

Electroretinogram - Flicker Fusion Frequency in Albino Trout

The role of the retinal epithelial pigment (REP) in the visual process is not completely understood. The REP is considered to shield the scotopic elements, the rods, from bright light in the light-adapted state. This occurs as a result of the retinomotor responses in most teleosts, the Anura and most birds¹. In the teleosts the REP reacts to light and temperature differently than do the rods and cones²⁻⁵. The REP in the unexposed eye responds to photic stimulation of the contra-lateral eye⁶. BROWN⁷ has recorded rapid evoked potentials from the REP. These potentials could be recorded from dry REP and are temperature dependant⁸.

Albinos are obviously excellent subjects in investigations of the REP's role in vision. The retina of the older albino trout differs in structure from that of the normal trout. There are fewer cones and the inner layers are poorly developed in the albino⁹. The absorption spectrum of the scotopic pigment of the albino rainbow trout, obtained photometrically by CROUZY and ALI¹⁰, corresponds to that obtained by WALD¹¹ with the extracted pigment of the normal rainbow trout (*Salmo gairdneri*).

Although the electroretinogram (ERG) of albinos has been recorded and found to be different from that of normal subjects¹², their ERG - Flicker Fusion Frequency (FFF) has not been studied. In this report the ERG - FFF of light- and dark-adapted albinos are presented and compared with those of normal trout.

Albino and normal brook trout, *Salvelinus fontinalis* (Mitchill) were obtained from the Provincial Government's hatchery at St. Faustin and were acclimated to the temperature at which the experiments were to be conducted. The fish were 12-15 cm long (total length). All the experiments were carried out between July and November 1967. The techniques for anaesthetization, creating stimulus light and recording ERGs were similar to those which we have described earlier¹³.

Before recording the ERG - FFFs of light-adapted fish, they were exposed to flickering light (30 c/sec) of the same intensity as that which was to be used in the experiment for 3-5 min. There was a background light throughout the experiment (6 ft-c). Thus, the fish were in the same state of light-adaptation. At each intensity the ERG - FFFs were recorded twice, once when the frequency of the stimulus

light was increased and again as the frequency was decreased.

In the case of the dark-adapted fish, they were dark-adapted for at least 2 h prior to the experiment. The fish were fixed to the tray and the electrodes placed in their positions under deep red light (25 W). The fish was left in total darkness for 5 min between each experiment at every intensity. Recording at each intensity lasted 7-10 sec. This was done to keep the fish in a completely dark-adapted state. At each of the intensities the FFFs were recorded twice with 5 min interval between the experiments.

At light intensities lower than 100 ft-c the ERG - FFFs of the albino are higher than those of the normal trout (Figure 1). The lower the intensity, the greater is the difference. The Maximum Fusion Frequency (MFF) is also attained at a much lower intensity in the albino (15 ft-c) than in the normal trout (250 ft-c). Within the range of light intensities used, the albino might be considered to show a decrease in the ERG - FFF when the intensity exceeds 100 ft-c. While this is not statistically significant, the trend is evident in the case of the albino and not in that of the normal. Such a decrease has not been observed in other normal fish¹³⁻¹⁵.

In the dark also the albino's ERG - FFF values are higher than those of the normal's at each of the light intensities

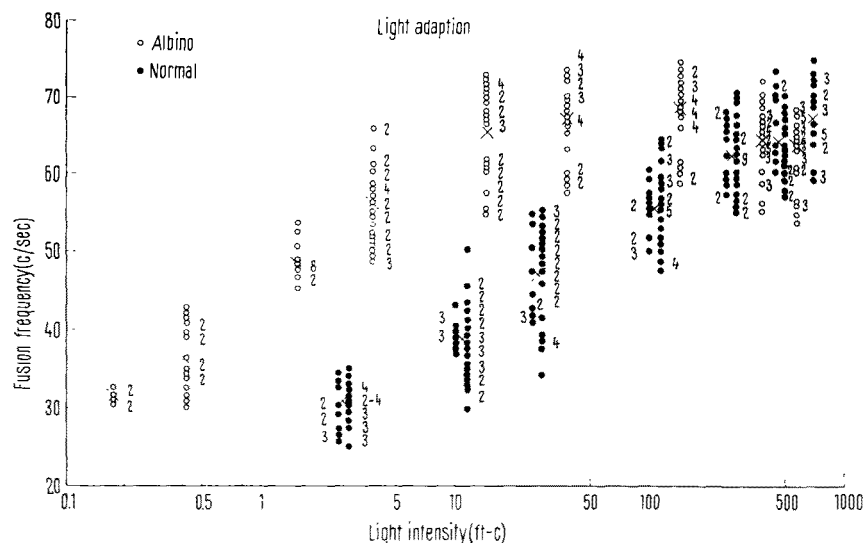


Fig. 1. ERG-FFF values for 5 each of light-adapted albino and normal brook trout (*Salvelinus fontinalis*) at various light intensities. Only the left eyes were used and 6 experiments were made at each intensity. The crosses indicate the means.

- ¹ G. L. WALLS, *The Vertebrate Eye and its Adaptive Radiation* (Cranbrook Inst. Sci. Bull. 19, Bloomfield Hills, Michigan 1942).
- ² M. A. ALI, *Can. J. Zool.* 37, 965 (1959).
- ³ M. A. ALI, *Can. J. Zool.* 39, 511 (1961).
- ⁴ M. A. ALI, *Can. J. Zool.* 40, 561 (1962).
- ⁵ M. A. ALI, *Naturwissenschaften* 19, 471 (1964).
- ⁶ M. A. ALI, *Rev. Can. Biol.* 23, 45 (1964).
- ⁷ K. T. BROWN, *Nature* 207, 1249 (1965).
- ⁸ K. T. BROWN and P. W. GAGE, *Nature* 211, 155 (1966).
- ⁹ M. A. ALI, *Can. J. Zool.* 42, 1158 (1964).
- ¹⁰ R. CROUZY and M. A. ALI, *C. r. hebdom. Séanc. Acad. Sci., Paris* 261, 4509 (1965).
- ¹¹ G. WALD, *J. gen. Physiol.* 25, 235 (1941).
- ¹² A. E. FRILL and G. B. LEE, *Arch. Ophthalmol.* N. Y. 69, 32 (1964).
- ¹³ M. A. ALI and H. KOBAYASHI, *Rev. Can. Biol.* 26, 341 (1967).
- ¹⁴ I. HANYU and M. A. ALI, *Science* 140, 662 (1963).
- ¹⁵ I. HANYU and M. A. ALI, *J. cell. comp. Physiol.* 63, 309 (1964).

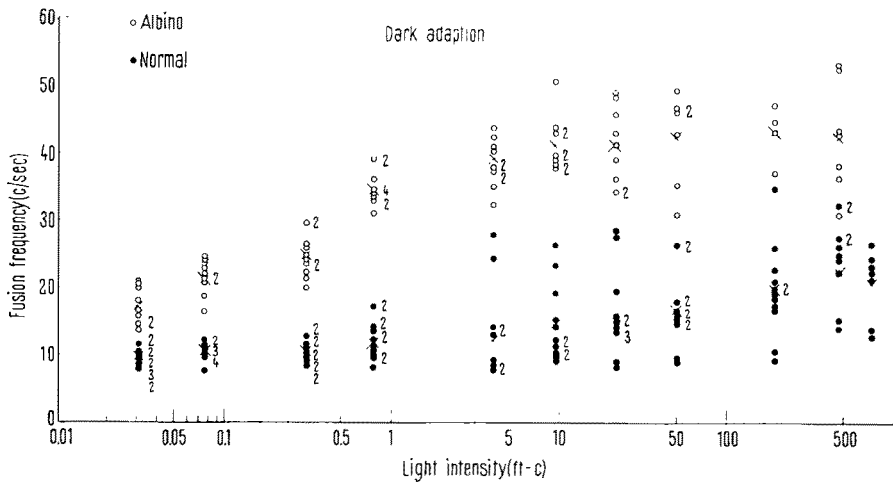


Fig.2. ERG-FFF values for 6 each of dark-adapted albino and normal trout. Only the left eyes were used and 2 experiments were made at each intensity. The crosses indicate the means.

used (Figure 2). The MFF is attained at 10 ft-c in the case of the albino while it is attained at about 460 ft-c in the normal trout's case. The shift from rods to cones occurs at intensities higher than 0.3 ft-c in the albino while it does so at intensities higher than 4.0 ft-c in the case of the normal trout (Figure 2).

It is evident from these results that the lack of REP in the albino is primarily, if not wholly responsible for the differences observed (Figures 1 and 2). Due to the absence of the pigment, not only a greater amount of light enters the eye, but this light impinges on the visual cells twice, as a result of its reflexion by the sclera. In the normal trout the light will be absorbed by the REP and not reflected. In the light-adapted state the rods of the normal fish are shielded by the REP while in the dark-adapted state at least the cone outer segments are masked by the REP. This will also reduce the amount of response. In the albino, both in the light- and dark-adapted states, the rods as well as the cones will be responding and to almost twice the quantity of light. The cones, of course, will respond only to intensities which exceed their threshold which may be safely assumed to be between 0.1 and 1.0 ft-c on the basis of earlier investigations with related salmonids^{2, 3, 18}.

Résumé. Les Fréquences de Fusion de l'Electrorétinogramme (FFF) ont été enregistrées chez les truites mouche-tées (*Salvelinus fontinalis*) normales et albinos. Chez les poissons adaptés à la lumière ainsi qu'à l'obscurité les valeurs sont plus élevées chez les albinos que chez les animaux normaux. Les valeurs maximum de FFF sont aussi atteintes à des intensités moins élevées chez les albinos. Ces différences sont attribuables à l'entrée d'une plus grande quantité de lumière ainsi qu'à sa réflexion par la sclérotique, ce qui est due au manque de pigment épithelial rétinien.

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An Improved Bioassay Method for Kinins

Recently, naturally occurring vasoactive polypeptides, kinins, have been supposed by many researchers to play important roles under pathological conditions such as acute pancreatitis¹, carcinoid syndrome², various allergic diseases^{3, 4}, or inflammations⁵. However, there are few reports giving direct evidence that kinins are involved in such diseases.

The following problems make it difficult to study the kinins: (1) they exist in extremely small amounts in plasma and in tissue; (2) they are rapidly destroyed by kininase which is present simultaneously in the blood or in the fluid.

In 1963, BINIA et al.⁶ used a dog's hind-quarter as an organ preparation for the bioassay of plasma kinins. However, this method was unsuitable, since the preparation was not sensitive and produced no vasodilatation with amounts of kinin of less than 10 ng. In the present study, a 20 times more sensitive method for the assay of kinin is presented utilizing the hind-quarter of a rabbit.

Albino rabbits of both sexes weighing 3.0-4.0 kg were used. The animals were anaesthetized by an i.v. injection of urethane (1.0 g/kg body weight). The carotid artery was exposed and a polyethylene cannula was inserted. One femoral artery was then isolated and cannulated

¹ A. P. THAL, E. E. KOBOLD and M. J. HOLLENBERG, *Am. J. Surg.* 105, 708 (1963).

² J. A. OATES, K. MELMON, A. SJOERDSMA, L. GILLESPIE and D. T. MASON, *Lancet* 1, 514 (1964).

³ R. E. MANCINI, H. HUIDOBRO, E. F. COLLAZO and R. MONASTIRSKY, *Proc. Soc. exp. Biol. Med.* 123, 227 (1966).

⁴ K. ABE, N. WATANABE, N. KUMAGAI, T. MOURI, T. SEKI and K. YOSHINAGA, *Experientia* 23, 626 (1967).

⁵ H. ZACHARIE, J. MALMQUIST, J. A. OATES and W. PETTINGER, *J. Physiol.* 190, 81 (1967).

⁶ A. BINIA, J. C. FASCILOLO and O. A. CARRETERO, *Acta physiol. latinoam.* 13, 101 (1963).