71. *The Origin of the So-called Cone Potential*

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(Comm. by S. KATSUNUMA, M.J.A., May 13, 1958)

There exists a considerable literature[1)-6)] on the so-called cone potential,[7)] but the problem, from where does it originate, has not yet been solved.[8)9)] The present experiment is devoted primarily to solve this problem by a histological approach, dealing with some charac- of this potential.

Method. The inverted retina was prepared from a carp eye (cyprinus carpio) described by the author elsewhere.[10)] For marking the tip of the ultra-microelectrode, an electrode filled with a saturated lithium-carmine 3 Mol-KCl solution was prepared by the same technique as the usual one filled with 3 Mol-KCl solution. The electrodes with a resistance of about 2 to 5 MΩ were preferred to the present experiments.

After the response was recognized by inserting of this electrode into the retina, a direct current was supplied from 100 volt battery through a Ringer-gelatine bridge to the electrode in such manner that it assumed a negative polarity (Fig. 1). A few granules of carmine could be isolated successfully from the tip of the electrode by a current of 30 μA flowing for 30 to 60 sec. After repeating this procedure at many points in the retina, the retina was fixed by Bouin solution, and then serial paraffin sections were made by the usual method, without staining.

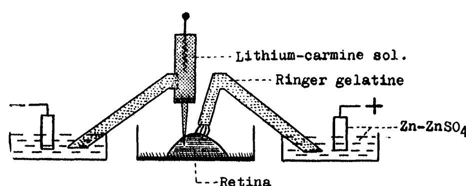

Fig. 1. Diagrammatic representation of the method

For the other observations of responses, 3 Mol-KCl filled ultra-microelectrodes with a resistance of 10 to 30 MΩ were used.

Light stimulation was given by a flash bulb with a flash duration of 100 μsec and an intensity of 30 lumen per sec. Recording and viewing were performed with the aid of a cathod follower D-C amplifier and a two beam oscilloscope system.

Experimental results. In the present experiments 35 specimens, successfully marked by carmine granules, were obtained. Eight of those are illustrated in Fig. 2 together with a diagrammatic representation of the position of the granules in each specimen. In these prep-

arations the carmine granules may be found on the outer plexiform layer (D, G), on the horizontal cell layer (E, F, H), within the inner nuclear layer, or on the boundary region between the inner nuclear layer and the inner plexiform layer (A, B, C). The distribution of the

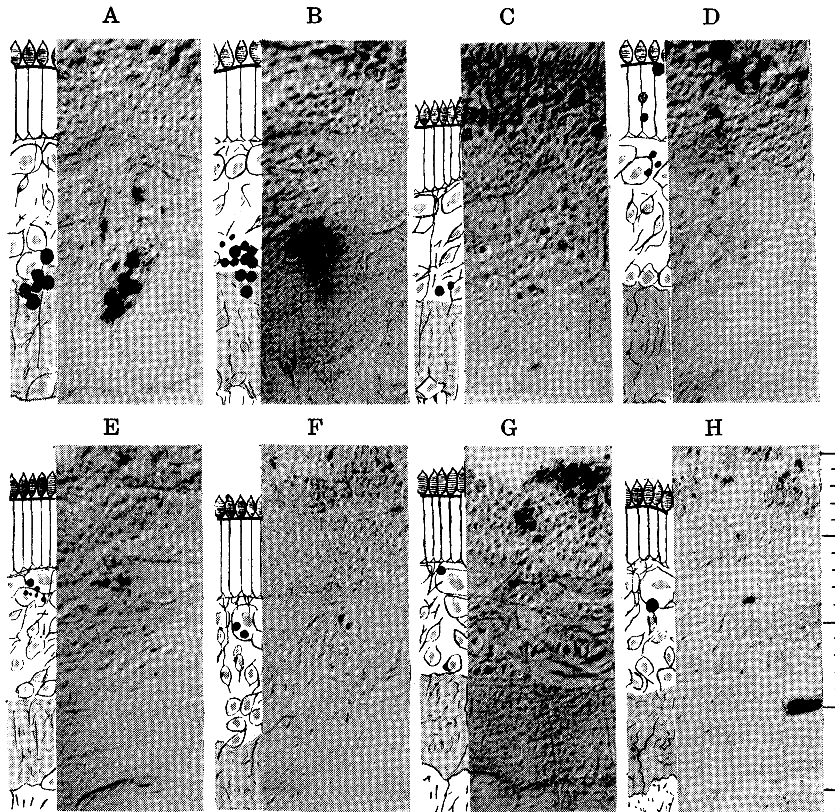

Fig. 2. Eight sections showing the positions of lithium-carmine granules together with those schemata

granules in the retina obtained from all the specimens is illustrated in Fig. 3. These results clearly support the contention that the so-called cone action potential originates from within the layer of the secondary neurone in the retina. It is of particular interest that the positions of most of the granules are found grouped in two separate regions of the layer. One is in the horizontal cell layer and the other, the large bipolar and amacrine cell layer. However, this electrophoretical method could not solve completely the problem as to whether or not the potential was of an intracellular nature; therefore, some additional observations were performed in order to solve this problem. The responses to light flashes were obtained from as many points as possible within an area of two cubic millimeters on the retinal surface. Many of these responses showed waves of approximately the same magnitude

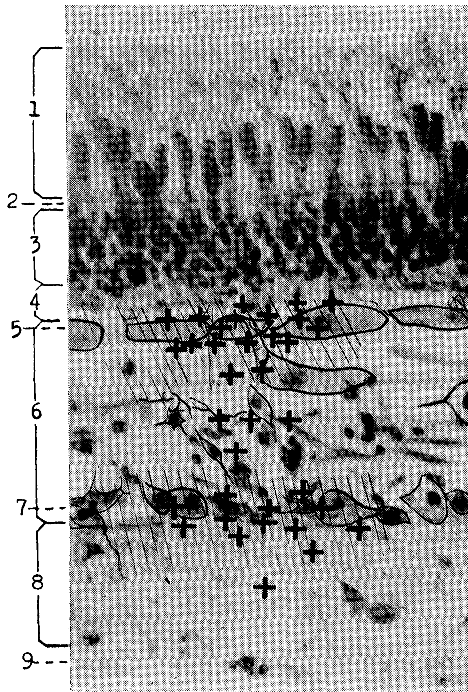

Fig. 3. Distribution of the lithium-carmine granules forced into the retinal tissue from the tip of the electrodes. A cross showing the position of a granule
The designation of the layers: (1) rod and cone layers, (2) outer limiting membrane, (3) outer nuclear, (4) outer plexiform, (5) horizontal cell, (6) inner nuclear, (7) amacrine cell, (8) inner plexiform, (9) ganglion cell layer

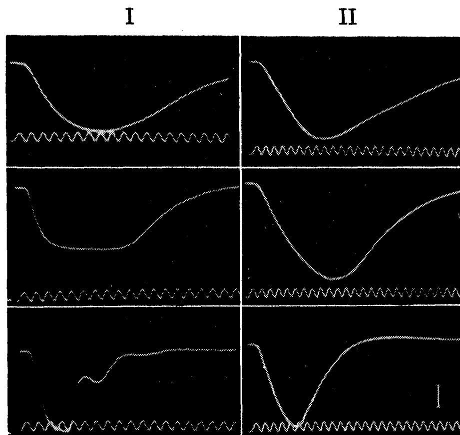

Fig. 4. Different type of the responses to light flash taken from two series of the experiments (I and II). Time: 61 cycles, Calibration: 10 mV

but having variations in their frequency or period of oscillation. The representative responses taken from two series of experiments are shown in Fig. 4. The variation, of a response type from within a small area, indicates that these responses are recorded from within a small compartments individually electrically isolated from each other. The potential change, during the insertion of an electrode into the retina, was traced on a long recording film which showed the registration of the electrode depth for every 10 μ of penetration, as illustrated in Fig. 5. In all the specimens whose potentials were recorded, it was recomfirmed that, the negative potential always appeared instantaneously at the same depth as the appearance of the action potential. This negative potential is considered to be equal to the resting potential of the cell membrane and signifies that the electrode has been inserted into cell. In addition to these obser-

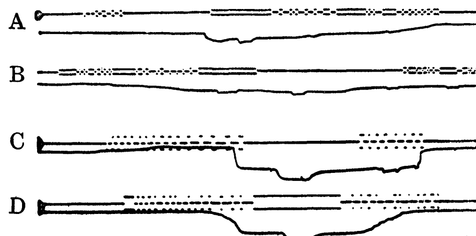

Fig. 5. Potential changes during the insertion (A and C) and the extraction (B and D) of a microelectrode. Upper trace in each record showing the registration of the electrode depth for every 10 μ

vations in carp retina, an attempt was made to measure this action potential using the retina of several other kinds of fish, namely: *mugil linnaeus, lateolabrax japonicus, sparus aries, kareius bicoloratus, halichoeres poetilopterus, girella punctata, hexagrammos tilesius*, and *epinephelus fario*. From the retina of *mugil linnaeus, lateolabrax japonicus, sparus aries*, and *kareius bicoloratus*, it was easy to record an essentially similar action potential as from the retina of carp. But from the others, it was very difficult to obtain an conclusive results in spite of very careful manipulation. This result leads to supposition of a structural difference in the retina between the former and the

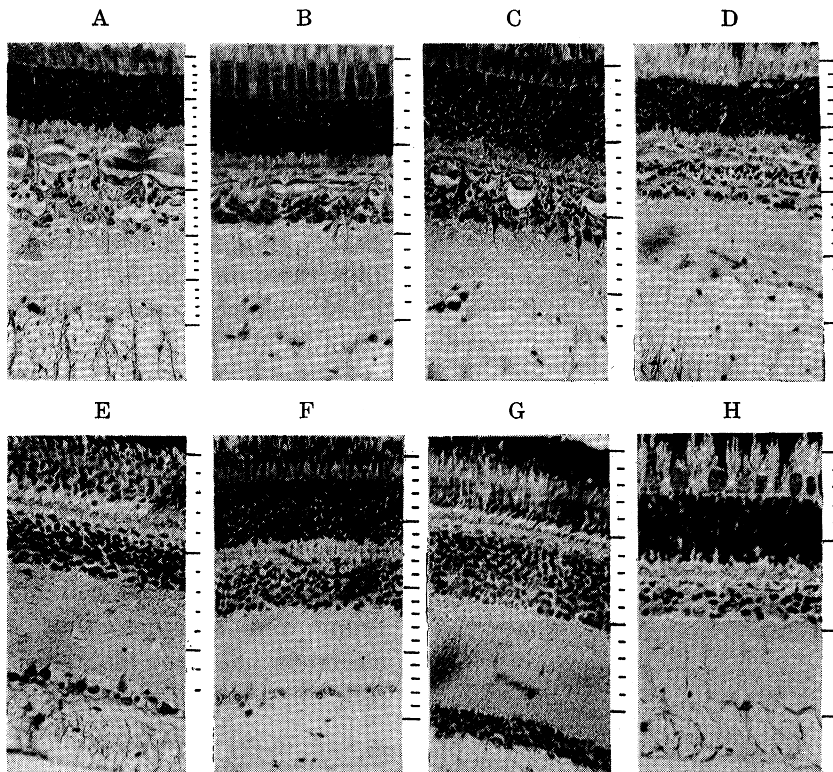

Fig. 6. Hematoxylin-eosin preparation of retina of eight kinds of fish; *mugil linnaeus* (A), *lateolabrax japonicus* (B), *sparus aries* (C), *kareius bicoloratus* (D), *halichoeres poetilopterus* (E), *girella punctata* (F), *hexagrammos tilesius* (G), *epinephelus fario* (H)

latter. Retinal sections of 10 μ in thickness were made from all the fishes and were stained by a hematoxylin-eosin solution. These sections clearly demonstrated that the cells in the inner nuclear layer of the retina were always large in the former, but on the contrary always small and more crowed in the latter. This may be easily seen on the micrographs in Fig. 6.

Discussion. MacNichol, Macpherson, and Svaetichin[11] have already made an attempt of the histological determination of the region which produced "the cone potential", using a microelectrode filled with crystal violet solution.[11] The crystal violet solution was electrophoretically forced into the retinal tissue around the tip of the microelectrode; however, it is felt that the solution diffuses over too great an area for the accurate location of the tip in the order of micron. The lithium-carmine solution used in the present experiments could be easily seen in the form of a small red granule superimposed on the yellow background of the specimen stained by the picric acid of Bouin solution, and seemed to show a more accurate location of the electrode tip in the micron order than the crystal violet. In many cases, most of these granules were found at both ends of the inner nuclear layer.

On the other hand, it was recomfirmed that the hyperpolarized potentials, obtained at depths of from 70 μ to 150 μ from the surface of the cone layer were always accompanied with the resting potential of about 40 mV and individually showed different types of waves. Even when analogous action potentials sometimes could be obtained independent from the resting potential as above, they were always lower in amplitude and frequently showed a depolarized potential.

Hematoxylin-eosin preparations of the retina made from many fishes showed noticeable differences in the inner nuclear layer. In general, the fish whose retina consists of an inner nuclear layer crowded with small cells is considered to have a greater discrimination to light than the fish having uncrowed large cells. This fact tends to indicate that the possibility of successful recordings is due only to the structural characteristics of the inner nuclear layer and the larger the cells in the inner nuclear layer, the greater the ease with which the potentials are obtainable. Accordingly, it can be concluded that, as far as the large hyperpolarized potential is concerned, it is intracellular in nature and is obtained from the inner nuclear layer of the retina.

Now, the question may be raised as to the characteristics of the hyperpolarized potential, because, as far as was previously known, the intracellular action potential of the neurone always showed depolarization. But, from the consideration that the potential phenomena produced in the active tissue are attributable to ionic transmission, all intracellular potentials may not always show depolarization during activation. On the other hand, the horizontal and amacrine cells are considered to have characteristics of a glia cell, and to be different from the neurone in the strict sense of term.[12] From the points of view, it is likely that the hyperpolarized potential may be produced from the horizontal and amacrine cells. This consideration may be further supported by the fact that the lithium-carmine granules forced

into the tissue from the tip of the microelectrode are mainly distributed along the horizontal and amacrine cell layers in the specimens of the present experiments.

Summary. In order to determine the accurate location of the origin of the so-called cone action potential, the histological approach was taken combined with an electrophoretical technique using a lithium-carmine filled ultra-microelectrode. Further, the data, concerning the problem whether or not the potential was intracellular, were discussed. From these results, it was pointed out that the so-called cone potential could not be attributed to a cone cell, but appeared to be the intracellular potential of the horizontal and amacrine cells.

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