FLUORESCEIN DYNAMICS IN THE EYE

by

JOSE CUNHA-VAZ & DAVID MAURICE

(Lisbon & Los Altos)

Fluorescein has been very widely used in the study of aqueous humour dynamics (LUGOSSY, 1959) and qualitative methods of assessing absolute and relative flow rates by this means have been developed (GOLDMANN, 1950; JONES & MAURICE, 1966; ANSELMI, BRON & MAURICE, 1968). However, a full understanding of the dynamics of the movement of fluorescein between the anterior chamber and the blood is lacking. For example, it has to be assumed in these methods that the exchange of the dye between the aqueous humour and the blood is almost entirely by a process of passive diffusion across the anterior surface of the iris.

It was recently shown (CUNHA-VAZ & MAURICE, 1967) that fluorescein was removed from the vitreous chamber by an active transport mechanism that was present over the entire retinal surface and probably extended as far forward as the posterior iris surface, and that this mechanism can be saturated by competitive inhibitors of organic ion transport. The present paper describes measurements of the movement of fluorescein between the chambers of the eye and the blood, either with the active mechanism operating or with it suppressed, when the ion is moving virtually only under the influence of diffusion and bulk fluid flow. This comparison gives additional insight into the normal intraocular dynamics of this dye.

METHODS

Young coloured rabbits, principally of the Dutch strain, were used as the experimental animals.

Fluorescein administration

Intra-ocular injections were made under superficial anaesthesia. Not more than 20 μl.

of solution containing fluorescein or inhibitor were injected centrally into the vitreous body through a 30-gauge needle as described by MAURICE (1957).

Systemic injections of fluorescein were made intraperitoneally or, more usually, into a marginal ear vein. When it was desired to maintain a steady level in the blood, a schedule for repeated injections was developed which finally took the form of an initial injection of 8 ml of a suitable concentration followed by 1 ml injections at 10 min. intervals.

Fluorescein measurements

Samples of blood were taken from a cut in the marginal ear vein into a 10 or 25 µl. 'Microcap' pipette and discharged without centrifugation into a convenient volume of saline; readings were taken in the supernatant when the cells had sedimented or been centrifuged down. When intravenous injections of fluorescein were given, the blood was sampled from the vein on the opposite side, and was taken immediately before an injection when these were repeated.

Fluorescein concentrations were determined with the slit-lamp fluorophotometer of MAURICE (1962). The animals were kept under sedation with Promazine hydrochloride (about 5 mg/kg intramuscular) while they were being examined.

For a representative measurement of the concentration in the vitreous body the maximum value of the fluorescence immediately behind the lens was chosen. The pupil needed to be dilated with atropine and phenylephrine and the angle between the arms of the instrument reduced, though not so much as described by CUNHA-VAZ & MAURICE (1967). It was not necessary to fit the animal with a contact lens or to change the objective of the microscope as explained in the same paper.

The measurements in the anterior chamber were taken from a central area near the cornea. When its fluorescence was very low compared to that of the vitreous body, the lowest readings were accepted.

Free fluorescein was determined in the blood by dialysis at 37° C as described previously (CUNHA-VAZ & MAURICE, 1967).

RESULTS

Intravitreous fluorescein

When small doses of fluorescein, normally 20 µl 0.05% solution, were injected into the vitreous body, the dye was seen to be localised at first but to immediately begin spreading. Readings behind the lens rise and reach a steady value, around the 10^{-5} g/ml level, in about one hour. In the aqueous the rise is delayed and maximum values are not reached till 4-5 hours after the injection.

Without inhibitor. The concentration in the vitreous began to fall rapidly

after reaching its maximum value. This fall was logarithmic with a half period of about 2½ hours ($k_v = 4.2 \times 10^{-3}$/min. in 7 animals). On occasion, the animal was killed at the end of an experiment, the entire vitreous body collected, and the average fluorescein concentration within it was measured. This was found to be 30-40% of the maximum concentration behind the lens, as estimated in vivo.

The concentration in the aqueous humour, measured in vivo, ranged from one-thousandth to one-hundredth of that in the vitreous body just behind the lens, the median ratio being 0.0025 in 12 eyes. As a result of the considerable radiation of green light from the vitreous body, especially when the pupil was dilated, it was difficult to be certain of readings made in the anterior chamber, but on a number of occasions they were confirmed by measurements made on the aqueous humour after it had been withdrawn into a syringe. There was a suggestion that dilation of the pupil increased the anterior chamber level, but this point was not investigated further.

The concentration in the aqueous humour appeared to undergo a fall parallel to that in the vitreous body, but it was too low for this to be established with certainty.

Inhibited. When a competitive inhibitor was present in adequate concentration, either iodopyracet injected together with the fluorescein or benemid injected systemically, the rate of loss from the vitreous body was much reduced. The fall was still apparently exponential with a half period of about 13 hours ($k_v = 1.0 \times 10^{-3}$/min. in 5 eyes). The average concentration in the vitreous was found to be about 60% of the maximum post-lenticular value.

The concentration of fluorescein in the aqueous humour was very much raised above that in the unpoisoned eye, being found to vary from 1½ to 28% of the maximum post-lenticular value in 10 experiments, with 15% as the median value. No difference between the effect of one inhibitor and another was observed in this small series. The rise could be strikingly demonstrated by injecting fluorescein into an eye without inhibitor, and later treating the animal with systemic benemid (fig. 1).

Systemic Fluorescein

Without inhibitor. The entry of fluorescein into the anterior chamber was observed after a single intravenous injection of 1 ml 10% fluorescein, using a

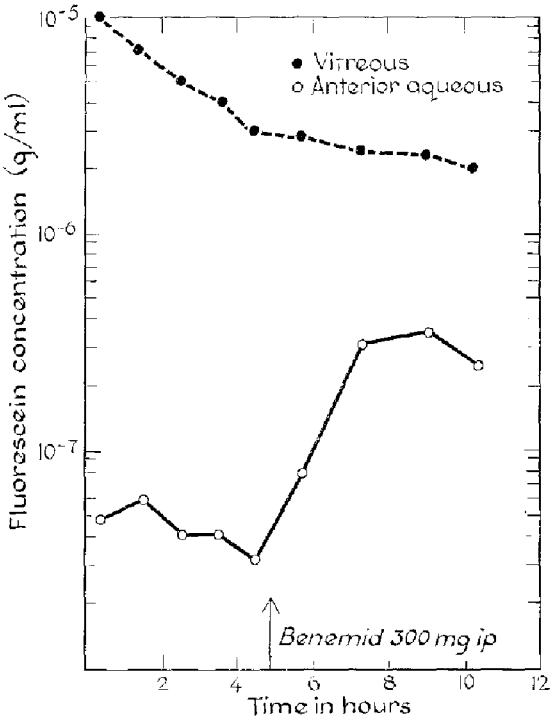

Fig. 1

Changes in fluorescein concentration in ocular fluids before and after intraperitoneal administration of benemid. 20 µl of 0.06% fluorescein injected in vitreous 2½ hr. before zero of time-scale.

slit-lamp with blue light. In some cases, time-lapse cinephotography of the eye was carried out. A stream of warm air blown over the head of the animal reduced convection currents in the anterior chamber and allowed the first appearance of the dye to be located.

Shortly after the iris stained green, a fluorescent cloud was seen to arise from its surface. No particular fluorescent stream emerging from the pupillary border could be distinguished but after the aqueous humour was stained throughout, an uncoloured fluid could be seen entering from the lowest part of the pupil. This 'black' aqueous humour could be seen entering as long as the anterior chamber was stained green. It tended to appear in bursts, separated by a minute or two, a bubble of unstained fluid developing in the anterior chamber over a

period of seconds in the temperature equilibrated eye, and then remaining stationary while it mixed slowly into the anterior chamber by diffusion. After interrupting the warm air, convection reasserted itself and the incoming black stream rose close to the front surface of the lens and, swirling around, rapidly mixed with the aqueous humour as a whole.

By turning the rabbit on its back, the stream of black aqueous could be made to come from the top of the pupil, that is the same anatomical position as before.

The concentration of fluorescein in a freshly formed bubble of black aqueous could be estimated with little trouble by employing the smallest window of the fluorometer. This was found to follow a very similar curve to that of the total blood fluorescein, and in six out of seven animals the ratio of the concentrations in the aqueous and the blood assumed a value of 3-4 $\times$ 10^{-3} (fig. 2).

Inhibited. When the eye was affected with a systemic or local inhibitor of fluorescein transport, the aqueous entry from the posterior chamber may still be identified, though in the period shortly after a single injection it stands out a brighter green than the general level in the anterior chamber. A period in which it cannot be distinguished follows, and finally when the blood level has dropped sufficiently, it may again be seen as a darker volume against the general background. Its concentration was 2-5 $\times$ 10^{-2} times that in whole blood in five well-inhibited eyes.

Steady State Levels

The entry of fluorescein into the anterior chamber is faster in the inhibited than in the uninhibited eye but comparative measurements on the dynamics of fluorescein entry did not promise to show meaningful differences, and attention was concentrated on the ratio of the aqueous humour and blood levels when a steady state was reached. Attempts were made to keep the blood level constant and in the initial experiments one or more intraperitoneal doses were given, but better control was achieved by repeated intravenous injections. When benemid was given systemically, the fluorescein was lost at a slower rate from the blood (CUNHA-VAZ & MAURICE, 1967) and was not injected so frequently. The concentration of the injected dye ranged from 10^{-4} g/ml in isotonic saline to 10^{-1} g/ml in water.

The blood level could sometimes be kept steady for some hours by this

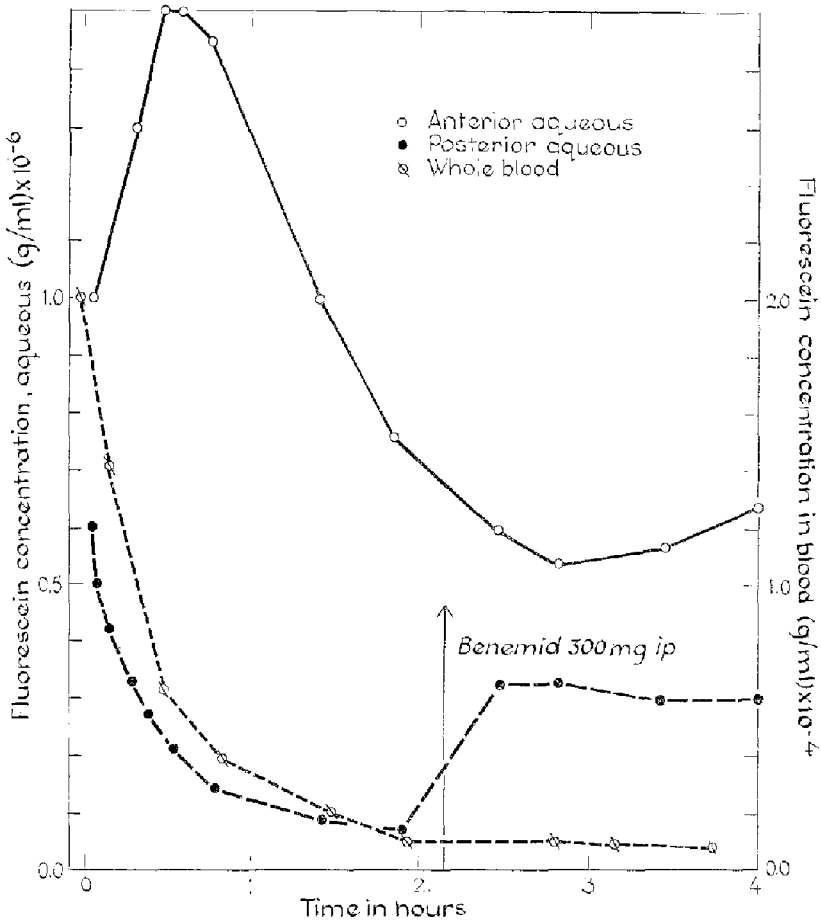

Fig. 2

Changes in fluorescein concentration in aqueous humour and whole blood before and after intraperitoneal administration of benemid. 1 ml 10% fluorescein injected intravenously 10 min. before zero of time-scale.

method and after the first hour the aqueous humour remained constant; in these cases there was no problem in analysing the results. On other occasions the experiment had to be rejected because the blood level altered throughout; sometimes, however, if this took the form of a gradual decline, the steady state ratio could be taken to be that at the moment when the aqueous level was at its maximum value.

Steady state values. The ratio of the fluorescein concentration in aqueous humour to that in whole blood at the steady state condition is plotted in fig. 3 for different blood levels. In untreated animals the value of this ratio is seen to remain in the range 0.01-0.03 for blood levels below 10^{-4} g/ml but to rise at higher concentrations.

In animals that had been treated with systemic benemid or had received intravitreal injections of inhibitor, the ratio was considerably raised. A previous injection of saline into the vitreous body did not alter the value of the ratio from that in the control eye in two experiments.

Free fluorescein. The results of the dialysis experiments are also exhibited in fig. 3. They show that the ratio of fluorescein in the dialysate to that in whole blood lies in the range of 0.2-0.3 below a total concentration of 10^{-4} g/ml but above this the ratio increases rapidly.

DISCUSSION

Previous Investigations

FORBES & BECKER (1960) and BECKER & FORBES (1961) carried out experiments very similar in design to these reported here, but used radioactive iodopyracet as the test material, and followed its rate of loss from the vitreous body with an external counting system. The results were similar to those found with fluorescein, as were the values of the ratios of the concentrations in the anterior chamber and vitreous body in the normal and inhibited eye.

BECKER & FORBES (1961) successfully treated the transport of iodopyracet out of the vitreous body in terms of saturation kinetics, and showed that it could be expressed by the Michaelis-Menten equation, and there is little doubt that the behaviour of fluorescein would be similar. In the case of this dye the concentration changes in the vitreous body and posterior and anterior aqueous humour can be followed in a single animal and this allows a more complete analysis of the dynamics to be carried out.

BLEEKER, VAN HAERINGEN, MAAS & GLASIUS (1968) obtained a value of 0.03 for the aqueous to blood plasma steady-state ratio for fluorescein. This is compatible with the values in fig. 3 corresponding to whole blood.

Normal eye

Vitreous-blood exchange. The dynamics of the loss of fluorescein from the

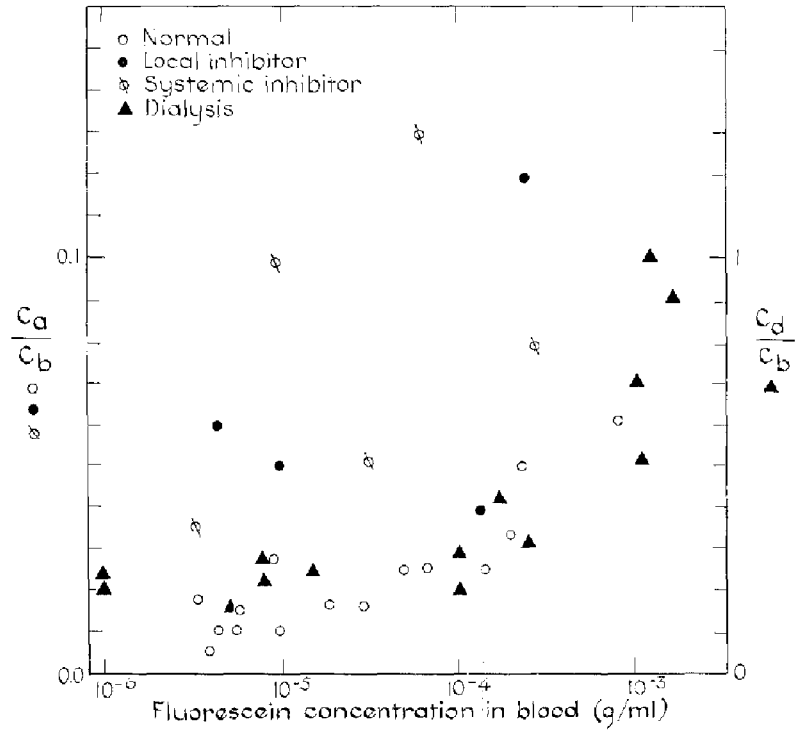

Fig. 3

Steady-state ratios between aqueous humour and whole blood (left ordinate) and dialysate and whole blood (right ordinate).

vitreous body can be understood readily from the observations of the distribution of dye, already published (CUNHA-VAZ & MAURICE, 1967). The concentration gradients, which are set up as a result of an active transport mechanism, are consistent with a rapid efflux across the entire posterior retinal surface and a less pronounced one forwards towards the anterior chamber.

For either of the geometrical approximations to the form of the vitreous body considered in that paper, the time constant for the loss of fluorescein would be given by $\frac{\pi^2 D}{\tau^2}$ (CRANK, 1956) where D is the diffusion constant, and τ is the distance between the lens and the retina, where zero concentration is assumed. Inserting a representative value for τ, 6½ mm, leads to a time constant of

8.5×10^{-3}/min compared to the observed value of 4.2×10^{-3}/min. This implies that either the diffusion of fluorescein is slowed in the vitreous, or that the active transport mechanism at the retinal surface is not sufficient to bring the concentration of fluorescein down to zero. The latter is in accordance with the in vivo measurements of the concentration gradients within the vitreous cavity (CUNHA-VAZ & MAURICE, 1967).

Vitreous-aqueous exchange. It might be expected from the molecular weight of fluorescein that the rate of diffusion into the anterior chamber should cause the concentration there to lie between 20 and 7% of that in the vitreous body, the figures found for Na and albumin (MAURICE, 1957; 1959). In fact, a value of less than 1% was determined. This is not a result of a physical obstruction to diffusion as is shown by the effects of inhibitors on the ratio, but, as suggested by CUNHA-VAZ & MAURICE (1967), it is probable that the entire excretory mechanism extends forward over the posterior surface of the iris.

Blood-aqueous exchange. When fluorescein is administered systemically, the removal of the dye by the posterior iris surface from the fresh secretion of the ciliary body could account for the low fluorescence of the fluid entering through the pupil (MAURICE, 1967).

If this were the true mechanism, on the other hand, it might be expected that the pools of posterior aqueous humour that irrupt into the anterior chamber would show a fluorescence that was patchy and at a level dependent on how long the fluid had been accumulating behind the iris. Careful observation shows, however, that their staining is uniform and consistent, implying that the fluid secreted by the ciliary body has a fluorescein content no higher than that in the 'black' aqueous entering through the pupil. Further experiments are required to be certain how far forward the excreting system extends.

There is no question, however, that the greatest entry of fluorescein from the blood into the anterior chamber is across the front surface of the iris. The conventional equation for the exchange between blood and anterior aqueous humour is:

$$\frac{dC_a}{dt} = k_o (C_h - C_a) + k_d (C_p - C_a) \qquad (1)$$

where C_a, C_h and C_p are the concentrations of free fluorescein in the anterior

and posterior aqueous humour and the plasma, and k_o and k_d are the transfer coefficients for outflow and diffusion.

It follows that when $\frac{dC_a}{dt} = 0$:

$$\frac{k_d}{k_o} = \frac{C_a - C_h}{C_p - C_a} = \frac{r_{ab} - r_{hb}}{r_{db} - r_{ab}} \qquad (2)$$

Where r_{ab}, r_{hb}, and r_{db} are the ratios of the fluorescein concentrations in the anterior and posterior humours and in the dialysate to that in whole blood, in the steady-state.

Introducing the experimentally determined figures for these ratios leads to an average value of 0.06 for k_d / k_o, rather below that arrived at by GOLDMANN (1950) on the same basis.

Inhibited eye

Loss from vitreous body. The slow rate of loss of fluorescein injected into the vitreous body of the inhibited eye corresponds to the shallow gradients of concentration from the lens to the retina found under the same conditions in earlier experiments (CUNHA-VAZ & MAURICE, 1967). These gradients indicate that the greater part of the loss would be forward into the anterior chamber.

If this were the case, the loss coefficient from the vitreous body, k_v, can be calculated from the value, 0.16/day, found experimentally for serum albumin which is certainly lost only by the anterior route (MAURICE, 1959). On the assumption that fluorescein diffuses seven times more quickly than albumin through the vitreous body, in accordance with their free diffusion rates, the value of k_v for the dye may be estimated to be 0.8×10^{-3}/min. This is close to the average figure obtained experimentally 1.0×10^{-3}/min and supports the view that the greater part of the fluorescein leaves the vitreous body across its interface with the posterior chamber.

Vitreous-aqueous exchange. In the inhibited eye the concentration in the aqueous humour in many cases rises to the value 10–15% of that in the vitreous, which would be expected for a molecule of the size of fluorescein. Similar levels were found when larger amounts of fluorescein were injected into the vitreous body so that the concentration there rose above about 10^{-4} g/ml. At these and higher levels, the anterior chamber holding a concentration above 10^{-5} g/ml

becomes bright green, while with only ten times less in the vitreous body the aqueous concentration drops below 10^{-7} g/ml and is scarcely visible, even in the slit-lamp. This would account for contradictory observations as to the behaviour of intravitreously injected fluorescein that have been published.

Blood-aqueous exchange. The brighter staining of the fluid passing through the pupil of the inhibited eye, favours the view that there is normally a secretion of fluorescein out of the freshly formed fluid.

In principle, the use of Eq. 2 should permit a value for k_d/k_o to be derived in the inhibited eye, and thus indicate whether the rise in C_h was sufficient to account for the change in C_a. Inserting average values in the equation gave a larger ratio than in the normal eye, but the large scatter of the results suggests that non-specific breakdown of the blood-aqueous barrier might take place. In the individual experiment (fig. 2) there was little rise in the ratio on inhibition.

SUMMARY

Fluorescein injected into the vitreous body is transported rapidly out of the eye in the posterior segment. Injected in excess or in the presence of competitive inhibitors it leaves more slowly, by way of the anterior chamber.

Systemically administered fluorescein reaches higher levels in the anterior chamber when inhibitors are acting on the eye. Direct observation shows that the concentration in the newly secreted aqueous humour also is increased.

RÉSUMÉ

La fluorescéine injectée dans le corps vitré est transportée rapidement hors de l'oeil par la voie postérieure. Lorsque l'injection se fait avec un excès en fluorescéine ou en présence d'inhibiteurs compétitifs, la sortie est plus lente et le colorant passe par la chambre antérieure.

Après une injection intrapéritonéale ou intraveineuse la fluorescéine atteint des taux plus élevés dans la chambre antérieure, lorsque des inhibiteurs agissent sur l'oeil. L'observation directe montre que la concentration est également augmentée dans l'humeur aqueuse nouvellement sécrétée.

ZUSAMMENFASSUNG

In den Glaskörper injiziertes Fluoreszein wird schnell durch die hinteren Ab-

schnitte aus dem Auge transportiert. Wenn es im Übermaß oder in Gegenwart konkurrierender Hemmstoffe injiziert wird, verläßt es das Auge langsamer durch die Vorderkammer.

In den Körper eingeführtes Fluoreszein ergibt ein höheres Niveau in der vorderen Kammer, wenn Hemmstoffe auf das Auge wirken. Direkte Beobachtung zeigt, daß die Konzentration auch im frisch sezernierten Kammerwasser erhöht ist.

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Instituto de Alta Cultura & Institute of Ophthalmology, Judd St., London.

(D.M., Division of Ophthalmology Stanford University Medical Center, Stanford, Calif.)