Determination of tear volume and tear flow

S. Mishima,* A. Gasset, S. D. Klyce, Jr., and J. L. Baum,**

The dynamics of tear flow were studied for human subjects using fluorescein as an indicator. A new fluorophotometer attachable to a slit lamp was designed to determine fluorescein concentration of tears in situ. After the instillation of about 1 μl of fluorescein solution (1.0 Gm. L⁻¹) into the cul-de-sac, its concentration in the tears was found to decay in a single exponential pattern. In most cases, the turnover rate was initially fast and became slower after about five minutes. The initial faster turnover rate was interpreted as the result of stimulation of lacrimation due to the application of solution, and the subsequent slower decay interpreted as the physiologic turnover. The initial turnover showed individual variation and was lower in older than in younger persons. The physiologic turnover rate was fairly constant among normal subjects, the average being about 16 per cent min⁻¹. The tear volume in the cul-de-sac was measured with two methods. The dilution method consisted of instilling 16.2 μl of fluorescein solution (0.10 Gm. L⁻¹) sampling after blinking and determining the dilution ratio. This method was found to be subject to large errors due to lacrimation. The second method involved the construction of a semilog plot of concentration decay after the application of a known amount (about 1 μl) of fluorescein solution (1.0 Gm. L⁻¹), the extrapolation of the decay curve to zero time, and the computation of the tear volume from the zero time concentration. The latter method gave very consistent results. The average tear volume obtained was 7.0 ± 2.0 μl, with no significant difference between age groups, sexes, and fellow eyes. This value agreed well with the probable tear volume calculated from anatomical considerations. The combination of the tear volume and turnover rate determinations gave an average tear flow of 1.2 μl min⁻¹ with a range of 0.5 to 2.2 μl min⁻¹. The tear volume was found to increase with increasing tear flow. Since the tear volume obtained with the zero time method corresponds to an initial faster tear flow, the normal tear volume with a normal tear flow was estimated from the volume-flow relationship; the average normal tear volume was 6.2 ± 2.0 μl.

The rate of human tear flow under normal conditions is an elusive quantity to measure. Irritation to the eye and psychologic stimuli can cause rapid fluctuations in the normal tear flow. Schirmer¹ first determined

---

From the Corneal Research Unit, Institute of Biological and Medical Science, Retina Foundation, Boston, Mass.

This investigation was supported in part by Grant-in-aid No. C 276 of the National Council to Combat Blindness, Inc., New York, and in part by Public Health Service Research Grant B 02229 from the National Institute of Neurological Diseases and Blindness, United States Public Health Service.


**Special Fellowship No. F11 NB 1309 from the National Institute of Neurological Diseases and Blindness, United States Public Health Service. Present address: Department of Ophthalmology, New York University, New York.
this quantity in patients with their lacrimal sacs extirpated, measuring the time when tears first overflowed the cul-de-sac. After considering the amount of evaporation during this time, he estimated the amount of tear secretion to be 0.5 to 0.75 Gm. in 16 waking hours. This value corresponds to 0.6 to 0.8 µl min. Schirmer also established a test for tear flow determination using a strip of filter paper. This test, which now bears his name, has been modified by many authors. Because of its simplicity, it is an excellent diagnostic tool for detecting reduced lacrimation. However, it does not provide an estimation of normal tear flow, since the insertion of filter paper into the cul-de-sac stimulates reflex lacrimation.

Another way of estimating tear flow has been to observe the disappearance of dyes instilled into the cul-de-sac. Improving this technique, Nover and Jaeger used fluorescein as an indicator. The technique was also used by Kirschner, who reported values for normal tear flow close to those of Schirmer. However, this method was not capable of showing dynamic changes in the rate of tear flow since the fluorescein concentration was not determined in situ. Moreover, the quantity of fluorescein solution used was so large that the method may have produced irritation. By following the concentration decay of fluorescein in the tears, the turnover rate of the indicator could be evaluated. To calculate this turnover rate, the authors assumed that the change of concentration follows an exponential decay with a single turnover rate for the experimental period—an assumption never proved. In order to translate the turnover rate into actual volume of tear secretion, the volume of tears in the cul-de-sac at a given time must be known. Since no previous experiments had given this value, the total tear volume had been assumed to be about 20 µl.

Zintz and Schilling seem to be the first investigators who attempted the determination of the tear volume in the cul-de-sac. They instilled a known amount of Congo red solution and took a sample of the mixture after the subject blinked. By determining the ratio of dilution, the tear volume was computed.

The present investigation deals with an improved technique to determine both tear volume and turnover rate on an individual in a brief period. The principle involves the determination of the dilution of fluorescein by the tears and the rate of its concentration decay. Fluorescein concentration in the tears was determined in situ with a newly designed fluorophotometer which allows the study of dynamic changes in tear flow.

The fluorophotometers

1. **Objective fluorophotometer.**
   a. **Construction.** An objective fluorophotometer was constructed according to the principle described by Maurice. The apparatus consists of a slit lamp equipped with a mercury lamp (Toshiba SHL 100 UV, Tokyo) and a filter (Kodak Wratten No. 34 violet), and a photometric microscope. A mirror with a transparent window was placed at the first image plane of the microscope. Light from the fluorescent solution passed from this window through a filter (Kodak Wratten No. 61 green) to the photomultiplier (Toshiba 7306, Tokyo). This combination of filters suppresses scattered light from ocular tissues, allowing only fluorescent light to reach the photomultiplier. The photomultiplier signal was amplified and detected with a synchronized detector system. The final output was read with a microammeter placed within the microscope.

   The fluorophotometer gave a linear response to fluorescein concentrations between 100 and 0.02 mg. L.

   b. **Preparation.** A darkroom was used for fluorescein concentration determinations. A standard fluorescein solution (concentration 1 mg. L. ) was prepared. A reading for this solution was obtained before and after each determination of the tear fluorescence to correct for any change in the sensitivity of the apparatus.

Downloaded from iovs.arvojournals.org on 03/19/2019
A \[ L_1 \] and \[ L_2 \], a Huygens type eyepiece; \[ F \), two rectangular prisms with a small round mirror in the interface; \[ D \), diffusing plate; \[ S \), iris diaphragm; \[ F \), and \[ F_t \), Kodak filters, Wratten No. 61 green; \[ L_s \), the lens to focus the pinhole \((S)\) at the pupil of the observer’s eye.

**2. Subjective fluorophotometer.**

*a. Construction.* The principle of this apparatus is based upon the subjective matching of the brightness of fluorescein in the tears with that of a small standard field. A diagram of this fluorophotometer appears in Fig. 1. Two right-angle prisms were cemented together along their hypotenuse and placed in a Huygens type eyepiece (magnification: \( \times10 \)). A small circular area (0.6 mm in diameter) was silvered at the interface of the prisms and was illuminated from the side to provide the standard field for brightness comparison. The size of this field was chosen in order to approximate the apparent size of the cross section of marginal tear strip observed with the microscope. The side illumination consisted of a small lamp, a diffusing plate, an iris diaphragm, and filters (Kodak, Wratten No. 96, neutral density, and No. 61 green). A micrometer was attached to the iris diaphragm to control the light intensity reaching the small silver mirror. A correctly placed lens focused the iris diaphragm pinhole at the pupil of the observer. The instrument was attached to a Haag Streit slit lamp (Model 360) in place of an eyepiece, as shown in Fig. 2.

*b. Calibration of the subjective fluorophotometer.* The light source of the slit lamp and that of the lamp in the fluorophotometer came from the same power supply, the voltage being regulated to \( 5 \pm 0.1 \) volts. A filter (Kodak, Wratten No. 34 violet) was placed before the slit lamp and another filter (Kodak, Wratten No. 61 green) before the eyepiece (Fig. 1). This combination eliminated violet scattered light from the ocular tissues.

Prior to subjective matching of fluorescence to standard field, the observer’s eye was dark adapted for 30 minutes. The subsequent procedure was carried out in a darkroom.

A small chamber was constructed of a plexiglass plate and a rod to simulate the shape and size of the marginal tear strip. The chamber was filled with fluorescein solution of known concentration (pH 7.2), and was viewed with the slit lamp and the fluorophotometer using an observation-illumination angle of 60 degrees. The cross-sectional image of the fluorescein solution was placed alongside the standard comparison field, and the brightness of this field changed until the boundary between the field and the solution disappeared. Brightness matching was done with different slit widths of the slit lamp, the results being plotted in Fig. 3. To minimize error...
in transferring these results to the computation of fluorescein concentration in the tears, the calibration was made using that part of the chamber which approximated the cross-sectional area of the marginal tear strip. If the cross-sectional area was varied during calibration, the error was estimated to be within 10 per cent of the concentration measured. The optimum range for the fluorescein concentration was between 200 and 5 mg L⁻¹.

c. Preparation for tear fluorescence determination. The right eye of the observer was dark adapted for 30 minutes prior to the subsequent procedures. A fluorescein solution (concentration 10 mg L⁻¹) was injected into the calibration chamber described above, and the brightness was matched by adjusting the slit width with the micrometer set at 7.50 (see Fig. 3). Then a series of readings was taken with the micrometer to determine which calibration curve was to be used. For tear fluorescence determination, the procedure described for calibration was used.

Determination of the turnover rate of the tears

The turnover rate of fluorescein in the tears was determined for healthy male and female subjects ranging in age from 20 to 89 years.

1. Procedure. The subjects were instructed beforehand in how the determination was to be done and were asked to behave naturally. Approximately 1 μL of fluorescein solution was instilled on the upper lateral bulbar conjunctiva with a PE 10 polyethylene tube (Intramedic, Clay-Adams, Inc., New York) attached to a micrometer syringe. The concentration of fluorescein used was 1.0 Gm L⁻¹ when not otherwise stated. Immediately following instillation, the subjects were asked to blink quickly several times and to roll their eyes to induce good mixing of the solution with the tears. The fluorescein concentration in the lower marginal tear strip was then determined at intervals. When the objective fluorophotometer was used, it was necessary that its transparent window have about five times the cross-sectional area of the tear. This reduced error caused by the subject's eye movements, allowing the observer to maintain the tear image inside the window to take a reading. Therefore, it was necessary to assume that the cross-sectional area of the marginal strip remained fairly constant during the course of the experiment. This assumption was not necessary when using the subjective fluorophotometer.

2. Results and comments. Changes in the fluorescence of the marginal tear strip after instillation of fluorescein solution are plotted in Figs. 4, 5, and 6. In nearly all cases, the decay of fluorescence could be interpreted in terms of a single exponential. Let the initial concentration be C₀; then concentration at time (t) is given by C = C₀ exp (-kt), k being the turnover rate. It is expressed in this paper in per cent per unit time (minute). The turnover

![CALIBRATION OF FLUOROMETER](image)

Fig. 3. Calibration of the subjective fluorophotometer with known fluorescein concentrations (grams per milliliter) and different slit widths of the slit lamp. The slit width becomes narrower from left to right curves.
rate can easily be obtained from a semilog plot of concentration versus time. Construction of a nomogram facilitated the calculation. In some cases, the decay could be expressed by a single turnover rate which remained constant past the sensitivity of the technique (Fig. 4). This occurred most often when the subject claimed irritation at the time of instillation, producing a very high turnover rate. In the majority of cases, however, the initial rapid decay was followed by a slower decay after 4 to 5 minutes (Fig. 5). Such a decreased decay was marked when the subject claimed least irritation due to instillation. One individual will show one, two, or more changes in rate of turnover depending upon the amount of irritation.

The change in concentration decay, therefore, was not interpreted as an initial imperfect mixing of the fluorescein solution with the tears. Apparently, instillation of solution as small as 1 μl caused some stimuli for lacrimation, resulting in faster initial decay, which became normal with time. It is interesting to note that the turnover rate could change from 10 per cent per minute to almost 80 per cent per minute without being noticed by the subject. Such variation in turnover rate did not cause overflow of tears from the cul-de-sac. Reaction to the instillation seemed to be fairly constant in
the same individual at different times, but
the difference among individuals was very
large.

The tear flow was found to increase sud-
ddenly due to a variety of subtle stimuli
(Fig. 6). These included coughing, sneez-
ing, wind, and many other factors, all of
which produced a more rapid decay in a
matter of a few seconds. It was also found
that the more intense the stimulus, the
more rapid a decay was encountered.

3. Physiologic turnover rate. In view of
the above findings, a standard method to
determine the physiologic turnover rate of
the tears was chosen. About 1 μl of fluo-
rescein solution (concentration 10 Gm. L.−1)
was instilled on the upper lateral bulbar
conjunctiva, and the subject was asked to
behave naturally. After 5 minutes, the
determination of fluorescein concentration
was started with the subjective fluoropho-
tometer. Two or three successive measure-
ments were taken to avoid error in match-
ing, and such measurements were done at
intervals of about 1 or 2 minutes.

The turnover rate determined 5 minutes
after instillation for a normal indoor en-
vironment showed a fairly narrow range of
individual variation among the normal sub-
jects, the average being about 16 per cent
min.−1 (Table I). Between the age groups
20 to 45 and 50 to 89 years no significant
difference was found, the averages being
16 and 18 per cent min.−1 respectively. Both
the objective and subjective fluorophotom-
eters gave practically the same results
(Table I).

**Tear volume determination**

1. **Principle.** When a known amount
(m) (in microliters) of fluorescein solution
with known concentration (C₀) is in-
stilled into the cul-de-sac and is well mixed
with the tears of a volume, V (in micro-
liters), producing a concentration (C), the
tear volume is given by

\[ V = m \left( \frac{C₀}{C} - 1 \right) \]

This equation is valid only when the con-
centration of the tears (C) can be deter-
mined initially without losing the instilled
fluorescein and at the same time insuring
good mixing. The above condition consti-
tutes a problem in the determination of
tear volume, since the tears are constantly
secreted and drained fairly rapidly.

2. **Procedures.**

a. **Dilution method.** The technique used
was in principle similar to that of Zintz
and Schilling. A known amount of fluo-
rescein solution (100 mg. L.−1) was instilled
into the upper lateral part of the cul-de-sac.
The amount of fluorescein instilled was
made constant by drawing up 12.0 mm. of
solution into a PE 20 polyethylene tubing
(Intramedic) with the aid of a micrometer
syringe. The volume was calculated to be
16.2 ± 0.1 μl by weighing the volumes of
mercury occupying known lengths of tub-
ing. This volume was chosen as a compro-
mise to minimize stimulation and to facili-
tate subsequent sampling.

After having the subject blink about ten
times and move his eye, a sample of tear-
solution mixture (about 1 μl) was taken
from the lower fornix with a PE 10 poly-

**Table I. Turnover rate**

<table>
<thead>
<tr>
<th>Age</th>
<th>Objective fluorophotometer</th>
<th>Subjective fluorophotometer</th>
<th>Physiologic fluorophotometer</th>
<th>Subjective fluorophotometer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Turnover rate (% min.−1)</td>
<td>No. persons (de-term.)</td>
<td>Turnover rate (% min.−1)</td>
<td>No. persons (de-term.)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>20-45</td>
<td>41</td>
<td>15-68</td>
<td>9</td>
<td>(10)</td>
</tr>
<tr>
<td>50-89</td>
<td>38</td>
<td>12-72</td>
<td>26</td>
<td>(25)</td>
</tr>
</tbody>
</table>

Downloaded from iovs.arvojournals.org on 03/19/2019
ethylene tube (Intramedic). The sample was then introduced into a capillary space made by separating two cover glasses at a distance of about 0.1 mm. The fluorescence of the sample was then determined with the objective fluorophotometer. The volume necessary for the determination was approximately 0.03 μl, and therefore determination of the sample could be done quickly and easily without influence of evaporation. The fluorescence of the original solution was likewise determined, and the ratio of dilution calculated. The time between instillation and sampling was about 15 seconds.

b. Zero time method. About 1 μl of fluorescein solution with concentration of 1.0 Gm. L⁻¹ was instilled as described before. The amount of fluorescein solution was determined each time by measuring the length of solution in PE 10 polyethylene tubing (Intramedic), the volume being calibrated to be 0.81 ± 0.01 μl per 10 mm. by weighing mercury. Immediately after instillation and blinking, the concentration of fluorescein in the lower marginal tear strip was determined at intervals with the subjective fluorophotometer. In almost all cases, the decay of concentration in the initial 5 minute period followed a single exponential pattern (Figs. 4 and 5). The semilog plot of concentration against time was extrapolated to zero time. Since this concentration decay was shown not to depend upon mixing in this technique, the zero time intersect was interpreted to be the tear concentration under ideal conditions, i.e., complete mixing and no loss of fluorescein from the tears. From the zero time concentration, the ratio of dilution and thus the tear volume may be calculated.

3. Results.

a. The dilution method. The tear volume was determined for both men and women aged 20 to 45 years. The determination was repeated on different days for each individual. The average of the determinations for 10 men and 3 women was 16 μl, with a wide range from 5.3 to 66 μl. Values obtained for the same individual also varied considerably—as much as from 9 to 66 μl. As has been pointed out above, irritation causes a tremendous increase in tear secretion in a few seconds; the scattering in the data was obviously due to stimulation of lacrimation. Irritation due to sampling was especially significant, and the technique of sampling quite difficult to control. Since the instillation of even 1 μl causes considerable increase in tear flow, the amount of solution used here was considered too large to be free of vigorous stimulation.

b. Zero time method. Tear volume was calculated with the zero time method for 16 men and 21 women aged between 20 and 89 years. The average tear volume for all subjects was 7.0 ± 2.0 μl (standard deviation), with a range of 4.0 to 13 μl, which is very narrow compared with the former method. Since a time course of decay was used in this technique, any increase of tear flow theoretically does not affect the computation of zero time concentration. In practice, however, an accurate determination was very difficult when the initial concentration decay was over 100 per cent min⁻¹ and the result had to be discarded. An error might also be brought about in the fitting of a straight line to the semilog plot of the data (Figs. 4 and 5) and the consequent extrapolation to zero time. The probable maximum error in fitting a line was calculated for 17 determinations, and the standard deviation was found to be ± 1.9 μl, with a maximum range of ± 3.3 μl. Careful instillation of a very small amount of fluorescein solution rarely resulted in more than a 100 per cent min⁻¹ initial decay, thus favoring this technique.

Average tear volumes for age and sex groups are shown in Table II. The difference in the tear volume between right and left eyes for ten individuals is also shown in Table II, in terms of the variance of difference. In all three categories, no significant difference was found.

4. Evaluation of the methods: Probable tear volume, based on anatomical considerations. Considering the above findings, the zero time method was deemed the best
Table II. Tear volume (µl)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Average (S. D.)</th>
<th>No. persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6.7 ± 2.0</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>7.1 ± 2.2</td>
<td>21</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-45</td>
<td>7.4 ± 1.3</td>
<td>16</td>
</tr>
<tr>
<td>50-89</td>
<td>6.6 ± 2.3</td>
<td>21</td>
</tr>
<tr>
<td>Eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>7.7 ± 1.6*</td>
<td>10</td>
</tr>
<tr>
<td>Left</td>
<td>7.9</td>
<td></td>
</tr>
</tbody>
</table>

*[(right - left) / Number of cases] %

Technique for determining the tear volume. It should be stressed, however, that a small scattering of the data in this method does not guarantee that it gives the tear volume accurately. One may be sure from observation that a short time after instillation the fluorescein is well mixed with the tears along the marginal tear strips; however, one cannot be certain that the fluorescein is well mixed with the tears under the palpebral conjunctiva and in the fornices. Non-uniform mixing produces unpredictable errors—a most undesirable situation in any quantitative determination. Attempts to mix the fluorescein with the tears by pulling the lids and by vigorous blinking after instillation produced excessive tearing, which made the determination of tear volume impossible. In order to evaluate the experimentally determined quantity, the probable tear volume was estimated from anatomical considerations.

a. Volume of tear film in the normal palpebral fissure. Since the thickness of the tear film was previously measured to be 6.5 µm, the volume of tear film in the open palpebral fissure may be calculated from the area. Thirty eyes of normal men and women were photographed with a single lens reflex camera with the proper extension tubes to produce a x 1 magnification. From 1 by 1 prints the area of the palpebral fissure and the area of the lacrimal lake were cut out separately. A standard area of 200 mm. of graph paper was likewise photographed, printed, and cut out. The weighing of the cutout pieces and comparison with the weight of the standard field gave the apparent area of the lacrimal lake and the rest of the palpebral fissure. The palpebral fissure was assumed to have a single horizontal curvature, the curvature in the vertical plane being neglected. The radius of curvature was calculated to be an average of 15 mm. by separately measuring the distances between the inner and outer canthi and corneal apex. Along the horizontal meridian, the reduction in the actual area to the apparent area in the photograph was calculated for every vertical segment 1 mm. wide and integrated throughout the length of the horizontal meridian. The area of the fissure plus the area of the lacrimal lake was calculated, giving an average value of 172 ± 32 mm. The total volume occupied by the tears in this area is therefore 1.1 ± 0.2 µl.

b. Volume of tears in the marginal tear strips. The cross-sectional area of the marginal tear strip was estimated. About 1 µl of fluorescein solution (1 per cent) was instilled into the tear strip, and this was observed with a slit lamp at specular reflection using an illumination-observation angle of 60 degrees. The apparent size of the image was compared with a transparent square grating in the eyepiece, and its size and shape reproduced on graph paper. The actual depth of the tear strip was calculated, using an index of refraction of 1.33 for the tears. This distance was also estimated by measuring the distance between the posterior edge of orifices of the Meibomian glands and the posterior edge of the lid. The cross-sectional area of the marginal tear strip was calculated to be approximately 0.05 mm. The length of the lid margins was measured using 1 by 1 photographs and was found to be 57 mm. on the average. The tear volume in the marginal tear strip was then estimated to be 2.9 µl on the average.

c. Tear volume under the palpebral conjunctiva and the fornices. This quantity was the most difficult to estimate. To determine
the state of tears in this area, i.e., the existence of reservoirs and the thickness of the fluid layer, a small mirror was inserted in the anterior chamber of colored rabbits. Using a slit lamp and the combination of filters mentioned before, the surface of the palpebral conjunctiva was observed from behind with the presence of fluorescein solution (10 per cent) in the cul-de-sac. The tears were observed to curve around the lid margin from the marginal strip and then to form a layer of uniform thickness between the bulbar and the palpebral conjunctiva. No large tear reservoirs were observed, and the tear layer seemed to have a thickness close to that of the precorneal tear film, although one cannot be certain of its exact dimension. It was therefore assumed that this tear layer was about 6.5 μ thick. The total area of the conjunctiva covered with this tear film was calculated on the assumption that the fornix extends to the equator of the eyeball, being a sphere of radius 12 mm. The tear volume of this whole area was then calculated to be 5.6 μl. Subtracting the volume in the open area, a value of 4.5 μl was obtained for the tear volume under the palpebral conjunctiva and the fornices.

d. Probable normal tear volume. Addition of the estimated tear volumes above gives a total of 8.5 μl, the open portion of the cul-de-sac having 4 μl. Therefore, experimental values would be expected to be 4 μl, if there were no mixing of fluorescein with the tears under the palpebral conjunctiva and fornices. If there were complete mixing in this area, one would expect to obtain an experimental value close to 8.5 μl. Most of the results of the zero time method in fact did fall between these two values, giving an average of 7.0 ± 2.0 μl.

The above calculation of the probable tear volume involved some uncertainties. It is also very probable that the tears in the deep fornices escaped good mixing with fluorescein. However, the good agreement of the values shown above seem to indicate that the zero time method gives a reasonable estimate of the tear volume.

5. Relation between tear volume and tear flow. It is of interest to determine if the tear volume changes with variation in the rate of tear secretion. A plot was made (Fig. 7) using a logarithmic scale both for tear volume and the initial rate of secretion (initial reaction for instillation of fluorescein solution). A correlation analysis revealed a positive correlation between the tear volume and the rate of secretion with a confidence level of over 95 per cent. Thus one may say that the tear volume increases slightly with an increase of tear secretion.

Discussion

The zero time method of volume determination interferes very little with normal tear dynamics, and therefore can be applied before the determination of turnover rate with a brief interim. Thus, the combination of methods offers a reasonable estimation of the amount of tear secretion. The average normal secretion was calculated to be 1.2 μl min. Considering individual variation in turnover rate, one may say that this value lies approximately between 0.5 and 2.2 μl. Schirmer and Kirschner assumed many factors in their computation of normal tear secretion; nevertheless, their results seem to agree with the present values. Kirschner reported that the normal tear flow decreased with increasing age. It is probable that his results were an average of complex changes in tear flow and involved inaccuracies in the determination of fluorescein concentration. Therefore, it was felt that this relationship must be reinvestigated. However, the number of determinations among age groups of the present investigation was not large enough to allow any definite conclusion to be drawn.

The average value of tear volume obtained by the zero time method does not strictly represent the normal tear volume with normal tear flow. Since instillation of fluorescein was shown to increase tear flow

*The correlation analysis was made for logarithmic conversion of values of the tear volume and the initial turnover rate. The turnover rates were then converted to rate of tear flow (in microliters per minute) in Fig. 7. Logarithmic values of tear volume showed a normal distribution.
Tear volume and tear flow

initially, and since an increase in tear flow results in a slight increase in tear volume, the average normal tear volume would be less than the experimental value. Normal tear flow was determined after the initial reaction subsided. The probable normal tear volume with the normal tear flow of 1.2 \( \mu l \) min.\(^{-1}\) may be obtained from Fig. 7, the average being estimated to be 6.2 ± 2.0 \( \mu l \) as compared with the experimental value of 7.0 ± 2.0 \( \mu l \).

Zintz and Schilling\(^{13}\) used a method similar to the dilution method of the present study and reported large tear volumes (30 to 60 \( \mu l \)), especially in young persons. Such large tear volumes were frequently obtained in young persons in the present study using the dilution method. However, the zero time method never gave values of more than 15 \( \mu l \) with a reasonable initial decay. Since it has been shown that the instillation of only 1 \( \mu l \) of solution caused a considerable increase in the initial tear flow, one must expect that this will cause a serious error in tear volume determination with the dilution method. A calibrated amount of saline solution ranging from 10 to 28 \( \mu l \) was instilled into the cul-de-sac to visualize the state of the tears at different known volumes. When 10 \( \mu l \) of normal saline was added to the normal tear volume, the eye appeared quite watery, as indicated by a noticeable increase in the volume of the marginal strips. When 28 \( \mu l \) of normal saline was added, a considerable amount overflowed at the lacrimal lake. Most of the increased tears were drained in a few seconds by blinking. These findings indicate that the cul-de-sac cannot hold an additional amount of more than about 25 \( \mu l \) unless the solution is added slowly, with blinking to allow for drainage. If the lid was pulled out to accommodate a larger volume, the result was copious lacrimation, which produced a considerable error in determining the normal tear volume.

A decrease in tear volume with increasing age was reported by Zintz and Schilling. Since their method involved errors due to reflex lacrimation, as discussed above, this age dependence might be due to an error inherent in the techniques. The present material is not large enough to give any definite conclusion, but no large difference in tear volumes was found between young and old age groups (Table II). They also showed that tear volume–age relation was similar with the age dependence of the Schirmer test. This test measures normal tear flow plus the additional reflex lacrimation caused by insertion of filter paper under the lids. Therefore, it is possible that the volume-age variation was due to the difference in reaction to stimuli among age groups.

It was found that the tear volume increases with increasing tear flow. However, this increase was very slight, and the eyes never looked watery within a range of secretion of up to 10 \( \mu l \) min.\(^{-1}\) If the drainage rate did not change with this increase of tear secretion, the tears would overflow within 3 minutes, since the maximum capacity of the cul-de-sac in an upright position to hold tears seems to be around 30 \( \mu l \). The fact that the volume increases only slightly indicates that the rate of drainage must increase with increasing rate of secretion; a fine regulatory mechanism of tear drainage does exist.

The main force of tear drainage is attributed to the pump action of the lacrimal sac associated with blinking.\(^{10-13}\) The observation that saline added to the cul-de-sac drained very quickly by blinking can be understood from Nagashima’s experiment.\(^{16-37}\) Since the lacrimal punctum opens into the marginal strip, the drainage force must be a pressure difference between the lacrimal sac and the tear strip. As can be visualized from the concavity of the surface of the marginal strip, the interspace between the lids and the bulbar conjunctiva acts as a capillary, and the surface tension of the tears makes the pressure in the strips lower than that of the atmosphere. An increase in tear volume causes the concavity of the tear strip surface to decrease, leading to a less negative pressure there. Thus, it is expected that the pressure gradient between the mar-
ginal strip and the lacrimal sac increases with increasing tear volume, the result being an increase in drainage. This describes a regulatory mechanism of drainage which increases the effectiveness of the lacrimal sac as a pump. However, this mechanism, due to surface tension, will saturate when the tear volume increases to a point at which the surface of the marginal strip does not show any concavity. Above this point this mechanism cannot increase drainage, the result being an overflow of tears. Since the maximum capacity of the cul-de-sac in an upright position seems to be around 30 \( \mu l \), the rate of secretion which will cause overflow will be around 100 \( \mu l \) min\(^{-1}\) in a normal drainage system, as judged from Fig. 7.

It was found previously\(^2\) that about 3 \( \mu l \) hr\(^{-1}\) cm\(^{-2}\) of evaporation occurs from the normal tear surface under standard indoor conditions. The tonicity of the human tears is dependent upon the dynamic inter-relationship of the rate of tear secretion, tear volume, rate of evaporation, and rate of water movement from the aqueous induced by the hypertonicity of the tear film.\(^{15,21}\) It is possible to formulate a theoretical relationship of these quantities and to calculate the tonicity increase in the tears of open human eyes from the average values of the present study. The rate of evaporation from the average area of the palpebral fissure (172 mm\(^2\)) corresponds to 0.085 \( \mu l \) min\(^{-1}\). The normal tear flow is 1.2 \( \mu l \) min\(^{-1}\) and the normal tear volume 6.2 \( \mu l \). The apparent osmotic water permeability of the whole rabbit cornea (2.18 \( \times 10^{-3} \) \( \mu l \) min\(^{-1}\) cm\(^{-2}\) m osmole\(^{-1}\)) was used (recalculated from Mishima and Maurice\(^2\)). This calculation led to a tonicity increase of 1.04 times the original. If water movement from the cornea is neglected, the tonicity increase was 1.07. The human cornea is thicker than that of the rabbit, and therefore the permeability would be less. Assuming the original tear tonicity to be 0.9 per cent saline equivalent,\(^{22-24}\) the average tear tonicity for open human eyes would be expected to lie between 0.94 and 0.96 per cent saline equivalent. This agrees with the result of a direct determination (0.953 per cent) of unstimulated tears.\(^2\)

It must be emphasized that, when using this method to determine turnover rate of the tears, one must be sure of a normal epithelium. Fluorescein diffusion is very slow through the normal cornea and the conjunctival epithelium compared to the loss due to tear flow. However, when there is erosion of the epithelium, the method will give a falsely high turnover rate, since fluorescein will be lost via the cornea and aqueous humor easily across a damaged epithelium. Errors due to this factor must always be considered in the application of the method to pathologic cases.

The fact that the tear flow may increase easily to 100 per cent min\(^{-1}\) or more upon instillation of solution has clinical significance. The time of contact of the instilled solution may be less than expected, depending upon the amount of stimulation and the individual threshold for stimulation. By bathing the eye with solutions of various tonicities at 37° C., it was found that 0.9 per cent saline was the most comfortable. By varying the temperature of the bathing solution, it was found that the solution temperature which caused the least irritation was body temperature. These factors
seem to be important in the topical application of solutions on the eye.

The authors wish to express their gratitude to Drs. C. H. Dohlman, D. M. Maurice, I. Fatt, W. K. McEwen, and B. O. Hedbys for their helpful contributions through discussions. Acknowledgement is also due to Drs. Emori and Okuhara for their valuable help in construction of the objective fluorophotometer.

REFERENCES

Discussion

Dr. W. M. McEwen. Several years ago, from the data then available, I estimated that the normal tear flow was probably about 10 μl per millimeter. Just recently, Noris found a value of 16 μl per millimeter.

In view of Dr. Mishima's paper, we now have to change our thinking and accept a value of about 1 μl per millimeter, a value very near the original value of Schirmer.

The present work of these authors is a definitive paper on tear volume and rate of secretion. It is difficult to obtain accurate data on such a
minute and labile system. They were wise to use several different methods to obtain their results. Their meticulous and precise work also shows that higher values obtained by others can be explained by irritation.

I congratulate them on a good, firm paper.

REFERENCES
