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Calculation of the permeability of the blood-retinal barrier to fluorescein

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Abstract. A method is presented, for calculation of the permeability of the blood-retinal barrier to fluorescein which is based upon stimultaneous determination of the free fluorescein concentration in plasma and the fluorescein concentration profile in the vitreous body. By aid of a simplified mathematical model of the eye the blood-retinal barrier permeability is calculated automatically on a computer from corresponding values of the fluorescein concentration in plasma and in the vitreous body. The present method eliminates some of the factors of uncertainty, which have been present in earlier applied fluorophotometric methods, thus contributing to increasing the exactness of the fluorophotometric method for the estimation of the permeability of the blood-retinal barrier to fluorescein. Apart from the permeability of the barrier, the diffusion coefficient for fluorescein in the vitreous body is also estimated by the present method.

Introduction

The retinal cells are separated from the bloodstream by a unique barrier system called the blood-retinal barrier. This barrier is made up by two epithelial cell systems, i.e., the pigment epithelium and the endothelial cells of the retinal vasculature (Cunha-Vaz 1979). Under normal circumstances the barrier is tight, only permitting passage of biologically important substances (glucose and certain amino acids), while all other water-soluble substances such as fluorescein are almost totally detained by the barrier. However, several retinal diseases are connected with an increase in the permability of the barrier (breakdown of the blood-retinal barrier). Quantitation of the permeability of the barrier to fluorescein has been carried out during the last 8 years by vitreous fluorophotometry. The method is based on a determination of fluorescein in the vitreous body by a slit lamp fluorophotometer after i.v. injection of a standard dose of fluorescein. Even though the method in its first presentation has given valuable results, the exact calculation of the permeability and a comparison of data from different laboratories have been complicated by a number of uncertain factors: individual unknown variations of the plasma fluorescein concentration, binding of fluorescein to plasma proteins, metabolism of fluorescein, variations in optical resolution of the equipment used, variations in time of measurement in the vitreous body after fluorescein injection, and difficulties in the exact positioning of the concentration profile in the vitreous in relation to the retina and lens.

The present paper presents a standardized method for calculation of the blood-retinal barrier permeability to fluorescein, which eliminates or reduces the above-mentioned factors of uncertainty. The method focuses especially upon fluorescein in plasma, the determination and definition of the fluorescein concentration profile in the vitreous body, and formulation of a mathematical formalism, which permits the permeability of the blood-retinal barrier to fluorescein to be calculated from corresponding values of fluorescein in the plasma and vitreous body. This method is described in the present paper, while a more detailed presentation is given elsewhere (Lund-Andersen and Krogsaa 1982; Lund-Andersen et al. 1982; Larsen et al. 1983; Lund-Andersen et al. 1985).

Fluorescein in plasma

Fluorescein (14 mg/kg body weight) was injected i.v. over a 1-min period. Blood samples were obtained from another, previously placed catheter before injection and 5, 15, 30, 60, and 120 min after wards. In order to separate free fluorescein from protein-bound fluorescein, the plasma was ultrafiltrated (Lund-Andersen and Krogsaa 1982; Lund-Andersen et al. 1982). The free concentration in the ultrafiltrate, as well as the total plasma fluorescein concentration, was determined by conventional fluorophotometry – the latter after suitable dilution of the plasma; the filter combination used ensured that only about 10% of the fluorescence signal was due to fluroescence from fluorescein metabolites. Accordingly, the fluorescence obtained from the plasma and vitreous body is, in the following, interpreted as reflecting fluorescein alone.

Figure 1 shows the free fraction of fluorescein in ultrafiltrate versus time after injection. It appears that the free fraction is increasing. This phenomenon is an in vivo phenomenon, the mechanism of which is not fully understood at present. However, the fact that the free fraction is not constant necessitates a determination of the free-fluorescein concentration over the entire period of examination, rather than calculating this value from measurements of total plasma fluorescein under the assumption that there is a constant ratio between free and bound fluorescein.

Figure 2 shows the time course of the free fluorescein concentration in plasma after an injection of fluorescein in a normal male volunteer. The initial period indicated

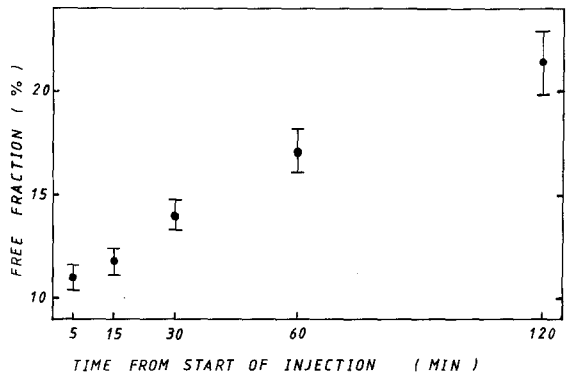

Fig. 1. Free fraction of fluorescein in plasma versus time from start of injection of fluorescein. The free fraction was obtained by ultrafiltration and is expressed in percent of total concentration in plasma. Results are means ± 2 SEM of 21 determinations (2 normal volunteers and 19 patients). The values from the patients and volunteers did not differ (by permission of Lund-Andersen et al. 1982)

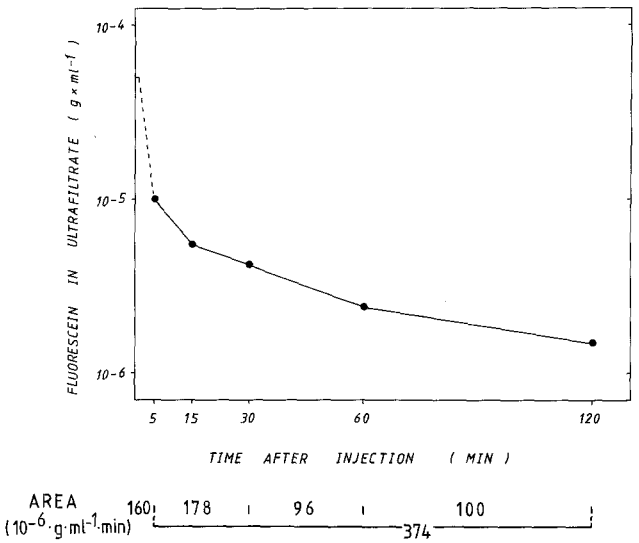

Fig. 2. Concentration of free fluorescein (*ordinate*) versus time (*abscissa*) in plasma from a normal male volunteer. Fluorescein was injected at time 0. The first blood sample was obtained at time 5 min, the following 15, 30, 60, and 120 min after injection. The concentration during the first minute of injection was not directly measured, but calculated (see text), and this part of the curve is accordingly indicated by *dotted lines*. The directly observed values each represent the means of 3 determinations with SEM smaller than the symbol. In the lower part of the figure the area under the curve is shown, partly as the fractional areas and partly as the sum

by a dotted line represents the estimated concentration in the bolus when the 10% fluorescein solution was injected over a 1-min period, assuming a cardiac output of 5 l, hematocrit of 43%, free fraction of 15% and neglecting recirculation and extraction in the pulmonary circulation. The area under the curve, including the bolus, is shown in the lower part of the figure. It appears that the area under the bolus is about 30% of the total area. Since the area under the curve represents the amount of fluorescein to which the blood-retinal barrier has been exposed, the area under the bolus has to be taken into consideration by calculation of the permeability of the barrier, as will be the case in the present paper. The area under the curve shows only a smal-

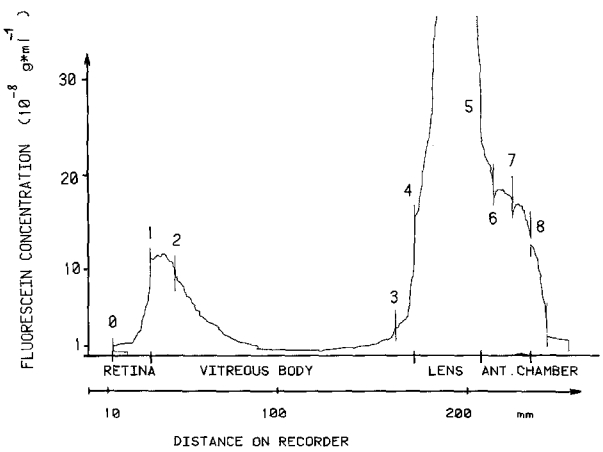

Fig. 3. Fluorescein concentration in the vitreous body and anterior chamber of the eye from a patient with proliferative retinopathy 60 min after injection of fluorescein. The fluorescence corresponding to the lens reflects autofluorescence of the lens. The *vertical marker points* indicate the following positions of the optic fiber: *1*) retina in focus, with blue slit in the middle of the optic fiber; *2*) optic fiber free of the blue slit on retina; *3*) optic fiber at the postérior lens surface; *4*) posterior lens surface in the middle of the optic fiber; *5*) anterior lens surface in the middle of the optic fiber; *6*) optic fiber at the anterior lens surface; *7*) optic fiber at the posterior corneal surface; *8*) posterior corneal surface in the middle of the optic fiber

ler variation (within 10%) when the determination is repeated on the same person over a period of half a year. However, the interindividual variation is of a considerable magnitude. Thus measurements on 100 patients with diabetes, sclerosis disseminata and arterial hypertension have shown interindividual variations of a factor of three between the areas under the curve. The reason for this variation, which is also present for the total fluorescein concentration, is at present not fully understood. The phenomenon, however, stresses the importance of plasma fluorescein determinations for each individual clinical examination.

Determination of the fluorescein concentration profile in the vitreous body

The concentration profile of fluorescein in the vitreous body was determined by a slit lamp fluorophotometer, in principle as described by Cunha-Vaz, but with some modifications, as described in detail elsewhere (Lund-Andersen et al. 1985). A foot switch makes it possible on the X axis to mark positions of the optic fiber in relation to structures in the eye. The equipment is coupled on-line to a minicomputer in parallel with the X–Y recorder.

The minicomputer is connected to a large central computer, with graphical terminal. The fluorophotometer shows a linear relationship between concentration and current over two decades. The signal-to-noise ratio was optimized by application of a chopped light system so that a concentration of 1.10^{-9} g/ml was determined significantly. The spatial resolution showed that less than 5% of the fluorescence from the retina and choroid was measured 1.5 mm from the retina. The concentration profile was measured before, 30, 60, and 120 min after injection of fluorescein. Automatic calibration was performed before every measurement.

Figure 3 shows a typical concentration profile obtained on a diabetic patient with proliferative retinopathy 60 min

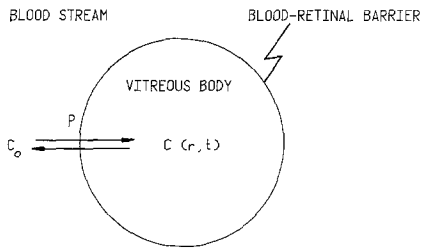

Fig. 4. Simplified model of the eye used for automatic calculation of the permeabity of the blood-retinal barrier to fluorescein (P). (C_o) indicates concentration of free fluorescein in plasma, C (r, t) concentration of fluorescein in vitreous body at the position (r) at time (t)

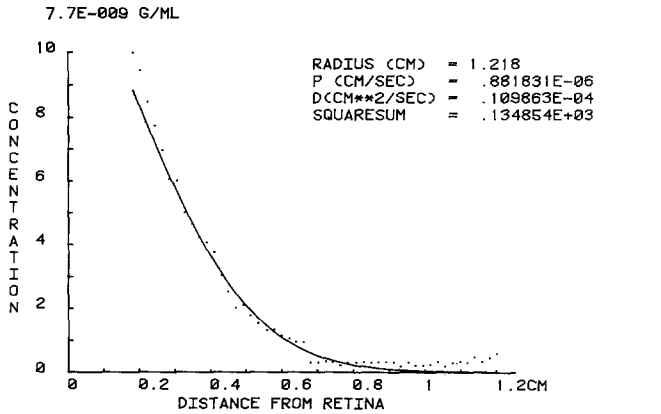

Fig. 5. Graphic illustration of the directly measured concentration profile in the vitreous body indicated by *dots* after calculation of its placement in the vitreous body and substraction of the fluorescence before injection of fluorescein. Data originate from Fig. 3. The fully drawn *line* represents the profile corresponding to the best fit of model to experimental data. The permeability P and diffusion coefficient D appear in the right upper corner of the figure, together with the statistical square sum parameter. The curve fraction between marker point 1 and 2 is not included (cf text)

after injection of fluorescein. The numbered vertical marks indicate certain positions of the optic fiber, as explained in the legend of Fig. 3.

Placement of the concentration profile in the vitreous body and calculation of the radius of the eye are performed by application of the marker points and knowledge of the ratio between movement of slit lamp and movement of the focus plane in the eye, as described by Krogsaa et al. (1984). The fluorescence signal obtained before injection is subtracted automatically from the signal obtained after injection.

During calculation of the permeability the part of the concentration profile placed between marker points 1 and 2 is not used. This is due to the fact that this part is not well defined since it reflects both fluorescence from the retina and choroid and quenched fluorescence from the extracellular space of the retina. The computer model described in the following works independently upon that fraction of the curve and extrapolates the concentration profile back to the retinal level.

Calculation of the blood-retinal barrier permeability

Calculation of the permeability of the blood-retinal barrier requires a combination of the plasma concentration curve (Fig. 2) with the concentration profile in the vitreous body (Fig. 3). This combination is performed by curve fitting using a simplified model of the eye. The model is schematically shown in Fig. 4. It appears that the blood-retinal barrier is compared with a homogeneous spherical shell, which separates the bloodstream from the vitreous body. Fluorescein can penetrate the barrier in both directions with permeability P, and fluorescein moves in the vitreous body only by simple diffusion, with diffusion coefficient D. The equation combining the concentration in the vitreous body (C (r,t)), distance from centrum (r), radius (a), permeability (P), plasma concentration (C_o), time (t), and diffusion coefficient in the vitreous body (D) is given in Eq. (1)

$$c(r,t)=\int_o^t c_o(t-s)\cdot F(r,s;a,D,P)ds, \tag{1}$$

$$F(r,s;a,D,P) = \frac{aP}{r\sqrt{D}}\left[G\left(\frac{a-r}{2\sqrt{D}},s;k\right)-G\left(\frac{a+r}{2\sqrt{D}},s;k\right)\right]. \tag{2}$$

$$G(x,s;k)=e^{-x^2/4s}/\sqrt{\pi}s-k\cdot e^{k(x+k\cdot s)}\operatorname{erfc}(k\sqrt{s}+x/a\sqrt{s}) \tag{3}$$

where $k=P/D-D/a$ and erfc is the complementary error function. Radius (a) is determined experimentally. P and D thus have to be determined from the experiments.

The development of the equations is described in more detail by Larsen et al. (1983).

The mathematical formula is coupled on a computer to which the plasma concentration curve and the concentration profile in the vitreous are transmitted. The computer changes P and D until the best fit between model concentration profile and the directly observed concentration profile is obtained. The values of P and D appear on a graphical terminal (shown in Fig. 5; see figure legend). The present method forms the basis for examination of the permeability of the blood-retinal barrier to fluorescein in our clinical examinations. The method furthermore enables an estimation to be made of the fluorescein diffusion coefficient in the vitreous body.

Comments on the simplified model and the interpretation of P

The model is a considerable simplification of the actual situation, especially since it does not take into consideration the anatomical asymmetry of the eye corresponding to the differences between the anterior and the posterior part of eye. However, the model is usable in cases in which the contribution of fluorescein from the anterior segment is in significant, such as in the example shown in Fig. 3.

In the present formula the transport across the barrier is stimulated by a single symmetrical permeability factor. This is a considerable simplification since the transport across the barrier is a result of permeation according to the electrochemical gradients and possible active transport processes. However, to date there is no knowledge about these processes and their mutual significance for the resultant transport. Accordingly, at present it seems reasonable to use an operational permeability factor to describe the per-

meability of the barrier. However, in the biological interpretation of P it is necessary to take these facts into consideration. Thus a change in electrical potential across the barrier alone will lead to change in P.

Even though the present method eliminates several of the methodological problems apparent in previously applied methods, the exact biological interpretation of P is a key problem in the interpretation of the fluorophotometric data and is not yet solved. Further work and experiments are necessary in order to elucidate this key problem.

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