Fluorescein diffusion in the human optic disc

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The characteristics of the transcapillary transfer of fluorescein dye in the optic disc of healthy individuals has been studied. A diffusible fluorescein dye and a nondiffusible reference substance, indocyanine green (ICG), which was assumed to remain in the capillaries, were injected into the circulatory system. The time courses of the concentrations of the two dyes in the optic disc were determined by simultaneously recording the fluorescence intensity of fluorescein and the infrared absorption by ICG with a fundus reflectometer. The difference between the fluorescein concentration curve and the reference ICG curve is a measure of the accumulation of fluorescein in the disc tissue. Our measurements indicate that fluorescein dye does not diffuse across the capillaries in the optic disc. The accumulation of fluorescein in the disc only starts at about one minute after the injection and seems to be due to diffusion of the dye from the surrounding choroid. The time constant of this diffusion process was found to be approximately one minute.

Key words: fluorescein angiography, fluorescein diffusion, optic nerve circulation, dye dilution technique, fluorescence of disc, indocyanine green angiography, blood-brain barrier.

A sufficient rate of exchange of metabolites across the capillaries is essential to maintain normal tissue function. Diseases that affect the optic disc circulation, such as glaucoma or papilledema, lead to a visual deficit presumably through alteration of the normal rate of transcapillary exchange. The measurement of the parameters that characterize this exchange is therefore of utmost importance.

In a theoretical paper, Goldmann recently suggested using fluorescein as a tracer to measure the rate of exchange of metabolites between the capillaries of the optic disc and the nerve-head tissue. He treated the transfer of fluorescein from the capillaries into the disc tissue as a two-compartmental diffusion process. In his model, the transit of fluorescein into the tissue is characterized by a single parameter, the time constant $1/M$ of the transfer process. $1/M$ can be determined if the time course of the concentration of the fluorescein in each compartment, i.e., the capillaries and the disc tissue, can be measured. This is not possible in the optic disc, since these two compartments are intermixed. Goldmann attempted to overcome this difficulty by using two pro-
Fig. 1. Dye dilution curves recorded with a fundus reflectometer from the superior temporal branch of a retinal vessel of a normal subject after injection of a mixture of 500 mg. of fluorescein and 50 mg. of indocyanine green. The time scale is in seconds for the first part and in minutes for the remainder. The vertical scale is in arbitrary units of concentration. When comparing the fluorescein curve (dashed line) to the ICC curve (solid line), three parts can be identified. The first part, A, is the first passage of the dyes, where both curves are practically identical. In the second part, B, the rate of decay of the fluorescein concentration is slightly faster than that of ICC. This can be explained by the fact that fluorescein is distributed both in the plasma and in the extracellular fluid, whereas ICC is distributed only in the plasma. In the third part of the curves, C, the rate of decay of ICC is slightly faster than that of fluorescein due to the fact that the elimination of ICC by the liver is faster than the elimination of fluorescein by the kidneys.

We propose an alternative approach which uses a two-tracer method. This method consists of injecting a combination of two dyes into the circulation: fluorescein, which is diffusible, and ICC, which is nondiffusible. The rate of accumulation of fluorescein in the disc tissue is determined from the difference between the time courses of the concentrations of these two dyes. This method has been applied to study fluorescein accumulation in the optic disc tissue of normal individuals.

General considerations

Let us first consider the time course of the concentrations of fluorescein and ICC in the blood after a sudden injection of a mixture of these two dyes into the circulation. These can be obtained by recording with a fundus reflectometer: the intensity of fluorescent green light emitted by fluorescein and the change in intensity of infrared light due to absorption by ICC. The recordings are made directly from a retinal vessel. We assume, and shall later demonstrate, that the fluorescence intensity and the infrared density (logarithm of intensity) changes are proportional to the concentration of the respective dyes. Fig. 1 displays representative fluorescein (dashed line) and ICC (solid line) concentration curves, recorded from a retinal artery. The time course of the concentration of fluorescein is very similar to that of ICC.

If we now perform our measurements on a part of the optic disc tissue that is perfused by a mesh of capillaries permeable to fluorescein, and therefore will permit a local accumulation of fluorescein, we will record a fluorescein curve, $C_f(t)$, which should differ from the ICC curve, $C_{ic}(t)$. $C_f(t)$ will be a summation of two components: one represents the intravascular fluorescein, $C_f(t)$, and the other corresponds to the fluorescein which has accumulated in the tissue, $C_{f,t}(t)$. Thus

$$C_f(t) = C_f(t) - C_{f,t}.$$  
Considering the similarity between the intravascular concentration curves of ICC and fluorescein, we can state as a good approximation that $C_f(t)$ is equivalent to $C_{ic}(t)$. Therefore,

$$C_f(t) = C_{ic}(t) - C_{ic0}(t),$$

where the concentrations are normalized with respect to the concentration of the injectate.

Method

Seven healthy volunteers, 25 to 37 years old with normal vision and no ocular abnormalities, served as subjects. Injections were made in the antecubital vein. A mixture of 50 mg. of indocyanine green (ICC) and 500 mg. of sodium fluorescein was injected in approximately two seconds. The injections were followed by a saline flush. The passage of the dyes in the eye was documented photoelectrically with our fundus reflectometer. With this instrument, the fluorescence intensity of fluorescein, $I_f$, and the changes in infrared optical density of ICC, $\Delta D^{IR}$, are simultaneously recorded photoelectrically from a small spot (200
Fluorescein diffusion in optic disc

Fig. 2. Relation of fluorescence intensity, $I''$, to fluorescein concentration and of the infrared density changes, $\Delta D''$, to indocyanine green concentration in the optic disc tissue. Four injections (each lasting two seconds) were given to the same normal subject on various days. Recordings were made with a fundus reflectometer from a point on the temporal side of the optic disc. The intensity of light at the peak dye concentration of the respective curves was plotted against the amount of dyes in the injectate. The linear relation that was obtained signifies that, in the present range of concentration, the intensity of light is directly proportional to dye concentration.

Basic to our method is the assumption that $I''$ and $\Delta D''$ are directly proportional to the concentrations of the dyes. We have validated this assumption experimentally for the optic nerve. The results are shown in Fig. 2.

Results

Fig. 3 displays typical dilution curves recorded from a normal optic disc in the region indicated on the fundus drawing. The fluorescein dilution curve is practically identical to the ICG curve during the first passage. After the first passage, however, the fluorescein curve rises continuously instead of decaying. This is the result of accumulation of fluorescein dye in the tissue under the measuring spot. The rate of increase of fluorescein dye in the tissue can be obtained as described above by subtracting the ICG curve from the fluorescein curve. The result of this subtraction is the dashed curve in Fig. 3 and represents the time course of accumulation of fluorescein.
in the optic nerve tissue. Fluorescein starts to accumulate in the tissue only after the passage of the main fluorescein bolus and increases continuously to reach a plateau at about 7 to 10 minutes after injection. We fitted this curve to an exponential function of the type

\[ I_p (1 - e^{-\beta (t - t_0)}) \]

where \( I_p \) is the intensity measured at the plateau, \( 1/\beta \) is the time constant of this diffusion process, and \( t_0 \) is the time at the beginning of the dashed curve. A good fit is obtained when \( 1/\beta \) approximately equals one minute.

We also measured the fluorescence intensity quasi-simultaneously from both the center and the periphery of the optic disc in one patient by alternately moving the excitation spot from one site to the other. We then computed the difference between both intensity recordings. The time variation of this difference, plotted in Fig. 4, implies faster peripheral accumulation.

**Discussion**

This paper describes a new method of determining the rate of entry of fluorescein into the optic disc. With this method, the time course of the concentration of the fluorescein in the capillaries of the nerve is deduced from the time courses of the concentration of the simultaneously injected dyes. By subtracting the time course of the concentration of ICG from that of the total fluorescein concentration, we ob-
tain directly the time course of the fluorescein located in the disc tissue and hence can avoid pressing on the eye. We found that the time constant for the diffusion of fluorescein into the optic disc is approximately one minute.

Our method eliminates estimating the concentration of fluorescein in the disc capillaries from measurements in a retinal artery. Such a procedure may be feasible in the state of health but will lead to a major error if, for example, a local reduction of the disc capillary blood flow is not accompanied by a similar impairment of the retinal circulation.

Our method, however, is not free of inaccuracies. In setting the intravascular fluorescein concentration, \( C_f(t) \), equal to intravascular ICG concentration, \( C_{ICG}(t) \), we introduce some error in the estimation of the time constant of fluorescein accumulation. However, after examining the close correspondence between the time course of the two dyes in blood, we are satisfied that this error does not exceed 20 per cent.

We also wish to point out that the amplitude and time course of the ICG curve permit us to study changes in the intravascular circulation at the nerve head. This capability should prove useful in following specific pathology associated with altered circulation in the nerve head.

Having approximated the time constant of diffusion at one minute (dashed line, Fig. 3), we now wish to investigate the origin of the fluorescein accumulation. The most natural assumption is that the fluorescein has diffused out of the capillaries of the optic disc. If this is the case, we would expect that during the first passage of the fluorescein dye, which is on the order of a third of a minute, a substantial amount of fluorescein would accumulate in the tissue. This would alter the shape of the fluorescein curve during the first passage. In particular, a marked delay in the peak of fluorescein concentration as compared to

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**Fig. 6.** Same as in Fig. 5, but for \( R = 50 \). Sections A, B, C, and D are solutions for time constants \( 1/M = 1, 2, 20, \) and 40 minutes, respectively. The time scale is in seconds.
the corresponding peak of ICG would be observed.

To test the effect of the fluorescein diffusion on the fluorescein curve, we solved the differential equation associated with Goldmann's model (Equation 1 in the appendix) for various time constants, \( 1/M \). Figs. 5 and 6 indicate that for a time constant of one minute, the shape of the fluorescein dilution curve for the first passage should be very different from the actual recorded curve. For example, the peak fluorescence intensity should be reached 10 to 17 seconds later than for ICG. Moreover, once peak intensity has been reached, the fluorescein curve should remain much higher than the ICG curve. Clearly, this is not the case in our recordings. The computed curves fit the first part of the recorded fluorescein curves better when the equation is solved for time constants from infinity down to 40 minutes. We can thus conclude that the accumulation of fluorescein in the disc is not the result of its diffusion across the walls of the disc capillaries. Therefore, we must look for another source for this accumulation.

We note that fluorescein accumulates faster in the periphery of the disc. This suggests that fluorescein is entering the optic disc from this area. This view is consistent with the findings of Grayson and Laties' in monkeys. Using a fluorescence microscopy technique, they demonstrated that fluorescein within the optic nerve was confined to the blood vessels only. In addition, they found small amounts of fluorescein in the periphery of the nerve and postulated that the origin of this fluorescein is the choroid. The possible routes by which fluorescein could reach the optic nerve from the choroid have been discussed by Cohen. 7

Our conclusion that fluorescein does not diffuse across the capillaries of the optic disc is further supported by electron microscopic findings: the capillaries of the optic disc do not have fenestrae in the basement membrane and their endothelial cells possess tight junctions. In this respect, these capillaries are similar to other capillaries of the central nervous system and share with them a special characteristic of transcapillary transfer, generally termed the blood-brain barrier.

Our conclusions are valid only for that part of the optic disc from which fluorescence can be recorded effectively. Because of the strong light scattering by the tissues and the high optical absorption of the blood, it may be that the area from which we record fluorescence does not extend down to the level of the lamina cribrosa except in the physiologic cup. Further investigations are necessary to determine the actual depth from which fluorescein fluorescence can be recorded effectively in the disc. Ernest and Archer 7 described four stages of disc fluorescence during fluorescein angiography. Their arterial and venous stages correspond to the passage of the main bolus in the dilution curve in our recordings. Their late fluorescence stage coincides with the part of our curve that is characterized by a diffusion constant of approximately one minute. Our photometric method did not confirm Ernest and Archer's suggested initial retrobulbar fluorescence stage.

Fluorescein angiography after acute glaucoma and in conditions such as papilledema reveals early leakage of the dye into the optic nerve tissue. This is due to impeded microcirculation, which results in capillary endothelial damage and disappearance of the normal blood-brain barrier. Further experiments are now underway to determine if our method is suitable for quantifying the severity of such conditions. We are also attempting to determine if our method can be used to detect changes in the integrity of the blood-brain barrier in less severe circulatory disturbances of the optic disc, such as simple glaucoma.

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REFERENCES


Appendix

In Goldmann's theory, the diffusion of fluorescein into the optic disc is described by the following differential equation:

\[
\frac{1}{M} \frac{dC'_t}{dt} = C'_f - C'_t
\]

where \(C'_t\) represents the concentration of fluorescein in the feeding arteries and \(C'_f\) represents the concentration of fluorescein in the tissue of the optic disc. 1/M is the time constant of the transfer process. The problem is to determine 1/M from measurements of \(C'_t\) and \(C'_f\).

Let us assume that fluorescein is injected into the circulation and that the concentration time curve of the fluorescein in the capillaries of the disc is the one presented in Fig. 1. During the first passage, the concentration of fluorescein in such vessels, \(C'_f(t)\), can be expressed mathematically as

\[
C'_f(t) = K(e^{-\alpha t} - e^{-\alpha t'})
\]

We fitted our recorded curves from the optic disc to this function by substituting the values 1/8 for \(\alpha\), and 1/4 for \(\alpha\). \(K\) is the constant of proportionality. Let us assume that the volume of the interstitium (\(V'\)) of the optic disc under the measuring spot is 10 to 50 times larger than the volume of the capillaries perfusing this tissue (\(V_v\)) and that the fluorescein which diffuses out of these vessels is instantaneously diluted in the total volume of the interstitium. Let us represent the ratio \(V'/V_v\) by \(R\). Substituting \(C'_f\) by \(N_t'/V'\) and \(C'_t\) by \(N_t'/V_v\) into Equation 1 gives:

\[
\frac{1}{M} \frac{dN'_t}{dt} = \frac{N'_t}{V'} - \frac{N'_t}{V_v}
\]

\(N'_t\) and \(N'_t\) represent, respectively, the number of fluorescein molecules in the volume of the vessels, \(V'_v\), and in the volume of the interstitium of the tissue, \(V'\). \(V'_v + V'\) is the total volume under the measuring spot. Since \(V' = R \cdot V'_v\), we can write:

\[
\frac{1}{M} \frac{dN'_t}{dt} = R \frac{N'_t}{V'} - \frac{N'_t}{V'_v}
\]

This equation was solved by computer using

\[
N'_t(t) = K (e^{-\alpha t} - e^{-\alpha t'})
\]

The constant of proportionality \(K\) can be taken as 1 without loss of generality. Fig. 5 displays some of the computer solutions for various time constants 1/M when the ratio \(V'/V_v\) was assigned the value 10. Fig. 6 presents similar curves for \(R = 50\).

In writing equation 3, we are aware that equating the capillary concentration of fluorescein to the concentration in the arterial blood does introduce an error, as discussed by Pappenheimer, Benkin, and Borroco. This error, found to be very important for highly diffusible tracers with small molecular weights, such as water or NaCl, decreases with increasing molecular weight. For fluorescein (MW = 332.5), the magnitude of the error cannot be determined at this stage, but is thought to be sufficiently small.