Protein in rabbit lacrimal gland fluid. Darlene A. Dartt,* and Stella Y. Botelho.

A variety of proteins have been identified in tears, which are composed of secretions from all the orbital glands. It has been assumed that the lacrimal gland was the source of these proteins because there is indirect evidence that the lacrimal gland can secrete protein in response to cholinergic stimulation. In the present study, the concentration of protein was determined in lacrimal gland fluid, i.e., the fluid collected directly from the main excretory duct of the lacrimal gland, uncontaminated by secretions from the other orbital glands. The concentration of protein in the lacrimal gland fluid was higher than that in plasma at lacrimal gland fluid flow rates between 2 and 10 μl/min, but it was the same as plasma at flow rates slower than 2 μl/min and faster than 10 μl/min.

The mean concentration of protein in unstimulated rabbit tears was found to be 12.6 μg/μl by Bouvinda et al.1 The protein concentration in stimulated rabbit tears is unknown, but Dohlman et al.2 found 8.1 μg/μl protein in stimulated tears and 22.7 μg/μl protein in unstimulated tears in normal human subjects. Any of the orbital glands could be the source of protein in tears. The evidence that the lacrimal gland can be the source of the protein in tears is as follows. The concentration of sialic acid (most likely derived from alpha globulins and/or mucoids) in lacrimal gland fluid, uncontaminated by secretions of other orbital glands, increases as lacrimal gland fluid flow increases in response to a cholinergic agonist, pilocarpine.3 Mucopolysaccharides have been identified in excised human lacrimal glands.4 Protein is released by exocytosis into the medium bathing isolated lobules, suspensions containing 95% secretory cells, and isolated fragments of rat lacrimal glands, exposed to a cholinergic agonist, carbamyl choline.5,6 Since this evidence is indirect, we thought it important to determine whether the in vivo lacrimal gland does, in fact, secrete protein, by determining the concentrations of protein in lacrimal gland fluid, uncontaminated by fluid from the other orbital glands, at various flow rates.

Materials and methods. The experiments were performed upon five male New Zealand white rabbits weighing 3.3 ± 0.1 kg (mean ± S.E.) because the lacrimal gland of this species has one functioning main excretory duct.7 Each rabbit was anesthetized with intravenous sodium pentobarbital (209 to 240 mg in divided doses), ventilated through a tracheostomy tube with a respirator (Harvard 607); and monitored by continuous recording of femoral artery pressure and Lead I EKG on an ink-writing polygraph (Grass 7B). Each sample of lacrimal gland fluid was collected in a preweighed (Mettler H20 balance) length (3 to 6 cm) of polyethylene tubing (P90), which had been slipped over the distal end of a tapered glass cannula (0.3 to 0.5 mm tip o.d.; 9 to 12 mm length) in the main excretory duct of the lacrimal gland. At the end of each collection period, the preweighed polyethylene tubing was removed, reweighed, sealed at both ends with parafilm, and stored at −15° C until analyzed for protein by the microanalytical spectrophotometric method of Lowry et al.8 as modified by Oyama and Eagle,9 with 1.0 mg of rabbit serum albumin (Miles Laboratories) in 1.0 ml of deionized water used as the standard protein. At the end of each experiment, a 3 ml sample of blood was removed from the femoral artery, heparinized, and centrifuged to obtain a 20 μl sample of plasma, which was stored at −15° C in a sealed length of polyethylene tubing until analyzed for protein. Protein analyses were made in duplicate on the lacrimal gland fluid samples, which had been diluted 1:40 with deionized water, and on the plasma samples, which had been diluted 1:2 with 1N NaOH and then 1:20 with deionized water. The volumes of the undiluted samples of lacrimal gland fluid were determined by dividing the sample weights by the density of an artificial lacrimal gland fluid and found to vary from 2 to 20 μl. Average lacrimal gland fluid flow rate was calculated by dividing the sample volume by the sample collecting time, which had been measured during collection with a rapid-reset, digital elapsed-time indicator (Technilab 11). Minimal flow rate was obtained by anesthetizing the cornea and conjunctiva with 50 μl of 0.5% proparacaine HCl, and various flow rates above minimal were obtained by injecting 1 to 40 μg of a cholinergic agonist, acetylcholine (ACH), into the lacrimal gland arterial supply, which had been isolated by ligating the ipsilateral internal carotid, external maxillary, lingual, superficial temporal and posterior occipital arteries.10

Results. The concentration of protein (μg/μl) in lacrimal gland fluid was 40.0 ± 5.3 (n = 4) at flow rates <2 μl/min; 68.4 ± 4.6 (n = 11) at flow rates from 2 to 3 μl/min; 76.9 ± 1.7 (n = 11) at flow rates from 4 to 6 μl/min; 77.5 ± 1.1 (n = 4) at flow rates from 7 to 9 μl/min; and 57.3 ± 6.7 at flow rates >10 μl/min (Fig. 1). In plasma the concentration was 53.1 ± 3.9 μg/μl (n = 5).

Discussion. The results of the present study provide direct evidence that the lacrimal gland does secrete protein in response to a cholinergic
Fig. 1. Concentration of protein in lacrimal gland fluid, uncontaminated by fluid from other orbital glands. Closed circles, Samples collected without stimulation with ACh; open circles, samples collected during flow stimulated with various doses of ACh injected into the arterial supply of the lacrimal gland. Each point is the average of two determinations on a single sample of lacrimal gland fluid. The dashed line is the concentration of protein in plasma.

agonist, a finding which is compatible with the fact that preformed protein is released into the medium bathing isolated fragments of rat lacrimal glands in the presence of carbonylcholine. ACh could release presynthesized protein in storage vesicles or increase the rate of synthesis and release of newly formed protein. Although there is no evidence, ACh could increase the permeability of vascular and secretory membranes so that there is increased movement of protein from plasma to fluid. The present study was not designed to provide an answer to the question of how ACh stimulates protein secretion in the lacrimal gland. However, in this study two facts suggest that ACh releases preformed protein. (1) The concentration of protein in lacrimal gland fluid was higher than in plasma at flow rates between 2 and 10 μl/min. (2) The collecting time for ACh-stimulated samples was less than 5 min regardless of dose. Such a mechanism would be consistent with the finding that there is exocytotic release of preformed protein in isolated single lobules and secretory cells exposed to cholinergic agonists. On the other hand, ACh may also increase the filtration of protein, since the concentration of protein in lacrimal gland fluid is the same as that in plasma at flow rates slower than 2 and faster than 10 μl/min.

It is to be noted that the concentration of protein in unstimulated lacrimal gland fluid is three times that which has been reported by Bonavida et al. in unstimulated rabbit tears. There are at least two possible explanations for this discrepancy. First, the low value in tears may be a false value, since Bonavida et al. also used the spectrophotometric method of Lowry et al. and, unless an additional clarification step had been performed, the opacity of the Harderian gland fluid in tears could have produced an underestimate of protein concentration. Second, since the rabbit lacrimal gland contributes only about 10% of the volume of unstimulated tears, if the lacrimal gland is the major source of protein in tears at minimal flow rates, the relatively protein-rich fluid from the lacrimal gland would be diluted by the greater volume of relatively protein-poor fluid from the other orbital glands. We believe that it is important to identify the proteins in lacrimal gland fluid and in fluid from the other orbital glands by analyzing uncontaminated samples, which have been collected from cannulae placed in the various excretory ducts.

We thank E. McAvoy and A. Kleinzeller for their help in setting up the method used to determine protein.
Key words: lacrimal gland, lacrimal gland fluid, protein, tears, cholinergic stimulation

REFERENCES