Electroretinogram changes after fluorescein injection: a new method to evaluate blood-retinal barrier dysfunction. MAKOTO TAMAI AND KATSUYOSHI MIZUNO.

When a retina with an impaired blood-retinal barrier (BRB) is illuminated with blue light (480 nm) after intravenous fluorescein (F) injection, photoreceptors are presumed to be stimulated not only by the original blue light but also by a green one (520 nm) that is emitted by the fluorescein. In the present study, the value of the electroretinogram (ERG) was examined before and up to 10 min after injection, and the values were significantly larger than those of normal subjects at 0.25 (p < 0.001), 1 (p < 0.05), 2 (p < 0.005), 3 (p < 0.05), and 10 (p < 0.01) min after injection. It may be concluded from these results that a comparison of the ERGs before and after F injection can be useful in demonstration of impairment or destruction of the inner and outer BRB.

Quantitative vitreous fluorophotometry has made it possible to detect one of the earliest changes of the blood-retinal-barrier (BRB). The breakdown of the BRB in diabetes before the appearance of retinopathy has been reported by means of this method.¹ ² This technique was further extended to detect the earliest onset of retinitis pigmentosa.³ These studies demonstrated an increased leakage of fluorescein (F) into the vitreous from the inner and/or outer BRB 1 hr after intravenous F injection. It can be assumed that the retina would be heavily bathed with leaked F before its escape into the vitreous, and quantitative detection of leaked F within the retina proper seems to be impossible to detect by fluorophotometry. We therefore attempted to estimate the degree of leaked F contents in the retina and/or vitreous by means of recording electrical responses of retinal elements, whose activity would be elicited not only by the excitatory (480 nm) but also by the secondarily emitted fluorescent light (520 nm). This paper describes a method for following the electroretinogram in normal subjects and diabetic patients without overt retinopathy who were injected with F.

Materials and methods

Subjects. Two groups of patients were examined to test the clinical usefulness of the F-ERG, to standardize the procedure, and to determine its value for evaluating involvement of the BRB in the diabetic patient.

Group I. Normal subjects (17 eyes of 15 individuals; 30 to 70 years old) who had no history of systemic and ocular diseases and had normal corrected vision

Group II. Diabetic patients without overt retinal involvement (13 eyes of 7 cases; 49 to 74 years old)

These patients were being treated for diabetes and their blood sugar was being followed at the Diabetes Clinic of the University Hospital, Tohoku University Medical School. The duration of diabetes ranged from 4 months to 5 years. Patients in this group had 1.0 corrected visual acuity, and had normal ophthalmoscopic and F angiographic examinations. The discovery of any diabetic retinal changes automatically excluded a patient from this group.

ERG and F-ERG. Panthops M-II (Biophysi

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References


Fluorescein-ERG in normal subjects

Fig. 1. F-ERG in a normal subject. The value of the peak-to-peak amplitude of the a- and b-wave was 255 µV with 517 nm and 135 µV with 480 nm light before F injection. After administration of F, the 480 nm light value reached maximum (145 µV, 107.4%) at 5 min and minimum (120 µV, 88.9%) at 10 min. The value with orange light at 10 min was 209 µV, 82.1% of that before F injection.

Medical S.A., France) was used to record the ERG. The stimulating light of orange (1800 lux) and blue (150 lux) was 5 msec in duration and 4 Hz in frequency. The blue light was produced with a narrow band (480 nm) filter. The orange light was produced with a filter that cut off wave lengths below 517 nm. This light was used for getting the uniform adaptation level and checking the ERG that was elicited with the light of longer wave-lengths than that of the absorption spectrum of F. Both were obtained from a halogen lamp of 2000 lux and interference filters (Asahi Bunko, Tokyo). Forty-eight responses were averaged. These two kinds of stimulating light were transmitted by two optical fibers to a pair of stimulation binoculars that were set at the same distance for each eye. The stimulation tubes of the binoculars had a frosted glass of 5 cm in diameter and were placed 5 cm from the top of the corneas.

Subjects were fitted with contact lens electrodes and reclined on a bed in dim fluorescent light (15 lux on the bed). Maximum dilation of the pupil was obtained in both eyes by instillation of 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin phosphate; Santen Pharmaceutical Co., Osaka, Japan). They were asked to fix their eyes on the stimulation tubes. The control responses to orange and blue light were recorded.
before F injection. After intravenous injection of 10 ml of 5% sodium fluorescein, the averaged ERG with blue light was recorded six times: just after injection (0.25 min) and 1, 2, 3, 5, and 10 min later. Finally, the averaged ERG to the orange light was recorded once again.

The amplitude of the b-wave (trough of a-wave to peak of b-wave) was measured and compared to the amplitude that was recorded before F injection in both blue and orange light stimulation, respectively. This relative amplitude at each time point between normal subjects and the diabetics was statistically evaluated by the Mann-Whitney method. Furthermore, the enhancing effect was followed for much longer periods (up to 60 min) after F injection in several patients. Various degrees of enhancement were observed, but the discomfort produced by contact lens electrodes in the dark room over such a long period was so severe that the experiments were not continued for longer than 10 min.

Results. ERGs of a normal subject without
Fluorescein ERG in diabetes without overt retinopathy

Fig. 3. A, Distribution of values of F-ERGs before and after F injection in diabetic patients without overt retinopathy. B, Mean and standard deviation. o, Normal subjects shown in Fig. 2. B. In the diabetic patients, ERG was significantly enhanced at every recording point except at 5 min after injection.
cataract are presented in Fig. 1. The amplitude of b-wave was 255 \mu V with orange and 135 \mu V with blue light stimulation, respectively. During the 10 min recording sequence, the amplitude of the blue light–stimulated F-ERG increased or decreased slightly. It was 145 \mu V at 5 min, and 120 \mu V with blue and 209 \mu V with orange light at 10 min. These values were 107.4% and 88.9% of those recorded before F injection, respectively. The value with orange light stimulation at 10 min was 82.0% of the control value. The relative amplitude of the F-ERG of all cases in group I is shown in Fig. 2, A. Fig. 2, B, shows the mean value and standard deviation of each recorded point. The value with orange light was 87.5% ± 16.6 at 10 min.

The relative amplitudes of the F-ERG in 13 eyes of 7 cases in group II are shown in Fig. 3, A and B. In most cases, the relative amplitudes with blue light were above the 100% level for the entire recording period after injection. A statistical comparison of these amplitudes to those of normal subjects showed significant differences at all recording points except at 5 min (0.25 min p < 0.001; 1 min p < 0.05; 2 min p < 0.005; 3 min p < 0.05; and 10 min p < 0.01). The value with orange light was 87.5% ± 11.8 (mean ± S.D.) at 10 min, which showed no differences from that of normal subjects.

Discussion. Abnormal function of the BRB is closely correlated with retinal vascular and uveal diseases.\(^4\) Vitreous fluorophotometry has been recently developed as a quantitative method to estimate a degree of alteration of the BRB.\(^1\) The results presented in this paper show that impairments of the BRB can be detected sooner with the F-ERG than with fluorophotometry done 1 hr after F injection. The changes in the ERG (peak-to-peak amplitude of a- and b-wave) during the 10 min after intravenous F injection were small in normal subjects, who were expected to have no leakage of the dye, but the F-ERG was enhanced in diabetic patients without overt retinopathy.

A study of ERG amplitudes after injection in diabetic patients and in normal subjects (Fig. 3, B) reveals two important points. First, the enhancing effect of F injection on the ERG occurs in diabetes before overt retinopathy can be seen. Thus the breakdown or impairment of the BRB and leakage of F from the inner BRB occurs earlier in this disease than is apparent with conventional methods of examination. The present results strongly support those that have been described for vitreous fluorophotometry.\(^1\) Second, the enhancement of the amplitude appears very swiftly. Even immediately after F injection, the value is larger than that of the normal (p < 0.01). This may mean that photoreceptors or the associated retinal tissues are rapidly bathed by leaked F before its escape into the vitreous.

At present we have no data on how much of the original blue light was absorbed by the leaked F within the retina and/or vitreous and what fraction was re-emitted. Although there are still some problems to be resolved, it may be concluded that the F-ERG could be a useful clinical tool for supplementing vitreous fluorophotometry and for evaluating early changes in the BRB.

We extend our heartfelt thanks to Drs. D. I. Hamasaki and P. J. O'Brien for their critical reading of the manuscript, and to Miss S. Hirakawa for her secretarial assistance.

From the Department of Ophthalmology, Tohoku University School of Medicine, Sendai, 980 Japan. This work was supported by Grant-in-Aid for Scientific Research (No. 5482996) of the Ministry of Education, Science, and Culture of Japan. Submitted for publication Feb. 7, 1980. Reprint requests: Makoto Tamai, M.D., Department of Ophthalmology, Tohoku University School of Medicine, Sendai, 980 Japan.

Key words: blood-retinal barrier (BRB), inner BRB, outer BRB, electroretinogram, fluorescein injection, diabetes mellitus

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Electro-oculograms (EOGs) were recorded from 14 patients with a malignant melanoma of the choroid, nine of