Although loss of epithelium may be primarily responsible for the loss of the antigen, the fact that stroma also contains the antigen must be considered. We did find the protein in an extract prepared from corneas from which the epithelium had been removed. We might postulate that stromal antigen diffused into the medium after the epithelium was lost. We did not find the antigen in the medium we tested, but the amount present at any one time could have been too small to detect, even in concentrated medium.

The observation that cultured corneas appear to have taken up serum proteins from the medium is perhaps more important. CaR very closely resembled bovine serum in its electrophoretic pattern and seemed to contain a greater concentration of serum proteins than we found in CA. Precipitin lines were heavier and more numerous. CaR contained the rabbit serum used in the culture medium, and sections from representative corneas stained with FL-RG. If cultured corneas are to be used in experimental or clinical transplantation situations, it would seem advisable to determine the possible immunologic effect of the additional proteins present. It would also be advisable to determine whether corneas cultured in medium containing serum from the potential host species would show increased survival time. Recent evidence indicates that corneas cultured in medium containing rabbit serum had a slower rejection time than we found in CA.

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Key words: immunodiffusion, immunoelectrophoresis, corneal antigen, isoelectric focusing, fluorescent antibody, cultured corneas.

REFERENCES


Conjunctival goblet cell density in normal subjects and in dry eye syndromes.

ROBERT A. RALPH.

Serial sections prepared from biopsies of the deep tarsal portion of the inferior nasal conjunctival fornix in normal subjects and in patients with various dry eye syndromes were analyzed with respect to the goblet cell densities. When compared to normal subjects, individuals with keratitis sicca, Stevens-Johnson syndrome, ocular pemphigoid, and acute alkali burn all demonstrated progressively lower goblet cell densities per millimeter of epithelial surface. These disease entities can, therefore, be considered goblet cell-deficient syndromes.

The conjunctival goblet cell density may reflect the severity of local disease in the mucin-deficient dry eye syndromes. Diminution in number of goblet cells has been noted in ocular pemphigoid and avitaminosis A, while in keratoconjunctivitis sicca, Norrd1 has described an apparent increase in mucus content of the tear film. Average goblet cell counts in normal subjects were first cited in 1910 by Virchow,2 who reported 10 goblet cells per millimeter on specimens from the upper orbital and tarsal areas, and 15 goblet cells per millimeter from the lower orbital and tarsal regions. From flat preparations of the entire conjunctiva, Kissing3 determined that the highest goblet cell counts, excluding the semilunar fold, were along the lower nasal oblique meridian. Examination of ten preparations, advancing centrifugally from the cornea along this meridian, resulted in an increasing goblet cell count reaching a peak along the tarsal conjunctiva.
close to the fornix. Only the region of the semilunar fold had a count which exceeded that of the inferior nasal quadrant. When Kessing's average counts of flat preparations from the lower nasal conjunctiva are adjusted to correspond to six micron (cross) sections, the goblet cell density is approximately 10 per millimeter.

**Methods.** Conjunctival biopsies in the present series were obtained from the deep tarsal portion of the inferior nasal fornix following instillation of proparacaine 0.5 per cent, and application of cocaine 4 per cent. A three by four millimeter rectangle of tissue was spread, epithelial side up, on a small square of cardboard, and immersed in 95 per cent ethanol for fixation. Serial sections, six microns in width, were prepared after mounting the fixed specimen in a paraffin block. A periodic acid-Schiff (PAS) stain was then applied. Using an ocular micrometer, the number of complete goblet cells per millimeter of epithelial surface was counted. Fifteen separate sections were counted and averaged for each individual.

The normal subjects in this series consisted of seven individuals averaging 65.3 years of age, who were undergoing keratoplasty for corneal scarring or bullous keratopathy, and who had never demonstrated signs or symptoms of the dry eye syndromes.

Samples were obtained from patients in several of the dry eye categories. In each case, the distribution of males and females was approximately equal.

For statistical calculations, variances among the individual values in each group were substantially equalized, and the distributions were normalized by transformation to logarithmic functions. The resulting values were then submitted to a one-way analysis of variance from which were derived the statistical relationships.

**Results (Table 1).** The average goblet cell count in normal subjects was 8.84 per millimeter (Fig. 1). In the keratitis sicca group, the average was 2.11 per millimeter. Progressively smaller populations of goblet cells were noted in the Stevens-Johnson, ocular pemphigoid, and acute alkali...
Fig. 2. Conjunctiva in ocular pemphigoid. Epithelium is devoid of goblet cells in this specimen. PAS. ×50.

burn cases (Fig. 2). The average goblet cell count among normal subjects was significantly greater than for any of the disease groups ($p = 0.001$). The average of the keratitis sicca patients was significantly larger than that of the Stevens-Johnson group ($p = 0.05$) and that of the ocular pemphigoid patients ($p = 0.01$). The difference between the averages for the Stevens-Johnson and ocular pemphigoid patients was not significant. Since all values in the alkali burn cases were zero, the statistical analysis was not extended to this group.

Discussion. It is of interest to note the relative decrease in goblet cell density in keratitis sicca patients when compared with the normal subjects. Sicca patients for years have been thought to have an excess of mucin along with their decreased aqueous tear production. Clumps of PAS-positive material found in juxtaposition to the epithelial surface in these cases may represent mucin which failed to wash away as a result of reduced aqueous tear output.

In fresh alkali burns, no goblet cells were observed because the conjunctival epithelium was entirely absent.

Further investigations are necessary in order to evaluate fully the role of mucin deficiency in the dry eye syndromes. The present work suggests that there does exist a correlation between goblet cell density and the disease categories studied, enabling these entities to be classified roughly according to the degree of goblet cell deficiency.

Lack of a statistically significant difference between goblet cell averages for the Stevens-Johnson and ocular pemphigoid patients implies that goblet cell densities alone are not sufficient to differentiate these two groups from each other.

Keratitis sicca, Stevens-Johnson syndrome, ocular pemphigoid, and acute alkali burns of the eyes can be thought of as goblet cell-deficient syndromes. Whether they are in the absolute sense also “mucin-deficient” syndromes cannot be answered definitively by the present report, for sources of mucin other than the goblet cell may be operative. Thus, the need for continued research along these lines is recognized.

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Subsensitivity to pilocarpine in primate ciliary muscle following topical anticholinesterase treatment. PAUL L. KAUFMAN AND ERNST H. BÁRANY.

Accommodative responses to intramuscular pilocarpine were determined in four surgically aniridic vervet monkeys, before and after eight weeks of daily unilateral topical treatment with echothiophate iodide. The echothiophate-treated eyes maintained maximum myopia during the treatment course. However, after echothiophate treatment was stopped and the refraction had returned to baseline, a subsensitivity of the accommodative mechanism to pilocarpine became apparent. Normal sensitivity to pilocarpine did not return until four to five months after echothiophate treatment had been stopped.

Subacute or chronic systemic treatment with anticholinesterase (ChE) agents causes various non-ocular cholinergic end-organ systems to become subsensitive to direct acting cholinomimet-ics.1, 2 Topical anti-ChE treatment induces subsensitivity to cholinergics in the irides of several mammalian species.3, 4 We report here the occurrence of this phenomenon in primate ciliary muscle.

MATERIALS AND METHODS.

Animals. Four adult female vervet monkeys (Cercopithecus aethiops) weighing 2.5 to 3.5 kilograms had both irides totally removed to facilitate objective refraction (PLK, unpublished technique). The anterior chambers were free of cells and flare and the lenses were crystal clear on slit lamp examination at the start of the experiments two months postoperatively.

Refraction. The animals were anesthetized with intramuscular sodium methohexitol (Brietal, Lilly) 15 mg. per kilogram (refraction only) or methohexitol followed by intramuscular pentobarbital 30 to 35 mg. per kilogram (refraction under systemic pilocarpine). Refraction was performed with a Thorner refractometer.5 To improve the optics and expand the minus range of the refractometer, methylmethacrylate contact lenses of known minus power were placed on the corneas.

Pilocarpine testing. Deep intramuscular injections of pilocarpine-HCl solution were given in the thigh. Each eye was then refracted every four to eight minutes until a stable myopia was reached and began to fade. Maximum myopia was taken as the mean of at least two successive determinations on this plateau. Each animal received pilocarpine-HCl doses of 0.1, 0.2, 0.5, 1.0, 2.0, and 3.0 mg. per kilogram.6 At each session, the baseline refraction was determined, and one or two doses of pilocarpine were then administered. When two doses were given, the sequence was: 0.1 followed by 1.0, or 0.2 followed by 2.0, or 0.5 followed by 3.0. The second dose was not given until the effect of the first began to fade (generally 30 to 40 minutes separated the doses). Accordingly, the first dose was ignored, i.e., the second dose in the session, 0.2; 2.0, was considered to be 2.0 rather than 2.2 mg. per kilogram, etc. The pharynx was suctioned intermittently. At the end of a session, atropine sulfate 0.01 mg. (salt) per kilogram was injected intramuscularly. At least 48 hours separated the sessions, ensuring that the effect of the atropine had disappeared completely (PLK, unpublished observations). The system, consisting of experimenters, refractometer, animal, and drug gave reproducible results. For instance, monkey No. 1 was given intramuscular pilocarpine, 1.0 mg. per kilogram, three times before the start of topical treatment, and showed very similar responses in each eye on all three occasions (Fig. 1).

Anti-ChE treatment. Echothiophate iodide 0.25 per cent solution (PI), as commercially available eye drops (Phospholine iodide 0.25 per cent, Ayerst) was used. A control solution, identical except for the absence of echothiophate iodide was prepared (diluent). The monkeys were treated twice daily (approximately 9 a.m. and 9 p.m.) on weekdays and once daily on weekends. Never did more than 24 hours elapse between treatments. One eye of each monkey was treated with PI, the other eye with diluent. Separate droppers, each specially made to deliver only 4 µl per drop, were used for the two solutions. Two monkeys had PI applied to the right.