Communications

Fluorescence irido-corneal photography

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Fluorescein is widely used in ophthalmology for various purposes. Ehrlich (1882) observed that fluorescein introduced intravenously appeared in the aqueous humour. This phenomenon was employed for examination of the blood–aqueous barrier by Amsler (1946). Pflüger (1882) introduced a method of staining corneal erosion and ulceration by the use of fluorescein.

Coons, Creech, and Jones (1941) introduced a method of immunofluorescent staining, which is now used in the diagnosis of ocular herpes simplex virus and adenovirus infection, and Novotny and Alvis (1961) introduced a method of fluorescence fundus photography, which has enabled us to study the retinal circulation, vascular disorders of the retina, and other retino-choroidal diseases.

Fluorescence irido-corneal photography is a modification of fluorescence fundus photography (Mitsui and Matsubara, 1968). We have found this procedure very useful in the analysis of vascularization of the cornea and iris.

Method of photography

Any apparatus for slit-lamp photography equipped with a bright electronic flash may be used. Among them the Nikon-zoom photo-slit lamp seemed to be the best. The Zeiss photo-slit lamp may also be used.*

Two filters were attached to the photo-slit lamp: a blue/violet-excitiser filter† was attached to the housing of the electronic flash and a blue/violet-absorption filter‡ was placed in front of the film.

The patient was injected intravenously with 10 ml. 5 per cent. solution of sodium fluorescein. The injection was carried out as fast as possible, at least within 10 seconds. Possible side-effects of the injection, usually a slight nausea, were prevented in most instances by the intramuscular administration of metoclopramide§ 10 mg. (2 ml. of 0·5 per cent. solution) 10 minutes before the photography. Slit-lamp photographs of the cornea or iris were taken at intervals after the injection of fluorescein.

The slit of the slit lamp was opened to its maximum, so that a discoid illumination was obtained. The exposure dial of the camera was set either to that for photography under electronic flash, which was usually indicated by X, or to 1/30 sec. The maximum loading was given to the generator of the

* The minimum interval of serial photography depends on the time for the maximum charging of the generator. The time for the maximum charging is about 10 sec. in the Nikon and 25 sec. in the Zeiss apparatus.
† A cobalt filter which allows chiefly blue and violet rays to pass. A Fuji gelatin filter No. 18 was used in the present experiment, which had a similar absorption curve to that of the Kodak Wratten filter No. 47.
‡ A green filter which allows green fluorescence to pass but cuts off blue/violet rays. Fuji gelatin filter No. 17 was used, which was similar to Kodak Wratten filter No. 58. A yellow filter may also be used, but the contrast of corneal vessels is lower by a yellow filter, as the iris image appears brighter. It should not be used, therefore, for Caucasiuns with light-coloured irides.
§ Primperan © manufactured by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan.
Results

BEHAVIOUR OF CAPILLARIES AT THE NORMAL LIMBUS

Fig. 1 is a schematic illustration of the normal limbus as observed by fluorescence corneal photography. The first side section illustrates the anatomy of the limbal capillary plexus modified from Vogt (1930). The other four sections show diagrammatically the chronological sequence of the fluorescence corneal photographs of the limbus. The second section from the left shows the earliest finding, which may be termed the prevascular phase. The upper part of the section, shown as black dots, represents the conjunctiva, which has its own fluorescence or autofluorescence. The lower part represents the cornea in which nothing is seen because the fluorescein has not yet reached the limbal vessels. This prevascular phase lasts for 15 to 20 seconds. Fluorescein then reaches the conjunctival vessels and the vascular phase appears as shown by the next two sections of the illustration. In the normal limbus the blood stream is seen only in some of the capillaries. Therefore, the fluorescence appears as strands of cotton waste attached to the limbus. In the early vascular phase (third section), the fluorescein has not yet leaked from the capillaries, but in the late vascular phase (fourth section), fluorescein leaks from the capillaries into the surrounding tissues. The leakage is, however, so diffuse that we cannot say that any particular blood vessels are responsible for it. The vascular phase lasts for a few minutes. The fluorescence then disappears from the blood vessels and the postvascular phase shown in the last section of the illustration is reached. During this phase the fluorescence gradually approaches the centre of the cornea.

![Diagram](image)

**FIG. 1 Diagrammatic representation of normal limbus observed by fluorescence corneal photography**

Fig. 2 shows actual pictures of the normal limbus as observed by fluorescence corneal photography. 10 seconds after the fluorescein injection during the prevascular phase (a), the conjunctiva shows its autofluorescence and the cornea looks black. (b) shows the same limbus 20 seconds after the injection during the early vascular phase, the limbal capillaries are seen as cotton waste attached to the limbus. (c) shows the same limbus 40 seconds after the injection during the late vascular phase; diffuse leakage of fluorescein is apparent at the limbus, but the capillaries are still fluorescent. 3 minutes after the injection during

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* Set the selector switch for the flash generator at position 4 (Nikon) or IV (Zeiss).
† For the Zeiss instrument photographic lens 1 x was used.
‡ When a yellow filter is used for absorption filter, the exposure will be correct, and ordinary development is applicable.
§ Konidol-super § produced by Konishiroku Photo Ind. Co. Ltd., Tokyo, was used. The ratio of the high speed development was 2 or 3 x.
Fluorescence irido-corneal photography

**Fig. 2** Fluorescence corneal photographs of normal limbus

(a) Prevascular phase (10 sec. after fluorescein injection)
(b) Early vascular phase (20 sec.)
(c) Late vascular phase (40 sec.)
(d) Postvascular phase (3 min.)

In the postvascular phase (d), no capillaries are seen and diffuse fluorescence is approaching the centre of the cornea. The dark portion at the upper edge of each photograph is the conjunctiva beyond the circular area illuminated.

**Differentiation of microscopic pannus from inflamed limbus**

Fig. 3 shows a schematic illustration of pathological limbal findings during the early vascular phase. On the left is the normal anatomical structure, and next a fluorescence photograph of the normal limbus. The next section illustrates the fluorescence phot-

**Fig. 3** Diagrammatic representation of limbus observed by fluorescence corneal photography in various conditions during the vascular phase
graph of an inflamed limbus. In normal eyes blood circulation is seen only in some of the limbal capillaries, but in inflamed eyes the limbal capillaries dilate with resulting congestion in the limbal capillary plexus and fluorescence photography demonstrates the whole anatomy of the limbal plexus, as in the first section. Fig. 4 shows an example of an inflamed limbus caused by anterior uveitis. Attention must be paid to the fact that there is no special leakage of fluorescence from congested capillaries.

FIG. 4. Fluorescence corneal photograph of inflamed limbus in a case of anterior uveitis (30 sec.)

The fourth section from the left in Fig. 3 illustrates an early microscopic pannus. The characteristic feature of progressive vascularization when examined by fluorescence photography is an obvious leakage of fluorescence from the apex of the vessel. Fig. 5 shows a chronological sequence of fluorescence photographs of a progressive pannus. (a) is the early vascular phase, 30 seconds after fluorescein injection, with leakage of fluorescence from the apex of neovascularization (arrows). (b) shows the same limbus after 50 seconds in the late vascular phase; apart from the diffuse leakage of fluorescence

FIG. 5. Fluorescence corneal photographs of progressive pannus after fluorescein injection
(a) after 30 sec.
(b) 50 sec.
(c) 110 sec.
(d) 210 sec.
at the limbus, there are many focal leakages of fluorescein in the cornea, corresponding to
the apex of neovascularization. (c) shows the same limbus after 2 minutes, in the transition
from the vascular to the postvascular phase. (d) shows the postvascular phase after 4
minutes. In (c) and (d) characteristics of progressive pannus still persist, and in (d) some
vessels appear as negative shadows, as will be discussed below.

Another difference between an inflamed limbus and progressive pannus may often be
observed in the postvascular phase. In early or active pannus the corneal blood vessels
often, if not always, appear during the postvascular phase as negative shadows in an
intense surrounding fluorescence (Fig. 6). The leakage of fluorescence from the apex is
so sharp that it reminds us of the iridium tip on a gold pen nib. In an inflamed limbus,
however, such negative shadows rarely appear during the postvascular phase. Conjunc-
tival vessels may appear as negative shadows even in normal eyes and in that situation
cannot be regarded as a pathological sign.

![Fig. 6 Progressive pannus in the postvascular phase, showing negative shadows of the vessels](image)

**BEHAVIOUR OF REGRESSIVE PANNUS**

The last two sections of Fig. 3 illustrate the findings in regressive pannus and pannus sequel.
In the former a slight leakage of fluorescein is still seen at some places along the vessels,
but not at their apex (Fig. 7a). In the latter no focal leakage of fluorescein can be seen
(Fig. 7b).

![Fig. 7 Regressive pannus (a) and pannus sequel (b) during the vascular phase](image)
Fig. 8 illustrates the limbal findings in a case of herpetic corneal ulcer observed by fluorescence corneal photography. The photographs were taken at one-week intervals from the top to the bottom. The left hand pictures \((a_1, b_1, c_1)\) show the findings in the vascular phase and those on the right \((a_2, b_2, c_2)\) the postvascular phase. At first there is an obvious leakage of fluorescein from the apex of the pannus in the vascular phase \((a_1)\) as well as a definite negative shadow of vessels in the postvascular phase \((a_2)\). One week later the pannus shows a considerable extension into the cornea, but the leakage of fluorescein and the negative shadow have become less pronounced \((b_1, b_2)\). During the...
next week, extension of vessels has ceased, the leakage of fluorescein is very small, and no negative shadows appear (c1, c2). Clinical improvement followed the disappearance of the leakage seen with fluorescence photography.

**BEHAVIOUR OF THE IRIS**

In a normal brown iris, little fluorescence appears in the iris after the intravenous injection of fluorescein. The entry of fluorescein into the aqueous humour is slight so that it does not interfere with the corneal photography.

In an inflamed iris, however, the fluorescence appears in the iris in the vascular phase and a considerable leakage of fluorescein takes place into the aqueous during the post-vascular phase. Fig. 9 shows a case of iritis 40 seconds after the fluorescein injection. The surface of the iris is strongly fluorescent with the typical appearance of an inflamed limbus. The fluorescence later began to leak into the aqueous, but it was half an hour before the aqueous fluorescence completely obscured the image of the iris and pupil.

The findings in neovascularization in the iris, or rubeosis iridis, are characteristic. In the early vascular phase, the image of the capillaries appears on the iris like shreds of cotton waste. In the late vascular phase a considerable leakage of fluorescein into the aqueous begins. The leakage is often so rapid and profuse that the fluorescence from the aqueous covers the image of the iris in the course of a few minutes. Fig. 10 (overleaf) shows the findings in rubeosis iridis 50 seconds after the fluorescein injection. At a capillaries on the iris are distinctly seen as cotton waste. At b the leakage of fluorescein from the capillaries is obvious. At c the leakage of fluorescein is so rapid that the capillaries are already obscured and 5 minutes later the anterior chamber was filled with brilliant fluorescence. This patient was suffering from secondary glaucoma. The rubeosis was not visible by ordinary slit-lamp examination; nevertheless, fluorescence photography detected the presence of neovascularization in the iris. The rubeosis became visible by slit lamp one month later. Rubeosis iridis was also predicted by fluorescence photography in cases of Vogt-Koyanagi’s syndrome, haemorrhagic glaucoma, and diabetes.

When rubeosis can be seen with the slit lamp, fluorescence photography shows the capillaries even more plainly. When rubeosis has disappeared after successful treatment,
fluorescence photography continues to show capillaries on the iris for a considerable length of time.

Discussion and conclusion

When the limbus is inflamed, there is congestion in the limbal capillary plexus. In its first stage an extension of the capillaries into the cornea (microscopic pannus) is difficult to differentiate from an inflamed limbus, even by slit-lamp examination, but fluorescence corneal photography enables the conditions to be distinguished from each other. For this purpose, the finding of the limbus in the vascular phase is of great value. Leakage of fluorescein at the apex of the capillary loop indicates a progressive extension of the corresponding vessel. The leakage at the apex is sometimes so sharp that it resembles an iridium tip on a gold pen as suggested in Fig. 6. A focal leakage of fluorescence from an inflamed limbus does not occur unless an extension of the vessel takes place.

The localization and degree of the leakage not only enable microscopic pannus to be differentiated from inflamed limbus but also indicate the activity of various kinds of corneal vascularization. Clinical improvement is often seen after a decrease or disappearance of leakage in the fluorescence photographs. Fluorescence corneal photography may therefore be used to predict the course of corneal inflammation which is accompanied by vascularization.

Fluorescence iris photography can detect an increase in the permeability of the uveo-aqueous barrier; it can predict rubeosis iridis long before its demonstration by the slit lamp and will also reveal a past history of rubeosis. It may therefore be useful in the analysis of disorders of the iris and of some glaucomatous conditions.

References

Ehrlich, P. (1882) Dtsch. med. Wschr., 8, 35