INVESTIGATIVE OPHTHALMOLOGY

A new objective slit lamp fluorophotometer

Stephen R. Waltman* and Herbert E. Kaufman**

A new readily available slit lamp fluorophotometer is described. The use of fiber optics and a sensitive photomultiplier tube made its construction feasible. Only minor easily removed modifications are needed to convert existing slit lamps to this instrument. It is objective, portable, and easy to use.

Key words: slit lamp fluorophotometer, instrumentation and methodology, fluorescein, concentration, permeability, corneal endothelium, aqueous humor, endothelial permeability.

Measurement of the time rate of change of fluorescein concentration in the various ocular tissues permits the in vivo study of various parameters of ocular physiology. Tear flow, epithelial and endothelial permeability, and drug penetration into the anterior segment are some of the parameters that can be evaluated. These studies can be performed only if an accurate, reliable, slit lamp fluorophotometer is available. Several previous investigators¹ ² have built fluorometers in order to study the dynamics of ocular fluorescein turnover. Because of the difficulties in constructing and using these instruments, investigations utilizing them have been limited. This is evidenced by the relative scarcity of investigations employing this type of device. This prompted us to construct the simple, portable fiber optic fluorophotometer described below.

Methods and materials

A model 360 Haag Steit slit lamp was modified as described below to convert it to an objective fluorophotometer. Slit lamp. The incandescent bulb normally present in the slit lamp is used as the source of illumination. A filter holder containing the removable, exciting filter is mounted on the arm in front of the light source. The lamp and microscope arm are locked at an angle of 65° for measurements.

Microscope. The original Haag Steit microscope is unaltered except for replacement of the right eyepiece with a modified removable one. The modified eyepiece contains a fiber optics probe designed so that it can be superimposed on any area of the image of the optical cross section.
of the anterior segment.* In this way the fluorescein concentration of that area can be measured. The sensor tip of the probe, which is 0.15 mm. in diameter, is in focus simultaneously with the optical section. Fig. 1 is an artist's conception of the view through the modified eyepiece. Fig. 2 is a cross section of the eyepiece.

The light from the monitored area is transmitted via a fiber optics bundle in the eyepiece to a larger bundle and hence to a sensitive photomultiplier tube. Before reaching the tube the light passes through a barrier filter and the fluorescein concentration is then quantitated.

**Electrical system**. The photomultiplier tube (EMI 9502) is a low dark current, 13 diode Venetian blind type, with a rated gain of $29 \times 10^6$ at 1,500 volts applied voltage. Between 1,250 and 1,560 volts are applied to the tube when it is in use. The sensitivity increases at higher voltage but so does the background noise. The output from the photomultiplier is fed into a fiber optics bundle in the eyepiece to a larger bundle and hence to a sensitive photomultiplier tube. Before reaching the tube the light passes through a barrier filter and the fluorescein concentration is then quantitated.

The choice of a suitable exciting and barrier filter is important so that light scattered from the eye or reflected from the anterior corneal surface does not affect the readings. Baird Atomic* interference filters are used. The exciting filter passes 70 per cent of the light between 4,600 and 4,800 Å and none above 5,000 Å. The barrier filter passes light between 5,000 and 5,400 Å only.

The instrument is operated in a darkened room or with a red safelight.

**Results**

The calibration and linearity of response of the instrument were measured using standardized solutions in glass test tubes. The area measured was 0.1 to 1.0 mm. behind the edge where the exciting light strikes the solution. The depth of the solution from which the readings are made was important with concentrations above $10^{-3}$ mg. per milliliter because 10 per cent of the incident blue light is absorbed by a layer of fluorescein at this concentration, 10 mm. thick. At higher concentrations it is necessary to read the solution just behind the edge to get an accurate response.

The instrument gives a linear response to fluorescein concentrations between $5 \times 10^{-6}$ and $5 \times 10^{-2}$ mg. per milliliter. This response can be obtained without the use of neutral density filters or any auxiliary light source. The use of a larger fiber optics sensor allows the minimum detectable concentration to be lowered 3-fold, but since this extends into the range of the natural fluorescence of the ocular tissues it is not often used. The meter may be read with an accuracy of 1 per cent except on its most sensitive setting at high applied voltages. Here it is possible to determine the reading to $\pm 3$ per cent in a few seconds. This reduced sensitivity is due to random fluctuations in the noise output from the photomultiplier tube.

A possible source of error in reading the fluorescence of the tissues of the anterior segment is the return of light from the iris or lens. The iris or lens may scat-
After the green light emitted by the fluorescein to the photometer, or some of the exciting blue light may be returned through the aqueous and cornea to excite further fluorescence. Measurements of the total fluorescence of the anterior segment are affected by these variables in eyes with blue but not with darker irides. Corneal readings and aqueous readings are little affected if the aqueous is read midway between the cornea and iris.

The values for the transfer coefficient ($K_{oa}$) from cornea to aqueous, which is a measure of endothelial permeability to fluorescein, are in agreement with those of Starr, who used Maurice's instrument. In 10 young normal adults we found an average $K_{oa}$ of 0.48 per cent per minute. This yields an endothelial permeability to fluorescein of 0.011 cm. per hour which is in agreement with Maurice's reported values. Our value for the natural fluorescence of the cornea is $8.5 \times 10^{-6}$ mg per milliliter, which is approximately half the value reported by Maurice and is most probably due to the narrower bandpass filters used in our instrument.

**Discussion**

Our instrument offers several advantages over currently available fluorophotometers while retaining the sensitivity and accuracy of Maurice's instrument. It is light and can be transported to various places within an institution by a single courier. It is commercially available and easily assembled. It is used with the model 360 Haag Streit slit lamp and can be adapted to other instruments. It requires no special power source and uses the light source of the slit lamp. The area being measured is in constant view and upright. With little practice, the sensing device is easily kept superimposed on the cornea. Using the chart recorder, the device is easily operated by a single person. The one disadvantage of the instrument is that it must be operated in a darkened room or with a red safelight. This is only a minor annoyance and is easily tolerated considering the advantages of the instrument over those currently available. It appears to be as sensitive and at least as accurate as Maurice's device, and is certainly more easily obtained and used.

The artistic ability of Mr. Ron Norvelle is appreciated.

**REFERENCES**