Electrophysiological Evidence for a Neural Stage in Dark Adaptation[1]

Evidence has been presented which suggests that the time course of dark adaptation is not completely explained by the rhodopsin regeneration as postulated by the photochemical theory of HECHT[2]. This evidence is based on electrophysiological and psychophysical data (for references see [3]).

A means of testing this hypothesis has been made available by the findings of BROWN and WATANABE[4,5], who recently succeeded in isolating the late receptor potential in cat and monkey retinas by occluding the retinal artery. This procedure, which abolishes the *b*-wave component of the electroretinogram (ERG), leaves intact the receptor component (PIII in the terminology of GRANIT). The receptor layer of these retinas is maintained by the choroid circulation. Furthermore, recent investigations[6] have given evidence that the *b*-wave component of the ERG is generated at the level of the inner layer of bipolar cells. In the present paper, the amplitude variations of the *b*-wave and of the late receptor potential are compared during dark adaptation. It will be shown that these amplitudes vary independently in a manner which indicates a neural stage of dark adaptation.

The experiments were performed in cats anaesthetized with pentobarbital (35 mg/kg). The retinal artery of one eye was photocoagulated and the pupils of both eyes were dilated with atropine. The ERG responses were detected through a glass pipette, filled with 1% agar dissolved in Ringer's solution, which was inserted into the vitreous body through a hole near the limbus of the sclera. The indifferent electrode was on the back of the eye. Pulses of light, 2 msec in duration and of 50 lux intensity, were presented to the eye by an electronically driven Sylvania glow tube (type 1130).

The organization of the experiments was as follows: after 15 min of dark adaptation, the retina was exposed to a bleaching illumination of approximately 4000 lux for 3 min. The ERG response was then tested in one of the eyes with single flashes at intervals ranging from 5–30 sec and for periods as long as 20 min. This procedure was then repeated for the other eye. Figure 1 shows samples of the ERG responses from the photocoagulated eye (Figure 1A, B) and from the normal eye (Figure 1C, D). The lower responses in each case (Fig. 1B and 1D) were recorded after 5 sec of dark adaptation, and the upper ones (Figure 1A and 1C) were obtained after 30 sec. The receptor response is clearly present after 5 sec while, at the same time, the *b*-wave response of the normal eye is not readily detectable.

In Figure 2, the amplitude of the *b*-wave and of the late receptor potential (measured, in each case, from the base line to the maximum) is reported for the first 100 sec of dark adaptation. The ordinate indicates the amplitude of the responses as 1% of a reference response taken after 10 min of dark adaptation. Notice that while the receptor response is initially present with a magnitude of 15–25% of the reference response, the *b*-wave response is almost undetectable for at least the first 10 sec of dark adaptation. For the first 60–80 sec of dark adaptation, the late receptor potential response remains proportionally larger than the *b*-wave response. After about 100 sec the relative values of the 2 responses converge quickly, and after 2–3 min of dark adaptation they are nearly identical.

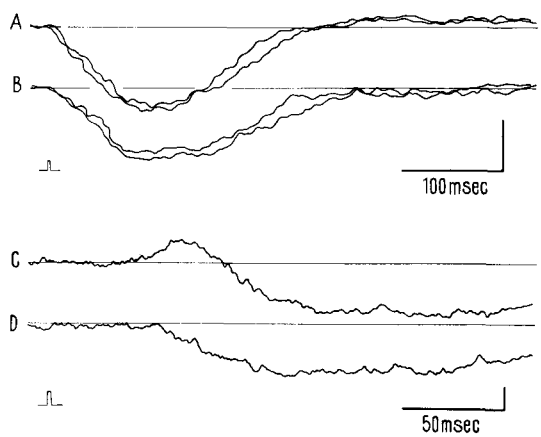

Fig. 1. Early stages of dark adaptation of the late receptor potential and *b*-wave of the electroretinogram. Recordings of the late receptor potential (A and B) and *b*-wave (C and D) are shown for two stages of dark adaptation. The records reproduced in B and D were obtained 5 sec after the commencement of dark adaptation. Those shown in A and C were obtained at 30 sec. The reproducibility of the results for the late receptor potential is emphasized by the similarity of the 2 responses recorded in A and B during different dark adaptation sequences. The vertical bars represent equal amplitudes for each pair of records and the pulses in all cases were of 2 msec duration.

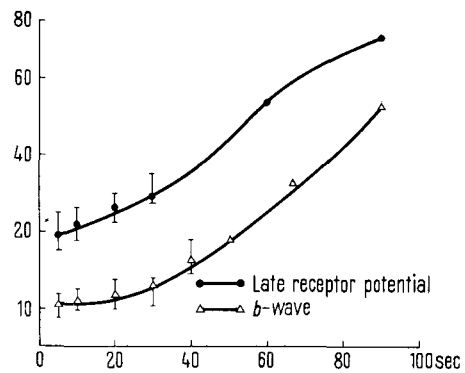

Fig. 2. Dark adaptation of the late receptor potential and *b*-wave of the electroretinogram. The amplitude of the late receptor potential and of the *b*-wave were measured from the base line to the maximum and plotted as % of a reference response obtained after 10 min of dark adaptation (ordinate). These amplitudes are plotted as a function of time (in sec) after commencement of dark adaptation. Up to 40 sec, the points represent the means of the results of 3 experiments and here the vertical bars show the total range of these results. For later times, a single point from one experiment only is plotted. See text for further explanation.

[1] This research has been sponsored jointly by the Office of Scientific Research, EOAR, through the European Office, Aerospace Research, United States Air Force, under Contract No. AF 61 (052)-830.

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Our results show clearly that the strong attenuation of the sensitivity of the eye in the early part of dark adaptation is not only due to the loss of receptor sensitivity. It is also due to a neural process occurring in the retinal visual pathway, either between the receptors and the inner nuclear layer or within the inner nuclear layer itself.

Riassunto. Risposte elettroretinografiche sono state registrate dall'occhio di gatto. Sono state studiate le variazioni di ampiezza dell'onda *b* e del potenziale del ricettore (isolato tramite fotocoagulazione dell'arteria retinica) durante l'adattamento all'oscuro. L'analisi dei risultati ha dimostrato l'esistenza di uno stadio nervoso nell'adattamento all'oscuro con sede anatomica fra il ricettore e lo strato dei granuli interni.

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Thymidine H^3 Incorporation in the Nurse Cells of Amphigonic and Parthenogenetic Ovaries of *Megoura viciae* (Hom. Aph.)

Autoradiography has shown that the nucleus of the nurse cells of some insects incorporates thymidine H^3 during the ovocyte growth. This has been attributed to continuous endomitotic divisions. Such researches were restricted, however, to nurse cells of amphigonic insects.

It therefore appeared particularly interesting to extend the study of the functioning of nurse cells to species of aphids. In such species both amphigonic and parthenogenetic female individuals are present. The former carry ovaries with large polyploid nurse cells and the latter are viviparous and carry ovaries with very small nurse cells which are diploid as a rule and always the same size. Only in *Aphis fabae* parthenogenetic individuals were tetraploid nurse cells shown to be present[1]. The present research was carried out on *Megoura viciae*, which is easily bred in controlled environment.

Amphigonic and parthenogenetic females of the species at various stages of development were injected in the abdomen with 0.1 μC of thymidine H^3. At intervals of time ranging from 1 h to 4 days after the injection, the animals were fixed and embedded in paraffin. The slides were subjected to autoradiographic processing, using the 'stripping film' technique. Some were stained with the Feulgen reaction before autoradiography, and some with the Unna method after autoradiography.

A very clear thymidine H^3 incorporation was observed in the nuclei of the differentiating nurse cells of very young amphigonic females. Incorporation can also be distinctly observed in fully developed nurse cells which are connected by a cytoplasmatic bridge to the growing ovocyte (Figure 1).

Not all the nuclei, however, were affected, as previously shown in *Dytiscus*[2]. It should also be pointed out that, once the process of vitellogenesis is over, no further incorporation of thymidine occurs in the nuclei of the nurse cells.

In the ovaries of parthenogenetic females, in which ovocytes at various stages of vitellogenesis and embryos at various stages of development are present, a very clear incorporation of thymidine in the nuclei of the nurse cells occurs (Figure 2). Incorporation is not synchronous throughout the nurse cells; cells with an unlabelled nucleus may be found side by side with others which have actively incorporated thymidine in one and the same ovary. So DNA synthesis is asynchronous in these as in polyploid nurse cells.

Conclusions. In the amphigonic females of *M. viciae* an active nuclear incorporation of thymidine H^3 occurs during growth which may be attributed to continuous endomitotic divisions. However, even when the nurse cells are functioning fully, and when the nuclei appear to have achieved maximum development, incorporation of the thymidine H^3 continues, and, as was seen in other insects, does not affect all the nuclei. Incorporation would therefore seem to be due not to the continuance of endomitotic divisions but rather to the synthesis of metabolic DNA. On the other hand, even if one supposes that in the nurse cells of the amphigonic ovary endomitotic processes continue right up to the end of vitellogenesis of the amphigonic winter egg, this is quite out of the question so far as the parthenogenetic ovary is concerned.

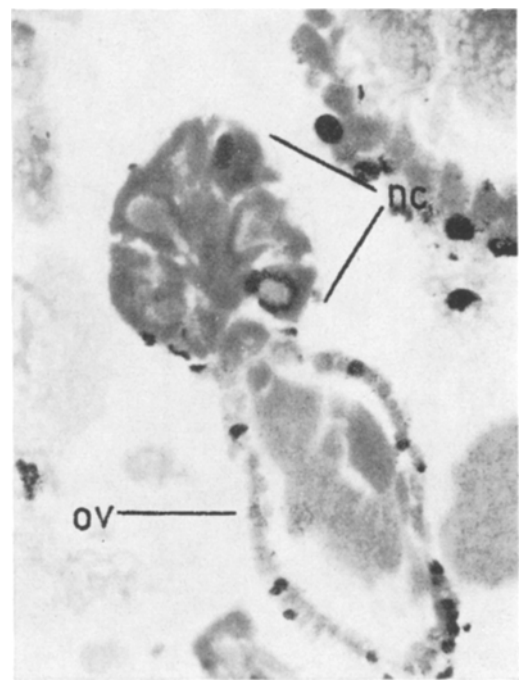

Fig. 1. Amphigonic ovary showing thymidine H^3 incorporation in polyploid nuclei of nurse cells. Notice the unlabelled nucleolus. Stained by Unna. × 310. n.c. = nurse cells; ov = ovocyte.

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