Selected proteins were quantitated after collecting samples of the tears by using two sampling techniques. Tears from the same individual were collected via adsorption by Schirmer filter paper strip from the unanesthetized, inferior, conjunctival sac and were compared with tears collected by a capillary tube (taking care not to touch the conjunctiva), after stimulation of tearing by irritation of the nasal mucosa with ammonia vapor. Tear samples were quantitated immunochromatically for two typical lacrimal proteins, lysozyme and lactoferrin, and three typical serum proteins, albumin, transferrin, and IgG. Tear analysis of all constituents were performed on a single sample of tears collected by each method from the same individual. Normal subjects without ocular pain or discomfort comprised a sample of 12 subjects ranging in age from 19 to 57 years and consisting of 9 men and 3 women. Concentrations of lysozyme and lactoferrin in samples collected by either method were not significantly different. In contrast, the concentration of albumin, IgG and transferrin collected by Schirmer filter paper technique was significantly higher ($P < 0.01$) than the concentration in tears collected by the capillary tube technique. A highly significant increase in serum proteins was seen when the Schirmer filter paper strip was used to collect tears compared to tears collected without mechanical stimulation of the conjunctiva. Invest Ophthalmol Vis Sci 25:374-377, 1984

The current availability of sensitive analytical techniques has stimulated interest in the identification, characterization, and quantitation of tear proteins. Accurate quantitation of proteins found in tear fluid is not only important in understanding the physiologic properties of tears, but also affords valuable diagnostic opportunities for the clinician. One of the basic requirements in this area of investigation is an appreciation of the effects of collection techniques on the concentration of tear constituents.

A number of investigators have measured the total protein concentration and specific components of human tears. Values obtained vary widely, indicating that a number of variables are operative in the release of substances into the tear fluid as well as their concentration. Van Haeringen noted in his review of the clinical biochemistry of tears that many contradictory results could be traced back to differences in specimen collection, and he pointed out an essential difference between tear fluid collected by capillary tubes and absorbent material: namely, filter papers and cellulose sponges are more likely to have mucus and epithelial cells sticking to the surface, thus affecting results. Josephson and Lockwood found that mild trauma to the conjunctival sac would produce measurable levels of serum albumin, gamma globulin, and transferrin, proteins not usually found in the nontraumatized conjunctival sac. McClellan and coworkers, in contrast, found no evidence of serum leakage when the eye was rubbed for 3 min, producing mild ocular trauma. Sapse et al found IgG in stimulated but not in unstimulated tears. Frey et al compared irritant-induced tears with emotional tears and noted an overall increased protein concentration in emotional tears but found no consistent differences in the serum proteins. Liotet et al found that inflammatory reaction induced an increase in the serum albumin and immunoglobulin fractions. Stuchell et al noted a difference in tear lactoferrin and lysozyme levels when tears were collected from the inferior fornix by two different methods, utilizing in one method a 2 x 6 mm filter paper strip (Periopaper®) placed for five seconds, and in the other method a 5 x 35 mm Schirmer filter paper strip placed for 3-5 min. Normal subjects were found to have an increased concentration of both proteins when reflex tearing was caused by the larger filter paper strip.

In view of the numerous observations on the effect of collection techniques on tear chemistry, a study was undertaken to document the quantitative effects on several tear proteins of two different standardized collection techniques in the same individual.

After stimulation of tearing by irritation of the nasal mucosa with ammonia vapor, selected proteins were quantitated in tear samples collected by adsorption with a Schirmer filter paper strip that irritates the unanesthetized conjunctiva and compared with tear samples collected by capillary tube (taking care not to touch the conjunctiva). Concentrations of two typical lacrimal proteins, lysozyme and lactoferrin, and three typical serum proteins, albumin, transferrin, and IgG, were determined. Tear analysis of the above constituents was performed on a single tear sample collected by each method in order to avoid multisampling errors.

Materials and Methods. Commercially prepared antisera to albumin, transferrin, and IgG, as well as standards for the serum proteins, were obtained from Calbiochem Behring Corporation (La Jolla, CA). Antisera to lactoferrin and lysozyme were obtained from Dako/Accurate Chemical of Long Island, New York. Human lactoferrin standard was obtained from Cappel Laboratories (Cochnavelle, PA). Lysozyme was a generous gift of Dr. Robert Caulfield of the Columbia University. Agarose low mw was obtained from Bio-Rad Corporation (Richmond, CA). Other chemicals were of reagent grade and obtained from Fisher Scientific Company.
The patient population consisted of 12 laboratory personnel who had not complained of ocular pain or discomfort and had no recent history of ocular disease. Informed consent was obtained according to guidelines of the Institutional Review Board. Contact lens wearers were not used as subjects. Subjects' ages ranged from 19 to 57 and consisted of 9 men and 3 women.

Tear collections were made randomly with respect to the two methods of tear collection at least 1 week between the two tear collection methods being compared in the same individual. One method used the standard Schirmer filter paper strip, which was inserted between the globe and the lower eyelid in the standard fashion. The filter paper strip was left in place until approximately one-half of the paper was wet. Volumes of tears were determined by means of a standard curve constructed with Schirmer tear test strips, a microliter syringe, and a 1% lysozyme solution as previously described or by differences in the weight of the Schirmer filter paper strip before and after wetting. The unwet portion of the paper was discarded, and the saturated portion of the filter paper strip was placed in a 2 ml microcentrifuge tube that was frozen and maintained at −80°C until the time of analysis. Extraction of tear proteins was performed by a standard method, and the concentrations were determined by standard kinetic assays.

**Fig. 1.** Serum proteins detected in tears collected by capillary tube with nasal stimulation (N) and by Schirmer filter paper strip (S).
fluid and analysis of the tear fluid eluate was done as previously described. Briefly, this was done by using 0.01 M TMED-0.029 M acetic acid buffer pH 5.0 to extract tears from the wet portion of the Schirmer filter paper strips. The amount of TMED-acetic buffer was calculated to provide a 1:10 ratio of tears to buffer, and then an aliquot was taken to give a 1:75 dilution. The concentration of albumin, transferrin, and IgG were determined at the 1:10 level of dilution or further diluted 1:75 when concentrations were too high for accurate quantitation. Lysozyme and lactoferrin were quantitated at the 1:75 level of dilution. The proteins, lactoferrin, albumin, transferrin, IgG and lysozyme were quantitated by previously described electroimmunochemical methods. The second method of tear collection was by nasal mucosal stimulation. Ten milliliters (10 ml) of 30% ammonium hydroxide was placed in a 50-ml Erlenmeyer flask. The flask was fitted with a two-hole stopper. A flexible plastic tube was inserted into one hole, then the subject would place the tube into either the right or left nostril and gently inhale the vapors, avoiding direct irritation of the eye. Subjects did not report eye irritation, and leakage of fumes into the eyes was minimized by gently compressing the nostrils around the nasal delivery tube. The reflex tears produced were collected by capillary action using a 25- or 50-μl capillary pipette as the secretions became visible along the lower lid. The secretions were collected carefully, taking care not to touch the conjunctiva. The capillary tubes were emptied of their contents by gently blowing the secretions into a 2-ml minicentrifuge tube and then stored at -80°C until the time of analysis. The samples were diluted and analyzed in a similar manner as the Schirmer filter paper strips. The results were reported in mg/dl, and statistical treatment of the data was by Student's paired t-test.

Results. By appropriate dilution, we were able to perform five separate protein analyses on each tear sample obtained. The proteins examined were grouped as those found predominantly in serum—albumin, transferrin and IgG (Fig. 1), or as those found predominantly in lacrimal gland secretions—lactoferrin and lysozyme (See Fig. 2).

When the concentrations of lysozyme and lactoferrin in the extracts from the Schirmer filter paper strip were compared with the concentration of tear proteins with the capillary tube technique, there were no statistical differences, 220 ± 79 mg/dl (range 79-328 mg/dl) and 213 ± 48 mg/dl (range 94-281 mg/dl), respectively, for lysozyme and 310 ± 145 mg/dl (range 125-589 mg/dl); and 340 ± 97 mg/dl (range 120-503 mg/dl) for lactoferrin (See Fig. 2). In contrast, the concentration of albumin after elution from the Schirmer filter paper test strip was significantly higher (P < 0.01) than the concentration of albumin collected by the capillary tube technique, 124 ± 142 mg/dl Schirmer (range 0-450 mg/dl) and 1.8 ± 6.0 mg/dl (range 0-29 mg/dl). Transferrin values for Schirmer filter paper test strip samples were also significantly higher (P < 0.01) than those collected by the capillary tube method, 4.5 ± 7.3 mg/dl (range 1-21 mg/dl) versus values below

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**Fig. 2.** Tear proteins collected by capillary tube with nasal stimulation (N) and by Schirmer filter paper strip (S).
detection limit of 1 mg/dl respectively. The serum protein IgG showed the same pattern as albumin and transferrin, a significantly higher ($P < 0.01$) concentration of IgG found with the Schirmer filter paper strip tear sample compared with the values found in the capillary tube obtained tears, $23.6 \pm 41.1$ mg/dl (range 0–152 mg/dl) versus values below the limit of 1 mg/dl.

**Discussion.** Much of the current interest in tears lies with identifying the proteins, electrolytes, lipids, and other components that, together, form the tear film. Understanding the nature of the tear film can lead to more meaningful analysis of tears in normal subjects, patients with various diseases, and those wearing contact lenses. Although there are a large number of proteins associated with the tear film, some of these proteins may not be indigenous to the tear film, but only are introduced as a result of mechanical or chemical irritation, permitting transudation into the tears. The purpose of this research was to study two methods of collection, one that is known to cause reflex tearing by direct irritation and another method that causes irritation of the nasal mucosa producing reflex response by the lacrimal apparatus with minimal local irritation. Our results support the findings that a marked and significant increase in serum proteins is seen when the Schirmer filter paper strip is used to collect the tears, compared with the results seen with reflex tearing without local irritation. In contrast, no significant differences were noted between the levels of the lacrimal proteins, lactoferrin, and lysozyme with the two collection methods.

Although significant differences were noted in mean values of serum proteins between the two collection methods, the range of serum protein values in tears was quite large. Some patients had a rather low but still significant increase of serum proteins in the tears, while some patients had a very high level of serum proteins that resulted in a rather large variation amongst the subjects tested. These subjects had no complaint of eye disease nor any obvious signs of eye disease. The integrity of the ocular surface may be markedly different due to genetic makeup or subclinical changes that are considered normal variation.

Stuchell et al$^8$ collected tears from the surface of the eye using different size filter paper strips and different collecting times: they found an increased concentration of lysozyme and lactoferrin in reflex tears compared to basal tears, probably reflecting the increase in flow rate. The present study demonstrates that varying compositions of tear fluid are produced at reflex tear flow rates by tear collection techniques using filter paper strips and most likely similar methods utilizing sponges. Both techniques touch the ocular surface. It is clear that the tear film is affected not only by tear flow rates and volume,$^8$ but also by factors stimulating the ocular surface. Mechanical stimulation should be avoided if one is to study the nature of the tear film with minimal contamination due to serum leakage. We do not know the contributions of the serum proteins to the protection of the ocular surface, nor do we know the protein-protein interaction between the lacrimal gland secretions with that of the serum.

**Key words:** human tears, collection methods, albumin, lysozyme, lactoferrin, reflex tears, IgG, transferrin


**References**