Fluorescein angiography and ocular hemodynamics

Effect of ocular pigments on choroidal fluorescence during induced ocular hypertension

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The appearance of fluorescein in the fundus of albino and pigmented rabbits was documented using a wide-angle fundus camera. Serial photographs were taken during decrease of intraocular pressure from levels above systolic ophthalmic artery pressure. In all the albino rabbits, the fluorescein first appeared in the choroid and only later in the retinal vessels. Contrary to this, in all the pigmented rabbits the dye seemed to appear in the retinal vessels before any choroidal fluorescence could be detected. This difference in the filling pattern is caused by retinal and choroidal pigments, which mask the choroidal circulation in pigmented animals. The simultaneous photoelectric recording of dilution curves after injection of a mixture of fluorescein and indocyanine green further confirmed that this differential appearance time does not have any hemodynamic significance, but is a purely physical phenomenon.

Key words: fluorescein angiography, wide-angle fundus camera, ocular hemodynamics, induced ocular hypertension, dye dilution curve, appearance time.

A number of reports dealing with the effect of induced ocular hypertension on the retinal and choroidal hemodynamics have been published recently. Similar methods were used in all these studies: the appearance time of fluorescein in the retinal vessels and in the choroidal circulation was documented by a photographic technique. The conclusions, however, were not uniform. One group of investigators repeatedly reported that the choroidal vessels collapse at significantly lower intraocular pressures (IOP) than the retinal vessels. Others, however, were unable to confirm these observations, or even obtained contradictory results.

The purpose of this communication is first to present some theoretical considerations indicating that retinal and choroidal pigments delay the detection of choroidal fluorescence, especially at high levels of IOP, and second to report confirming experimental evidence.
The passage of fluorescein dye in the ocular circulation can be recorded by measuring the intensity of the green fluorescent light emitted by the dye. Assuming that the intensity of fluorescence is proportional to the concentration of the dye, a graphic display of the changes in intensity with time is a dilution curve (Fig. 1). The dilution curve (A) in Fig. 1 represents a dilution curve at normal IOP. When the IOP is elevated, a reduction of flow results, and the dilution curve stretches along the time axis (curve B). Since less fluorescein enters the eye at high IOP, such a curve is always lower than one recorded at normal IOP. Curves similar to (A) and (B) have been photoelectrically recorded with a fundus fluorophotometer in animals and in humans.

When fluorescein dilution curves are recorded with a photographic technique, the intensity of fluorescence is first transformed into a density of silver grains on a negative. By densitometric measurements in serial negatives a density/time curve can be constructed.

Fig. 2 analyzes such a transformation. In quadrant 3, dilution curves identical to those of Fig. 1 have been drawn with the time axis pointing down. In quadrant 2, a curve, which is called the "characteristic" curve of the film, shows the optical density, or darkening, of the photographic negative as a function of intensity. If we assume a constant exposure time, the relation between the intensity of fluorescence in logarithmic units and the optical density on the photographic negative (D) is essentially linear (line S-R), except in the initial part of the curve, the so-called fog (line Q-S), where any change in intensity does not affect the density. The intensity level at point S can therefore be regarded as the threshold intensity level of that particular film. This threshold is represented in quadrant 3 by the line S-L.

The density curves which are obtained after processing the film are displayed in quadrant 1. In this quadrant, the density (D) of the photographic negatives is plotted against the time in seconds. Curves (A) and (B) are density curves under normal and high IOP, respectively. T and T represent the first appearance of fluorescence density in the respective curves. The dotted lines from P to P' demonstrate how the density curves are constructed from the dilution curves of quadrant 3. It can be seen that all the information regarding intensities below the threshold level is lost during such a transformation. For this reason curves (A) and (B) have the same appearance time (T) in quadrant 3, while the appearance
Fig. 3. This figure compares the effect of the filter in front of the choroidal circulation on the first appearance of fluorescein at three levels of IOP: (A) at normal IOP’s, (B) at high IOP’s (C) at IOP’s which approach ophthalmic artery systolic levels. The upper curves are choroidal. Those which are drawn with broken lines are hypothetical choroidal curves that would have been obtained if there were no filter. The actual choroidal curves (in solid lines) are drawn under these curves, and are obtained when an optical density (k) is introduced. The corresponding retinal density curve is shown in the lower part of each figure. By comparing Figs. A, B, and C, one can see that, as a result of the pigment filter, a progressive delay in the appearance time (A and B) and even complete disappearance of the choroidal curve (C) takes place. This happens despite a constant time relationship between the appearance of the hypothetical choroidal curve and the corresponding retinal curve.

time for the corresponding curves in quadrant 1 (T_a and T_b) is delayed. The delay depends on two major factors: the threshold intensity level and the shape of the curve. The flatter the curve and the higher the threshold level, the greater the delay.

Before comparing fluorescein appearance time in the retina and the choroid, another factor must be considered. The retina and the choroid, with their various pigments, transmit blue and green radiation poorly, in effect functioning as a high-density filter in front of the choroidal vessels. This filter lies behind the retinal blood vessels located near the surface of the retina. Such a filter greatly reduces the intensity of both the blue excitation light and the fluorescent light emitted from the choroid. Measurements by Geeraets and co-workers imply that this filter reduces the fluorescence intensity by at least a factor of 100. Fig. 3 demonstrates that the effect of such a filter on choroidal density curves consists of downward displacement by a constant distance (k) which is equal to the optical density of the filter. As a result, the appearance times of the choroidal curves T_a and T_b are further delayed (the first delay of T_a and T_b having taken place in the transformation of intensity to density). This second delay, like the first one, depends on the steepness of the dilution curve, but mainly on the optical density (k) of the pigment filter. The choroidal curves may even disappear completely because of this filter, leading to the erroneous conclusion that there is no choroidal flow (Fig. 3, C).

Materials and methods

I. Wide-angle fluorescein angiography. Eight albino and six pigmented rabbits were used in this experiment. General anesthesia was induced by intravenous injection of sodium pentobarbital (25 mg. per kilogram); pupils were dilated with 1 per cent cyclopentolate hydrochloride and 10 per cent phenylephrine hydrochloride. A reservoir of normal saline was connected to the vitreous via a polyethylene tube and a 23-gauge needle that was inserted through the pars plana. By elevating the saline reservoir, the IOP was set to a level just above systolic ophthalmic artery pressure, as determined by direct ophthalmoscopy. Fluorescein (1 c.c., 5 per cent) was then quickly injected into the marginal vein of the ear. The IOP was very slowly decreased while serial photographs were taken at the rate of two per second with a wide-angle fundus camera adapted for fluorescein angiography.

II. Photoelectric recordings of dye dilution curves in the choroid. Simultaneous recordings of fluorescein and indocyanine green (ICG) dilution curves were performed on four albino and four pigmented rabbits with a fundus reflectometer. This instrument was slightly modified for these types of measurements. Each injection consisted of a mixture of 1 mg. of fluorescein and 3 mg. of ICG in 0.2 c.c. of aqueous solvent. The preparation of the animals was as described above, but the IOP was elevated stepwise with measurements performed at different IOP’s.

Results

I. Wide-angle photography. In all albino rabbits, when the IOP was lowered from systolic ophthalmic artery pressure, fluorescein first appeared in the choroid and only later in the retinal vessels (Fig. 4). In all the pigmented rabbits, the sequence of filling appeared to be reversed (Fig. 5).

II. Photoelectric technique. Fig. 6 presents fluorescein and ICG dilution curves from the choroid of an albino rabbit for
three different IOP's. Similar curves were obtained in three additional albino rabbits. Fig. 7 presents curves from a typical pigmented rabbit. Analysis of these curves shows that in albino rabbits the dilution curves of both dyes are similarly affected when the IOP is increased. This applies for both the first appearance time of the dyes and for the height at the peak concentration. In the pigmented rabbits, however, the first appearance of fluorescein is delayed more than the first appearance of ICG. Also, the height of the fluorescein dilution curve is reduced more than the height of the corresponding ICG dilution curve as the IOP is increased. The time of peak concentration of fluorescein and ICG is equally affected by the IOP in both albino and pigmented rabbits.

Discussion

Although the rabbit does not possess true retinal vessels and is a poor experimental animal for study of ocular pathol-
Fig. 5. The mode of filling in a pigmented rabbit during decrease of IOP from levels above systolic ophthalmic artery pressure. (A) Fluorescein first appears in the disc and the retinal vessels. No choroidal fluorescence can be detected at this stage. (B) Half a second later faint choroidal fluorescence starts to appear at 12 o'clock (arrow). (C) and (D) Progressive filling of the retinal and choroidal vessels.

Fluorescein angiography performed during decrease of IOP from levels above systolic ophthalmic artery pressure shows that in albino rabbits fluorescein appears in the choroidal vessels before its appearance is detected in the retinal vessels. In pigmented rabbits fluorescein angiography under identical conditions gives completely opposite results, as retinal appearance precedes choroidal appearance. Assuming that no physiologic difference exists between pigmented and albino rabbits, this delay in the detection of fluorescence from the choroid of pigmented rabbits can only be...
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attributed to the filtering effect of the retinal and choroidal pigments. These pigments, acting as a high-density optical filter, delay detection of the first appearance of choroidal fluorescence.

The results of simultaneous detection of fluorescein and ICG curves further reveal the filtering effect of retinal and choroidal pigments. Such pigments influence the detection of fluorescein much more strongly than the detection of ICG, because the optical density of the retinal and choroidal pigments is much lower in the infrared region than in the blue and green regions.

The detection of the first appearance time of fluorescein in the choroid is affected by the following physical factors: the intensity of the incident blue light, the spectral density of the retinal and choroidal pigments, the concentration of the dye, and the sensitivity of the detection system. Therefore, the time of first detection of the dye is not a good indicator of the appearance of the dye in the eye. These physical factors affect the mean circulation time to a lesser extent than the first appearance time. However, in analyzing dilution curves it is more convenient to use the time of peak dye concentration \( T_{\text{max}} \), as has been done before. Despite the fact that its hemodynamic relevance is unclear, \( T_{\text{max}} \) is independent of the physical characteristics of the eye and the detection technique. The fact that \( T_{\text{max}} \) for pigmented rabbits is always identical for fluorescein and ICG indicates that the apparent delay in the first appearance time of fluorescein is solely the result of physical factors.

From the above considerations, it is now clear that a method which employs conventional fundus photography and regular processing of the film is apt to find a delay in choroidal appearance time. Such a delay is not appreciable at normal IOP, where the dilution curve is very steep. However, if, for any reason, the curve becomes flat, a greater delay occurs in the choroidal appearance time than in the retinal appearance time (Fig. 3).

If we apply this explanation to human experiments, we can now understand why several investigators consistently found a delay in choroidal appearance of the dye,
or even lack of appearance, at high IOP's. One way of decreasing this delay in the detection of the choroidal fluorescence is to reduce the optical density ($k$) of the filter. Another way is to lower the threshold sensitivity level of the detection system. Archer, Ernest, and Krill used these possibilities. They chose albino patients, in whom the density of the filter in front of the choroid is greatly reduced, and also used special film processing techniques. As a result they found that, at very high IOP, the appearance time of choroidal and retinal fluorescence was equal. The best way, however, to decrease choroidal delay is to increase the steepness of the initial part of the dilution curve. This may be achieved by increasing the fluorescein concentration and the sharpness of the bolus by using intra-arterial injection. Swietliczko and David, using this technique in monkeys, found that choroidal fluorescence precedes retinal fluorescence even at diastolic ophthalmic artery pressure.

Our photoelectric recordings of indocyanine green and fluorescein dilution curves provide proof that the delay in choroidal fluorescence is, in fact, due to the filter effect of the retinal and choroidal pigments. Furthermore, indocyanine green detection demonstrated that retinal circulation was obliterated while choroidal circulation persisted at extremely high IOP.

Thus, all the investigators who employed techniques which enhance the fluorescence detection were unable to demonstrate choroidal collapses at IOP which do not occlude retinal circulation. Furthermore, some evidence indicates that in normal eyes, when IOP is elevated to levels in the vicinity of systolic ophthalmic artery pressure, dyes appear first in the choroid rather than in the retinal vessels.

Best, Toyofuku, and Galin performed fluorescein angiography during induced ocular hypertension in patients with retinitis pigmentosa. Their most frequent findings were a reversal of the so-called "normal" relative opening pressure in the choroid and in the retina. However, these patients also exhibit marked depigmentation, which allows more direct visualization of the choroidal pattern. It is possible that part of this "reversal" is simply due to a reduction in the optical density of the pigment filter in front of the choroid.

Our experiments show that, with the present techniques, precise determination of fluorescein appearance time in the choroid is impossible. Furthermore, comparison of the times of appearance of fluorescein in the retina and choroid, without taking into account the optical properties of the ocular pigments, may lead to erroneous interpretation. Indeed, experiments which compare the appearance of fluorescein in the retina and the choroid at high levels of IOP actually provide more information on the sensitivity of the detection system and the amount of fundus pigmentation than on the ocular hemodynamics.

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