


The Rabbit Lacrimal Gland in Vitamin A Deficiency
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Purpose. To investigate the morphology, ultrastructure, and protein secretion of the vitamin A-deficient rabbit lacrimal gland.

Methods. The lacrimal glands of vitamin A-deficient rabbits and age-matched controls were fixed, processed by standard methods, and examined by light and transmission electron microscopy. Protein secreted by the lacrimal gland was analyzed using gel filtration chromatography and electrophoresis.

Results. By light microscopy, the glands of experimental and control rabbits were indistinguishable. Electron microscopy showed little effect of vitamin A deficiency on the lacrimal acini, although occasional pyknotic nuclei were observed. The intralobular ductal epithelium was unaffected. Protein concentration and composition were essentially unchanged in lacrimal gland fluid of vitamin A-deficient rabbits compared to controls.

Conclusions. The rabbit lacrimal gland is minimally affected by vitamin A deficiency, suggesting species differences between rabbits and rats in the vitamin A requirements of the lacrimal gland. Normal lacrimal gland structure and function in vitamin A deficiency allow for the prompt secretion of retinol on the restoration of vitamin A to the diet. Invest Ophthalmol Vis Sci. 1994;35:1249–1253.

Among the many tissues that require vitamin A for control of cellular differentiation are the corneal and conjunctival epithelia, which become keratinized and undergo squamous metaplasia in retinoid deficiency.1,2 Because of the apparent dryness of the ocular surface epithelium in vitamin A deficiency, there has been a long-standing interest in the effects of vitamin A deficiency on tear secretion and the lacrimal gland.3 The role of vitamin A in lacrimal gland function has taken on added significance since the demonstration that the lacrimal gland secretes and metabolizes vitamin A.4,5

Bron,6 using light microscopy, demonstrated degeneration of the extraorbital lacrimal gland in the vitamin A-deficient rat. More recently, Hayashi et al8 reported ultrastructural abnormalities of the lacrimal gland in vitamin A-deficient rats. In contrast to these observations, we have observed that lacrimal gland function in the rabbit is essentially unaffected by vitamin A deficiency, as assessed by fluid and electrolyte secretion.7 Because this suggested that the structure of the vitamin A-deficient rabbit lacrimal gland should also be normal, a study was undertaken to examine the lacrimal glands of vitamin A-deficient rabbits histologically and ultrastructurally.

MATERIALS AND METHODS. Animals. Vitamin A-deficient New Zealand white rabbits (n = 9) were prepared as previously described.1,2 The rabbits were weighed weekly, and the eyes were examined by slit-lamp microscopy for xerophthalmia. The animals were maintained until they reached an established endpoint defined as weight plateau (mean ± SE = 2.17 ± 0.06 kg) followed by weight loss (final weight = 1.96 ± 0.08 kg) and development of stage 3 to 4 xerophthalmia1,2 in both eyes. At this point, serum retinol levels are less than 3 μg/dL. The vitamin A-deficient rabbits and four age-matched control rabbits (3.8 ± 0.12 kg) were anesthetized with intramuscular ketamine (30 mg/kg) and xylazine (5 mg/kg) and euthanized by an intravenous overdose of sodium pentobarbital. The lacrimal glands were then removed from each animal.

Histology and Electron Microscopy. Part of each gland was fixed in 10% neutral-buffered formalin for light microscopy, whereas the remainder was fixed in 0.2 M sodium phosphate-buffered, 8% glutaraldehyde for transmission electron microscopy. Paraffin sections of formalin-fixed glands were stained with hema-

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Submitted for publication April 30, 1993; revised July 26, 1993; accepted August 19, 1993.

Proprietary interest category: N.

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toxylin and eosin or Alcian blue (AB, pH 2.5), periodic acid-Schiff (PAS). Lacrimal gland specimens fixed in glutaraldehyde were postfixed in 2% osmium tetroxide and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy.

**Protein Secretion.** Eight additional vitamin A-deficient rabbits (1.96 ± 0.12 kg) were prepared as described above. Five of these rabbits were anesthetized with ketamine and xylazine, and lacrimal gland fluid (LGF) was collected as previously described.\(^4\)\(^7\) The LGF total protein concentration was measured by the Bradford dye-binding method (Bio-Rad, Richmond, CA) using bovine serum albumin as a standard. The LGF also was analyzed by gel filtration chromatography using a Superose 12 FPLC column (Pharmacia, Uppsala, Sweden) and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 12.5% gels and the Bio-Rad Mini-Protean II system (Bio-Rad). Photographs of the gels were scanned with a Sharp JX-920 scanner (Sharp Electronics Corp., Mahwah, NJ) and Adobe Photoshop software (Adobe Systems, Inc., Mountain View, CA).

The three remaining vitamin A-deficient rabbits (1.8 ± 0.05 kg) were repleted with vitamin A by oral administration of a single dose of 0.5 mg retinol dissolved in corn oil, and returned to a normal rabbit chow diet. After 7 to 14 days, when xerophthalmia had cleared and the rabbits had gained weight (final weight, 2.2 ± 0.03 kg), the protein analysis experiments described above were performed and the lacrimal glands were fixed and examined by light microscopy. The protein analyses also were performed on LGF of normal rabbits. All procedures in this study adhered to the ARVO Resolution on the Use of Animals in Research.

**RESULTS. Histopathology of Lacrimal Glands.** By light microscopy, no morphologic differences in the lacrimal gland were evident among vitamin A-deficient and age-matched control rabbits. The lacrimal gland acini of the deficient animals appeared normal, and no inflammatory or necrotizing lesions were evident in any of the sections examined. There was no evidence of squamous metaplasia of the intralobular ductal epithelium in any of the lacrimal glands (Fig. 1A,B).

Epithelial cells in serous acini of normal and vitamin A-deficient animals exhibited AB/PAS-positively stained neutral mucosubstances in the middle and apical portions of cells. Lacrimal glands from vitamin A-repleted rabbits were also indistinguishable from normal glands (data not shown).

**Electron Microscopy.** The acini of lacrimal glands of control and vitamin A-deficient rabbits consisted of columnar to high cuboidal cells that contained numerous, membrane-bound, electron-lucent, secretory granules (Fig. 1C,D). The acinar cells also had prominent Golgi complexes associated with the secretory granules, and well developed rough endoplasmic reticulum located basally in the cell. The basally located nucleus in these cells had a uniform ovoid shape, moderate amounts of peripherally marginated heterochromatin, and one or two prominent nucleoli. The lacrimal acinar cells of the vitamin A-deficient rabbits generally were indistinguishable from those of controls; however, there were occasional cells with shrunken, electron-dense, pyknotic nuclei and dilated cytoplasmic organelles, indicating individual cell degeneration and necrosis (Fig. 1D).

The epithelium lining the lumina of the intralobular ducts also was similar in morphology in both vitamin A-deficient rabbits and controls. Most of the cuboidal epithelial cells lining the ductal lumen contained several membrane-bound, markedly electron-dense, spherical granules in the apical cytoplasm. These cells also had a prominent microvillar luminal surface. No degeneration or metaplasia of the ductal epithelium was observed in vitamin A-deficient animals (Fig. 1E,F).

**Protein Secretion and Composition.** These experiments on LGF protein were undertaken based on the observation of abundant secretory granules in the lacrimal acinar cells of vitamin A-deficient rabbits. The total protein concentration of LGF collected from vitamin A-deficient rabbits was 18.4 ± 2.28 mg/ml (mean ± SE, n = 5). This value was not significantly different from the protein concentration of LGF in normal rabbits, 18.4 ± 1.23 mg/ml (n = 8) or vitamin A-repleted rabbits, 16.0 ± 2.59 mg/ml (n = 3, one-way analysis of variance, P > 0.05).

Analysis of LGF protein by gel filtration chromatography revealed no apparent differences among the proteins secreted by lacrimal glands of normal or vitamin A-deficient animals (Fig. 2). When LGF proteins of control, deficient, or vitamin A-repleted rabbits were separated by electrophoresis (Fig. 3), some variation among the samples was observed, especially in the higher–molecular-weight proteins; however, as shown by scans of representative samples, no major or consistent differences that could be attributed to nutritional status were observed (Fig. 4).

**DISCUSSION.** The results of this study show that the structure of the rabbit lacrimal gland acini was minimally affected by vitamin A deficiency, and that the intraglandular ducts were normal in spite of other signs of hypovitaminosis A in these rabbits.

The abundance of secretory granules observed in the acinar cells of vitamin A-deficient rabbits suggested normal protein synthesis and secretion. This was confirmed through experiments that demon-
FIGURE 1. Light and electron micrographs of control (A,C,E) and vitamin A-deficient (B,D,F) rabbit lacrimal glands. No morphologic differences were apparent by light microscopy (A,B). Arrows, serous acinus; M, mucous acinus; D, intralobular duct. Tissue sections were stained with hematoxylin and eosin. Electron micrographs of acinar cells (C,D) show abundant secretory granules (S). A few cells in vitamin A-deficient rabbits (D) had pyknotic nuclei and swollen endoplasmic reticulum (arrows). No differences are seen in the intralobular duct cells (E,F) of normal and vitamin A-deficient rabbits. Scale bars: (A,B) 20 μm; (C) 0.4 μm; (D) 0.3 μm; (E,F) 0.5 μm.

Striated no consistent changes in the protein composition of LGF of these rabbits. These data are in agreement with previous observations that tear secretion (Schirmer test) is normal and that lacrimal gland fluid secretion and electrolyte composition are minimally affected by vitamin A deficiency in rabbits. We also have reported that vitamin A-deficient rabbits have normal levels of retinyl ester hydrolase activity and retinol-binding protein mRNA expression in the lacrimal gland.

These observations on rabbit lacrimal gland are in marked contrast to the effects of vitamin A deficiency on rat exorbital lacrimal gland. As reported by Hayashi et al, in the vitamin A-deficient rat, the lacrimal
acini are reduced in size and secretory granules are essentially absent. The Golgi apparatus, rough endoplasmic reticulum, and nuclei are abnormal, and the acinar lumina are obstructed. Both rabbit and rat lacrimal glands stain positively for neutral mucosubstances; however, although this staining is lost in the vitamin A-deficient rat, PAS staining remained unchanged in the rabbit lacrimal gland.

The reason for the difference in vitamin A dependence between rat and rabbit lacrimal gland is unknown. A basic difference between the glands is the extraorbital location of the rat lacrimal gland studied by Hayashi et al and the orbital location of the rabbit gland; however, the glands are functionally similar and both secrete retinol. We have identified a potentially important difference between the lacrimal glands of rabbits and rats with respect to retinoids. Cellular retinoic acid-binding protein (CRABP) and CRABP mRNA are present in the rat exorbital lacrimal gland, but are not detectable in the rabbit lacrimal gland. Given the role of retinoic acid in cellular differentiation, this suggests a difference between rat and rabbit lacrimal glands in the function of retinoic acid in maintenance of cellular differentiation.

The results of this study raise the question of the relevance of the rabbit and rat models to lacrimal structure and function in human vitamin A deficiency. Tear secretion appears to be essentially normal in vitamin A-deficient humans. Sommer and Emran reported decreased tearing in 24% to 27% of children with xerophthalmia due to vitamin A deficiency; however, they defined decreased tearing as less than 15 mm wetting/5 min in the Schirmer test with topical anesthesia. The generally accepted Schirmer score with anesthesia for diagnosis of dry eye is less than 5 mm wetting/5 min. Other reports do not support Sommer and Emran's observation. Bron stated that adequate tears are present in vitamin A deficiency and suggested that the tear film is unstable due to a patho-
logic ocular surface. Specific case reports support this opinion. In a case of self-induced vitamin A deficiency in an adult, Fells and Bors\(^{11}\) reported plentiful tears, and Sullivan et al\(^{12}\) reported two cases of vitamin A deficiency in alcoholic men, both of whom had Schirmer test results of greater than 22 mm of wetting.

No recent pathology reports on the human lacrimal gland in vitamin A deficiency are available. In the early literature on hypovitaminosis A, Wilson and Dubois\(^{13}\) reported a fatal case of keratomalacia in an infant, apparently due to vitamin A deficiency. They stated in their necropsy report that the lacrimal glands were negative. This case, as well as the reports of normal tear secretion, suggest that, in terms of lacrimal gland function, the rabbit model of vitamin A deficiency is more relevant to human hypovitaminosis A than the rat model.

The lacrimal gland can be compared to the liver, which, although it has a major role in vitamin A metabolism, is minimally affected by hypovitaminosis A. For this reason, the liver is able to respond immediately to vitamin A repletion by secretion of the retinol-binding protein complex for delivery of vitamin A to peripheral tissues. In the same way, the lacrimal gland of rabbits, and possibly humans, is unaffected by vitamin A deficiency, and can respond promptly to vitamin A repletion by secretion of vitamin A in lacrimal fluid, restoring this nutrient to the tear fluid.

**Key Words**
lacrimal gland, retinoids, tears, vitamin A, xerophthalmia

**Acknowledgments**
The authors thank Donna Pearce and Anna Fekete, Department of Ophthalmology, Medical College of Wisconsin, Milwaukee, Wisconsin, for their expert electron microscopy. Megan Dennis and Martha Quigley of the University of Pittsburgh also provided technical assistance.

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