Fluorescein Studies of Diabetic Retinopathy*

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[With Special Plate]

The use of fluorescein for assessing arterial insufficiency in limbs and viability of strangulated intestine, no such toxic reactions have been reported (Goodman and Gilman, 1955). Now, however, we deem it a wise precaution in all patients to give an intravenous test dose of 0.5 ml. of the 5% solution.

Method

Photographic Technique

A full description of the method is to be found in previous reports (Dollery et al., 1962, 1963) and the arrangement is seen in Fig. 1 (Special Plate). The patient is seated at the retinal camera. Preliminary colour photographs are taken of three areas selected for special study. Then the 50-cm-long polythene catheter is introduced through a needle into the median antecubital vein, and when inserted to its full length the tip is lodged in the upper part of the superior vena cava. A preliminary injection of saccharin determines the catheter-to-tongue time, which is a guide to the arrival in the retina of subsequent injections of fluorescein.

The camera-back with the colour film is replaced by one loaded with high-speed black-and-white film and equipped with a green filter. The observer focuses on the selected area of the retina with the white viewing light before sliding a blue filter into the common pathway of the viewing light and electronic flash light. He watches the retina with this blue light while his assistant injects 5 ml. of 5% fluorescein into the catheter and begins counting. After 8 to 20 seconds the green fluorescence of the dye appears in the retina, moving swiftly along the arteries. A rapid series of five or six photographs at 21-second intervals records the passage of the dye through the arteries, capillaries, and the veins in the area of the retina in focus. Two further injections of fluorescein can be made at the one session to study other areas of the retina.

The fluorescence photograph is projected on to a white card and a drawing made. Projection of the colour slide of the same quadrant on to this drawing enables a correlation of the visible lesions with those revealed by fluorescence photography.

Precautions

In more than 300 fluorescein studies of hypertensive and diabetic patients we have encountered only one untoward reaction, probably due to hypersensitivity.

The patient was a 61-year-old woman with diabetes controlled by diet and tolbutamide. Four minutes after receiving her first 5-ml injection of fluorescein she developed a temporary loss of consciousness, lasting about two minutes and associated with hypotension and bronchospasm. She recovered after an intravenous injection of 100 mg. of hydrocortisone and 250 mg. of amino-phylline. Occasional attacks of bronchitis with asthma had troubled her in the past, but there was no definite history of allergy, nor had she ever been given fluorescein. No cutaneous sensitivity was subsequently demonstrated. Other patients had received the same batch of fluorescein without incident, and on culture it was sterile.

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REFERENCES


Novotny and Alvis (1961) first reported the technique of fluorescence retinal photography, whereby fluorescein is injected into the circulation and photographed as it passes through the retinal vessels. Dollery et al. (1962) developed the method of using a venous catheter to inject the fluorescein into the superior vena cava rather than a peripheral vein, and in this way they obtained a sharper appearance of the dye.

This retinal angiography enables clinicians to delineate in their diabetic patients the abnormalities in the small retinal vessels which Ashton (1950, 1953) has clearly demonstrated at post-mortem examination by vessel injection studies, and which Cogan et al. (1961) have shown after other elements of the retina have been digested away by trypsin (Kuwabara and Cogan, 1960).

This paper deals with the results of studying the retinæe of 41 diabetic patients, using fluorescence retinal photography. All the patients had recognizable retinopathy, and clear media was the only selection criterion used.

that a mannitol diuresis exerts a protective effect against anoxia. Mannitol has been suggested as a prophylactic measure against the development of post-operative renal failure in patients at risk—for example, cross-clamping of the abdominal aorta during grafting (Barry et al., 1961). Moore (1963) also recommends its use in similar circumstances and suggests that it might prove of prophylactic value in deeply jaundiced patients undergoing operation. The results of these experiments strongly support this recommendation. An investigation into the effect of mannitol on sequential renal function studies in jaundiced patients undergoing operation is at present being made.

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of fluorescein five minutes before giving the main injections for photography.

The majority of our 41 patients have been studied as out-patients. With experience we have found fluorescein retinal photography a simple procedure which has been repeated several times in some of the patients, and which can form part of a routine assessment.

Results in Normal Fundus

The slug of dye enters the retinal arteries 8 to 20 seconds after injection. The first photograph shows the dye entering the four main arteries and their proximal branches, supplying the macula and other areas close to the disk (Special Plate, Fig. 2). The dye advances along the major arterioles to supply the peripheral retina, while the capillaries fill around the macula and close to the disk. Next, the minor venous tributaries draining these areas fill and streams of dye appear along the walls of the major veins running to the disk (Special Plate, Fig. 3 B). In these major veins the central core of blood comes from the periphery without fluorescein, for at this stage the dye is only just entering the peripheral capillary bed. This pattern of laminar flow is maintained throughout the length of the major veins, except where arteriovenous nipping creates turbulence (Dollery et al., 1963). In the late venous stage blood from the peripheral retina contains fluorescein and the entire width of the retinal veins is fluorescent as in Fig. 4 B, Special Plate (Dollery et al., 1962).

When the lens and vitreous are absolutely clear and the retina is in sharp focus it is sometimes possible to discern the fine reticular pattern of the capillaries, as in parts of Fig. 2. However, in most patients the capillaries filled with fluorescein present a ground-glass appearance.

Results in Diabetic Retinopathy

1. Microaneurysms

Fig. 3 A (Special Plate) is a routine photograph of a moderately severe diabetic retinopathy. The fluorescence photograph (Fig. 3 B) of the same area shows fluorescein in the arteries and capillaries and beginning to fill the veins. Numerous small fluorescent dots are shown against the faint ground-glass glow of the capillary bed. On careful superimposition all the recognizable microaneurysms on the colour photograph coincide with fluorescent dots. There are, however, many more fluorescent dots without any corresponding lesion on the colour photograph. Some are located beside or clustered around small haemorrhages, recognizable in the fluorescence photograph as small dark areas where the extravasated haemoglobin absorbs the light emitted from fluorescein traversing the underlying capillaries.

Number.—It is found that in patients with microaneurysms the introduction of this contrast material demonstrates many more microaneurysms than can be counted on a routine retinal photograph or with an ophthalmoscope. Of the 39 patients who had visible microaneurysms in the areas under study, 30 showed at least 50% more microaneurysms on the fluorescence photographs. Fig. 4 (Special Plate) is an extreme example of this discrepancy. Only 6 to 10 microaneurysms are visible in the left superior temporal quadrant, whereas in the fluorescence photograph over 1,000 microaneurysms have filled.

Arrangement.—In an advanced retinopathy with numerous microaneurysms, dot and blot haemorrhages, fluorescein photography often reveals groups of 8 to 20 dots in a small area drained by a single venule, resembling grapes bunched about a single stalk. Where the retinopathy is of mild to moderate severity the microaneurysms in the fluorescence photographs occur in scattered groups of two to six and the capillary bed has the even ground-glass appearance observed in normal
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Green emitted light.
Eyepiece.
Blue filter.
Viewing light.
Flash.
Blue light to excite fluorescence.

FLUORESCENCE PHOTOGRAPHY

Fig. 1

Fig. 2

Fig. 3A

Fig. 3B

Fig. 4A

Fig. 4B
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Fig. 5A

Fig. 5B

Fig. 6A

Fig. 6B

Fig. 8A

Fig. 8B
patients. In most patients microaneurysms do not appear to be closely related either to the arterioles or to the venules, except in those patients with severe retinopathy mentioned above.

**Duration of Filling and Abnormal Permeability.—**Microaneurysms appear to have varying durations of filling. In a rapid sequence of fluorescence photographs some dots disappear along with the drainage of the capillaries and the minor venous tributaries. Other dots continue to fluoresce for five to eight seconds after fluorescein has drained from minor venous tributaries in the vicinity, and throughout retain their clear outline and the same diameter. Yet other microaneurysms continue to glow for periods up to 10 minutes after filling with fluorescein. In successive photographs these fluorescing dots enlarge, their outlines become blurred, and finally a diffuse glow marks the site of the original discrete dot. In some diabetic patients fine white dots can be seen ophthalmoscopically in the retina, and Friedenwald (1950) has suggested these are thrombosed microaneurysms. Fig. 5 (Special Plate) shows some areas which are filled with fluorescein. Almost at once the fluorescein diffuses outwards so that the original dot becomes an area of diffuse glow, showing that these particular aneurysms are pathological and have abnormally permeable walls. Retinal arterioles appearing ophthalmoscopically as white lines are seen in patients with hypertension complicating diabetes. They prove, in many instances, to be patent, filling normally with fluorescein.

### 2. New Vessels

New vessels are a feature of severe diabetic retinopathy, and 23 patients had this lesion. The two types which are easily visible are the tufts of looped vessels projecting into the vitreous from a major vein or the optic disk, and the large new vessels following a convoluted and haphazard course across the surface of the retina, unrelated to the orderly, bifurcating pattern of normal retinal vessels. The fine new vessels seen in injection and trypan-digested preparations of the retina probably represent dilated capillary channels around areas where there is capillary closure (Ashton, 1959). These fine new vessels are seldom visible ophthalmoscopically.

When these fine new vessels are filled with fluorescein they become easily visible. In Fig. 6 A (Special Plate) retinal photography shows new vessels lying between patches of hard exudate, but in Fig. 6 B fluorescence photography reveals many more new vessels. In comparison with the orderly lattice of normal capillaries these new vessels appear larger and more convoluted and there is considerable segmental dilatation of the walls, especially where they abruptly change course. Alongside two arteries are clear zones bordered by fine new vessels, a pattern Ashton (1953) demonstrated with Indian-ink injection preparations. In the capillary bed, where it is not obscured by new vessels, small black spots may be seen devoid of fluorescein and unrelated to haemorrhages, and these are probably areas of capillary closure.

Fig. 7 (Special Plate) is another fluorescence photograph of new vessels situated mainly on the surface of the retina. The walls of many new vessels have blurred outlines compared with the normal retinal vessels, suggesting leakage of fluorescein through the vessel walls. This process of leakage or transudation is most marked in new vessels of the rete mirabile illustrated in Fig. 8 (Special Plate). This rete, arising from the right inferior temporal vein and its tributaries, fills with fluorescein in the early venous stage of the circulation of the dye. The vessel loops appear momentarily as narrow lines of fluorescence, but their apices rapidly become broad bands with blurred edges, indicating rapid leakage of fluorescein, as seen in Fig. 8 B, three seconds after the transit of the fluorescein. It shows a diffuse fluorescence glow in the vitreous adjacent to the looped new vessels, seen as black lines new empty of fluorescein.

New vessels near the disk fill early in the circulation of the dye because these vessels derive their blood supply from the arteries close to the optic disk. Some of the new channels appear to shunt blood from artery to vein, while other new vessels have a prolonged circulation time.

### 3. Retinitis Proliferans

Fifteen patients had retinitis proliferans, the most common lesion being a frond of fibrous tissue arising from the optic disk. Other lesions were flat sheets on the surface of the retina and small tufts situated away from the disk, some being astride arteries or veins. The vessels in the retinitis proliferans fill early in the arterial stage of the circulation of the dye, and, where the frond arises from the disk, the vessels appear to fill from the retinal arteries as they pass over the disk. Fluorescein in the vessels of retinitis proliferans passes quickly into the adjacent retinal veins. As in the new vessels, the fluorescein appears in the vessels of the retinitis proliferans initially as narrow lines with sharp edges, and almost immediately the edges become blurred and widened from leakage. The fluorescence glow rapidly extends throughout the mass of retinitis proliferans and into the adjacent vitreous and persists there for periods of at least an hour. Fig. 9 (Special Plate) illustrates the circulation of fluorescein through the vessels of the retinitis proliferans and the massive leakage of the dye.

Retinitis proliferans, in the form of plaques of fibrous tissue on the retina, behaves similarly. Where such plaques lie across a vein they can impede the return of fluorescein in that vein.

In vitro experiments comparing the intensity of light emitted from solutions of fluorescein in plasma and blood show a much greater emission from the plasma solutions as haemoglobin absorbs some of the emitted light. Similarly in Figs. 7, 8 B, and 9 B and C the emitted light from the fluorescein outside the new vessels is more intense than that from fluorescein within vessels which are not leaking.

### 4. Exudates

In the 13 patients with hard exudates, fluorescence retinal photography revealed no characteristic vascular pattern in relation to these lesions. Sixty out of approximately 300 individual patches of hard exudate were close to microaneurysms, many of which were leaking. However, other microaneurysms in the same patients were not associated with hard exudates. None of the exudates fluoresced in the way observed with soft exudates in hypertension (Hodge and Dollery, 1964). Fig. 10 (Special Plate) illustrates these features.

**Discussion**

With the ophthalmoscope it is difficult to distinguish fine red dots and vessels against the orange background of the retina. By introducing a contrast material into the retinal circulation fluorescence retinal photography reveals many more microaneurysms and fine new vessels than are visible ophthalmoscopically and narrows the gap between what can be seen in the living patient and the wealth of detail seen in trypan-digested preparations of the retina (Cogan et al., 1961). While the fine reticulum seen in the fluorescence photograph of the normal patient (Fig. 2) is made up of capillaries, we have never seen individual capillaries in diabetics. Fig. 2 was obtained under exceptionally favourable conditions where the media were clear and the fluorescein was introduced by an arterial injection (Dollery et al., 1963). When the fluorescein is introduced into the superior vena cava the slug of dye is diluted by passage through both sides of the heart and the pulmonary circulation. This and the presence of a very slight...
haziness of the media in a large proportion of diabetics made it difficult to visualize the capillaries in this study. Although, in Fig. 6 B, an arterial injection of fluorescein has shown up zones of capillary closure alongside arteries and scattered small areas of capillary closure (Ashton, 1953), we have been able to discern this phenomenon in only three patients after intravenous injections; usually it is not until microaneurysms and fine new vessels develop that we can show up lesions in the capillary bed with the present intravenous technique.

Besides providing fine anatomical details of the lesions in diabetic retinopathy, fluorescence photography gives information on the permeability of vessels. A proportion of the microaneurysms, segments of the new vessels in the retina, the new vessels projecting into the vitreous and in the retinitis proliferans, all show leakage of fluorescein. It is not surprising that these vessels have abnormally permeable walls because of the alterations in the basement membrane of the endothelium in the microaneurysms (Bloodworth, 1963) and the defective adventitial layers of the new vessels (Ballantyne, 1946; Gartner, 1950).

Hodge and Dollery (1964) have shown that on equilibrium dialysis up to 80% of fluorescein in plasma is bound to the proteins. They infer that this protein-binding is easily reversible because of rapid loss of fluorescein from the blood into the extracellular fluid after an intravenous injection. While it is certain from the photographic studies that free fluorescein (molecular weight of 361) is leaking from these microaneurysms and abnormal vessels, it is possible that fluorescein in the protein-bound form is leaking out as well.

Abnormally permeable vessels could explain the retinal oedema and vitreous haze encountered in some patients with diabetic retinopathy. Retinal oedema, for example, was observed ophthalmoscopically in the young girl whose retinal photographs (Fig. 6 A and B) show numerous fine new vessels with leaking segments. Vitreous haze occurs in patients with a retite mirabile or retinitis proliferans, as in the patients whose fundi are illustrated in Figs. 8 and 9. It is often unrelated to previous vitreous haemorrhage, it fluctuates throughout the day, and it is easily discerned by patient and clinician. Continued leakage of plasma proteins from the vessels of the retite mirabile and retinitis proliferans may cause this turbidity of the vitreous.

Information about the circulation rate in the retinal vessels is restricted to direct comparative observations of different vessels in the same field after each injection of fluorescein. We are able to recognize only prolonging filling of some microaneurysms and rapid shunting and delayed drainage of certain vessels, but only relative to neighbouring microaneurysms and vessels.

These preliminary studies have shown that fluorescence photography can reveal the large proportion of small-vessel abnormalities hitherto invisible in the living patient, and can also demonstrate the important functional abnormality of enhanced permeability of these vascular lesions. Further studies may prove useful in elucidating the life-history of specific lesions, as a guide to prognosis in individual patients and in assessing the response to various forms of treatment.

Summary

Retinal photography while fluorescein traverses the retinal circulation has been used to study 41 diabetics with visible retinopathy.

Microaneurysms and fine new vessels were usually shown in far greater numbers and detail than could be observed ophthalmoscopically and with routine retinal photography.

Leakage of fluorescein occurs from a proportion of microaneurysms and segments of the fine new vessels, and more pronounced leakage takes place from the large new vessels and into retinitis proliferans.

No constant or characteristic lesion was seen in retinal vessels adjacent to hard exudates.

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References


Forced Expiratory Time: A Simple Test for Airways Obstruction


This paper describes a study of a simple physical sign of diffuse airway obstruction undertaken to see how accurate it is in comparison with more refined methods. Diffuse airway obstruction is a feature of asthma and of the chronic non-specific disease which in Great Britain is commonly labelled as chronic bronchitis and emphysema. Clinical examination as usually practised is not a very good method of detecting or evaluating diffuse airway obstruction: first, because the classical techniques of inspection, palpation, percussion, and auscultation are usually taught with emphasis on the detection of localized or lateralized conditions; secondly, because physical signs which are generally sought, such as the barrel-shaped chest, poor expansion, and diminished cardiac and hepatic dullness, are related to hyperinflation which is secondary to obstruction, and, in any case, they may be present in elderly subjects without chest disease. Direct evidence of obstruction is usually elicited only by listening to the chest; even then the types of rhonchi

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