CLINICAL METHOD OF OBSERVING CHANGES IN THE RATE OF FLOW OF AQUEOUS HUMOUR IN THE HUMAN EYE*†

I. NORMAL EYES

BY

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It has been known since the time of Ehrlich (1882) that fluorescein, instilled into the conjunctival sac in routine clinical practice, stains the deep structures of the cornea and eventually appears in the aqueous humour. The relative concentration of this dye may be measured by the method devised by Amsler and Huber (1946) in their studies on the permeability of the blood-aqueous barrier. These two observations form the basis of a simple technique by which changes in the rate of flow of aqueous may be observed clinically. In order to avoid confusion, we have designated the procedure outlined below the "fluorescein instillation test" to distinguish it from the "fluorescein permeability test" of Amsler and Huber. This initial paper describes the technique and the results obtained in the normal human eye.

TECHNIQUE

One drop of 10 per cent. fluorescein is instilled into the conjunctival sac of each eye on three occasions at two-minute intervals, and after a further 15 min. the conjunctival sac and surrounding skin are copiously irrigated with normal saline. A trace of vaseline on the skin of the lids will prevent the skin being excessively stained. The eyes should be kept open during the 15-min. period in order that the dye may be distributed over the whole cornea by the action of the lids. If the epithelium is damaged, as by the application of a tonometer, an excessive amount of fluorescein may be taken up by the cornea, and it is therefore advisable not to record the ocular tension until the conjunctival sac has been irrigated.

The concentration of fluorescein in the aqueous is estimated by the method of Amsler and Huber (1946). It will be remembered that in this method, the minimum amount of light necessary for the observer to perceive the green flare in the anterior chamber is recorded by adjusting the current passing through the bulb filament of a Haag-Streit slit lamp. The apparatus is calibrated by a photometer before each test, and is checked at intervals throughout the experiment. It has been found that more accurate and consistent results are obtained when the observer has had two or three minutes dark adaptation before each reading.

Readings are taken at 10-min. intervals in the early stages, and thereafter at 15 to 30-min. intervals. The results are plotted on the Amsler-Huber chart, in which the concentration of fluorescein in the aqueous is expressed in ampères.

To a certain extent this method of expressing concentration in ampères is likely to be misleading, and we have therefore constructed a curve from which a given reading in ampères can be transposed into terms of an actual concentration of fluorescein (Fig. 1).

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For this purpose, readings of solutions of known concentrations of fluorescein contained in thin glass test tubes have been taken. In this way the Amsler-Huber chart can be converted to a curve which, although not claimed to be completely accurate, will give a better idea of changes in the concentration of fluorescein in the aqueous humour.

With practice, two observers will obtain readings within 0.1 amp. of each other; it is obvious from Fig. 1 that any experimental error arising in this way becomes of greater significance when the concentration of fluorescein is high. It is therefore better to conduct this test so that the highest concentration does not rise above 3 amps and the technique described above has been designed to that end.

**RESULTS IN NORMAL HUMAN EYE.**—The aqueous in contact with the cornea begins to take on a green fluorescence approximately 30 min. after the first drop is instilled, and the whole depth of the anterior chamber soon becomes homogeneously coloured. The concentration increases to a maximum in approximately 2 hrs, after which there is a slight but steady fall lasting from 12 to 18 hrs. In some cases, fluorescein was still present in the anterior chamber 24 hrs after instillation.

A typical curve in a normal subject is illustrated in Fig. 2. For practical purposes, the concentration curve rapidly reaches a maximum from which it falls...
slowly over the 12 hrs of observation. The shape of the curve was fairly constant in the normal eyes of man and rabbit, although the height of the curve varied in different eyes according to the amount of fluorescein taken up by the corneal epithelium at the time of instillation.

**Effect of Mydriatics and Miotics.**—In normal subjects, instillation of homatropine 1 per cent. or pilocarpine 2 per cent. did not alter the fluorescein concentration, neither as a general rule did eserine 0.5 per cent., except that in two subjects it produced a transitory and slight fall in concentration which returned to its former value in less than an hour. In these two exceptions, the ocular tension was also lowered by 2 mm. Hg Schiötz, indicating that in normal subjects the more powerful miotics may cause a temporary increase in the rate of flow of aqueous, and at the same time facilitate the passage of aqueous from the anterior chamber.

**Discussion.**—When the corneal epithelium is intact, there is little or no evidence of fluorescein in the cornea on slit-lamp examination, as it is masked by the greyish background of the corneal substance. Because it is difficult to obtain readings of the concentration of fluorescein in the cornea, it is impossible by this method to calculate the relative rates of flow of aqueous at different times in absolute values.

If a small area of epithelium is deliberately damaged and stained with fluorescein, the stained area can be seen to enlarge equally in depth as well as in breadth throughout the cornea, and to assume a spherical outline of staining beneath the epithelium. It appears, so far as can be seen by examination with the slit lamp, that the fluorescein penetrates through the corneal lamellae as readily as it permeates laterally between them. When this stained area reaches the endothelium the concentration in the aqueous rapidly reaches a high level.

After the maximum is attained, the concentration of fluorescein must represent a steady state between the concentrations in the cornea and aqueous, and because the latter is constantly being replaced, presumably at the rate of approximately 2 c.mm./min. (Goldmann, 1950), the concentration in the aqueous can never be as high as that in the cornea. The cornea acts as a reservoir for fluorescein, and its depletion results in a gradual fall of the concentration in the aqueous. The experiments described below suggest that this steady state holds when fluorescein is added to the system across the blood-aqueous barrier.

**Experimental Procedure**

The "fluorescein permeability test" of Amsler and Huber was performed on a normal subject, and on a second occasion, the "fluorescein instillation test" was carried out on one eye (left) of the same subject, and after the concentration maximum had been reached, 2 ml. 10 per cent. fluorescein were injected intravenously as in the conventional permeability test. The concentration in both eyes was then estimated at intervals for 30 min. and the two sets of curves compared.

It was found that, although starting and running at a higher level than its fellow, the curve in the "instilled" (left) eye ran approximately parallel with that of the "non-instilled" (right) eye. Fig. 3 shows the converted Amsler and Huber permeability curve in a normal subject, and Fig. 4 shows the combined "instillation" and "permeability" curves in the same subject. Fig. 3 shows that, after 30 min., fluorescein had crossed the blood-aqueous barrier to raise the concentration in the aqueous of the right and
left eyes to 3.5 and 4.2 parts per 100 million respectively. In Fig. 4, the left eye is seen to have had a concentration of 6.5 parts per 100 million before the permeability test was carried out; the intravenous injection raised the concentration in the left eye by a further 4 parts to 10.5 per 100 million, and in the right eye to 3 parts per 100 million. Thus approximately the same amounts of fluorescein crossed the blood-aqueous barrier of each eye on each occasion.

It follows that the presence of fluorescein in the aqueous does not influence the permeability of the blood-aqueous barrier to fluorescein.

Six out of ten normal persons conformed to this observation, and in the remaining four cases, the greatest difference from the expected result was 1.5 parts per 100 million, which is considered to be within the limits of experimental error.

**DISCUSSION**

The concentration of fluorescein decreases slowly over a prolonged period in the normal eye because a steady state exists between the concentration of fluorescein in the cornea and in the aqueous, combined with a continuous flow of aqueous humour. The slope of the curve would be unaltered if an additional quantity of aqueous left the eye without being replaced by fresh fluid, and would fall more sharply only if clear aqueous entered to dilute the fluorescein in the anterior chamber. Thus, if a period of increased flow of aqueous developed after the maximum concentration had been reached, the concentration would fall more steeply than normal; it would continue at a lower level until the rate of flow decreased again, when, provided that there was sufficient fluorescein present in the corneal reservoir, the concentration would rise.

If the rate of flow of aqueous was great when instillation began, one could anticipate that the concentration would not be high and that the subsequent
fall would be steeper than normal. Conversely, if the rate of flow of aqueous was completely arrested when instillation began, one might expect that the maximum concentration would be high, and that no subsequent fall in concentration would occur.

Fig. 5 shows the theoretically-derived curves calculated for a flow of aqueous of 2 mm$^3$/min., and for double and half this rate. The curves (a) and (b) are those to be expected should the rate of flow of aqueous change from 1 to 4 mm$^3$/min. or vice versa.

In order to investigate the effect of a totally stagnant aqueous, the following animal experiment was carried out.

**Experiment.**—The eyes of a rabbit were enucleated during nembutal anaesthesia and were immediately suspended for 30 sec. in 2 per cent. fluorescein solution so that only the corneae were in contact with the dye. The eyes were then copiously washed with normal saline and placed in liquid paraffin. No fluorescein leaked out into the paraffin as it is not soluble in this substance. Frequent readings of the concentration of fluorescein in the aqueous were made by removing the eye from the paraffin and observing it with the slit lamp. The paraffin did not interfere with the readings and the corneae remained clear for over 24 hrs.

The dye rapidly permeated the cornea, appearing in the anterior chamber in measurable quantities in 12 min. Initially the dye appeared in a layer behind the cornea, but gentle agitation of the eye in paraffin allowed the fluorescein to be distributed homogeneously throughout the anterior chamber.

After the maximum had been reached, the aqueous was washed out with normal saline through an oblique needle puncture at the limbus (Chandler, 1949). The concentration of fluorescein, which fell when the anterior chamber was irrigated, rose very rapidly when the irrigation ceased. Fig. 6 illustrates an
example of this experiment, the purpose of which is to show that, provided fluorescein has permeated the cornea to the endothelium, a stagnant aqueous will allow a rapid rise in the concentration of fluorescein.

It is unlikely that small differences in the rate of flow of aqueous will be detected by the fluorescein instillation test. For example, massage of the normal eye did not alter the concentration of fluorescein in the aqueous; similarly, tonometry carried out during the test does not materially influence the curve.

The curve in normal subjects fell slightly and steadily over a considerable period, during which meals were taken and various amounts of exercise carried out. This indicates that, within the limits of sensitivity of this test, the rate of flow of aqueous is kept relatively constant, being influenced, if at all, to an undetectable degree by the ingestion of food and changes in posture.

**SUMMARY**

Instillation of fluorescein into the conjunctival sac results in its appearance in measurable quantities in the aqueous. The concentration of fluorescein in the aqueous represents a steady state which depends on the amount taken up by the cornea and the rate of flow of aqueous humour.

The flow of aqueous results in the eventual disappearance of fluorescein from the cornea and anterior chamber, a process taking up to 24 hrs in the normal eye.

These features form the basis of a method of observing changes in the rate of flow of aqueous. The results obtained in the normal human eye under these conditions are described.

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**REFERENCES**


