

Fluorescein angiography of the anterior segment

Its value in corneal disease

D. L. EASTY AND A. J. BRON

Department of Clinical Ophthalmology, Moorfields Eye Hospital, London

In conjunctival and corneal diseases, invasion of the corneal substance by vessels is often the forerunner of more serious disease. The study of vessels in detail is of importance in the assessment of such disease processes and, in particular, in following their natural history and response to treatment.

Reports of fluorescein angiography of the anterior segment have generally concerned the microvasculature of the iris. Vannas (1969) described in some detail his technique of fluorescein photography, and demonstrated the angiographic findings in pseudoexfoliation of the lens capsule and in various types of glaucoma. The effects of iris disease on the fluorescein pattern have been reported by other workers; Cobb (1968) noted the fluorescence of vascular tufts at the pupillary margin, which he later noted were particularly common in myotonic dystrophy (Cobb, Shilling, and Chisholm, 1970). The only report concerning the fluorescence of the microvessels found in corneal disease has come from Mitsui, Matsubara, and Kanawaga (1969), who demonstrated the vascular patterns associated with trachoma and with herpes simplex keratitis.

We have recently started serial photography of the fluorescence of abnormal vessels in diseased corneae, and the corneae of some 250 patients have been examined. In this report, selected records from some of these patients will be presented and discussed and their value assessed.

Materials and methods

The Zeiss photo slit-lamp camera was modified in the following ways in order to make it suitable for recording fluorescein angiograms. The power pack of the photo slit-lamp camera was replaced by a modified Zeiss Fundus Camera power pack. With this arrangement and the number 4 flash setting, firing at 2.0–2.5 second intervals could be achieved. The excitation filter used was of the absorption type (Zeiss 30 : 14 : 13) and was placed before the flash source. More recently a Balzar interference filter (FITC 4) has been used with greater success. A Kodak Wratten 15 barrier filter was placed in front of the camera objective. In order to view fluorescence as it appeared barrier filters were included in the eye pieces. Kodak Tri-X film was used (ASA 400) and this was developed for 12 minutes in ID 11 at 68°F.

Observations

TRACHOMA

Fig. 1 shows the angiogram of a patient with inactive trachoma. There is a localized patch of pannus demonstrated in the venous phase of the angiogram. The complex

arcades of vessels are shown, and it can be seen that there is a mild leakage of fluorescein dye from the vessels of small calibre. The amount of leakage, though not great, is more than that which would be seen in normal limbal arcades. It is worth stressing that though the patient complained of minor irritative symptoms in this eye, the pannus in both eyes was clinically inactive; although superficial scarring was present, there were no infiltrates and there was no corneal oedema.

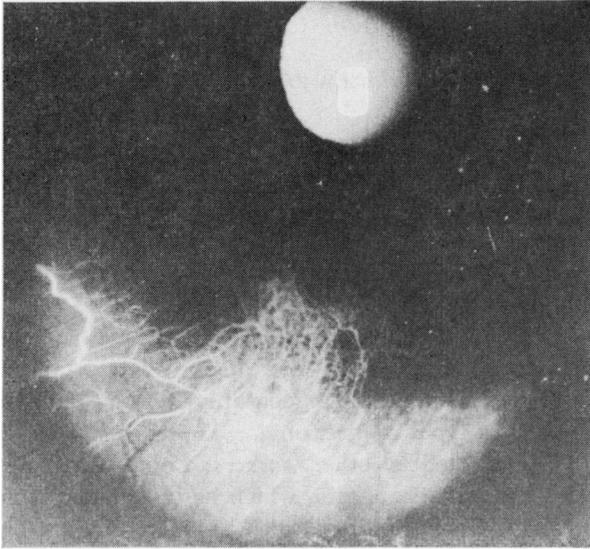


FIG. 1 *Microvascular anastomosis in the left eye of a patient with Grade IV trachoma*

A further point of some interest in this patient is that, despite a course of topical Predsol four times a day to the left eye for one week, no reduction occurred in the amount of leakage or in the form of the pannus. This contrasts, for instance, with the effect of steroids applied topically in a patient with a vessel-mediated homograft reaction, in which both reduction of leakage and regression of vessels occurred (Bron and Easty, 1969).

VESSELS IN CLEAR CORNEAL STROMA

The angiogram of vessels in the corneal stroma of an eye becoming phthisical after detachment surgery is demonstrated in Fig. 2. The vessels branch dichotomously and the branches of neighbouring systems interdigitate. Leakage occurs first from the terminal vessel loops, both afferent and efferent limbs, and is moderate in degree. It seemed likely that the case was one of anterior segment ischaemia, corneal vascularization having been recorded in this disorder (Sanders and Hoyt, 1969).

Why these vessels invade the optically clear corneal stroma is not known. Invasion in response to an ischaemic stimulus might be postulated. It might also be suggested that stromal oedema facilitates vessel entry. There is experimental evidence to show that aqueous humour production is reduced by interference with arterial supply to the ciliary body (Bárány, 1947), and ciliary body ischaemia with a reduction in the turnover of aqueous would be associated with a fall in aqueous oxygen tension. The resultant hypoxia of the posterior cornea could both produce oedema by interference with the endothelial pump and provide a stimulus for vessel ingrowth.

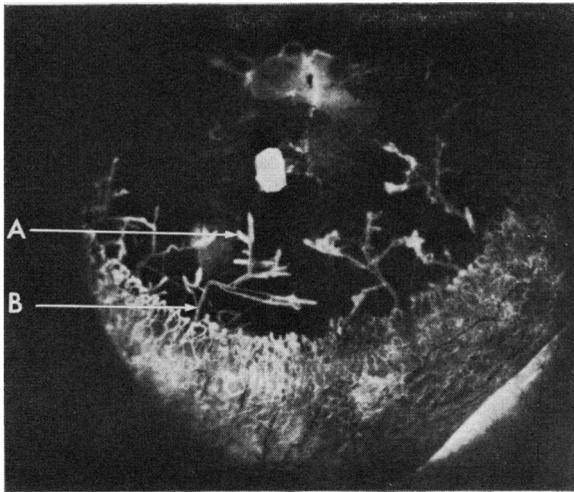
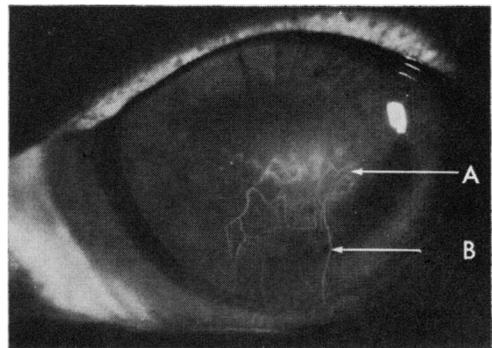
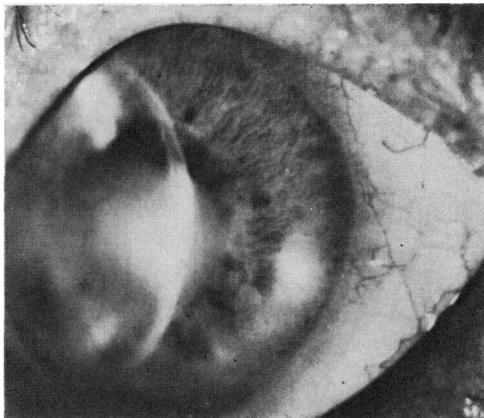


FIG. 2 *Fluorescein angiogram in a vascularized cornea after retinal detachment surgery (36 sec.). The eye was hypotensive*

- A. Apical leakage
- B. Fluorescing arterioles and venules in close apposition

ANGIOGRAPHY IN CLOUDY CORNEAE

Loss of corneal clarity may be due to corneal oedema, infiltration, or scarring, and to the deposition of material such as lipid. Whatever the cause of the opacity, fluorescein angiography often is able to demonstrate vessels which are difficult to photograph, or to observe clinically by slit-lamp examination. Fig. 3 demonstrates a central corneal lesion due to herpes simplex in a 56-year-old male patient with a long history of recurrent stromal disease in his left eye. The central cornea showed mild epithelial and stromal oedema but clinically there was no active keratitis. Angiography was performed shortly before corneal grafting. In Fig. 3*b* the vascular network is clearly shown in the venous phase of the dye transit (35 seconds). There is a definite difference in behaviour between the axial and the peripheral portions of the vessel network with respect to leakage. There is focal leakage of a mild degree from the terminal vessel loops, but none from the proximal arcades. There is thus a clearly-defined functional difference between the two areas. It may be noted that the survey photograph of the cornea completely failed to demonstrate the complexity of the vascular network (Fig. 3*a*).



- A. Area of focal leakage
- B. Venous tributaries

FIG. 3 (a) *Inactive stromal herpes simplex keratitis* (b) *Venous phase of angiogram (35 sec.)*

Fig. 4 (overleaf) shows the angiogram of stromal herpes affecting the upper cornea of the

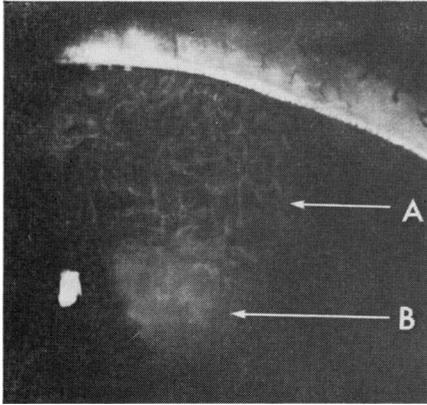


FIG. 4 *Vascular anastomosis in stromal herpes simplex keratitis (7 sec.)*

- A. Proximal vascular plexus
- B. Central zone of fluorescein leakage

left eye in a 60-year-old male patient. The affected cornea was grossly oedematous and vascularized, and the question whether active disease was present became important. In the fluorescein angiogram at 7 seconds the upper portion of the lesion can be seen to be well vascularized, with a complex arrangement of large venules and small arterioles. The illustration demonstrates moderate leakage towards the axial edge of the lesion. The degree of leakage is not as great as that encountered in the presence of the typical vasculitis seen in active herpetic keratitis. It is therefore likely that the stromal oedema is due more to endothelial dysfunction than to an active keratitis.

Lipid deposition may occur in the cornea in various conditions. It is a comparatively common occurrence and in the majority of cases it is difficult to understand the mechanism. Duke-Elder (1968) has summarized the literature and classified corneal lipid deposition into three fundamental groups. It may be primary, in which case it is deposited in an apparently normal cornea, or secondary, in which case it represents a terminal change occurring in degenerating cells. Under the term "lipid keratopathy" are grouped those cases in which the appearance of fat is preceded by corneal vascularization. It is noteworthy that the intensity of intravascular fluorescence and of fluorescein leakage may vary considerably from case to case.

Lipid deposition is a common occurrence after ophthalmic herpes zoster (Cogan, 1960). In a 57-year-old female with a crystalline deposition in a peripheral corneal scar

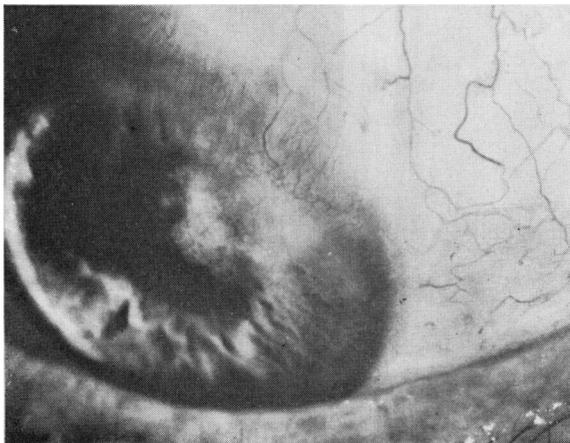


FIG. 5 (a) *Paralingual lipid keratopathy*

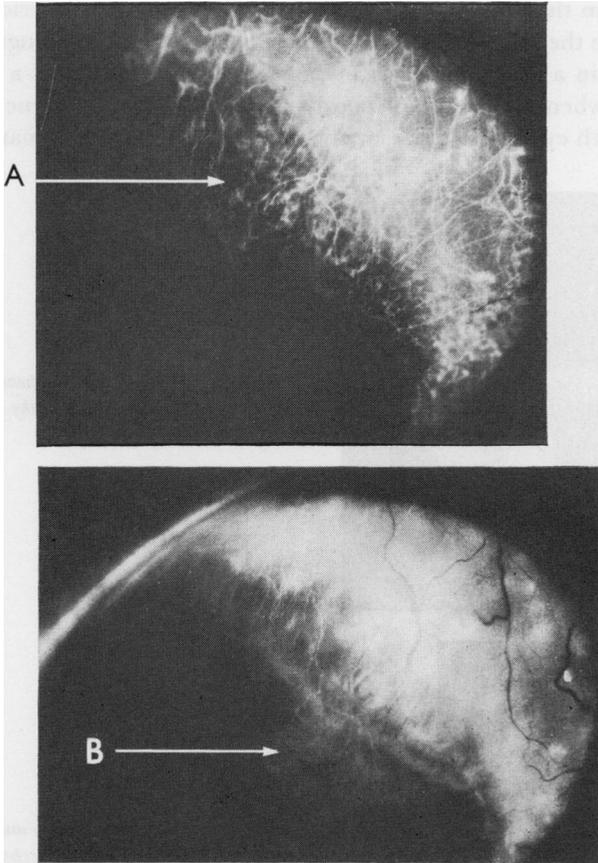


FIG. 5 (b) and (c) Angiography at 30 and 70 seconds respectively

A. Proximal zone of vascularization, showing early leakage

B. Distal zone of vascularization appearing as an area of faint leakage

in the left eye (Fig. 5a), the fluorescein angiograms demonstrated only moderate intravascular fluorescence at 30 seconds (Fig. 5b), and leakage into the stroma at 70 seconds was comparatively slight (Fig. 5c).

Fig. 6 shows a peripheral, post-zoster corneal scar with lipid deposition in the left eye of a 57-year-old female patient. The deposition was made up of a deep and a superficial

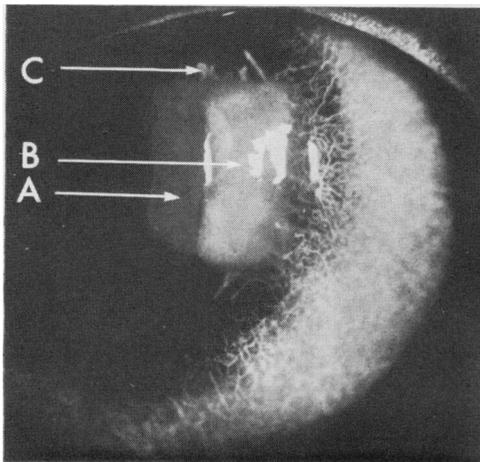


FIG. 6 Angiogram in a case of lipid keratopathy after an attack of herpes zoster ophthalmicus (22 sec.)

A. Deep lipid deposits

B. Superficial lipid deposits

C. Stromal vessels hidden by the pseudo-fluorescence of the superficial plaque

lamella, one lying anterior to the other. In this case the density of the anterior deposit has been sufficient to obscure the disposition of the deep set of vessels seen on angiography.

Fig. 7a shows the cornea in a 50-year-old man whose eyes were injured in a chemical explosion 16 years before, when a mixture of poorly nitrated dichlorobenzene and sulphuric acid splashed into both eyes. Diffuse scarring of the left cornea with marked lipid

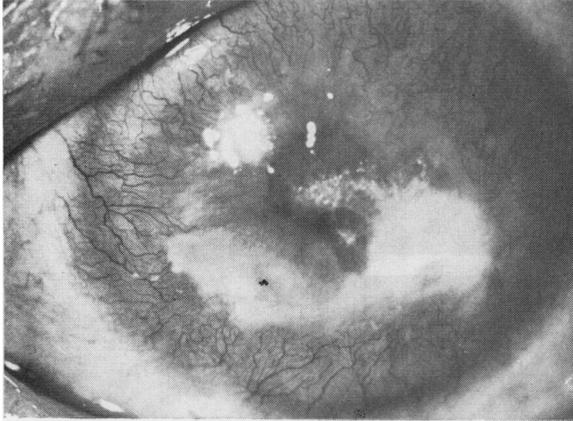


FIG. 7 (a) Corneal appearance in a patient with lipid keratopathy following a chemical injury

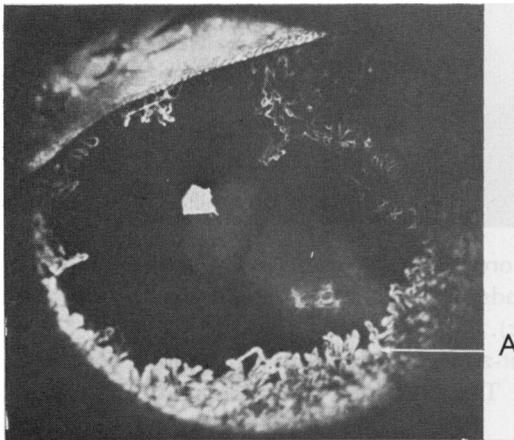
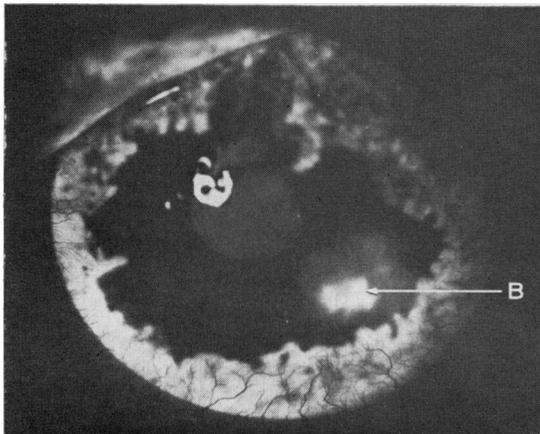


FIG. 7 (b) and (c) Early and late angiograms at 20 and 47 sec. respectively

A. Extensive superficial pannus



B. Intense leakage in stroma in relation to a lipid deposit

deposition occurred, but it was of interest in that this did not develop until some years after the original injury. The history is reminiscent of the natural history of mustard gas keratitis, and the similarity was borne out by the presence of aneurysmal dilatations of the veins of the conjunctiva. The angiograms are shown in Fig. 7*b* and *c*. The main feature of the early angiogram is an elaborate circumferential pannus. In the later angiogram an island of localized leakage of high intensity is seen in the lower temporal quadrant of the cornea. This corresponds to a plaque of dense lipid deposition. The apparent isolation of this vascular zone from the periphery is due to masking of the vascular supply by the corneal scar.

An interesting situation occurred in a 44-year-old female with extensive peripheral lipid deposition in both corneae of over 20 years' standing. On biomicroscopy, a number of radial vessels were visible, but the degree and complexity of the deeper vessels could not be appreciated. Fig. 8 shows the fluorescein angiogram taken 22 seconds after the initial injection. A fine and intricate plexus is clearly demonstrated. The capillaries

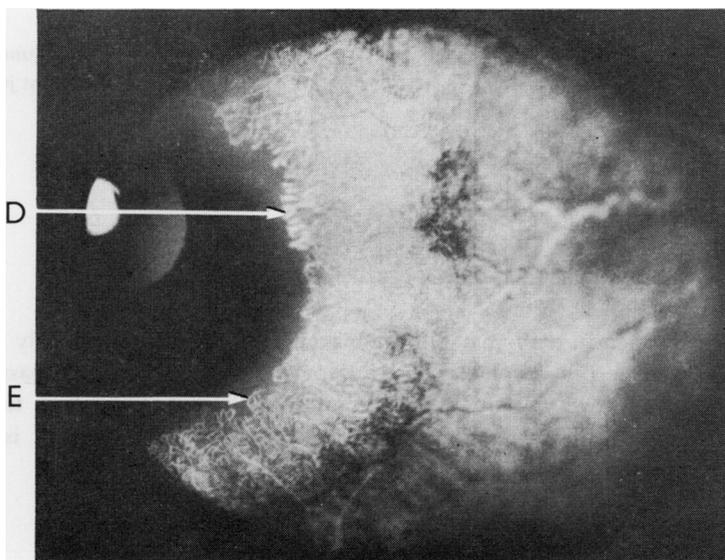


FIG. 8 *Fluorescein angiogram in the right eye of a 44-year-old woman with a dense lipid keratopathy (22 sec.)*

D. Early leakage in some areas
E. Fine capillary network

form arcades between the arterioles and venules leading to a regular and complex architectural arrangement. The angiogram is of interest because the intensity and speed of onset of the leakage were remarkable and the eye showed no signs of clinical activity. Furthermore, the angiogram suggests that the predominant source of supply to the capillary plexus is provided by the superficial perilimbal arterioles. It seems likely that the lipid has been deposited in a superficial pannus. Certainly the distinctive pattern with its regular architecture is entirely different from that seen in other cases of lipid deposition in the cornea so far described.

VESSELS AND CORNEAL GRAFTING

A study of vessels in the corneal graft situation is of importance; their presence is undesirable in the donor disc, chiefly because of their significance in the production of a homograft reaction. Fig. 9 (overleaf) shows a penetrating graft for a herpetic stromal abscess 16 days after surgery. The graft was performed through a previous lamellar graft. It was

optically clear at the time of examination and continued to remain so. The angiogram shows the disposition of vessels at the interface of the earlier graft. The irregular vascular ring is fed by radial vessels. Deeper vessels in the bed of the lamellar graft pass to the new graft interface, and there is a diffuse and moderate leakage, which however does not transgress the graft-host junction.

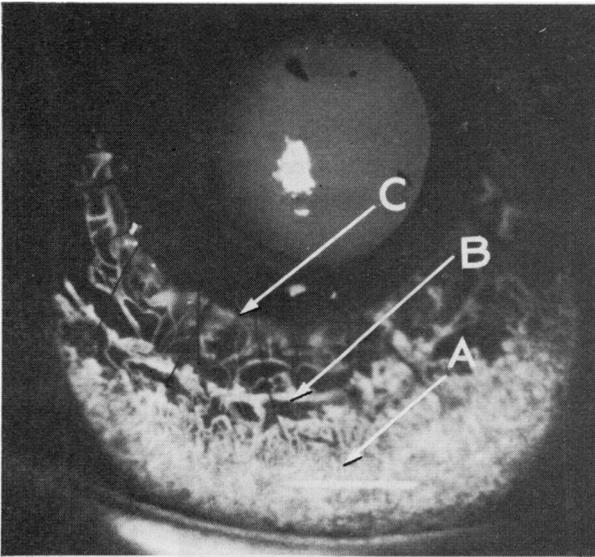


FIG. 9 *Vascularization at the interface in a 7-mm. penetrating graft after an herpetic stromal abscess (27 sec.)*

- A. Limbus
- B. Lamellar graft-host junction
- C. Penetrating graft-host junction

Fig. 10 shows the cornea in a 27-year-old male grafted $5\frac{1}{2}$ months previously for central scarring due to stromal herpes simplex. The angiogram shows the vessels passing to the graft-host junction. The eye was quiet on a regime of Predsol drops four times daily. It is seen that the vessels fluoresce faintly and although leakage occurs it is minimal. Vessels do not enter the graft itself.

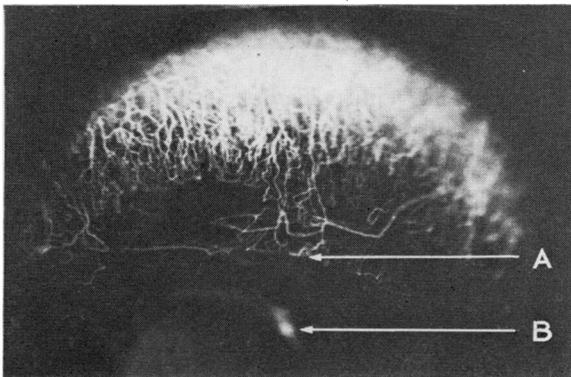


FIG. 10 *Angiogram of vessels reaching interface of a penetrating graft (24 sec.)*

- A. Position of interface
- B. Point of focal leakage from iris at pupil margin

In a 57-year-old female patient, who had been grafted on four occasions between 15 and 19 years ago for left herpetic keratitis, the final graft became cloudy after endothelial decompensation (Fig. 11*a*). The dense plexus of vessels in the recipient cornea is shown in Fig. 11*b*. Small vessel twigs may be seen passing into the temporal edge of the graft

between the twelve and 4 o'clock meridians. Leakage into the host cornea is pronounced. Leakage into the graft cornea, however, seems to be related to the presence of vessels in the graft itself (Fig. 11c); it is therefore seen within the temporal edge of the graft in relation to the vascular twigs, but it is virtually absent on the nasal side. Here, the host vessels do not reach the graft-host junction. The sharp contrast between the fluorescence of the host and donor corneae delineates the interface. This implies there is a definite barrier to diffusion across the interface.

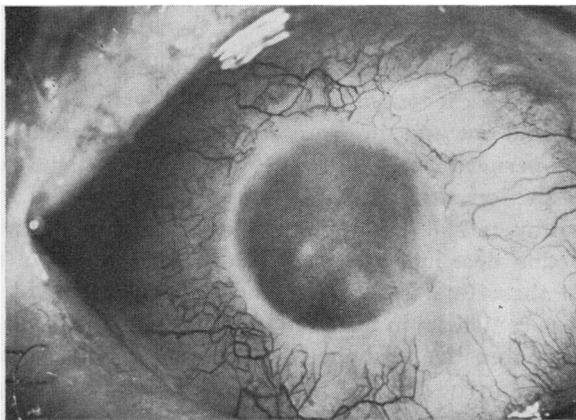
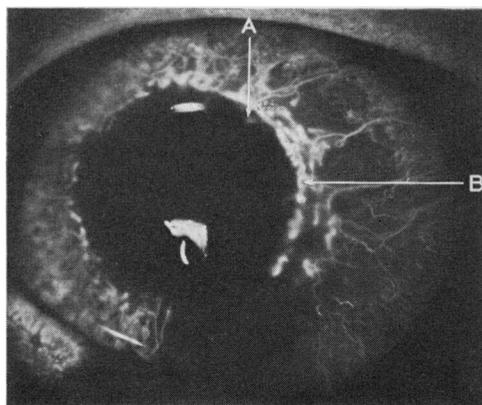
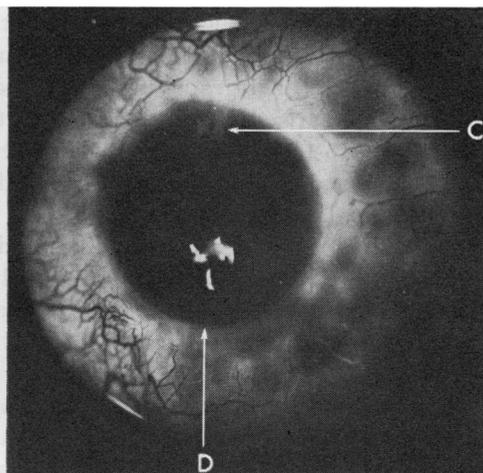


FIG. 11 (a) *Unsuccessful penetrating graft*



- A. Vascular tips in donor cornea
- B. Early and marked leakage at interface



- C. Leaking vessels in donor cornea
- D. Interface sharply demarcated

FIG. 11 (b) and (c) *Angiograms at 26 sec. and 1 min. 38 sec. respectively*

Discussion

In assessing the value of fluorescein angiography of the anterior segment, one must first determine what information it is able to provide. Fluorescein angiography of the retina reveals a stereotyped vascular system in which there are clear arterial, capillary, and venous phases of the dye transit. Against such a background, abnormalities in vascular pattern can be clearly demonstrated.

In fluorescein angiography of the cornea, the information that can be gathered is entirely different in nature. The cornea is an avascular tissue, and so the very existence

of vessels represents an abnormality. There is therefore no stereotyped pattern of normality in vascular anatomy with which to compare the abnormal. However, fine vascular plexuses which are difficult to record with accuracy by drawings or photography are clearly demonstrated by intravascular fluorescein and photography, though it is often more difficult to observe arterial and venous phases. This may be because the rate of circulation appears to be rapid and the arterial phase may be easily missed. At the same time, a diseased cornea may be invaded by many plexuses in which each vascular bed is distinct. Hence it is unlikely that arterial and venous phases will be in phase with each other, which adds to the problem of interpretation.

Leakage of dye from the vessels is a prominent feature of angiography of the globe and the vascularized cornea is no exception. The concept of damaged vessels leaking material into the tissues is an old one and the temptation to equate leakage of fluorescein dye with an increase of vessel permeability is difficult to resist. In the study of the microvasculature of the retina by the technique of fluorescein angiography, this interpretation is entirely justified, for healthy retinal capillaries are impermeable to fluorescein and leakage of the dye in the retina does indicate pathological permeability of these vessels. However, the behaviour of the retinal capillaries, and to some extent of the cerebral capillaries, differs from that of capillaries in tissues in other parts of the body (Hill, 1969). The capillaries of the skin, conjunctiva, kidneys, and peritoneum, for instance, readily allow free fluorescein to escape from the plasma. It has been demonstrated experimentally that the retinal vessels possess an active transport mechanism which prevents the movement of the fluorescein anion out of the vessel lumen (Cunha-Vaz and Maurice, 1966). This accounts, in part, for the impermeability of healthy retinal vessels to fluorescein during angiography; the behaviour of the conjunctival vessels and of vessels in the vascularized cornea suggests that they do not possess such a mechanism.

Up to 85 per cent. of fluorescein in the blood is bound to plasma protein, chiefly to albumin, while the rest is free and occurs chiefly in the anionic form at blood pH (Hodge and Dollery, 1964).

In pathological terms increased vessel permeability is characterized by the passage of colloids and cells from the blood stream into the tissues. Of the colloid particles, the smallest molecules will pass out more readily than the largest, so that albumin will pass out more readily than globulin. It is clear that the leakage of fluorescein from healthy conjunctival vessels must involve the loss of free fluorescein anions into the extracellular space. In the presence of inflammation, an increasing loss of albumin-bound fluorescein from the vessels would occur, in addition to the loss of free fluorescein.

Theoretically, there are two ways by which fluorescein may be lost from the capillary lumen: by diffusion along a concentration gradient, and by bulk flow of fluid, that is, as a result of a net loss of fluid from the intravascular to the extravascular compartment. The rate of diffusion will depend on the concentration of dye in plasma and on the resistance of the endothelial barrier to the diffusion process. Increase of endothelial permeability associated with inflammation not only allows protein-bound dye to diffuse from the vessel, but also reduces the resistance to diffusion of the free dye, so that both components are increased in the tissue fluids.

The contribution of bulk flow of fluid to the transfer of fluorescein across the vessel endothelium depends largely on the magnitude of the bulk flow in the body tissues. This in turn depends on the balance of hydrostatic and colloid osmotic forces acting inside and outside the vessel lumen. These factors determine the effective filtration pressure driving fluid across the capillary endothelium. The cornea differs from other tissues in that it

possesses a highly negative fluid pressure in the stroma in the deturgescenced state. The stromal fluid pressure has been estimated to be in the region of -40 mm.Hg in the living eye (Mishima, 1968), whereas the interstitial pressure in subcutaneous tissue is said to vary from zero to -7 mm.Hg (Guyton, 1966). For this reason the effective filtration pressure would be high across vessels in the cornea of normal thickness compared to vessels in other tissues, and bulk flow from these vessels may be expected to be higher as a result. However, it must be remembered that the stromal lamellae are tightly packed and form a definite barrier against the bulk flow of fluid. It must therefore be supposed that fluorescein escapes from vessels into the stroma by means of diffusion, and that transport by bulk flow is of minor importance. The cornea, therefore, does not differ from other tissues, in which the vessels lose small molecules by means of diffusion rather than by bulk flow (Pappenheimer, 1953).

In the inflamed cornea, the leakage of dye is often increased, and it is possible that this depends upon the increased flow rate, permeability, and total area of permeable endothelium. It has been shown that, in active inflammatory corneal disease, the leakage of fluorescein is intense, and that as the inflammation dies down the leakage is reduced. But there are certain situations in which the leakage is intense in the absence of apparent inflammatory disease. Patients with lipid keratopathy may demonstrate intense leakage without serious inflammation and it is possible that this may be related to the process of lipid deposition.

The corneal graft situation is one in which fluorescein angiography might be expected to be of value. In a straightforward corneal graft, host vessels start to pass towards the donor button but, instead of penetrating the graft, they either cease their centripetal growth before reaching the graft, or having reached it take a circumferential course along the graft-host interface. Fluorescein angiography shows whether fine vessel twigs enter the graft and provides a permanent record against which the further movement of the vessels may be assessed. The entry of host vessels into the graft is of importance, not only because of the immediate danger of a vessel-mediated homograft reaction but because at a later stage, when the eye is quiet and the graft clear, such vessels may still carry blood and may dilate in response to non-specific inflammatory stimuli. The graft is then exposed to the danger of a homograft reaction as a late event.

The interpretation of leakage from vessels in the neighbourhood of a graft requires careful consideration. There does seem to be some sort of barrier to the diffusion of dye within the stroma from host to graft. However, the barrier is not absolute and some dye does pass across the interface. This is particularly true when a circumferential vessel passes along the interface for some distance, an occurrence which is not uncommon. It would be easy to interpret the movement of fluorescein across the boundary as significant in the immune processes leading up to a homograft reaction. But it must be remembered that the dye is only a marker, either in the form of free anion, or bound to albumin. Whatever the form, when we are considering the efferent limb of the non-cellular component of the graft reaction, we are concerned with globulin, not albumin, and it is known that the resistance to diffusion of molecules through corneal stroma is greatly affected by molecular size (Maurice, 1969).

One form of leakage has been mentioned only in passing, but is seen quite frequently. Although leakage is usually most developed at the apical tips of arterio-venous loops or within more complex capillary plexuses, it is often seen along the larger vessel trunks, particularly the veins, in a late phase (Fig. 4). This perivenous leakage is probably a true loss of dye through the vessel walls (Zweifach, 1966), but other alternatives may be

considered. Lymphatics are absent from normal cornea except at the limbus, but they are known to be present in vascularized corneae (Collin, 1966) and it is therefore possible that protein-bound fluorescein may be removed from the cornea in part by this route and may become visible. It is also possible that dye may diffuse in the stroma from a site of intense vessel leakage, centrifugally along main vessel trunks passing to and from the lesion. It should not be difficult to investigate in histological material whether a perivascular space exists around such vessels.

Summary and conclusions

A technique of fluorescein angiography has been developed for the study of microvessels in the diseased cornea. Simple modification of the Zeiss photo slit-lamp camera allows photographs of good quality to be made at a frequency of one exposure every 2.0 to 2.5 seconds. Selected examples from some 200 patients are presented and discussed. It is concluded that the method is of value in accurately recording complete vascular patterns in the cornea, and in particular that it may be used in assessing changes in vascularity over a period of time. It has been used effectively in following the response of corneal disease to therapy. The significance of the leakage of fluorescein from microvessels is discussed, and it is pointed out that, while no simple conclusion can be drawn about this process when comparing cases, it may be of some value in following a condition in an individual case over a period of time. In the latter situation, it may reflect changes in the inflammatory activity of a disease process.

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