Sialic acid in rabbit lacrimal gland fluid.

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To date, there has been no attempt to determine which of the orbital glands contribute the sialic acid, which has been found in tears and tear mucoids. In the present study, sialic acid was found in the fluid collected directly from the lacrimal gland excretory duct, uncontaminated by the secretions of the other orbital glands (Nictitans, Harderian, conjunctival) as well as in the fluid secreted into the conjunctival sac by the other orbital glands, uncontaminated by lacrimal gland fluid. At all flow rates, the rate of secretion of sialic acid increased as flow rate increased in both fluids and the rate of secretion of sialic acid by the lacrimal gland was three times that by the other orbital glands. This is the first demonstration that a substance, which can be derived from either nonserum glycoproteins, such as the tear mucoids, or alpha-globulins, is a component of the secretions of the lacrimal gland, as well as of the secretion of the other orbital glands.

Sialic acid is a component of many conjugated proteins,1, 2 including glycoproteins found in the human lacrimal gland3 and in human tears.4, 5 Since the origin of sialic acid in tears is not known, the present study was undertaken to determine if the fluid collected directly from the excretory duct of the lacrimal gland, uncontaminated by secretions from the other orbital glands, contains sialic acid.

Material and methods. New Zealand white rabbits were used in this study because the rabbit orbital glands are generally considered to be analogs of human orbital glands, i.e., rabbit lacrimal-human lacrimal; rabbit nictitans-human accessory lacrimal (glands of Wolfring and Krause); rabbit Harderian-human tarsal; rabbit conjunctival-human conjunctival. In addition, it has been shown that the lacrimal gland in this species has only one functioning excretory duct.6 Adult male rabbits, weighing more than 3 kilograms were anesthetized with intravenous sodium pentobarbital (average initial dose, 30 mg. per kilogram), tracheotomized, and artificially resired by means of a Harvard pump. The right femoral vein was cannulated and used for subsequent anesthesia as required as well as for the administration of a cholinergic stimulator, pilocarpine HCI (0.4 mg. per kilogram), a relatively long-acting drug, which stimulates exocrine glands to secrete. The methods used to cannulate the lacrimal gland excretory duct and to collect samples have been published6 and are summarized as follows: ligatures were sutured in the upper and lower eyelids above and below the lateral canthus and gently weighted to evert the canthus to expose the orifice of the main excretory duct of the lacrimal gland, which can be seen as a slight dimple in the conjunctiva of the lower lid close to the lateral canthus. A piece of polyethylene (PE 10) tubing, either 3 cm. or 20 cm. long, was inserted into the orifice. After at least twenty minutes, during which the tubing was not disturbed to allow the tissue to seal tightly around the tubing, serial samples of lacrimal gland fluid were collected either by absorbing them onto preweighed pledgets of plastic sponge (Week-Cel, Edward Weck & Company, Inc.), which were forceps-held at the opening of the shorter cannula, or by allowing the fluid to fill the longer cannula. Volume of fluid was calculated either from the change in weight of the sponge or from knowledge of the inner diameter of and the length of fluid in the longer cannula. During the period that the lacrimal gland fluid was collected, the fluid, which was composed of the mixed secretions of the orbital glands, without the contribution of the lacrimal gland, accumulated in the ipsilateral conjunctival sac, from which it was either aspirated into a 1 ml. graduated syringe or absorbed onto a preweighed pledget of sponge. To calculate flow rates, the collection time was determined with a rapid reset digital, elapsed-time indicator, which was accurate to 0.1 second (Technilab 11). Within two hours after collection, all samples (in the longer cannulas, on the pledgets of sponge, and in the syringes) were placed in a freezer and kept frozen until assayed two weeks later. The thiobarbituric acid method of Warren7 as modified by Aminoff was used to determine sialic acid both before and after hydrolysis (with 0.1 N H2SO4 for one hour at 80° C.) of the samples, which had been defrosted at room temperature. The volume of the diluent (deionized water for the unhydrolyzed or H2SO4 for the hydrolyzed samples) was 30 to 100 times the sample volume (14 to 130 ml), a dilution which yielded absorbancies of 0.2 to 0.8 at 549 micra wave length on the Coleman-Hitachi 124 double-beam spectrophotometer. Assays were done of the diluents and of pledgets of nonsample-containing sponge, which had been treated in the same manner as the pledgets of sample-containing sponge.

Results. Results are given as mean values ± standard error. In each of two rabbits, the total...
gland fluid or evaporation of the fluid from the other orbital glands would have been greater and the difference between the two fluids would have been less than we found.

Discussion. The question, which we posed, has been answered since we have demonstrated that sialic acid is present in two components of tears, i.e., in the fluid secreted by the lacrimal gland, uncontaminated by fluid from the other orbital glands, as well as in the mixed fluid secreted by the other orbital glands, uncontaminated by lacrimal gland fluid. In fact, although the rate of secretion of sialic acid (μM/min.) increased in both tear components when the flow rate was increased with pilocarpine, at all flow rates it was two to three times greater in the lacrimal gland fluid than in the mixed fluid secreted by the other orbital glands. The secretogogue used in this study, pilocarpine, acts in the same manner as acetylcholine, the neural transmitter, which, when released from postganglionic efferent nerve terminals, stimulates secretory cells during reflex or psychogenic overflow tearing. Therefore, if sialic acid is derived from alpha-globulins, our findings indicate that the lacrimal gland would contribute more alpha-globulins to overflow tears than would the other orbital glands. On the other hand, if the sialic acid is derived from mucoids, the lacrimal gland would contribute more mucoids to overflow tears than would the other orbital glands. Because the sialic acid content of the fluids increased as flow-rate increased, the role of such mucoids may be to aid fluid flow by decreasing the surface tension of the ducts of the individual glands. Since it is generally considered that the lacrimal gland is the source of tear alpha-globulins and the conjunctival glands are the source of tear mucoids, it would be of great interest to determine the substance in the secretions of the individual glands, for which the bound and free sialic acids are derived.

Fig. 1. Concentration of total sialic acid in lacrimal gland fluid, uncontaminated by fluid from the other orbital glands (closed circles), and in mixed fluid from the other orbital glands, uncontaminated by fluid from the lacrimal gland (crosses). All samples were taken during unpredictable reflex stimulation. From these data, it is obvious that the amount of sialic acid secreted per minute increased as flow-rate increased. Thus, when the concentrations shown here are converted to content, the lacrimal gland secreted sialic acid at rates of 1.5 and 10.0 μM per minute when the fluid flow rates were 0.5 and 2.8 μl per minute, respectively; and the other orbital glands secreted sialic acid at rates of 0.3 and 2.0 μM per minute when the fluid flow rates were 0.5 and 3.0 μl per minute, respectively.

Key words: Lacrimal gland fluid, Orbital gland fluid, sialic acid, tears, mucoids, alpha-globulins, cholinergic stimulation, glycoproteins.

REFERENCES


Rabbit corneas were exposed to heterologous guinea pig anti-rabbit antibodies in the presence of complement and cytotoxic damage to the endothelium was demonstrated using para nitroblue tetrazolium. The possible role of such antibodies as "blocking antibodies" and as cytotoxic antibodies in the corneal graft rejection process are discussed.

The mechanism by which the donor corneal endothelial cell is destroyed by invading host lymphocytes has been demonstrated in rabbits, in humans, and the clinical presentation of the homograft rejection in humans in now well know. It is accepted that the host lymphocyte is primarily responsible for the destruction of the donor endothelial cell but the role of antibodies has not been determined.

Mansi, Ehrlich, and Polack1 bathed normal rabbit endothelium with heterologous duck antirabbit cornea serum and could not demonstrate a deleterious effect on normal endothelium, but they were able to demonstrate damage to regenerating endothelium.

Chandler, Gebhardt and Kaufman2 soaked whole rabbit corneas in heterologous antilymphocyte serum (ALS) and globulin (ALG), and succinylated antilymphocyte globulin (S-ALG), with and without complement. They were able to demonstrate that complement was necessary to produce cytologic damage to the endothelial cells and that antibodies that had the complement-fixing piece (Fc) chemically modified (S-ALG) were not cytotoxic in the presence of complement.

The purpose of this report is to demonstrate the presence of antibodies cytotoxic for rabbit corneal endothelial cells and to discuss the possible role of such antibodies in the corneal graft rejection process.

Materials and methods. Adult 2 to 3 kilogram New Zealand white rabbits were sacrificed by an overdose of intravenous pentobarbitol and the eyes were immediately enucleated. The cornea with a scleral rim was carefully removed and placed endothelial side up in a 10 c.c. beaker. The test reagents and tissue culture media were carefully injected down the side of each beaker to minimize trauma to the endothelium. The preparation of the guinea pig anti-rabbit lymphocyte serum has been described elsewhere.3

A total of 1.5 c.c. of fluid was necessary to completely cover each cornea. Twelve control corneas were separately incubated with either tissue culture medium (Minimum Eagles medium, Microbiological Associates), guinea pig complement (Microbiological Associates) undiluted and diluted, or antilymphocyte serum with a lymphocytotoxicity titer of 1:1024 (Table 1). In preliminary experiments we found the complement to be cytotoxic if it were diluted with normal saline or water, but that dilution with tissue culture fluid eliminated the cytotoxicity. Nine corneas were treated with equal volumes of undiluted complement and serum and two were treated with undiluted complement and ALS diluted 1:2 (Table 1).

The corneas were incubated for 30 minutes at 37°C C., the test fluids were aspirated and replaced with tissue culture fluid and incubated at 37°C C. for 30 minutes. Each cornea was then stained (paranitroblue tetrazolium),4 fixed in 10 per cent formalin for 24 hours, and mounted on glass slides as whole preparations.

Coded slides were evaluated by light microscopy. Staining involving more than 10 per cent of the cells was considered as evidence of cytotoxicity.

Table I. Effect of antilymphocyte serum and complement on corneal endothelial viability

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