in Different Mouse Strains

<table>
<thead>
<tr>
<th>Institute</th>
<th>Strain</th>
<th>Age (months)</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BALB/c</td>
<td>8</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>ACA</td>
<td>6</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>C57BL/10</td>
<td>4-5</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>5</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>6</td>
<td>2/5</td>
</tr>
<tr>
<td>2</td>
<td>BALB/c</td>
<td>6-9</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>3-4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>AKR</td>
<td>5</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>Af</td>
<td>4-5</td>
<td>0/4</td>
</tr>
</tbody>
</table>

just beneath the corneal epithelium. The corneal epithelium itself appeared flattened both in early (Fig. 2A) and end-stage opacities (Fig. 2B). Flattening of the basal cell layer was most prominent. The arrangement of the collagen fibrils near the deposits appeared disturbed. Inflamed corneas showed blood vessels often reaching the corneal center and numerous inflammatory cells, mainly polymorphonuclear cells, and fibroblasts (not shown). The corneas of sex- and age-matched BALB/c mice had no deposits. Kidney sections confirmed an ongoing nephritis in MRL/Mp lpr/lpr mice, while kidneys of MRL/Mp +/+ and BALB/c mice appeared healthy.

The nature of the phosphorus minerals in the corneas of MRL/Mp mice was analyzed by energy dispersive x-ray microanalysis. When healthy corneal tissue or clear parts of affected corneas were compared to lesional corneal tissue, the energy dispersive x-ray profile of the latter showed two additional peaks representing phosphorus and calcium (Fig. 3).

Serum Calcium, Phosphorus, and Parathyroid Hormone Levels

In the next set of experiments, we assessed whether the corneal calcium deposits correlated with increased serum levels of calcium, phosphorus, or PTH. In Table 3, it is shown that the mean calcium concentration in sera of male and female MRL/Mp lpr/lpr and BALB/c control mice did not differ significantly at weeks 10–16. In contrast to expectations, serum phosphorus levels in female MRL/Mp lpr/lpr mice were significantly decreased compared to the other three groups (P = 0.012).

Analysis of the serum PTH levels revealed that, at 4 weeks of age, MRL/Mp mice had levels that were comparable to those of 10 to 16-week-old BALB/c mice. At 12 weeks of age, PTH levels were significantly decreased in both male and female MRL/Mp lpr/lpr mice (P = 0.003). A longitudinal study, in which the MRL/Mp mice were tested at 4 and 12 weeks of age, showed that PTH levels had decreased significantly (P < 0.01) with a mean value of 0.33 pmol/L at 4 weeks and 0.20 pmol/L at 12 weeks (Fig. 4).

Tear Fluid Production

To assess whether the development of corneal lesions was associated with impaired tear production, Schirmer tests were performed in 14 MRL/Mp mice (7 male, 7 female) and 12 BALB/c control mice (6 male, 6 female) at 10 to 16 weeks of age. The amount of tear fluid produced showed a statistically significant gender-related difference in BALB/c mice (P < 0.01; Fig. 5). In contrast, MRL/Mp males and females produced equal amounts of tear fluid, but these amounts were significantly less than the quantities produced by BALB/c mice (P < 0.01). No significant difference
FIGURE 2. Photomicrographs of technovit-embedded cornea sections stained with hematoxylin and von Kossa. (a) A dark staining layer with a granular aspect is seen in the anterior stroma of a 7-week-old mouse, 1 week after onset of clinical signs. Note the thinning of the epithelium overlying the stained zone (original magnification ×64). (b) The anterior stroma of a 20-week-old mouse, 11 weeks after onset of the calcification, showing an interrupted zone of von Kossa-positive material. The epithelial thinning is still present (original magnification ×100).

was observed in tear fluid production by MRL/Mp mice with or without corneal opacities (data not shown).

Qualitative analysis of the tears by SDS-PAGE showed gender- and strain-related differences (Fig. 6). In male MRL/Mp and BALB/c mice, two separate bands at approximately 45 to 50 kD were most prominent. A protein band of approximately 95 kD (sometimes separated into two bands) was restricted to the tear protein profile of female mice. The differences in the protein profiles between male and female mice were comparable for MRL/Mp and BALB/c. Strain-related differences were less pronounced. Protein bands at 56 kD and 81 kD were present only in the profile of BALB/c mice.

DISCUSSION

In the current study, clinical, histologic, and functional aspects of the spontaneously developing corneal lesions in MRL/Mp mice were investigated. Corneal calcification is a common abnormality in MRL/Mp mice, as well as in other mouse strains that develop systemic autoimmune disease spontaneously. In this article, we studied the time course of the corneal calcification in MRL/Mp mice to determine whether this abnormality was part of the immunopathologic process. The main observations indicate that corneal calcification is a local phenomenon, occurring independently of the systemic autoimmune disease. First of all, the onset of corneal calcification clearly preceded the onset of systemic autoimmune lesions; and by week 12, the clinical signs of corneal calcification had stabilized. In the lacrimal gland, mild inflammation (without foci of mononuclear cells) may be observed in 4-week-old mice. Yet, it takes 4 to 5 months for the lacrimal gland inflammation to fully develop. Secondly, nonautoimmune-prone mice may develop similar lesions, albeit at a later age (this study) or after cholestanol feeding.17

Several investigators have denoted the corneal cal-
Spontaneous Corneal Calcification in Mice

TABLE 3. Serum Concentrations of Calcium, Phosphorus, and PTH in 10- to 16-Week-Old MRL/Mp ipr/lpr and BALB/c Control Mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Number of Mice</th>
<th>Calcium (mmol/l)</th>
<th>Phosphorus (mmol/l)</th>
<th>PTH (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL/Mp ipr/lpr</td>
<td>Female</td>
<td>5</td>
<td>2.14 ± 0.41</td>
<td>1.84 ± 0.88*</td>
<td>0.26 ± 0.05†</td>
</tr>
<tr>
<td>MRL/Mp ipr/lpr</td>
<td>Male</td>
<td>5</td>
<td>2.02 ± 0.33</td>
<td>3.15 ± 0.88</td>
<td>0.24 ± 0.07†</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Female</td>
<td>5</td>
<td>1.94 ± 0.36</td>
<td>2.73 ± 0.41</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Male</td>
<td>5</td>
<td>1.84 ± 0.52</td>
<td>2.81 ± 0.65</td>
<td>0.38 ± 0.06</td>
</tr>
</tbody>
</table>

* Female MRL/Mp mice had significantly lower phosphorus levels than the other three groups (P = 0.012).
† Male and female MRL/Mp mice had significantly lower levels of serum PTH compared to male and female BALB/c mice, respectively (P = 0.003).
PTH = parathyroid hormone.

Calcification seen in MRL/Mp mice as band keratopathy, thereby referring to the human counterpart of corneal calcification. An important question raised by the results of the time course study is whether corneal calcification in MRL/MP mice is indeed a suitable model of band keratopathy. Band keratopathy in humans is a clinical entity of calcification in the corneal epithelium. Epithelial calcification starts in the periphery of the interpalpebral region and is often associated with long-standing eye diseases, such as glaucoma and chronic uveitis. The calcium deposits in MRL/Mp mice are located only in the center of the cornea and histologically in the anterior stroma. Furthermore, hypercalcemia and hyperparathyroidism reported to be associated with band keratopathy were not found in MRL/Mp mice. In our view, the specificity of band keratopathy for the lesions seen in the MRL/Mp mice is therefore inadequate and confusing.

The mechanism underlying corneal calcification remains to be identified. The regions where calcification was present showed typical thinning of the overlying epithelium, particularly the basal layer. This might indicate that the material was of epithelial origin or related to altered activity of the overlying epithelial cells. A powerful inhibitor of calcification in vivo is pyrophosphate. Therefore, a decrease of pyrophosphate levels in the corneal stroma or epithelium by pyrophosphatase might permit local deposition of calcium phosphate minerals.

A blood-borne origin of the material seems unlikely because blood vessels were not observed in most of the corneas studied. As mentioned, there were no signs of elevated serum calcium levels, while serum PTH levels decreased during the course of corneal calcification. Fine and coworkers studied carbon dioxy-
ide laser-induced corneal calcification in rabbits and showed that extracellular corneal calcification, as seen in this study, differs in morphologic features from the intracellular form that is seen in hyperparathyroidism.15 Whether the corneal lesions in MRL/Mp mice represent a disseminated process of calcification and basement membrane changes, similar to what has been described for DBA/2 mice22 and certain rat strains,23 is unknown. However, two findings indicate that this is not the case. The renal tubular basement membrane changes observed occasionally in Fischer rats were not seen in the kidney sections of MRL/Mp mice examined as part of this study. In addition, a detailed study of the lesions in the oral mucosa of MRL/Mp lpr/lpr mice described amorphous material close to the epithelium, but these deposits did not correspond to calcium using the same staining method as in the current study.24

Another finding of the current experiments, although not related to the calcium deposits, is a significant difference in lacrimation between male and female BALB/c mice. Gender-related differences in the morphology of the lacrimal gland have been observed in a variety of species including the mouse, rat, guinea pig, rabbit, and man.25 Cornell-Bell and coworkers observed that the exorbital lacrimal glands of male rats produced significantly more IgA and secretory component in vitro than did glands from females. The milky aspect of the collected tear fluid suggests that admixture of Harderian gland secretion had taken place. Questions about the origin of the gender-related differences and what proportion the Harderian gland contributed to the lacrimation volume and protein patterns remain to be answered, but are beyond the scope of this study.

In conclusion, our findings suggest that corneal calcification in inbred mouse strains is a common phenomenon not restricted to autoimmune-prone mice. The phenomenon represents an interesting experimental system to study tissue calcification, but has limited applications as a model for band keratopathy in humans.

**Key Words**

cornea, mice, calcification, autoimmune mice, band keratopathy

**Acknowledgments**

The authors thank Drs. A. J. Otto (Netherlands Ophthalmic Research Institute, Amsterdam) and J. Bras (Department of Pathology, University of Amsterdam) for valuable advice and helpful suggestions, A. de Vos and J. de Feiter for technical assistance, and Mrs. W. C. A. M. Ompi-Janssen for secretarial assistance.

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