Histochemical Distribution of Carbonic Anhydrase in Rat and Rabbit Lacrimal Gland

B. Britt Bromberg,† Mary H. Welch,* Roger W. Beuerman,† Sek-Jin Chew,† Hilary W. Thompson,† David Ramage,* and Sherwood Githens**

Purpose. The purpose of this study was to examine the histochemical distribution of carbonic anhydrase (CA) in lacrimal glands from rats and rabbits; and to determine if age- and/or sex-related differences exist in the amount and distribution of CA in the rat lacrimal gland.

Methods. Lacrimal glands from young (3–12 wk) and aged (2–2.5 yr), male and female F344 rats and male rabbits were fixed in 1% paraformaldehyde and embedded in glycolmethacrylate. CA histochemistry was performed on 2-μm sections. The distribution of CA activity was determined by morphometric analysis.

Results. In rat lacrimal gland, CA activity was distributed in a discontinuous, mosaic fashion among the acinar cells. In tissue from young males and females as well as from aged females, about 10% of the acinar tissue displayed CA activity. Significantly more activity was present in tissue from aged male rats. CA was present in the ductal lumina, suggesting that it is a secretory product of the acinar cells. In rabbits, CA activity was associated with the basolateral membranes of the terminal acinar cells only.

Conclusions. In rat, the presence of CA activity in certain acinar cells and in ductal lumina suggests that CA is actively secreted by the lacrimal gland. An age-related increase in the amount of CA activity in the male glands exists that may be under gender-specific hormonal influences. In the rabbit lacrimal gland, the membrane-associated CA found uniquely with the terminal acinar cells suggests that these cells have special transport functions associated with the primary secretion of lacrimal fluid. Invest Ophthalmol Vis Sci 1993;34:339–348.

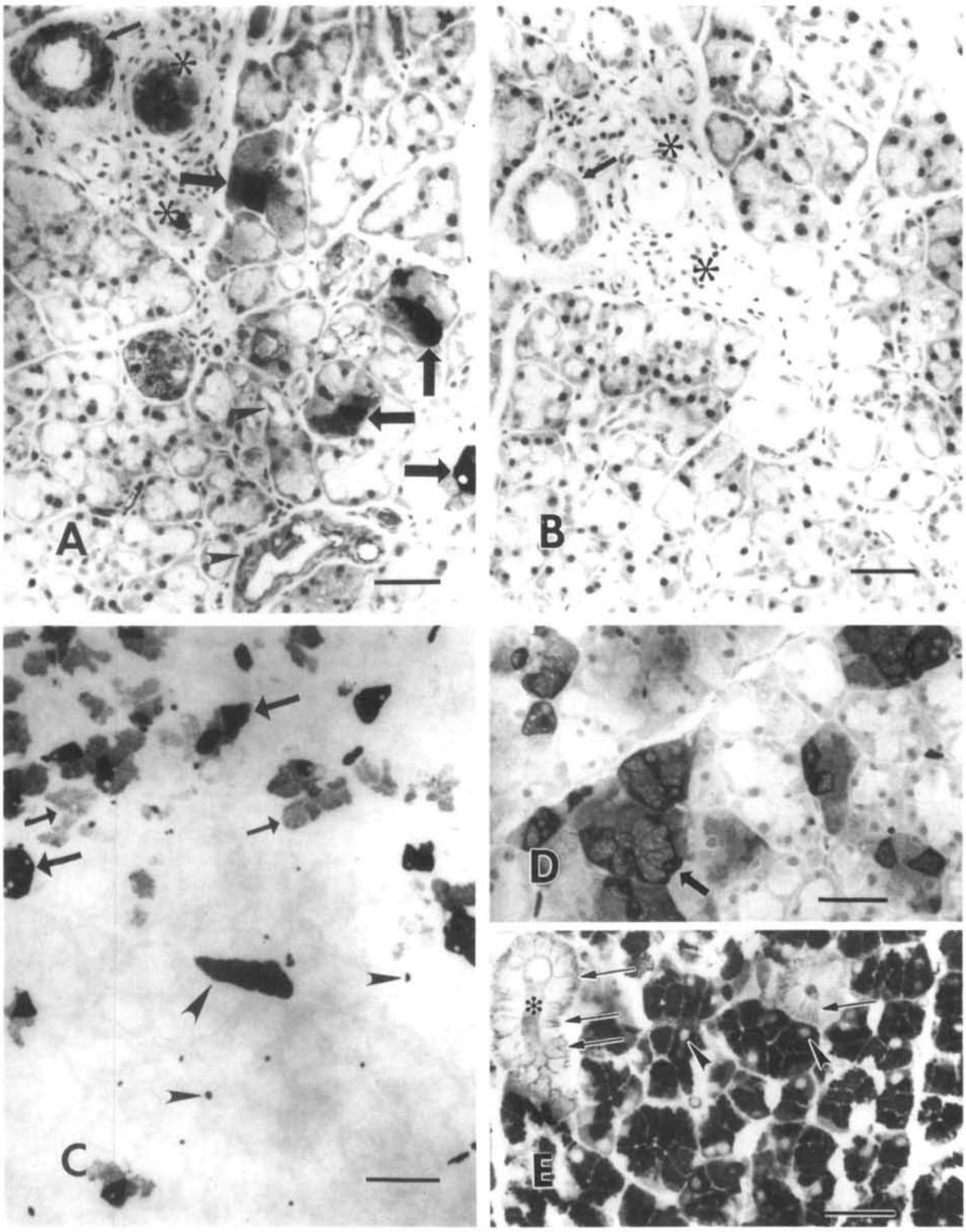
Carbonic anhydrase (CA) is a widely distributed cellular enzyme that exhibits at least seven different isozymic forms, CAs I through VII.[1] CA facilitates the reversible hydration of CO_2 and the subsequent generation of H^+ and HCO_3^-. These ions then participate in unidirectional and coupled transport processes that ultimately result in the transepithelial movement of one or more electrolytes and the coupled flow of water.[2] The production of these ions for participation in transport phenomena generally is ascribed to the cytoplasmic isozymes CA I and CA II or to the membrane-associated isozyme CA IV.[2–4]

Roles for H^+ and HCO_3^- in lacrimal secretion may be inferred from the presence of symport and antiport moieties identified in similar exocrine glands such as the salivary glands[5] and in the lacrimal gland itself.[6,7] Very few studies, however, have directly assessed the role of CA in lacrimal gland function. Although acetazolamide, a CA inhibitor, did not reduce basal tear flow rates in rats,[8] it did in patients after ocular surgery.[9] Further, by enzyme histochemistry, CA was found in monkey and rabbit lacrimal glands.[10] Attempts to identify CA directly by immunohistochemi-

*From the *Department of Biological Sciences, University of New Orleans, New Orleans, and the †Laboratory for the Molecular Biology of the Ocular Surface, Louisiana State University Eye Center, Louisiana State University Medical Center, New Orleans, Louisiana.*
This research was supported by U.S. Public Health Service, National Institutes of Health grants EY-04158 and EY-04074.
Submitted for publication: February 10, 1992; accepted June 22, 1992.
Proprietary interest category: N.
Reprint requests: B. Britt Bromberg, Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148.

cal application of antibodies to CA I and CA II in rodent lacrimal tissue yielded inconsistent results, however.[11] A few acinar and intercalated duct cells in mouse lacrimal gland reacted positively, but no CA I or CA II reactive cells were identified in the acinar population of the rat lacrimal gland.

We have examined by enzyme histochemistry the presence of CA in the lacrimal glands of rats and rabbits. CA activity was identified in only about 10% of the acinar cells in lacrimal glands from young rats. Its association with the apically located secretory vesicles of some acinar cells and its presence in ductal lumina suggests that the acinar cells synthesize and secrete CA as an exocytotic product, most likely the isozymic form CA VI. Acinar CA activity in rat lacrimal gland appeared to vary according to age and gender. In addi-



tion to the acinar CA activity, a membrane-associated form of CA, possibly CA IV, also was present in the interlobular duct epithelial cells of the aged rats and in the terminal acinar epithelial cells of the rabbit lacrimal gland.

MATERIALS AND METHODS

Animals

Male and female F344 rats and male New Zealand albino rabbits (2–4 lb) were used in these studies. Rats were killed with intraperitoneal injections and rabbits with intravenous injections of sodium pentobarbital. All animals were treated humanely in accordance with the May 1992 ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Histochemistry

Lacrimal glands (exorbital gland for the rat) were quickly excised and cut into fragments while immersed in fixative composed of 1% paraformaldehyde (Polysciences, Inc., Warrington, PA) in 0.1 mol/l phosphate buffer, pH 7.4. After 3 to 4 hr at room temperature, fixation was continued with fresh fixative overnight at 4°C. Except for sectioning and histochemistry, which were done at room temperature, the tissue was maintained and processed at 0° to 4°C. After fixation, the tissue was washed in phosphate buffer, partially dehydrated through 80% ethanol, and infiltrated with JB-4 Plus embedding medium (Polysciences, Inc.). Polymerization was accomplished in a nitrogen atmosphere. Sections (2 μm) were cut on a modified AO 820 microtome (American Optical; Leica Inc., Deerfield, IL) adapted for use with glass knives.

CA histochemistry was performed as described by Githens et al's[12] modification of Hansson's procedure.[13] Briefly, sections mounted on glass microscope slides were dipped for 2 sec into incubation media containing 5.85 mmol/l KH_2PO_4, 157 mmol/l $NaHCO_3$, 53 mmol/l H_2SO_4, 8.75 mmol/l $CoSO_4$, and 0.5% Triton X-100, pH 5.9. The incubation medium was maintained in a CO_2-enriched atmosphere. After dipping, the slides were held in front of a current of moving air for 28 sec. Dipping and aerating were repeated 13 to 15 times. Aeration facilitates the evolution of CO_2 produced locally at sites of active CA.[14] With the loss of CO_2, these sites become alkaline, causing the precipitation of $CoPO_4$, which, in turn, is visualized by incubation in 0.5% $(NH_4)S$. Control sections included incubation with 20 μmol/l acetazolamide or aeration in a CO_2-enriched atmosphere. Acetazolamide inhibits the enzyme activity,[2] whereas the CO_2 atmosphere prevents the evolution of CO_2 generated locally by active CA sites, and thus inhibits the local alkalinization and consequent precipitation of $CoPO_4$. Some sections were counterstained with nuclear fast red (NFR) before coverslipping. Sections were photographed using a Nikon M-35S camera (Nikon, Inc., Melville, NY) attached to an AO microscope (American Optical; Leica, Inc.).

Image Analysis

Histochemical CA activity was quantitated by measuring the area of reactive acinar cells in each section. For each slide, two to three noncontiguous fields were photographed using the ×10 objective. The photographic negatives of the stained tissue were viewed using a Videoscope model CCD 200E video camera (Video Scope International, Washington, DC) with a Pentax-A macro lens (Pentax Corp., Englewood, CO). Compensations for intensity variations, due to different development times and exposure settings of the film, were made empirically by adjustment of the gain and pedestal of the camera control. The images were captured on a 486/33 microcomputer with a Visionplus-AT overlay video capture board (Imaging Technology, Inc., Woburn, MA).

FIGURE 1. Discontinuous distribution of CA-positive cells in rat lacrimal gland. **(A)** CA-reactive cells (large arrows) are present in a minority of acinar cells in lacrimal glands from young male and female rats and in aged female rats. This example is from an aged female. A few positively stained duct cells are present in the interlobular duct (small arrow), whereas none are present in the intralobular duct (arrowhead). Red blood cells (RBC) in the vessels of the interlobular connective tissue have CA activity (asterisk). **(B)** A section parallel to that in **(A)** was incubated in the presence of 20 μmol/l acetazolamide. No positively stained cells are present. Landmark structures in **(A)** are marked similarly in **(B)**. **(C)** In the absence of counterstaining with NFR, unreactive cells, nuclei, and connective tissue elements are barely detectable. Strongly and weakly active cells (large arrows and small arrows, respectively) are present, as are RBCs in the centrally located venule (large arrowhead) and isolated capillaries (small arrowheads). **(D)** Occasionally, the strong basal CA activity is colocalized with the weak apical activity (arrow). **(E)** In contrast to rat lacrimal gland, CA histochemical activity in similarly prepared parotid gland is uniformly distributed among the acinar cells. Acinar cell nuclei appear as negative images (arrowheads). CA activity also is found in the lumen of the striated duct (asterisk) as well as associated with the basal and lateral membranes (arrows). **(A, B, D)** Counterstained with NFR; **(A–E)** bar = 50 μm.

Computer image enhancement and analysis were then performed with the Optimas program (Bioscan, Inc., Edmonds, WA). The threshold of the screen image was adjusted to reflect the two densities, strong and weak, of CA staining (see Results). Using these thresholds, screen objects were created, representative of the zones of stained tissue. After calibration for image dimensions, the individual frames were digitized and the areas of CA staining automatically measured. The results were exported to a spreadsheet file for calculation of the percent of staining relative to the total area of tissue in each frame (294×10^3 μm^2). Before data extraction, positively stained artifacts, such as red blood cells, were manually selected and digitally deleted from the images. Image capture, thresholding, and area measurement were performed in a single-blind manner.

Data Analysis

After the areas of strong and weak CA activities were determined for each negative as described above, the code was broken and age and sex data were entered into data files for analysis of variance (ANOVA) using the Statistical Analysis System (SAS).[15] Means and standard deviations were calculated by age and sex from the ANOVA, and preplanned statistical comparisons were made.

RESULTS

Rat Lacrimal Gland

In the rat lacrimal gland, the principal histochemical CA activity was located in the acinar epithelial cells (Figs. 1 and 2). Histologic control sections, incubated with 20 μmol/l acetazolamide (Fig. 1B), were unreactive when compared to sections incubated in the absence of acetazolamide (Fig. 1A), as were CO_2 control sections (not shown). In young animals (3–12 wk), only a minority of acinar cells, about 9% to 10% as judged by morphometric analysis, were reactive (Table 1). Among the CA-reactive cells, two levels of activity, strong and weak, were distinguished. The clear distinction among the strong, weak, and unreactive acinar cells is easily seen in the absence of any counterstain (Fig. 1C). In strongly reactive cells, comprising 4% to 5% of the total area from young animals (Table 1), the CA activity was localized to the basal cytoplasm (Figs. 1C, D) where the nuclei often could be seen in negative image. Another 4% to 5% of the acinar area in young glands had a weak level of CA activity (Table 1), usually localized to the apical cytoplasm (Fig. 1C). In favorable sections, both intense basal and weak apical activities were present in the same cell (Fig. 1D). Most positively stained acinar cells showed only strong or weak activities, however. The remaining acinar cells, constituting the majority of cells in young males, were characterized as unreactive, containing no histochemically detectable CA activity (Figs. 1A, C, D). In similarly prepared parotid gland (Fig. 1E), the distribution of CA activity was uniform among the acinar cells. This indicates that the mosaic pattern of CA activity in lacrimal gland was not a histochemical artifact. Lacrimal tissue from young (not shown) and aged female rats (Fig. 1A) had amounts (Table 1) and cellular localizations of CA that were very similar to those in young male glands.

In lacrimal glands from aged male rats (2–2.5 yr), there was an obvious increase in the histochemically detectable CA activity (Fig. 2A, Table 1). Increased activity was present in regions that exhibited relative histologic disorganization (Fig. 2A); however, these changes were not necessarily uniform throughout the gland. In some areas in which tissue disorganization had not occurred, the number of cells showing CA activity was apparently normal (Fig. 2B), that is, similar to that found in tissue from young animals.

Morphometric analysis showed that in sections from aged male glands, the strong and weak activities increased 1.5 and 2 times, respectively, over those in young males, and females of either age (Table 1). These changes were statistically significant (ANOVA, $P < 0.01$) whether calculated as absolute areas or as a percentage (ANOVA not shown).

CA also was present in locations other than the acinar cells in the rat lacrimal gland. Activity often was observed in the lumina of the intralobular, interlobular, and major excretory ducts (Figs. 3A, B). This suggested that some of the CA present in the acinar cells was the secretory CA isozyme, CA VI. In aged animals of both sexes, CA activity also was associated with the basal and lateral membranes of the interlobular duct epithelial cells (Figs. 1A and 3C). Red blood cells in

TABLE 1. Morphometric Analysis of CA Histochemistry in Rat Lacrimal Gland

		Area			
		Strong		*Weak*	
*Age/Sex**	*N*	$\mu m^2 \times 10^3$	%	$\mu m^2 \times 10^3$	%
YF	4	14.0 ± 1.6	5.1	13.1 ± 2.3	4.7
YM	5	12.6 ± 1.6	4.3	12.9 ± 2.3	4.4
AF	5	10.0 ± 2.1	4.7	15.8 ± 3.0	6.0
AM	7	19.8 ± 1.8†‡	7.3	26.6 ± 2.5§‖	9.8

* YF, young females (range 0.1–0.5 yr). YM, young males (range 0.1–0.2 yr). AF, aged females (range 2–2.9 yr). AM, aged males (range 2–2.25 yr).
† $P < 0.005$ compared to YM.
‡ $P < 0.001$ compared to AF.
§ $P < 0.001$ compared to YM.
‖ $P < 0.01$ compared to AF.

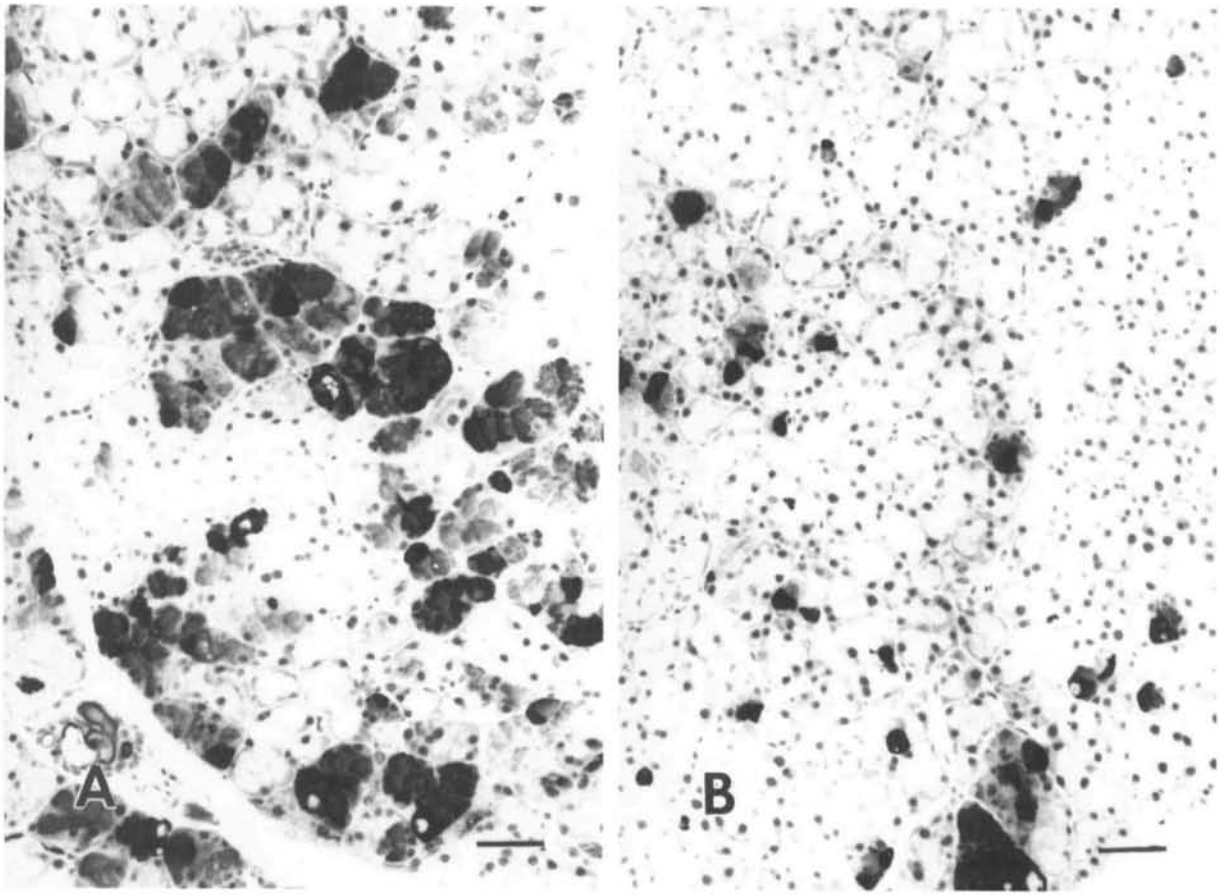


FIGURE 2. CA activity in aged male lacrimal gland. **(A)** In contrast to young male and young and aged female lacrimal gland, many more of the acinar cells show histochemical CA activity. Additionally, the acini are less well organized compared to the young tissue. **(B)** In another region of the same section as **(A)**, the organization of the acini and distribution of CA activity appears indistinguishable from that in young animals. Counterstained with NFR; bar = 50 μm.

capillaries and other blood vessels also were histochemically reactive (Figs. 1A, C, D).

Rabbit Lacrimal Gland

The histologic organization of the rabbit lacrimal gland is similar to that of the rat, except that the rabbit acini are more elongate, forming tubuloacinar structures. As in the rat lacrimal gland, only certain acinar cells in the rabbit gland had histochemically detectable CA activity. The positive cells, however, never appeared in the middle of the elongate acini (Fig. 4A). Rather, the reactive cells were found in what appeared to be the terminal cells of each acinus, or as free clusters of acinar cells, most easily interpreted as terminal acinar cells of acini cut in cross-section. Reactivity within these terminal acinar cells was restricted to the basal and lateral membranes and/or membrane-associated cytoplasm. Both CA-positive and CA-negative acinar cells were rich in their content of exocytotic vesicles. The CA-positive cells could be distinguished from other acinar cells by the paucity of the basal cytoplasm in the CA-positive cells (Fig. 4B). In contrast to the rat lacrimal gland, no cytoplasmic CA activity was found in the basal cytoplasm or associated with the exocytotic vesicles stored apically. In histochemical control sections, no activity was present (Fig. 4C), but the cells most likely to be reactive could be identified by the sparseness of the basal cytoplasm and the richness of the exocytotic vesicles.

CA activity could not be identified in intralobular or interlobular duct epithelial cells, nor was there any activity in the ductal lumina. Thus, in the rabbit, there was no evidence for any secretory CA. The only other sites to exhibit CA activity in the rabbit lacrimal gland were the capillary endothelial cells (Figs. 4A, B) and red blood cells (not shown).

DISCUSSION

The histochemical assay used in this study can detect CA activity resulting from all of the known isozymic forms, but it cannot reliably distinguish among them.[14]

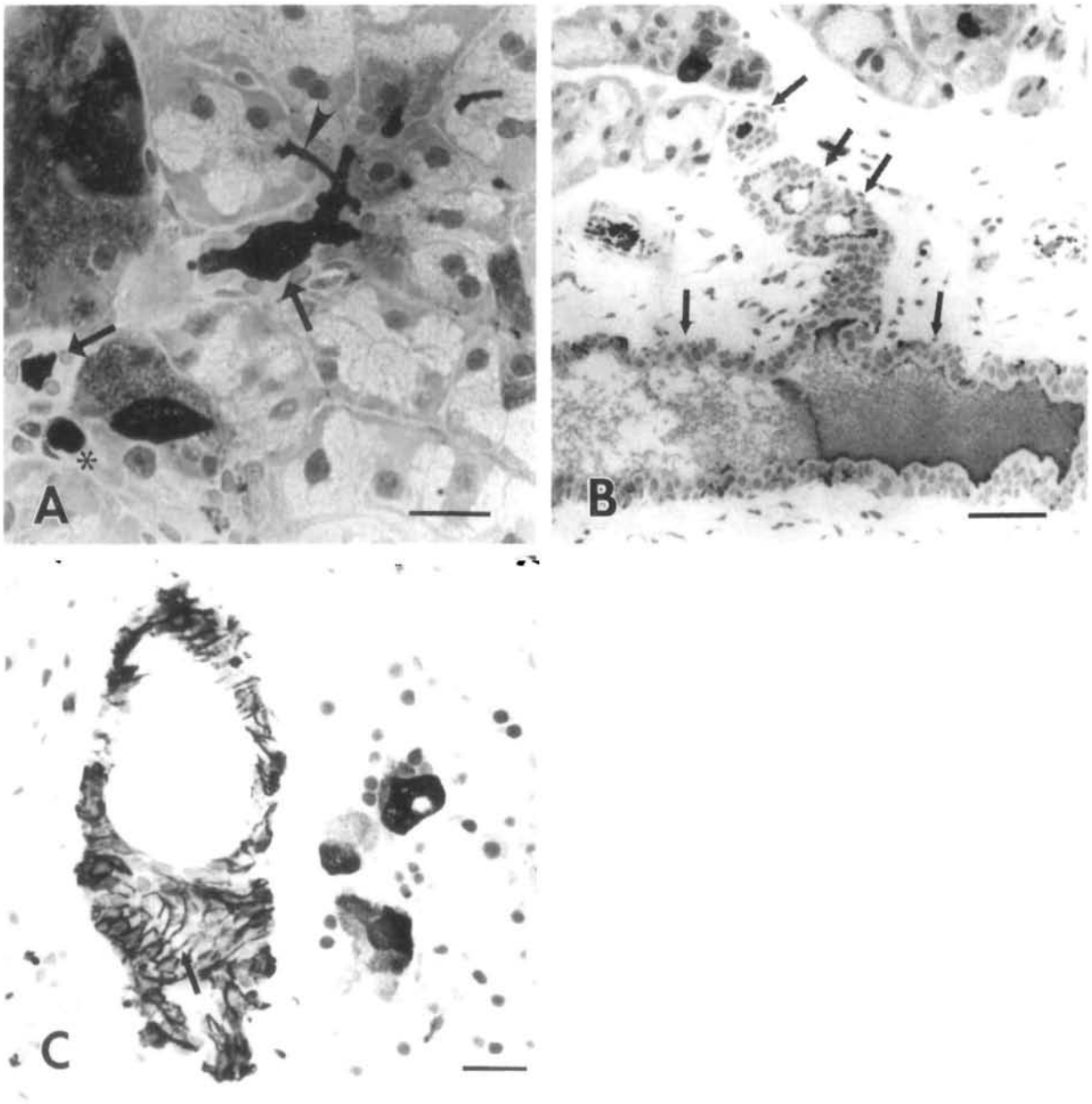


FIGURE 3. Ductal CA activity in rat lacrimal gland. Although not all ductal lumina are positive, CA activity may be found filling the lumina (arrows) of both intralobular **(A)** and interlobular and excretory ducts **(B)**. In **(A)**, CA within the intralobular duct (arrowhead) is associated with an apparently unreactive acinus. This luminal activity could be due to reflux from the larger duct during tissue preparation or from a CA-positive acinar cell not in the plane of this section (RBCs indicated by asterisk). **(C)** In aged rats, membrane-associated CA activity (arrow) often is present in or near the basal and lateral membranes of the interlobular duct epithelial cells. Counterstained with NFR; **(A,C)** bar = 25 μm; **(B)** bar = 50 μm.

Thus, the attribution of CA activity located in the apical cytoplasm of the rat acinar cells to CA VI and the membrane-associated CA in rat lacrimal duct and in rabbit terminal acinar epithelial cells to CA IV remains tentative, awaiting further biochemical characterization. It is possible that histochemically unreactive cells in both rat and rabbit lacrimal glands contain other CA isozymes in quantities or activities below the level of detection by the methods used here. Except where marginally detectable activity was initially present, it is unlikely that CA activity was lost due to fixation because it can be demonstrated even with up to 2.5% glutaraldehyde fixation.[14] It is conceivable that with the minimal fixation used here, soluble forms of CA (CAs I–III) could have been lost during dehydration in preparation for embedding the tissue. The initial activity of these isozymes would likely have been very low, however, because the abundant CA activity in red

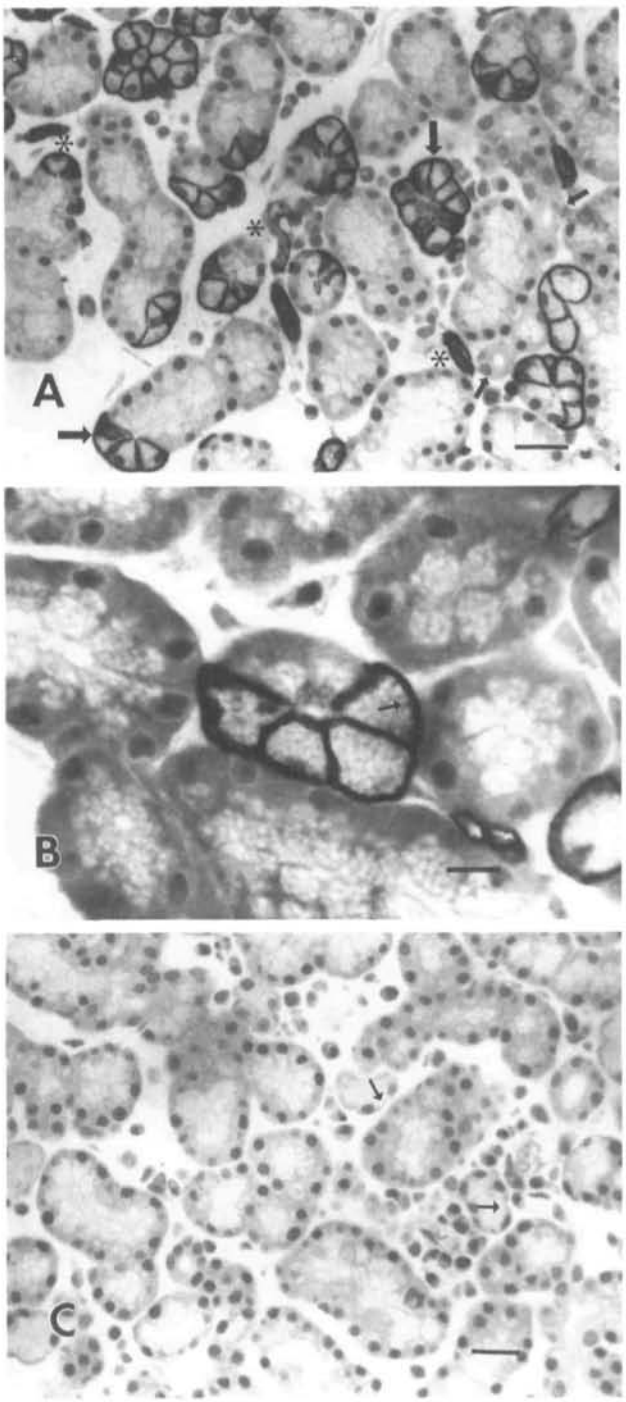


FIGURE 4. CA activity in rabbit lacrimal gland. **(A)** CA activity in rabbit lacrimal gland is associated principally with the basal and lateral membranes of terminal acinar epithelial cells (large arrow). Activity also is associated with the capillary endothelium (asterisks). Intralobular duct epithelium and lumen are negative for CA activity (small arrows). **(B)** Typically, the CA-positive acinar cells contain abundant exocytotic vesicles and have a relatively sparse basal cytoplasm (arrow) compared to the unreactive cells. **(C)** Acetazolamide (20 μmol/l) inhibits histochemically detectable CA activity. Cells likely to be CA-positive can be recognized by the sparse amount of basal cytoplasm (arrows). Counterstained with NFR; **(A,C)** bar = 25 μm; **(B)** bar = 10 μm.

blood cells suggests that significant cytoplasmic activity is retained by our procedures.

The absence of detectable CA in most of the rat acinar cells is somewhat surprising because the generation of H^+ and HCO_3^- are thought to be an integral part of the transepithelial transport of electrolytes and fluid accomplished by most secretory epithelia. Its absence, however, is consistent with the findings of Hennigar et al,[11] who could not identify by immunohistochemistry the cytoplasmic isozymes, CA I and CA II, in rat lacrimal gland even though they were present in other rat tissues. The prominent localization of CA activity in the apical cytoplasm of the acinar cells of the rat lacrimal gland suggests that this isozyme is the same as or related to the CA VI identified, along with its mRNA, in the parotid and submandibular glands of a variety of mammals, including humans.[16–19] This idea is further supported by the presence of CA activity filling the lumina of intralobular and interlobular ducts as well as the excretory ducts.

That only 10% of the acinar cells in the rat lacrimal gland contain detectable CA activity suggests that a functional heterogeneity exists among the acinar cell population. These data are consistent with physiologic evidence of heterogeneity based on differences in the specific activities of peroxidase secreted under the influence of various autonomic stimuli.[20,21] It is possible that secretion of lacrimal CA, like lacrimal peroxidase, is subject to selective stimulation by specific cholinergic, adrenergic, or peptidergic agonists. Experiments are underway to determine these relationships. A similar discontinuous distribution of CA activity occurs in the monkey lacrimal gland[10] and contrasts with its uniform presence in the acinar cells of the rat parotid gland (Fig. 1E; and see reference 16).

The basally located strong CA activity may represent nascent CA, still in the rough endoplasmic reticulum. This would be consistent with the pattern of lacrimal peroxidase synthesis as documented by Herzog and Fahimi.[22] In both cryostat and plastic sections, peroxidase histochemical activity in the basal cytoplasm often is greater than the apically located activity (personal observation). This suggests that packaged enzymes may be less reactive than are the unpackaged, nascent forms. Some acinar cells showed weak apical CA activity in the absence of detectable strong basal CA activity. Possibly, the reactive portion of the basal cytoplasm was excluded from these sections. Another possibility is that synthesis and packaging of the CA are temporally distinct and discontinuous events. Thus, after the newly synthesized CA has been packaged, no new CA would be synthesized until the cells have been stimulated to secrete their exocytotic load.

Age-related effects have been documented in the lacrimal gland clinically, physiologically, and morphologically.[20,21,23–28] There is a large variability in the histologic appearance of aged tissue, both between ani-

mals of a similar age, and even within individual lacrimal glands, as reported here. This is consistent with the high variability seen in physiologic data from aged lacrimal glands.[20,26] The increase in both strong and weak CA activities occurred in a parallel fashion. Insofar as this represents the acinar cell's ability to synthesize and package CA, these functions do not appear to be impaired. The physiologic significance of this increase is not yet known.

Gender-related differences in lacrimal glands are well established.[27-31] In intact animals, it is difficult to relate these changes to specific hormones such as testosterone or estrogen because the effects may be due to other gender-related hormones or combinations thereof.[32] In cultured lacrimal acinar cells, however, the synthesis and secretion of secretory component is directly enhanced by testosterone.[33] The increased presence of both strongly and weakly reactive CA acinar cells in tissue from aged males is another age- and gender-related effect of which the proximate cause remains to be determined.

The physiologic role for a secreted CA is not known. In rat lacrimal gland, the anion difference ($Na^+ + K^+ - Cl^-$) of fluid collected by micropuncture from the intercalated duct is about 43 mmol/l under resting and carbachol-stimulated conditions.[34] Bicarbonate ions may provide the residual anions. In fluid collected from the excretory duct, however, the anion difference ranges from 40 mmol/l at maximum flow rates to over 150 mmol/l under unstimulated flow. Analysis of HCO_3^- revealed a concentration of only 20 mmol/l, which was independent of flow rate. Possibly the secreted CA provides some means of trapping HCO_3^- in the ductal lumen,[17] which is then exchanged for another, as yet unidentified, anion present in the final secreted product.

Another possibility for the role of secreted CA is suggested by the observations of Lambert et al,[7] who found that 80% of the lacrimal acinar Cl^-/HCO_3^- antiporters were localized to intracellular membranes. Some of these may have been destined for transport to the basal and lateral surfaces of the acinar cells to participate in transepithelial electrolyte and fluid flux. Other Cl^-/HCO_3^- antiporters may have been associated with the membranes of the exocytotic vesicles, however, allowing the vesicles to serve as a source of intracellular HCO_3^- generated by the intravesicular CA. In fact, this may be the primary source of acinar cell HCO_3^-, given the apparent absence of immunohistochemically detectable levels of cytoplasmic isozymes CA I and CA II.[11]

The role of the membrane-associated CA activity found in the interlobular duct epithelium in aged rats of both genders and in the rabbit terminal acinar cells remains unknown. A CA isozyme, CA IV, has been identified in bovine respiratory epithelium[35] and in the apical and basolateral membranes of the renal proximal tubules,[36] where it is presumed to subserve a transport function. It also is present in the basal and lateral membranes of the striated ducts in submandibular (personal observation) and parotid glands (Fig. 1E) from young rats. The ducts of the lacrimal gland do not have the basal infoldings that give rise to the striations similar to those of the salivary gland ducts seen in light microscopy. Thus, the transport function accomplished by the salivary ducts probably has no exact homology in the lacrimal gland. The appearance of membrane-associated CA in interlobular ducts of aged rat lacrimal glands suggests that a transport function may be acquired that is not present to any large degree in the young animals. Its presence in the duct cells may represent a compensatory role for tasks normally accomplished by the acinar cells in the young animals. Eichhorn also found CA activity associated with the basal and lateral membranes of the rabbit lacrimal gland.[10] In contrast to our finding, however, all of the acinar cells showed membrane-associated staining in his study. Differences in fixation and/or section thickness may account for these contrasting findings. If the basolateral membranes of the nonterminal acinar cells have a membrane-associated CA, then its activity is less than that of the terminal cells under the histochemical conditions used here. The high activity of membrane-associated CA in the terminal portions of the acini suggests that special transport properties are associated with these cells and distinguish them from the other acinar epithelial cells. This again supports the notion of a heterogeneity among the lacrimal gland acinar cell population.

Acetazolamide is a commonly used drug for controlling elevated intraocular pressure resulting from glaucoma or after ocular surgery. In rats, acetazolamide reduced the peroxidase content of the secreted tears without affecting the basal tear flow.[8] This suggests that if secretion from the exorbital lacrimal gland was reduced, the tear volume was compensated by contributions from other ocular glands. In humans, the potential role of CA activity in the formation of tears was shown by the acetazolamide-induced reduction of tear flow in both eyes after unilateral cataract surgery.[9] To our knowledge, the CA activity in human lacrimal gland has not been reported; however, the histochemical distribution in monkey lacrimal gland[10] is very similar to that observed here in rat lacrimal gland. Thus, for determination of the role of CA in lacrimal gland physiology and its relationship to human tear formation, the rat lacrimal gland may serve as a useful model.

Key Words

aging, carbonic anhydrase, gender, histochemistry, lacrimal gland.

Acknowledgments

The authors thank Ms. Renee Kern for her work on the manuscript.

References

1. Dodgson SJ. The carbonic anhydrases: overview of their importance in cellular physiology and in molecular genetics. In: Dodgson SJ, Tashian RE, Gros G, Carter ND, eds. *The Carbonic Anhydrases.* New York: Plenum Press; 1991:3–14.
2. Maren TH. The general physiology of reactions catalyzed by carbonic anhydrase and their inhibition by sulfonamides. In: Tachyon RE, Hewett-Emmett D, eds. *Biology and Chemistry of the Carbonic Anhydrases.* New York: New York Academy of Sciences; 1984:568–579.
3. Maren TH. Current status of membrane-bound carbonic anhydrase. *Ann NY Acad Sci.* 1980;341:246–258.
4. Spicer SS, Sens MA, Hennigar RA, Stoward PJ. Implications of the immunohistochemical localization of the carbonic anhydrase isozymes for their function in normal and pathologic cells. In: Tachyon RE, Hewett-Emmett D, eds. *Biology and Chemistry of the Carbonic Anhydrases.* New York: New York Academy of Sciences; 1984:382–397.
5. Cook DI, Young JA. Fluid and electrolyte secretion by salivary glands. In: Forte JG, ed. *Handbook of Physiology Salivary, Gastric, Pancreatic, and Hepatobiliary Secretion.* Washington, DC: American Physiological Society; 1989:1–23.
6. Mircheff AK, Ingham CE, Lambert RW, Hales KL, Hensley CB, Yiu SC. Na^+/H^+ antiporter in lacrimal acinar cell basal-lateral membranes. *Invest Ophthalmol Vis Sci.* 1987;28:1726–1729.
7. Lambert RW, Bradley ME, Mircheff AK. Cl^--HCO_3^- antiport in rat lacrimal gland. *Am J Physiol.* 1988;255:G367–G373.
8. Van Agtmaal EJ, Thorig L, Van Haeringen NJ. Effect of acetazolamide (Diamox) on tear secretion. *Doc Ophthalmol.* 1985;59:77–80.
9. Rohrschneider K, Koch H-R. Auswirkungen von Acetazolamid (Diamox, Glaupax) auf die Tranenproduktion. *Klin Monatsbl Augenheilkd.* 1991;199:79–83.
10. Eichhorn M. The presence of carbonic anhydrase in orbital glands. *Histochemistry.* 1988;88:475–479.
11. Hennigar RA, Schulte BA, Spicer SS. Immunolocalization of carbonic anhydrase isozymes in rat and mouse salivary and exorbital lacrimal glands. *Anat Rec.* 1983;207:605–614.
12. Githens S, Finley JJ, Patke CL, Schexnayder JA, Fallon KB, Ruby JR. Biochemical and histochemical characterization of cultured rat and hamster pancreatic ducts. *Pancreas.* 1987;2:427–438.
13. Hansson HPJ. Histochemical demonstration of carbonic anhydrase activity. *Histochemie.* 1967;11:112–128.
14. Lonnerholm G. Histochemical localization of carbonic anhydrase in mammalian tissue. In: Tachyon RE, Hewett-Emmett D, eds. *Biology and Chemistry of the Carbonic Anhydrases.* New York: New York Academy of Sciences; 1984:369–381.
15. Freund RJ, Littell RC, Spector PC. Analysis of variance models of less than full rank. In: *SAS System for Linear Models.* Cary, NC: SAS Institute Inc.; 1986:85–140.
16. Carpentier P, Fournie J, Chetail M. Biochemical demonstration of carbonic anhydrase in the rat parotid secretion. In: Tachyon RE, Hewett-Emmett D, eds. *Biology and Chemistry of the Carbonic Anhydrases.* New York: New York Academy of Sciences; 1984:216–218.
17. Feldstein JB, Silverman DN. Purification and characterization of carbonic anhydrase from the saliva of the rat. *J Biol Chem.* 1984;259:5447–5453.
18. Fernley RT, Darling P, Aldred P, Wright RD, Coghlan JP. Tissue and species distribution of the secreted carbonic anhydrase isoenzyme. *Biochem J.* 1989;259:91–96.
19. Aldred P, Fu P, Barrett G, Penschow JD, Wright RD, Coghlan JP, Fernley RT. Human secreted carbonic anhydrase: cDNA cloning, nucleotide sequence, and hybridization histochemistry. *Biochemistry.* 1991;30:569–575.
20. Bromberg BB, Cripps MM, Welch MH. Sympathomimetic protein secretion by young and aged lacrimal gland. *Curr Eye Res.* 1986;5:217–223.
21. Bromberg BB, Cripps MM, Welch MH. Peroxidase secretion by lacrimal glands from juvenile F344 rats. *Invest Ophthalmol Vis Sci.* 1989;30:562–568.
22. Herzog V, Fahimi HD. Intracellular distinction between peroxidase and catalase in exocrine cells of rat lacrimal gland: a biochemical and cytochemical study. *Histochemistry.* 1976;46:273–286.
23. Walker R. Age changes in the rat's exorbital lacrimal gland. *Anat Rec.* 1958;132:49–69.
24. Damato BE, Allan D, Murray SB, Lee WR. Senile atrophy of the human lacrimal gland: the contribution of chronic inflammatory disease. *Br J Ophthalmol.* 1984;68:674–680.
25. McGill JI, Liakos GM, Goulding N, Seal DV. Normal tear protein profiles and age-related changes. *Br J Ophthalmol.* 1984;68:316–320.
26. Bromberg BB, Welch MW. Lacrimal protein secretion: comparison of young and old rats. *Exp Eye Res.* 1985;40:313–320.
27. Sashima M, Hatakeyama S, Satoh M, Suzuki A. Harderianization is another sexual dimorphism of rat exorbital lacrimal gland. *Acta Anat.* 1989;135:303–306.
28. Sullivan DA, Hann LE, Yee L, Allansmith MR. Age- and gender-related influence on the lacrimal gland and tears. *Acta Ophthalmol.* 1990;68:188–194.
29. Lauria A, Porcelli F. Leucine aminopeptidase activity and sexual dimorphism in rat exorbital lacrimal gland. *Basic Appl Histochem.* 1979;23:171–177.
30. Cornell-Bell AH, Sullivan DA, Allansmith MR. Gender-related differences in the morphology of the lacrimal gland. *Invest Ophthalmol Vis Sci.* 1985;26:1170–1175.
31. Pangerl A, Pangerl B, Jones DJ, Reiter RJ. β-adrenoceptors in the extraorbital lacrimal gland of the Syrian hamster characterization with [^{125}I]-iodopindolol and

evidence of sexual dimorphism. *J Neural Transm.* 1989;77:153–162.

32. Sullivan DA, Allansmith MR. Hormonal influence on the secretory immune system of the eye: endocrine interactions in the control of IgA and secretory component levels in tears of rats. *Immunology.* 1987; 60:337–343.
33. Hann LE, Kelleher RS, Sullivan DA. Influence of culture conditions on the androgen control of secretory component production by acinar cells from the rat lacrimal gland. *Invest Ophthalmol Vis Sci.* 1991; 32:2610–2621.
34. Alexander JH, Van Lennep EW, Young JA. Water and electrolyte secretion by the exorbital lacrimal gland of the rat studied by micropuncture and catheterization techniques. *Pflugers Arch.* 1972;337:299–309.
35. Whitney PL, Briggle TV. Membrane-associated carbonic anhydrase purified from bovine lung. *J Biol Chem.* 1982;257:12056–12059.
36. Brown D, Zhu XL, Sly WS. Localization of membrane-associated carbonic anhydrase type IV in kidney epithelial cells. *Proc Natl Acad Sci USA.* 1990;87:7457–7461.