A Method of Photographing Fluorescence in Circulating Blood in the Human Retina

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The physiopathology of the retinal vasculature would be better understood if more were known about blood flow in these vessels. Because of the unique quality of transparency in the eye, methods depending on direct observation of the retinal vessels seem especially inviting. Already reported by various authors are techniques for measuring the changes in caliber of retinal vessels,1 and methods of observing retinal blood flow by cinematography,2 and, in cats, by injecting trypan blue.3 Although useful, these methods have certain limitations, and additional means of observing retinal blood flow with increased visibility and definition are needed.

The purpose of this paper is to describe a method for the study of retinal blood flow in man by the use of intravascular fluorescein and retinal photography, and to report some preliminary observations made with this method.

Materials and Methods

The Zeiss fundus camera was used. It was equipped with an electronic flash, the maximum discharge of which allowed one photograph every 12 seconds. Light intensity of the electronic flash was set at position III, and retinal photographs were made of the luminescence of fluorescein as it passed through the retinal vessels. In order to do this, both activating and emitting wave lengths of blood-fluorescein mixtures were determined spectrofluorometrically. The optimal activating wave length was found to be 490 mμ, in the blue range of the visible spectrum; and the maximal emitting wave length was 520 mμ, in the green.

Kodak wratten filters no. 47 and no. 58, combined with a 3-mm. layer of 0.25 M copper sulfate, were accordingly inserted into the optical system (figs. 1 and 2) at appropriate points.

In order to modify the activating light, the blue no.-47 filter was placed in the path of the beam from the electronic flash and from the incandescent viewing source. This made it possible to see, as well as to photograph, the fluorescence as it developed and faded.

The green no.-58 filter with the copper sulfate layer was placed in the path of emitted light in order to absorb background illumination and increase the contrast between it and the transmitted fluorescence. The green no.-58 filter permitted approximately 5 per cent transmittance in the blue range, which gave a visible background in the retinal photograph irrespective of the fluorescence. The layer of copper sulfate solution was not essential, but slightly sharper negatives were obtained with it.

Ansco Super Hypan 35-mm. film was used; it was force developed for 10 minutes at 70° F. with UFG Ethol developer, placed in an acetic acid stop bath, and fixed for 10 minutes with Kodak acid fixer. Prints were made on Kodak Medalist F-2 or F-4 photographic paper, depending on the contrast desired.

In each patient, a control picture of the fundus was taken prior to injection of fluorescein. Then, in a darkened room, 5 ml. of Fluoresceite were rapidly injected into an antecubital vein and were followed by 5 ml. of normal saline to deliver a concentrated bolus of dye into the circulation. The first photograph was made when arterial fluorescence appeared, followed by serial photographs at 12-second intervals for approximately 3½ minutes.

Results

Normal Patients

The time from injection into the antecubital vein until visualization of fluorescence varied from 12 to 30 seconds, when a striking lumina

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*Fluoresceite is 5 per cent fluorescein in sodium bicarbonate, an injectable product of the C. F. Kirk Company, 521 W. 23rd St., New York 11, New York.
nescence appeared in the retinal vessels as the fluorescein passed through them.

Separate arteriolar and venous filling phases were present in the serial photographs (figs. 3 and 4). During and after the venous filling phase, a generalized background mottling developed, presumably representing the choroidal circulation. Near the end of the 3½ minute period of photography, contrast faded and the retinal vessels were difficult to see.

There were considerable differences in the circulation times from arteriolar to venous sides in different portions of the retina, the arteriovenous transit time being fastest in the region of the fovea, and slowest in the peripheral portions of the retina. An occasional arteriolar twig emptied more slowly than the others of similar size at comparable distances from the disk. One arteriolar-venous shunt was seen.

Stratified flow occurred in many of the larger vessels. In the larger arterioles the flow rate seemed more rapid in the central portion of the vessel than along the walls, since fluorescence appeared first and cleared first in the central portions. In the larger veins there was often a reversal of this pattern, the lateral portions of the venous stream first showing fluorescence, sometimes on only one side of the vein, as fluorescent blood entered the vein from small branches near the disk. These fluorescent streams tended to maintain their lateral position along the same side of the vein all the way to the disk. Two or three minutes after the injection, some larger veins continued to show more prominent residual fluorescence along the walls than in the central portions. It was not clear whether this represented a fluorescent plasma cuff or fluorescence of the vessel wall itself.

**Hypertensive and Diabetic Patients**

In addition to the same findings as in the normal eye, fluorescence in some hypertensive and diabetic patients showed smaller, more obscure vascular patterns that were difficult or impossible to see with the ophthalmoscope or in ordinary retinal photographs. New vessel formation was one of the most striking findings in these patients. Fluorescence appeared in microaneurysms, vasoproliferative areas, and tortuous small vessels as well as in the normal circulation (figs. 5 and 6). Fluorescence could not be seen in hemorrhagic areas, although it appeared early in some cotton-wool patches and remained throughout the period of photography. Some patches and edema residues failed to become fluorescent.

**Discussion**

Serial fluorescence-photography of the human retinal vasculature provides a dynamic record and increased visibility of the vascular pattern and blood flow by means of a simple technic.
Figure 3
Normal patient. Top. The arteriolar filling phase. Bottom. the venous filling phase. Arrows on the left indicate differential emptying of comparable-sized arterioles, and the arrow on the right indicates the rapid passage of the dye from an arteriole to a venule through a small vessel resembling an arterioloovenous shunt.

Activation by ultra-violet light was found to be unnecessary; filtered visible light avoided the potential hazards of ultra-violet exposure, and the natural fluorescence of the lens was no problem. The eventual appearance of fluorescein in the aqueous humor, however, contributed to the loss of definition in later serial photographs.

The background mottling, presumably from the choroidal circulation, which persisted throughout the period of photography, suggested that the rate of fluorescein turnover in the choroid may be slower than that of the retina.

At present, owing to the limitations of the electronic flash apparatus, pictures cannot be taken more often than every 12 seconds.

Figure 4

A new incandescent light source and a rapid film-changer may eventually permit exposure to be made in more rapid succession. Also, retinal cinematography would seem particularly adaptable to this method.

Further studies with the technics described here are being carried out to determine the rate of change of optical density of veins in the photographic negatives, to determine whether a relationship can be established between dye concentration and image density of the vessel. While such a relationship cannot give an estimate of retinal flow in absolute terms, it could provide a ratio between flow in control and experimental states, or between flow rates in different veins of the same eye.

Summary
A simple method, with use of intravenous fluorescein, was used for producing and photo-
FLUORESCENCE IN HUMAN RETINA

Figure 5
Hypertensive patient. Top. Made with Kodak Plus-X film before the injection of fluorescein. Middle. Made with Super Hypan film and shows the venous filling phase. Bottom. Taken 3½ minutes after injection. The arrow near the optic nerve head (middle figure) points to a cotton wool exudate that is becoming fluorescent and that develops marked fluorescence in the bottom figure. The second arrow (middle figure) indicates a region that shows only a few scattered hemorrhages in the nonfluorescent picture (top figure) but that develops definite fluorescence (middle and bottom figures), suggesting that capillaries throughout the region may have abnormally great permeability. Several other regions of increased permeability are evident.

Figure 6
Diabetic patient. Top. Made with Kodak Plus-X film before the injection of fluorescein. Middle. The arteriolar filling phase. The arrow points to a region of neovascularization and increased capillary permeability. Bottom. Venous filling. The arrow more distant to the disk indicates three microaneurysms that have become fluorescent. Graphing fluorescence in circulating blood of the human retina.

Separate arteriolar and venous filling phases, an arteriovenous shunt, sluggish choroidal circulation, stratified flow of fluo-
rescein, and rapid central retinal circulation times were observed in normal retinas.

Similar findings were seen in hypertensive and diabetic patients, and, in addition, neovascularization was clearly defined, and some cotton-wool patches, but not hemorrhages, were found to fluoresce.

Limitations and applications of the method are discussed.

Acknowledgment

Grateful appreciation is extended to John B. Hickam, M. D., and Fred M. Wilson, M. D., of the Departments of Medicine and Ophthalmology, respectively, for their assistance and criticism. The work of James Hartigan and others at Eli Lilly Company in determining spectrofluorometric data was most helpful.

References


THIRD OBSERVATION

Of the Preternatural or Morbid Sound of the Chest and Its General Import

X. To be able justly to appreciate the value of the various sounds elicited from the chest in cases of disease it is necessary to have learned, by experience on many subjects, the modifications of sound, general or partial, produced by the habit of the body, natural confirmation as to the scapulae, mammae, the heart, the capacity of the thorax, the degree of fleshiness, fatness, etc., inasmuch as these various circumstances modify the sound very considerably.—From On Percussion of the Chest. Published in 1761. Translated by John Forbes, M.D. In: Classics of Medicine and Surgery. New York, Dover Publications, Inc., 1959, p. 127.
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Circulation. 1961;24:82-86
doi: 10.1161/01.CIR.24.1.82

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