METHODS OF EVALUATION OF RETINAL CIRCULATION

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SUMMARY

The authors emphasize the need for the development of non-invasive objective methods for the study of retinal circulation under physiologic or pathologic conditions.

Clinical methods embrace the use of blue field entoptic phenomenon (that estimates leucocytes circulating in the peri-foveal capillary bed), Laser-Doppler speedometer (that allows the measurement of erythrocyte-velocity in retinal vessels) and methods based on the use of dyes, either by means of the direct estimation of their velocity (ophthalmoscopy, cineangiography and two-point fluorophotometry) or by measurement of the mean circulation time resorting to dilution curves (using angiography between 1 and 60 Hz, two-point fluorophotometry and Video-TV recording). The advantages and drawbacks of each of these methods are examined. Using a personal technique of two-point fluorophotometry (direct method) the authors present a study of reproducibility based on the evaluation of 5 normal subjects submitted to repeated injections. This study reveals that the minimum time of circulation in an arteriolar segment presents a coefficient of variation comprised between 2.8-4.5% which favours the good reproducibility of this method. This fact may be ascribed to the rise of intraocular pressure associated to the use of suction contact lenses. Although two-point fluorophotometry permits merely a relative measurement of retinal circulation speed, the method, in contrast to other available techniques, is readily applicable for pratical purposes.

I — INTRODUCTION

The assessment of retinal vessels' flow is one of the utmost aims of ophthalmologic investigation attempting the understanding of basic physiologic mechanisms, the physiopathology of retinal affections and the evaluation of therapy.

The number of Centers engaged in this study and the variety of methods employed, both in the clinic and in the animal experiments, undoubtedly shows that no single method is so far accepted without criticism.

The clinic calls for a non-invasive, easy to perform and easily reproducible method. The methods proposed are based on the determination of the velocity of *particles* carried by the bloodstream (erythrocytes, leucocytes and dyes injected in the systemic circulation) which has a volume difficult to assess but somehow directly correlated with the cut-sections of the artery and vein corresponding to the retinal territory under study.

II — EVALUATION METHODS OF RETINAL CIRCULATION

1 — BLUE FIELD ENTOPTIC PHENOMENON

When one stares at a glowing blue light *flying particles* come into sight. This phenomenon was described in the XVIIth century by Sauvages Duke-Elder (1962). SCHMIDT-GROSS (1954) interpreted these *particles* as being leucocytes circulating in peri-foveal capillaries (Figure 1). Riva and Loebl (1977) proposed the motions and distribution of leucocytes as means for the study of peri-foveal capillary circulation. These authors developed an Entoscope, already commercially available, (Medical Instrument Research Associates BF 100) in which the macula is illuminated 12° all around the fovea with blue light obtained by interference filter (maximum transmission at 430 nm) which favours the visualization of leucocytes. Using this method several pioneer works on the autoregulation of retinal circulation after variation of intraocular pressure and after inhalation of oxygen and carbon dioxide have already been published.

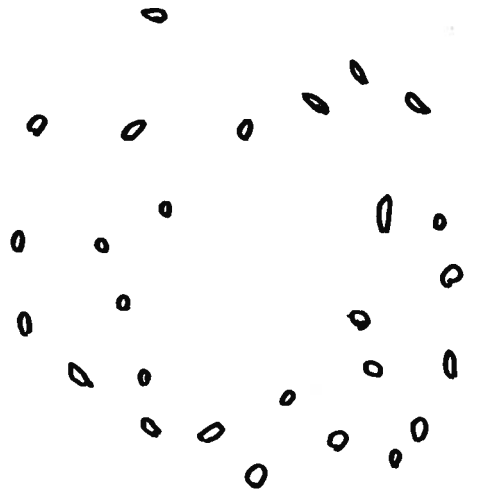

Fig. 1 — Blue field entoptic phenomenon. Configuration of circulating leucocytes.

With this method it is not possible to ascertain the flow volume of leucocytes but only the speed they attain in peri-foveal capillaries (0.8 mm/s). The subjectiveness of the method makes it amenable to criticism.

The velocity of the leucocytes may be calculated comparing the entoscopic perception with a computerized graphic simulator.

2 — LASER SPEEDOMETRY (DOPPLER EFFECT)

The Laser-Doppler speedometer is devised to measure the velocity or particles contained in a flowing fluid.

For this purpose Tanaka et al (1974) for the first time used a Laser of Helium and Neo in human practice in order to study retinal circulation.

The device is based on Doppler effect; the Laser beam when striking particles (erythrocytes in this case) of a flowing fluid is subjected to a change of frequency in proportion to the speed of the particle.

The light scattered by the erythrocytes is collected by an optic ending which conveys it towards the photocatode of a photomultiplier and an optic heterodyne where the fluctuation of frequency is recorded. There is a range of frequency fluctuation according to the several erythrocyte velocities (depending on their juxta-parietal or axial localization within the bloodstream).

Using an optic spectroscope it is possible to measure a spectrum of frequency fluctuation in which the highest frequency corresponds to high speed erytrocytes (axially located).

However, the frequency fluctuation is not only a function of the erythrocyte velocity but also depends on the angle between the Laser beam and the axis of the bloodflow and between the latter one and the scattered light.

For practical purposes the effects of the light geometry just described should be avoided which is not always an easy task.

It was attempted to overcome this setback either by using the parameter pulsatility, the relation between systolic and diastolic velocity, or using the so called *Differential Laser Doppler Method*, based on the combined effect of two beams, which provided rewarding results according to Okamoto et al (1980, 1982).

This is an expensive method (requires a He-Ne Laser and a computer) and it is not definite that the estimated erythrocyte velocity still is not affected by their rotatory motions. Likewise, the maximum velocities concerning vessels of the same caliber as appraised by Okamoto et al (1980) and Riva et al (1982) vary between 23mm/s and 50mm/s respectively. This method is therefore still far from general clinical acceptance.

3 — METHODS USING FLUORESCEIN

Retinal circulation has been studied with the help of dyes that permit the estimation of circulation times either through the use of direct methods (ophthalmoscopic, cineangiographic and fluorophotometric) or resorting to dilution curves (principle of Fick).

DIRECT METHODS

a) Ophthalmoscopic Methods

Used by Presencia in 1979, it consists in the timing of the time which lapses between the appearance of fluorescein in a retinal artery and in a corresponding vein after intravenous administration of the drug. The time lag would be proportional to the arteriovenous circulation time.

This method is quite simple though highly subjective. It should be also noticed that peri-papillary veins and arteries stain with fluorescein almost simultaneously.

b) Cineangiographic Methods

Fluorescein circulation time may be appraised using cineangiographic methods either by counting the number of squares during the filling of an arteriolar segment or watching the progression of the fluorescein bolus square by square. These methods were used in several centers.

The light intensity required for taking the film inicially achieved dangerous levels for the retina, but the implement of techniques resorting to strobe light sources and image intensifiers has recently mitigated the problem.

In order to administer an accurate bolus the intra-arterial route is required which compromises the use of this technique in human practice.

c) Two-Point Fluorophotometry

This method was inicially proposed by the school of Coimbra.

Using direct photometric techniques the minumum transit time between two sections of an artery 900 μ apart is estimated. The volume of this arterial segment is calculated by measuring the diameter of the segment and the flow volume across it as given by the formula:

$$F = \frac{v}{2t}$$

F = flow volume
v = arterial segment volume
t = minimum circulation time

The technique and its critics will be further on described (see Personal Contribution).

INDIRECT METHODS (USING DILUTION CURVES)

The method that uses dilution of dyes was first suggested in the XIX th century by the physicist Adolf Fick for the determination of cardiac output. It was later imployed in the assessment of bloodflow across organs or body parts. Blood samples must be collected from the afferent and efferent vessels pertaining to the region which bloodflow is under estimation.

It is generally accepted that a dye passing through the heart is homogeneously mixed in the bloodstream.

The mean time of retinal circulation is calculated through the analysis of fluorescein concentration curves plotted as a function of the time $\bar{C}$ (t) concerning artery and retinal vein after the administration of a systemic dose of fluorescein.

The concentrations of fluorescein in the artery and vein can not be directly assessed but only by measuring the intensity of fluorescein as a time function $\bar{I}$ (t), assuming this depends on the concentration of fluorescein

$$\bar{I}(t) = A\bar{C}(t)$$

being A a constant in every measurement.

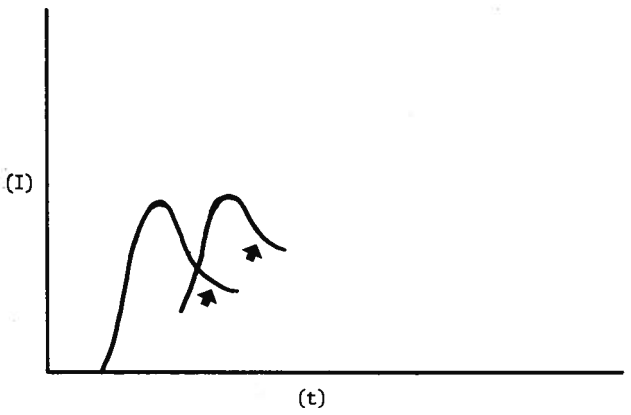

Fig. 2 — Concentration curve as a function of the time, obtained by densitometry at periodic intervals during the angiographic or cineangiographic series.
↑ = recirculation.

The mean circulation time (MCT) corresponds to the difference between the mean circulation time in the artery ($\bar{t}a$) and in the vein ($\bar{t}v$)

$$MCT = \bar{t}v - \bar{t}a$$

These mean arterial and venous circulation times are estimated according to fluorescein dilution curves in the artery and vein.

$$\bar{t}a = \frac{\int_0^x tIa(t)dt}{\int_0^x Ia(t)dt} \quad \text{and} \quad \bar{t}v = \frac{\int_0^x tIv(t)dt}{\int_0^x Iv(t)dt}$$

Generally the vessels chosen are the superior temporal artery and vein since this *superior temporal area* is considered to be a closed system which allows no escape of fluorescein from the regional vascular territory.

a) Densitometric Method

The arterial and venous curves are obtained by densitometry for each photograph either of fluoresceinic angiography or of cineangiography (Figure 2). The determination of the time taken by fluorescein to become apparent is deduced from the densitometric data obtained in each photograph.

Introduced in 1965 by Hickam and Frayser, the method was later ameliorated by Hickam et al (1966), Bulpitt et al (1971), Hill et al (1973), Evans et al (1973), Kohner (1974), Hill (1979) and fitted to the study of autoregulation and to the evaluation of pathologic conditions.

This method is rather laborious which makes it not feasible for clinical purposes. On the other hand, the dose of fluorescein injected in each exam (200-500 mg) does not allow more than two angiographic series to be taken per session.

b) Two-Point Fluorophotometry

This technique was introduced by Niesel et al (1973). It is used a slit lamp through which the ocular fundus is scanned by means of a contact lens. The slit lamp is connected to two optic fibers that can be focused on an artery and corresponding vein. The photosensitive plaques are likewise connected to two optic endings, two photomultipliers and to a photometer, being the fluorescein intensities as a function of the time, directly recorded following the injection of the dye.

The direct recording principle was improved by Ben-Sira and Riva (1973) who replaced the slit lamp by a modified Zeiss retinograph. Riva et al (1975, 1976, 1978) used this method in the study of diabetic circulatory abnormalities.

The inconvenience of the method derives from the fact that for each injection of fluorescein only one recording can be obtained and moreover small amplitude eye motions may affect the quality of the dilution curves.

c) TV Recording

Introduced in 1977 by Fonda et al, this technique consists in the video recording of fluorescein passage through retinal vessels using a TV camera equipped with a highly sensitive tube and connected to a retinograph.

Photosensitive plaques that pick up the intensity of fluorescein transmitted through an optic fiber to photomultipliers, photometer and recording oscilloscope are placed on the TV screen as well as on the artery and vein during the *playback* of the recording.

This method adds the advantages of photographic recordings to the easily obtainable curves inherent to direct photometric methods. Besides, it also permits the recording of several dilution curves pertaining to several vessels after a single injection of the dye.

The drawbacks of the method are concerned with the variables inherent to the video recording and to the automatic light by the TV set. On the other hand, the interface between the photosensitive plaque and the TV screen is another source of error because the first is separated from the latter by the thickness of the glass case. The cost of the system is equally significant.

Experimental works carried out by VanHeuven et al (1977) evidenced that this method provided flow values similar to those obtained by the invasive technique of the radioactive microspheres ($15\pm5\mu$).

d) Analysis of the Methods Using Dilution Curves

I) — Intensity and concentration of fluorescein in retinal circulation

It is generally accepted that fluorescein blood levels in retinal vessels are directly proportional to the emitted fluorescein intensity.

$$\bar{I}(t) = A\bar{C}(t)$$

A — Constant

However, A is not a constant and is subjected to temporal variations. On the one hand the distribution throughout the section of the vessel is not uniform and on the other hand the blue light of stimulation and the yellowish-green fluorescein are absorbed by the hemoglobin, axially located fluorescein in the bloodstream contributing with a smaller share for $\bar{I}(t)$ than juxta-parietal dye. As early as 1977, Behrendt assumed that in fluorescein angiography only juxta-parietal fluorescein was responsible for the fluorescence. This hypothesis was confirmed by Hill and Young (1976) who performed cineangiographic experiments whit fluorescein and indocianine green. The latter dye appeared earlier when infra-red angiography, which is less amenable to hemoglobin absorption, was used.

On the other hand the size of the photosensitive plaque may affect the venous dilution curve.

II) — Influence of recirculation on dilution curves

The fluorescein recirculation usually occurs before the completion of the curve corresponding to the first passage (Figure 2).

The correction is made by extrapolation assuming that the fall of concentration levels are exponential.

For Riva et al (1978) this correction is not possible in about 50% of the cases because the recirculation in the venous curve occurs almost simultaneously with the beginning of the descending slope of the curve. Riva uses the Stow and Hetzel method plotting the curve in function of the log-normal distrinution.

III) — Validity of the results in patients with abnormalities of the blood-retina barrier

According to the method of Fick for the appraisal of the mean circulation time, the retinal segment under study is considered to be a closed system i. e. it assumed that fluorescein does not spill off the vessels. This is not the case in most of the vascular retinopathies which compromise the accuracy of the results concerning bloodflow evaluation in these situations. On the other hand, neovascularization accompanied by several vitreous hemorrhage also hinders the build up of dilution curves.

III — TWO-POINT FLUOROPHOTOMETRY (PERSONAL CONTRIBUTION)

1 — TECHNIQUE

The two-point fluorophotometer is composed of a Haag-Streit slit lamp provided with a new light source (optic fiber connected to a ventilated 150W unit) and a system of filters, the stimulation filter, BALZER FITC-3 at the illumination arm, and the barrier filter, ILFORD 110 located in front of the photomultipliers.

The system of photometric detection is composed of a modified eye piece comprising two photosensitive plaques that may be juxtaposed on two arteriolar sections 900μ apart on the retina by way of a rotation of the eye piece. The photosensitive plaques are connected to two fibers, two photomultipliers and two photometers, the latter ones being also connected to a double beam recording osciloscope. The base time in the osciloscope is 0.1 s/partition.

The measurements are obtained after the setting of a vacuum contact lens that induces intraocular hypertension in the range of 30 mmHg.

The fluorescein time lag between the two sections is assumed to correspond to the minumum circulation time.

The diameter of the arteriolar segment is obtained from a fluoresceinic retinography performed while the patient is using contact lens.

2 — CRITICS TO OUR METHOD

a) Velocity profile

In our method when assessing the bloodflow across an arterial segment we took the mean time as being the double of the minimum circulation time assuming that the blood would behave as a Newtonian fluid displaying a parabolic erythrocyte velocity profile. This profile is, however, not yet fully characterized.

Studies performed with a Laser speedometer described the profile as being parabolic while with the help of cineangiography. Bulpitt et al (1973) found a somehow flattened profile, the so called *Shearing core*. In the latter case the mean time of circulation equals the minimum time (Figure 3).

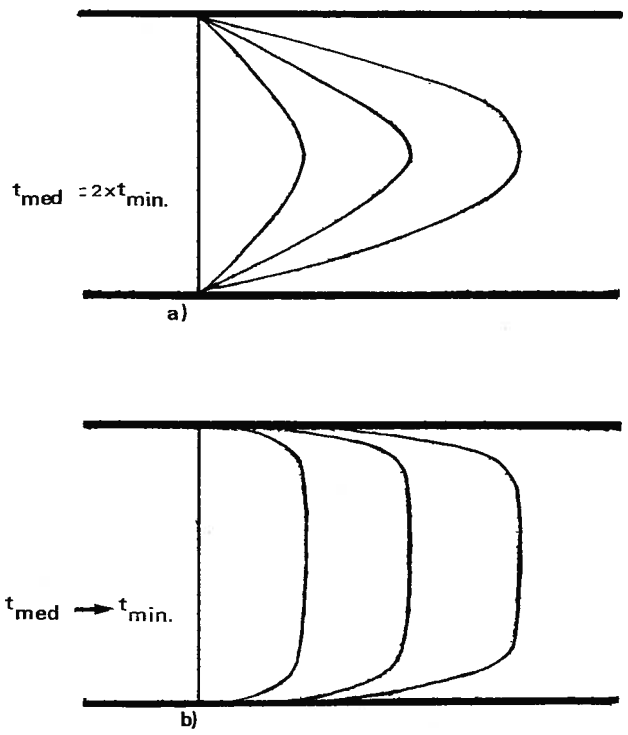

Fig. 3 — Erythrocyte velocity profile.
a) Parabolic shape
b) Flattened profile.

b) Reproducibility of the «time of appearance»

Riva (1979) and VanHeuven (1979) object to our study stating that they were not able to determine the appearance of fluorescein using direct fluorophotometric technique or by way of a video recording since the background noise of the photomultiplier was superimposed on the moment the fluorescein bolus was to become apparent. However, our method is not concerned with the measurement of the time of appearance of the fluorescein bolus on both sections of the artery but with the time which lapses between equal fluorescein intensity recordings on both sections of the artery. Likewise, Fonda et al (1976) using video recording concluded that what they call initial time (arbitrary time that fluorescein intensity attains 0.5 mm in the dilutionn curve) is a better reproducible parameter than the time of maximal intensity of the dilution curves used by Riva and VanHeuven.

c) Pulsatile nature of retinal arteriolar circulation

Bulpitt, Kohner and Dollery in 1973 resorting to cineangiography demonstrated the pulsatile nature of retinal arteriolar circulation. In vessels measuring between 30 and 80μ the systolic blood velocity was, therefore, 100-233% higher than diastolic velocity. Similar differences were found by Ebeli et al (1978), Feke et al (1978-1981) and Riva et al (1982).

As the determination of the circulation time according to our method is instantaneous (around 150ms) it may occur during systole or diastole and therefore be subjected to individual variations in the order of 3X.

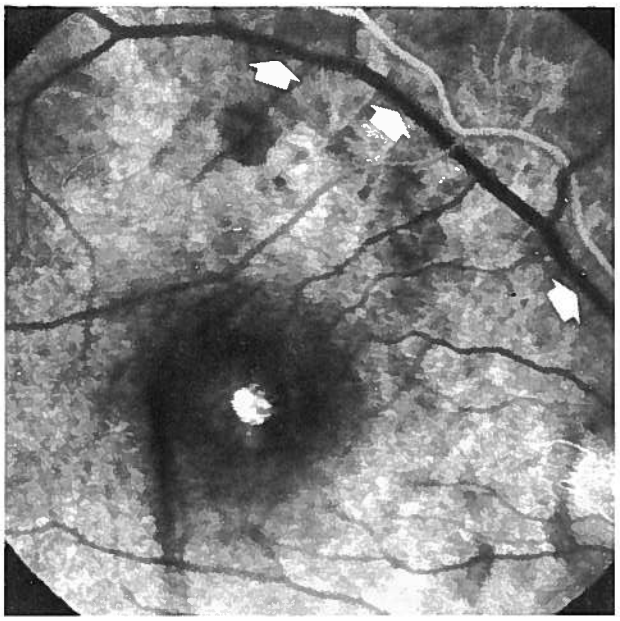

Fig. 4 — Arterial phase of an angiogram.
«The blood contained in the vein (arrow) blots out choroidal fluorescence».

We did not observe these variations in our patients maybe because we performed the measurements under conditions of raised intraocular pressure in the range of 30 mmHg caused by the suction lens and in these circumstances Langham and Tómey (1978) evidenced a reduction in the amplitude of the ocular pulse in contradiction to former manometric studies. On the other hand, we always inject fluorescein in small quantities (0.1ml of a 20% solution), instantaneously and during the systole by way of a reograph osciloscope. It is also known that the ocular pulse is synchronized with the heartbeat.

d) Influence of choroidal fluorescence

The adequate positioning of the photosensitive plaques on the arteriole prevents choroidal fluorescence from being picked up since erythrocytes are well impenetrable to stimulation or emission light (Figure 4).

In the case that small ocular movements may cause the photosensitive plaque to pick up some choroidal fluorescence, this should not be confused with arterial fluorescence.

It is noteworthy that when used fluorophotometry coupled to a Zeiss camera according to the method described by Riva we found it more difficult to perform small adjustments than when a slit lamp was used.

e) Difficulties experienced in clinical routine

Our technique was carried out by a team of 3 technicians. One of them works with the slit lamp, another injects the fluorescein while observing the osciloscope reography and the last one switches off the memmory of the osciloscope as soon as the device detects the presence of fluorescein since the time base of the osciloscope is 0.1/s.

The technicians mus be well trained to perform a team work and this fact if not strictly observed may hamper the easy clinical application of the method. However, when compared to other techniques this method may be considered as readily available for clinical purposes.

3 — ESTIMATION OF REPRODUCIBILITY

Five normal volunteers were studied by way of repeated (3-4) instantaneous injections of fluorescein (0.1-0.2cc of a 20% solution) administered synchronously with cardiac systole, amounting to 16 measurements.

Table 1 — Reproducibility of the method

	Age	T (msec.) Max	Min	A	CV (%)
A.L.	49 (4)	190	170	20	4.5
J.M.S.	29 (3)	150	140	10	3.9
E.F.V.	20 (3)	250	230	20	4.2
M.G.B.A.	27 (3)	210	200	10	2.8
E.L.A.	30 (3)	160	150	10	3.8

A = Amplitude

CV = Coefficient of variation $\frac{d}{\bar{m}'}$

Our results (Table 1) present amplitudes in the range of 20ms with coefficients of variation (relationship between standard deviation and average) between 2.8 and 4.5 which stands for the good reproducibility of the method.

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