Infrared fundus angiography

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This paper describes a technique for fundus angiography of the choroidal and retinal circulations by infrared absorption, using an intravenous dye, indocyanine green. The history and theory of intravascular dyes and of infrared photography in the examination of the ocular fundus and its circulation are first considered.

Vital staining dyes

Sorsby (1939) used intravenous Kiton green to produce permanent vital staining of the diseased human retina. Other workers have used vital staining dyes in experimental animals. Steinhausen and Loreth (1965) used Lissamin green in investigating the rat retina. Riehm and Podesta (1971) used both Patent blue (Disulphine blue) and Lissamin green in an examination of choroidal blood flow in an experimental preparation. They noted that these dyes diffused readily from the choroidal circulation. Amoils and Honey (1969) used Evans blue to produce staining of cryotherapy lesions in the rabbit retina. Kuwamoto (1971) used acridine orange, a fluorescent dye, to demonstrate retinal blood flow and staining of the poisoned rabbit retina, and Oliver, Zauberman, and Ivry (1970) used both Disulphine blue and Evans blue for staining retinal lesions.

The value of vital staining dyes has been demonstrated by these workers in the experimental animal, but the application of these techniques to the human fundus so far appears unrewarding.

Fluorescein and its limitations

Interest in dyes for use in the human fundus is centred in dyes which can be demonstrated within the intact circulation as well as leaking from it in disease. With the advent of fluorescein fundus angiography (Chao and Flocks, 1958) and the development of this technique for clinical use (Novotny and Alvis, 1961), an entirely new discipline has developed, and its various uses in eye research have been considered by Maurice (1967).

The usefulness of fluorescein is limited in two respects;

- (1) The size of the fluorescein molecule;
- (2) The wave length of emission of fluorescence.

The small molecular size and the partial binding to plasma proteins makes the fluorescein molecule readily diffusible. Thus it diffuses from the normal choroidal circulation and from the retinal circulation in diseases in which plasma proteins are not necessarily being lost from the circulation.

The emission wave length of fluorescein with its peak at 480 m μ is relatively short, so that some absorption takes place in the refractive media of the eye (Duke-Elder, 1954) and complete absorption occurs in the retinal pigment epithelium. The ocular media

absorb less at longer wave lengths and the retinal pigment epithelium becomes relatively transparent in the infrared at 800 $m\mu$.

Ideally the fundus angiographer would have at his command a selection of fluorescent dyes of widely differing molecular size (or protein-binding characteristics) and with differing emission spectra including emission in the infrared. With these he could investigate the nature of vessel leakage and examine the choroidal circulation through the intact pigment epithelium.

A protein-conjugated fluorescein molecule has been produced (Sollom, 1968), but the fluorescent properties of the molecule were reduced and the results were disappointing. Hodge and Clemett (1966) demonstrated two secondary emission peaks of fluorescein in plasma at longer wave lengths (530 $m\mu$ and 615 $m\mu$), but the fluorescence at these wave lengths is relatively weak.

Improved demonstration of the choroidal circulation with fluorescein has been achieved by excitation in the red at 606 $m\mu$ by Alessandrini (1971), but the main fluorescein emission still occurs at 480 $m\mu$ and so is largely absorbed by the pigment epithelium.

Indocyanine green

Fluorescent dyes are differentiated from the fundus structures by selective filtration both at the exciting and at the emitting wave lengths. This two-point discrimination permits a high degree of differentiation between the dye and the background.

Dyes visible by absorption can be differentiated only by a single filter that passes light of a wave length absorbed by that dye, so that structures containing the dye appear dark. This can not give a high degree of differentiation, except with a dye which absorbs at a wave length at which the fundal structures are transparent.

Indocyanine green absorbs heavily in the infrared with a maximum at 805 $m\mu$ in plasma (Fig. 1). This is at a sufficiently long wave length to differentiate the dye from the absorption spectra of the haemoglobins (Fox, Brooker, Heseltine, and Wood, 1956). With filtration to absorb all wave lengths shorter than 720 $m\mu$, the dye can be differentiated from other pigments in the fundus including the retinal pigment epithelium.

FIG. 1 Absorption spectra of indocyanine green and of the haemoglobins. The performance of infrared film and the absorption of the Wratten 88A filter are superimposed

Indocyanine is intensely protein-bound, chiefly to the albumin fraction, and thus behaves in the circulation as though it had the molecular size of a plasma protein and can be expected to leak from the circulation only at sites which are leaking protein.

This dye is taken out of the circulation by the liver to be excreted in the bile. The removal by the liver is very efficient so that the recirculation problem which occurs with fluorescein does not occur with indocyanine. The physical and physiological properties have been discussed by Fox and Wood (1960).

The intravascular use of indocyanine green was described by Fox and others (1956) for systemic circulation measurements and has since been in regular use by cardiologists. The fundus circulation was examined by reflection densitometry with indocyanine green by Collela and Pilkerton (1969) and its use in fundus angiography by intracarotid injection in monkeys and in man was described by David (1971) and Kogure, David, Yamanouchi, and Choromokos (1970).

Infrared fundus photography

Early studies in infrared fundus photography were reported by Kugelberg (1934) who recognized the relative transparency of the retinal pigment epithelium and by Feldman (1936). At this time there was little to be gained from black-and-white infrared photography of the fundus. Since then there has been improvement in the infrared sensitive films available, both black-and-white and colour. Ernest (1968) reported the use of Kodak Ektachrome Infrared Aero for what he called colour translation fundus photography, which was an appropriate title since he used a Wratten 12 filter which passes visible light down to a wave length of 500 m μ . Thus the image he obtained was made up from the visible spectrum as well as from the infrared. This same photographic technique was used by David (1971) and Kogure and others (1970) in conjunction with intracarotid indocyanine.

Methods

Consideration of the absorption spectra of indocyanine green and of the haemoglobins (Fig. 1) suggests that a sharp cut-off infrared filter holding back wave lengths shorter than 710 m μ will differentiate indocyanine from the blood pigments. A filter to cut out wave lengths longer than those absorbed by indocyanine is not required, since the sensitivity of infrared film extends only to 880 m μ . A preliminary frame made with this suggested filtration should show a featureless fundus as is also achieved with the double filtration for fluorescence angiography.

A Zeiss fundus camera was fitted with an infrared filter in the camera throat. A Wratten 88A filter was used for this purpose which cuts off sharply at 720 m μ (Fig. 1). A Wratten 25 which transmits visible red was fitted to the eyepiece to reduce possible focussing errors due to chromatic aberration. No filter was used in the illumination path of the fundus camera.

Results

Experiments were first made with black-and-white infrared film (Kodak). Indocyanine in intravenous doses of up to 50 mg. (1 ampoule) in 5 or 10 ml. was used without cannulation. The indocyanine could not be seen in the fundus by the operator of this apparatus, so a sequence of exposures was made. The resultant photographs did show the indocyanine, but contrast was disappointing.

Kodak Ektachrome Infrared Aero was then used with the same filtration and intravenous injections as had been used with black-and-white. Used in this way the infrared colour film is recording only in its infrared sensitive layer, which produces an overall red preliminary picture. The indocyanine then shows dark in contrast. With this technique enough contrast between the indocyanine and the background was obtained to

FIG. 2 *Early venous phase. Dark Caucasian subject with normal fundus. Ektachrome Infrared Aero. Wratten 88A filter. Intravenous indocyanine green 50 mg. in 7 ml.*

FIG. 3 *Late venous phase. Same as Fig. 2*

provide a useful demonstration of the choroidal circulation (reproduced here in black and white, Figs 2 and 3).

Other infrared filters which cut off at longer wave lengths than the Wratten 88A were tried. These included the Wratten 87 with a cut-off at 740 mμ, and the Ilford 813 with a cut-off at 750 mμ. The results obtained were not so good as those with the 88A, and it is not considered that a longer wave length cut-off represents any advantage. Although such filters further subdue to the background details, part of the spectral region of indocyanine absorption is also masked (Study of Fig. 1 will make this clear).

Discussion

A functional technique is presented for obtaining infrared pictures of the choroidal and retinal circulations with intravenous indocyanine green injections in man.

So far this technique has been used only on volunteers (ourselves and our colleagues) who are in a position to understand the possible toxic effects. Ethical problems need to be overcome in order to use the dye in patients. Fluorescein has a long record of safety in intravenous use so that it is difficult to promote any alternative form of fundus angiography unless it has a real advantage over fluorescein and is of comparable safety. Indocyanine green does have the advantage of demonstrating the choroidal circulation through the intact retinal pigment epithelium and of protein-binding to form a large molecule which will leak from the circulation only at sites which leak protein. This dye has proved safe in the hands of the cardiologists (Fox and others, 1956; Fox, Brooker, Heseltine, Essex, and Wood, 1957; Merriman, Wyant, Bray, and McGeachy, 1958; Fox and Wood, 1960) but, although total doses of up to 50 mg. intravenously were given in divided doses, it was not given as a single 50 mg. injection as we have used. Since we are using the dye in a new way we can not be entirely confident in its safety. Iodocyanine green does contain free iodine which implies a risk in iodine-sensitive individuals. There are also theoretical risks of overloading the protein-binding capacity of the plasma or of causing cerebral irritation by crossing the blood-brain barrier in subjects in whom this is defective.

Other dyes have been used intravenously in man with relative freedom from toxicity. Kiton green could cause nausea and vomiting (Sorsby, 1939) but was considered to be relatively free from toxicity by Whittet (1947). Coomassie blue, which is a protein-bound dye, has been given by intracarotid injection of 200 mg. in 10 ml. without producing electroencephalographic changes (Feindel, Garretson, Yamamoto, Perot, and Rumin, 1965), and was used in a dose of 2,000 mg. in one patient by Taylor and Thorp (1959), but Hoffman and Guz (1961) produced rigor and nausea in one subject with 924 mg. given over 63 minutes.

Disulphine blue has been used successfully in lymphangiography (Kinmonth, 1952, 1954) and by intracarotid injection (Engeset, Brennhovd, and Stovner, 1962).

Evans blue has been used for blood volume estimation, and was considered to be free from toxicity (Gibson and Gregerson, 1935), but one fatality has been attributed to this dye (Griffin, 1971).

We consider that further toxicological experiments are required before dyes other than fluorescein are used for intravenous fundus angiography. We had limited ourselves to intravenous doses of 50 mg. of indocyanine green, which is the minimum needed to demonstrate the choroidal circulation, but doses larger than this can be expected to give a better result. Toxicological examination of indocyanine green in the mode of administration for fundus angiography should be considered now.

Summary

A technique of infrared intravenous fundus angiography using indocyanine green is described. A Wratten 88A filter was used and the result recorded on Kodak Ektachrome Infrared Aero film. A useful demonstration of the choroidal circulation is obtained in the presence of a normal retinal pigment epithelium, but it is considered that ethical and toxicological problems must be overcome before putting this technique into routine clinical use.

We should like to thank Mr. M. D. Sanders for his encouragement to start this project and for providing us with the facilities to begin the work at the National Hospital for Nervous Diseases, London.

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