

On the Photo-perceptive Function in the Eye of the Hagfish, *Myxine garmani* Jordan et Snyder*

(With 13 Figures and 3 Tables)

By

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In Myxinidae, the eyes which are buried beneath the skin, are considered to be functionless (Jordan & Snyder '01). Behavioral response of the hagfish to light, however, was reported by Cole ('12), Gustafson ('35), Newth & Ross ('55), and Steven ('55). Newth & Ross ('55) gave a full account of the response to illumination in this fish and showed from the analysis of the reaction time that the photo-perception of the hagfish was essentially similar to that of other eyeless animals. They assumed that the photo-receptors of the hagfish might be located on the skin at the anterior end of the head and in the region of the cloaca.

The author ('63) succeeded in recording the action potential response to light from the so-called rudimentary eyes of the hagfish, *Myxine garmani*. The detailed results will be given in the present paper. In addition, histological observation including neuro-arrangement of the rudimentary eyes and behavioral experiments dealing with the blinded hagfish will be described.

MATERIAL AND METHODS

Hagfish, *Myxine garmani*, used in the present experiment were caught with a small trawl net about 2 miles off the coast of Yamaguchi Prefecture along the Japan Sea. The animals were kept for several days in the aquaria before the experiments. Body length of the animal used in the experiments was 50 to 60 cm. The experiments were carried out during the period from March to May in 1959.

a) Recording method of the action potential The eye from which action potential was recorded, was isolated by cutting the head, removing the skin, and taking out the eye with the surrounding 8 mm² of tissue. This was done in dim red light after the fish had been dark adapted for 2 hours. The experiments were carried out in an electrically shielded and darkened chamber. The stimulating light was

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admitted by a shutter fitted at the hole or one side of the chamber.

One end of a fine slender cotton wick moistened with saline was brought into contact with the surface of the eye. The other end of the wick was inserted into one arm of a U-tube containing the physiological salt solution. The physiological salt solution used was one for marine fish presented by Yamamoto ('49), and the concentration of the salts was as follows: NaCl 1.35 %, KCl 0.06 %, CaCl_2 0.025 %, MgCl_2 0.035 %, NaHCO_3 0.002 %. From the other arm of the U-tube, the action potential was led off by an electrode of Ag-AgCl type and after amplification was displayed on a cathode-ray oscilloscope. The grounded electrode was an Ag-AgCl plate covered with moistened cotton cloth, on which the eye was set. The time-constant of the A.C. amplifier is about 1 second. Shutter-release was controlled by a thyatron-relay apparatus connected with a single sweeper. The intensity of the stimulus light arbitrarily will be referred to as a 100 per cent or unit intensity. For the determination of spectral sensitivity the interference filters made by Shonan Optical Thin Film Co., Ltd. were used. The optical properties of these are well known. The method used here for the measurement of the spectral sensitivity is the same as that described in the previous report (Kobayashi '62).

b) Histological method The isolated eye was fixed with Carnoy's solution for 1 hour, washed with absolute alcohol and embedded in paraffin. Sections were stained with haematoxylin and eosin. To study the neurological arrangement of the retina, Powers' modified formula of Ungewitter's urea-silver impregnation technique was used.

c) Measuring method of reaction time in response to illumination The hagfish was kept under about 10 cm of sea water, in a wooden rectangular tank 100 cm long, 30 cm wide and 30 cm deep. The flow of sea water (18° – 20°C) was stopped during the experiment. The tank was kept in a dark room. In order to observe and record the behavior, the animals were illuminated by a red painted bulb provided illumination of 2 c. p. and placed 50 cm above the tank. It had been determined by previous testing that the animals did not respond to this intensity of red light. The stimulating light was provided by a 40 W. light bulb set 50 cm above the tank to give illumination of 220 lux. The spontaneous visible movement of the animal was used as index for the measurement of the reaction time. After an interval of 20 minutes for dark adaptation the animal was stimulated and its reaction time was noted. This value was averaged with similar tests obtained after a similar adaptational period. After determination of the reaction time in normal animal, the animal was anaesthetized with 5 % solution of urethan and the skin of the dorsal side of the head was incised longitudinally, to allow removal of the eye, and then the incised skin was sutured as it was before. After the operation, the animal was returned to running sea water. After the animal came out of anaesthesia the reaction time was again measured by the same manner with that in normal animal, and it was compared with that of the normal state.

RESULTS

a) Action potential recorded from the rudimentary eye of the hagfish

Wave-form and polarity Action potential induced in the dark adapted eye is shown in Fig. 1. The response to weak light (about 0.1 lux) produced a positive slow potential of monophasic form with rather long latency. The time course of the response was also very long. In the response to intense light (200 lux), however, the slow positive potential was preceded by the small negative one (Fig. 1, A). It is remarkable that such a negative potential in the response to intense light occurred in the dark adapted eye, when it was not seen in the fresh eye prepared approximately 20 minutes after the excision (Fig. 1, B).

Relation between the potential and the intensity of stimulus light

The eye was subjected to a series of stimulus lights of various intensities to obtain the threshold. The results are shown in Figs. 2 and 3, where the amplitude of the positive potential is plotted against the logarithm of the stimulus intensity. The amplitude of the positive potential remains almost constant when the stimulus intensity attains over 1 per cent of unit intensity, as shown in Fig. 3. At intensities below the minimum intensity eliciting the maximum amplitude response, the relation between the amplitude of the positive potential and the logarithm of the stimulus intensity, was found to be linear (Fig. 2). Moreover the amplitude of the negative potential increased linearly with the logarithmic increase of stimulus intensity, though the negative wave appears only in the case of stimulation with intense light. All records obtained from the several series of experiments are summarized in Fig. 3.

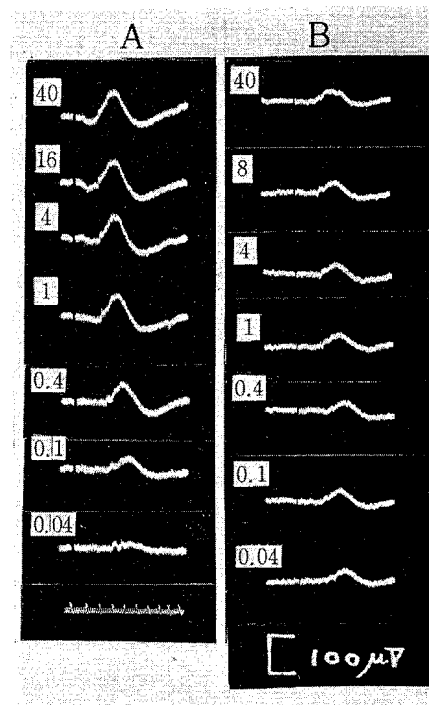


Fig. 1. Action potentials of hagfish eye for various intensity of light stimulus. The intensity of the stimulus light is represented as percentage of unit intensity. A, records in completely dark adapted eye. B, records taken immediately after the excision of the eye. Duration of test flash is 1/10 second. Polarity of the record is upward positive throughout this paper. Time mark, 1/6 second.

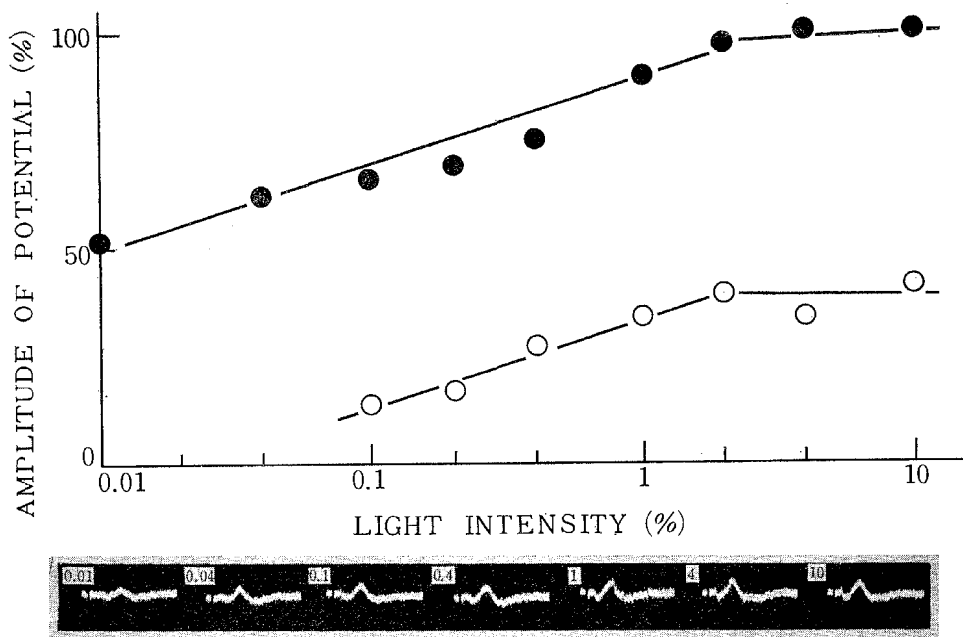


Fig. 2. Relation between the amplitude of the potential and the stimulus intensity. Filled circles, positive potential ; open circles, negative potential. Abscissa, log intensity of stimulus light. Ordinate, amplitude of the response in % of maximum one. Samples of record of response used for the measurement are reproduced at the bottom. The numerals on each record, the stimulus intensity.

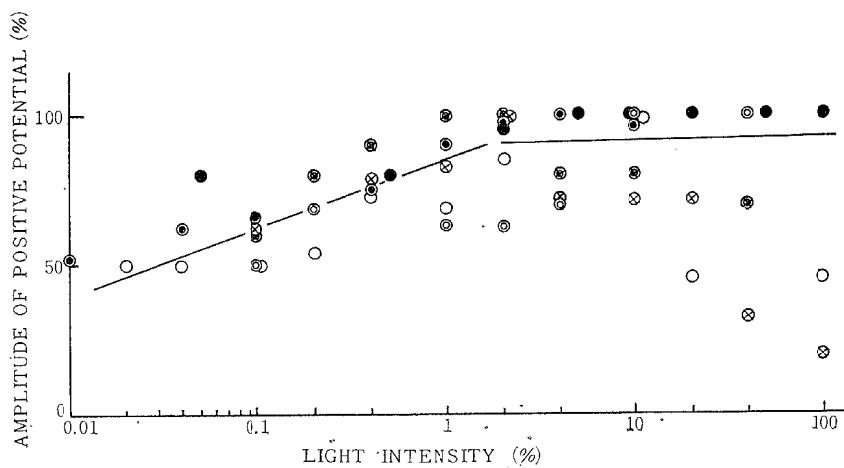


Fig. 3. Relation between the amplitude of positive potential and the stimulus intensity. All data obtained from experiments of six series are plotted. Other legends are same as in Fig. 2.

It appears that the latency of the response becomes shorter with the increase in stimulus intensity; even in the range where stimulus intensity elicits maximum amplitude of response.

Relation between the potential height and the duration of stimulation The shape and the amplitude of the response was not changed in any way by the variation in the duration of stimulus light, as long as its intensity was kept constant. This is shown in Fig. 4, where the eye is subjected to a series of stimulus lights of constant intensity with the various duration from 1 sec. to 1/300 sec. However, only in the shortest flash of 1/300 sec. is the amplitude of the positive potential lower than that with longer durations of the stimuli. This may be a manifestation of Bunsen-Loscow's Law.

It is noteworthy that no off-effect of the response appeared even after a stimulus of more than 1 second duration.

Light and dark adaptation The process of light adaptation was investigated on a dark adapted eye maintained under light of about 0.03 lux, using the size of ERG. The test flashes used were of the minimum intensity (about 10 lux) which produced maximum amplitude in dark adapted eye. The results are shown in A of Fig. 5. The sensitivity of the dark adapted eye began to decrease at once after an exposure to the background light, and soon it attained a state such that no detectable response was elicited by a stimulus of any intensity (ℓ - in Fig. 5,A). On the other hand, the change in threshold intensity was examined during the process of light adaptation (Fig. 6). With an exposure to the light of about 0.03 lux, the threshold intensity for eliciting the response rose rapidly as shown in Fig. 6,A. After about 5 minutes the threshold of the eye reached a maximum value. Several minutes after the eye was adapted to 0.03 lux, it was returned again to darkness, and the response to the test flash was recorded during the course of dark adaptation (d - in Fig. 5,A). The response amplitude was

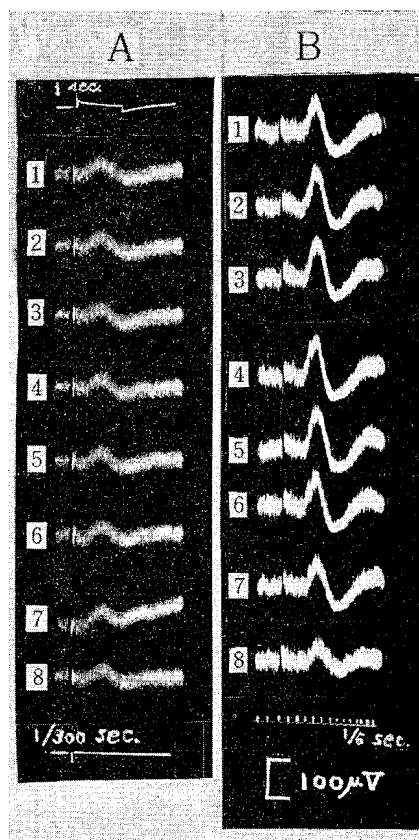


Fig. 4. Responses to different duration of the stimulus with constant intensity. Duration of the stimuli in 1 to 8 are 1, 1/2, 1/5, 1/10, 1/25, 1/50, 1/100 and 1/300 second, respectively. Intensity of the stimuli is 50 lux in A and 5 lux in B.

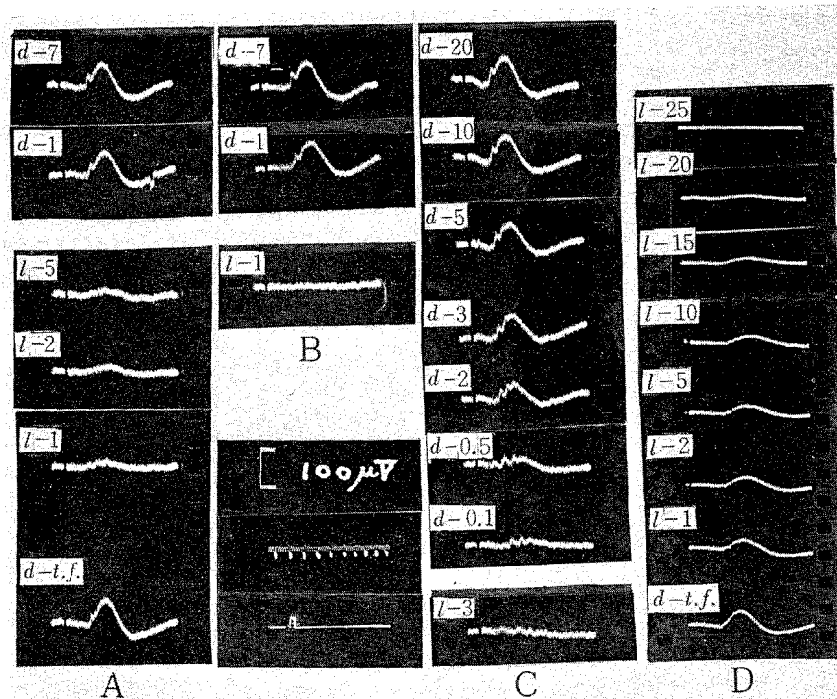


Fig. 5. Change in the ERG recorded in the course of time in light and dark adaptation. Intensity of the illumination used for light adaptation is about 0.03, 2, 20 and 0.1 lux in the case of A, B, C and D, respectively. Numeral on each record indicates the time in minute after beginning of the adaptation. *d*, dark adaptation; *l*, light adaptation; *d-t.f.*, response to test flash in dark adapted eye. In A, B and C, test flash was 1/50 second and 2% of unit intensity. In D, test flash, 1/50 second and 2 lux, is projected after the provisional cessation of the background illumination.

reappeared instantly after the eye was returned to darkness, although the eye seemed to take few minutes longer to regain the threshold intensity (Fig. 6, A').

If the eye was exposed to light of 2 lux, the sensitivity decreased rapidly so that there was no response after two minutes of the adaptation (*l-2*, in Fig. 5, B). As soon as the eye, adapted for 5 minutes to the light, was returned again to darkness, the sensitivity rapidly recovered; the response to the test flash reappeared after one minute (*d-1*, in Fig. 5, B), and the maximum sensitivity was regained five minutes after the beginning of the dark adaptation, as shown in Fig. 6, B'.

It is noticeable that in the light adaptation to 20 lux, the notch observed in normal response still remained even after the slow positive potential disappeared (*l-3*, in Fig. 5, C). The response having a maximum amplitude was obtained after the eye had been in darkness for 5 minutes (*d-5*, in Fig. 5, C). The threshold, measured after complete dark adaptation for six minutes is shown in Fig. 6, C'.

In the records of D in Fig. 5 the eye was adapted to the light of about 0.1 lux. The intensity of the test flash was about 2 lux, and the flash was projected after the background light was turned off. The records indicate the decrease in the potential, and the prolongations in the time-course and in the latency of response to light adaptation.

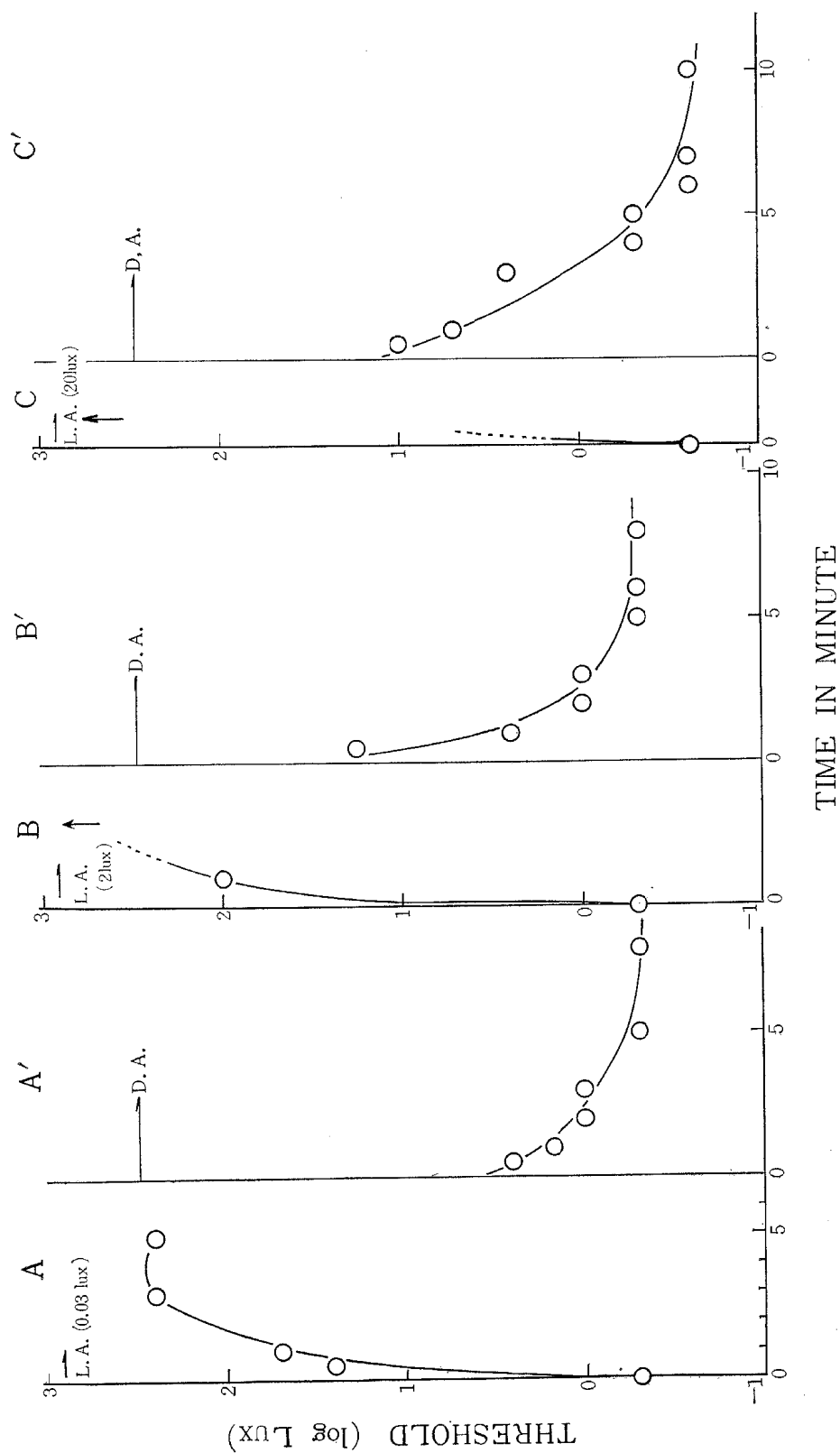


Fig. 6. Change in threshold of stimulus light for eliciting the ERG in the course of light (L.A.) and dark (D.A.) adaptation. Intensities of the illumination used for light adaptation are 0.03, 2 and 20 lux in the case of A, B and C, respectively. Arrows indicating upward show the remarkable increase of the threshold intensity, in which the ERG fails to appear at all.

The increase in sensitivity by dark adaptation was small when compared with that in other teleost fish eyes (Kobayashi '62), being only about 1.5—2.0 log units of the intensity.

Effect of potassium chloride and urethan It is a well known fact that the potassium ion suppresses the positive component of the ERG in vertebrates. As illustrated in Fig. 7,A, the hagfish also shows a decrease in positive potential within one minute after the application of 10 per cent KCl solution and in Fig. 7,B it shows that after the positive potential disappears, negative potential remains within the latency of about 350 msec. From these observations, it is evident that action potential found in the eye of the hagfish consists of selective components similar to the ERG of other vertebrates.

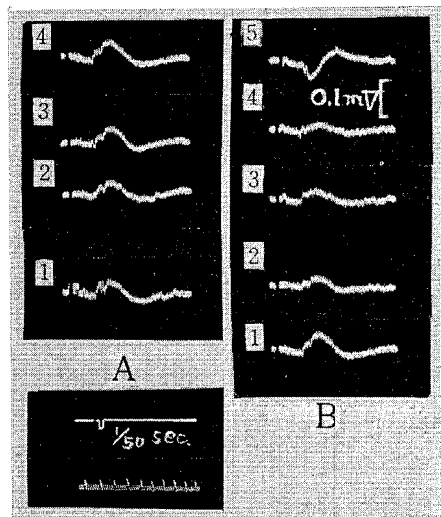


Fig. 7. Effect of 10% KCl sol. on ERG. A—1, control; 2, 1 minute after KCl application; 3, ten times in stimulus intensity, 1 minute after KCl application; 4, 5 and 6, 3, 4 and 5 minutes after KCl application, respectively, light intensity same as in 3. B—1, control; 2, 1 minute after KCl application; 3, ten times in stimulus intensity, 1 minute after KCl application; 4, same response as in 3, in fast sweep.

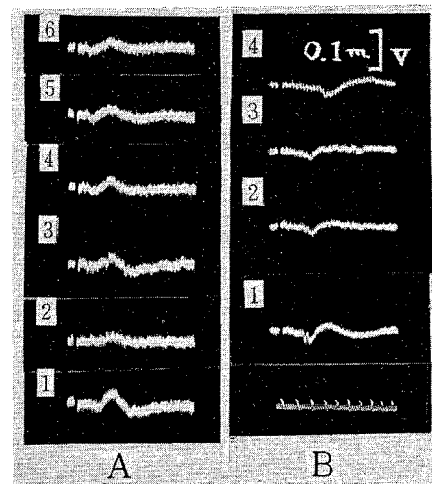


Fig. 8. Effect of urethan sol. on ERG. A, application of 3% urethan sol. — 1, control; 2, 3 and 4, 1.3 and 5 minutes after urethan application, respectively. B, application of 30% urethan sol. — 1, control; 2, 3, 4 and 5, 1, 3, 10 and 40 minutes after urethan application, respectively. Test flash is 1% of unit intensity and 1/50 sec. in duration.

Since the application of urethan solution was designed for the anaesthesia of the hagfish in the behavioral experiment, the effect of urethan on the eye was checked. Application of 3 per cent urethan solution did not suppress the action potential at all, but slightly accelerated the response (Fig. 8,A). Thirty per cent urethan solution, however, suppressed the response and amplitude of the positive potential decreased to half the initial height after 20 minutes. By pouring the physiological salt solution on the eye, the response was recovered completely in about fifty minutes (Fig. 8, B—5).

Effect of repetitive stimulation on the response The action potential records show that the eye of the hagfish did not respond to flicker stimuli. This fact suggests the existence of a long refractory period caused by the preceding stimulus. In order to determine the extent of the refractory period, the change in slow positive potential was investigated using slow repetitive stimuli. The investigation was made with the various intervals of off-duration in the repetitive stimulation which have 60 msec. of on-duration constantly. The suppression of the amplitude was seen well only after the second stimulus. The results in Fig. 9 were obtained by computing the ratio of the amplitude of the second response to the first, and plotting these values against the interval of the repetitive stimulus on the Y axis. The relative refractory period of the positive potential was observed to be very long, changing with the intensity of the stimulation. With the extrapolation of the curve in Fig. 9, the absolute refractory period may be assumed to be about 0.8, 1.8 and 4 seconds for stimulus

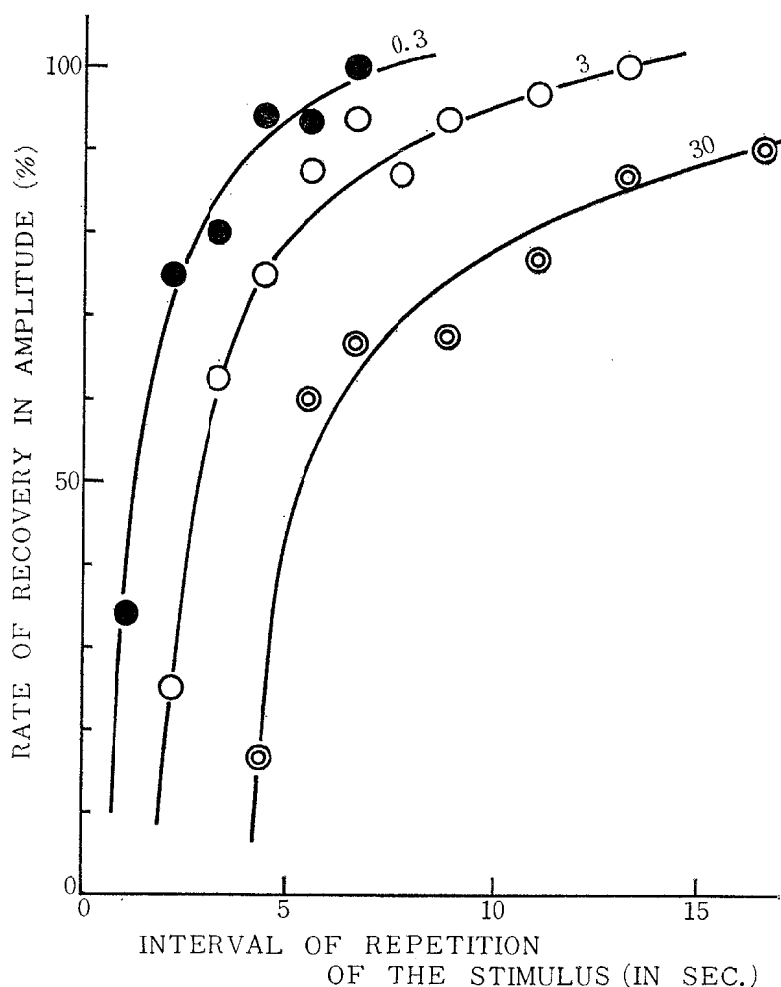


Fig. 9. Suppression of second response in repetitive stimulation. Abscissa, the time interval of repetitive stimulus in sec. Ordinate, the rate of the height of second response to that of first one. Intensity of the stimulus is given on each curve. Test flash is 1/10 sec. in duration.

light of 0.3, 3 and 30 lux, respectively.

Spectral sensitivity The spectral sensitivity curves are shown in Fig. 10. The sensitivity was plotted as the reciprocal of the minimum intensity required to produce the response. The sensitivity curve for the dark adapted eye is shown with solid line, and that for an eye adapted to weak light (0.2 lux) with broken line. These two

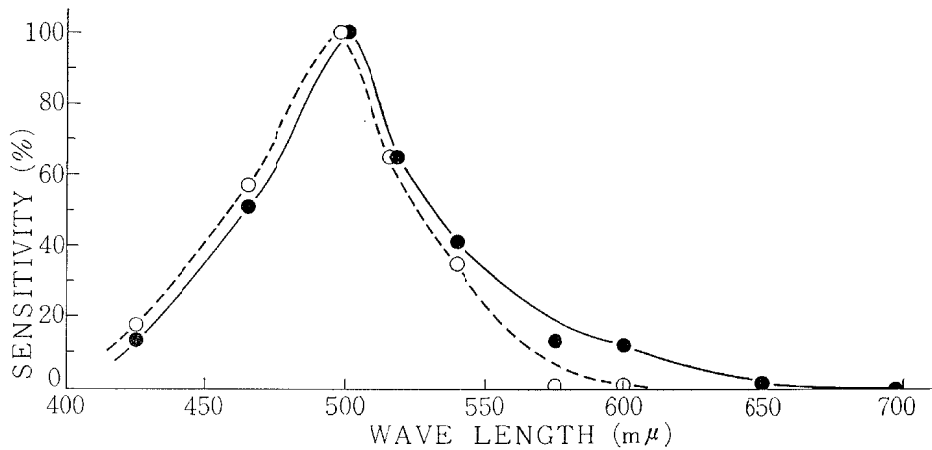


Fig. 10. Spectral sensitivity curve of the hagfish eye. Filled circles, in dark adaptation; open circles, in light adaptation. The sensitivity is represented as per cent of the maximum value.

curves are similar to each other in the shape having both their maxima at 500 mμ of the spectrum. "Purkinje shift" is not found in the eye of the hagfish. The sensitivity is generally found to be higher for shorter wave-length. In the dark adapted eye, the sensitivity to the light of the wave-length longer than about 575 mμ is below 10 per cent of the maximum one, and the eye was almost insensitive to the light of the wave-length longer than 650 mμ. Whereas in the light adapted eye it is below 2 per cent of the maximum one, and the eye is completely insensitive to that longer than 650 mμ.

Steven ('56) found also the maximum between 500 and 520 mμ of the spectrum in his behavioral experiments.

b) Morphological observation of the eye

The eyes of the hagfish lie beneath the skin on both sides of the head. The part of the skin over the eye forms a white opaque round window, lacking in melanin. Two string-like processes protruding from the anterior and posterior parts of the eye, attach to both ends of the window mentioned above, so that the skin can be moved by the contraction of the processes. The diameter of the eye is about 3 mm in horizontal section and contains a colorless transparent lens. This diameter is slightly longer than the diameter in a dorso-ventral section. As shown in Fig. 11, the outer structure of the eye vesicle seems to correspond with a cornea, and the lining of the vesicle, consisting of three distinct cell layers (R), is similar to the retinal layer of the usual eye. There is no structure like the falciform

process as observed in teleost fish. The lens is in contact with the corneal-like structure shown on the figure.

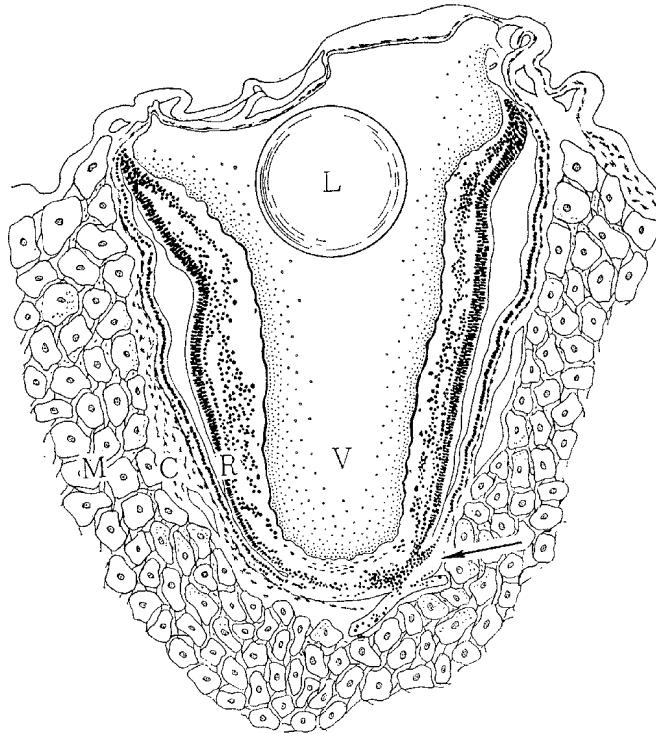


Fig. 11. Cross-section of rudimentary eye in the hagfish. M, muscle ; C, connective tissue ; R, structure like retinal layer ; V, vitreous body ; L, lens. Arrow indicates the structure assumed to correspond to *papilla nervi optici*. $\times 35$.

Details of the nucleated layer are shown in the photomicrograph (Fig. 12). In the outermost layer the cells are arranged in monocellular layer and have oval nuclei. It may be assumed from such an organization that the cells were produced from the outer wall of the optic cup and they are homologous to the pigment cells of the usual eye, although pigment granules are not developed. Below the "pigment" cell layer there is the receptor cell layer, which consists of 2 to 3 layers of the cells, having large oval nuclei. The length of outer segment of the receptor cell is in a range of 9μ to 12μ , and the breadth is about 1.5μ . Those of ellipsoid of the cell are about 3.6μ and 3μ , respectively. The breadth of myoid of the cell is about 2μ , but the length is different with individuals. The nucleus layer of the receptor cell is inferred to be homologous to the external nuclear layer, *i.e.* the nucleus layer of receptor cells of the retina of higher vertebrates. It appears, however, that the differentiation toward bipolar and horizontal cells has not so completely finished and the layer resembles the condition in the outer neuroblastic layer. In the innermost layer the nuclei are found layered or scattered. This layer may be homologous to the internal nuclear layer of the retina, because of two kinds of nuclei. It is assumable also that amacrine cells, ganglion cells and Müllerian fiber nuclei are not enough differentiated

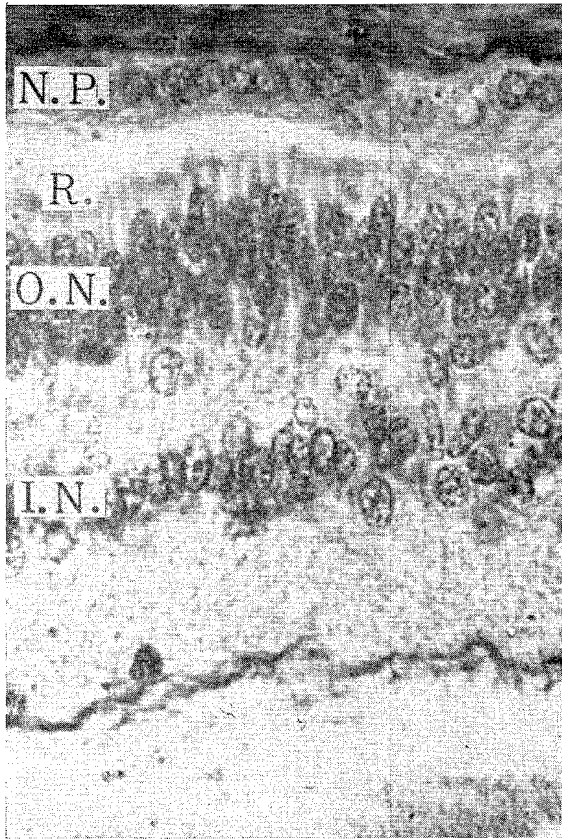


Fig. 12. Photomicrograph of the retinal layer like structure in the hagfish eye. R., Receptor ; O.N., outer neuroblastic layer ; I.N., inner neuroblastic layer. $\times 600$.

from the inside layer, which has remained in the state of development of the inner neuroblastic layer. The reticular tissue located inside the inner layer is in contact with vitreous humor through the internal limiting membrane. Therefore, the structure of the hagfish eye seems to correspond with the neuroblast stage. Figure 13 shows a photomicrograph of the hagfish retina prepared by the urea-silver stain besides a schematic presentation of its neurological arrangement seen in its preparation. It was certain that the optic nerve fiber was found being connected with the receptor cell nuclei in the arrangement.

In the fundus, stratified arrangements of the nuclei are absent, but a structure penetrating the retinal layer is observed. The penetrating point may perhaps correspond to the *papilla opticus nervi*

c) *Reaction time of the hagfish behavior responding to illumination, after removal of the eyes*

Although action potentials were recorded from the rudimentary eye of the hagfish, photoreceptive function of the eye cannot be confirmed unless the conduction of the impulse from the eye to the central nervous system can be confirmed. In order to extend the positive findings that the eye of the hagfish produces functional action potentials, behavioral tests for such response were made as well.

Experiment 1. Effect of excision of the eye

In the present experiments the behavioral response of normal hagfish to light was the same as that described by Newth & Ross ('55). Reaction time of the response to light was measured in two normal animals, which were subsequently blinded under urethan anaesthesia. In one animal, the spinal cord was transected in order to exclude the impulse conduction from the region of cloaca (Newth & Ross '55). The animals were considered sufficiently recovered from the anaesthesia 24 hours after operation. Then, the reaction time of the animal in response to the same illumination was again measured and it was compared with that before the operation. As shown in Table 1,

the results show the prolongation of reaction time in the "blinded" hagfish.

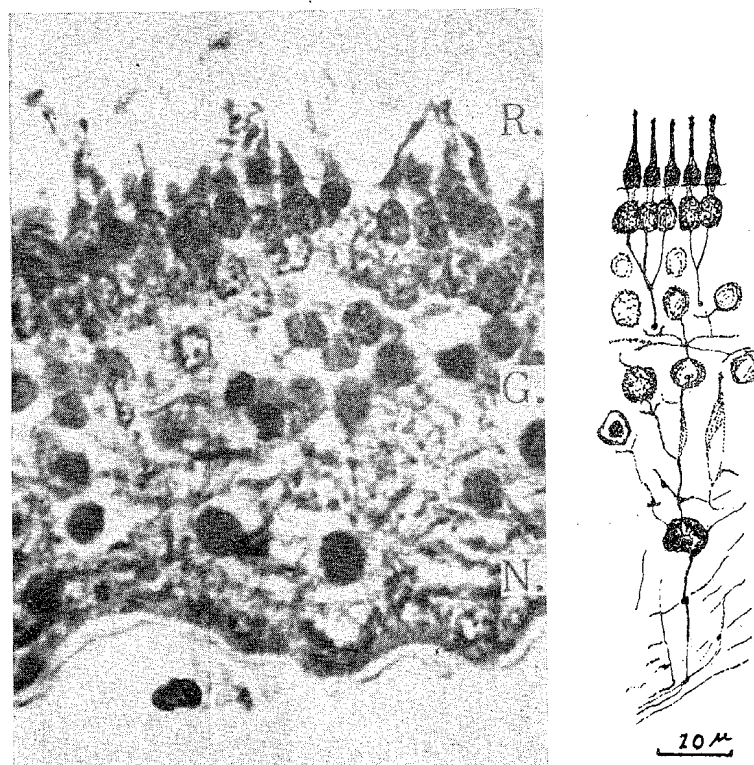


Fig. 13. Photomicrograph of the retinal layer like structure in the hagfish eye, prepared by urea-silver stain, besides a schema of the neurological arrangement therein. R., outer segment of the receptor cell ; G., nuclei of the neuroblast cells ; N., optic nerve fiber. $\times 600$.

Table 1. Reaction time of hagfish in behavioral response to light after removal of the eyes.

	Before operation		After operation	
	No. of trials	Mean of reaction time (second)	No. of trials	Mean of reaction time (second)
I*	5	14.85 ± 8.5	4	27.22 ± 11.2
II	4	7.4 ± 2.2	5	21.3 ± 8.5

* The spinal cord was transected.

Experiment 2. Effect of imperfect removal of the eye

According to Newth & Ross ('55) the hagfish in which its eyes were destroyed, still continued to respond to the local illumination on the head. It is suggestive in their experiment that the destruction of the eye might be faulty in the extent. In Exp. 1, the eye was removed instead of the destruction. Therefore the reaction time of the response of the hagfish from which the eyes were removed imperfectly, was investigated as a control in this experiment. The experimental procedure was the

same as that of Exp. 1, except the manner of the operation mentioned below. In one animal, only an eye of one side was removed and in the other the half of each eye in both sides was cut off, respectively. Table 2 shows clearly that any prolongation of reaction time is not seen in the responses of both animals. It is questioned here that the photo-sensitive function may depend on the extent in which the eye is partially isolated. In this experiment, however, it is seen that as long as even half of the eye remains, it is photo-sensitive functional.

Table 2. Effect of partial removal of the eye on the reaction time of hagfish, in response to illumination.

	Before operation		After operation	
	No. of trials	Mean of reaction time (second)	No. of trials	Mean of reaction time (second)
I *	5	14.4 ± 3.5	6	7.4 ± 0.9
II **	4	6.7 ± 0.8	6	6.5 ± 0.8

* An eye of only one side was removed.

** A half of each eye in both sides was cut off, respectively.

Experiment 3. Influence of experimental procedure on the measure

In the measurement of the reaction time, the influences resulting from the operation such as urethan anaesthesia, and the incision and the suture of the skin were investigated. Table 3 shows that neither the anaesthetic nor the surgical manipulations, other than complete removal of the eyes, had any effect on the reactions of the stimulated hagfish.

Table 3. Tests on influences of operative procedure to reaction time.

	Before operation		After operation	
	No. of trials	Mean of reaction time (second)	No. of trials	Mean of reaction time (second)
I *	4	10.6 ± 2.7	5	10.9 ± 3.2
II **	4	8.9 ± 1.0	4	7.8 ± 0.4

* The excision and the suture of the skin were performed under urethan anaesthesia.

** Only anaesthesia was performed.

DISCUSSION

The fact that action potential could be recorded from the rudimentary eye of the hagfish in this work, suggests that it is functional. Moreover it is interesting from the evolutionary view point that the properties of these action potentials resemble those of ERG of fish, particularly of elasmobranchs, though it is difficult to

understand that a-wave of the ERG appeared remarkably in dark adapted state. The fact that the ERG of the hagfish attains its maximum amplitude with relatively low intensity (Fig. 3) makes its ERG remarkably similar to that of the rod potential observed in the ERG of Batoidei, a typical benthonic fish (Kobayashi '62). Since the sensitivity in the completely dark adapted state is relatively high, and since the action potential is suppressed when light adapted (Figs. 5 and 6), it may be assumed that the photoreceptor system of hagfish is concerned only with the detection of light. Since the hagfish eye does not respond to flicker stimuli this buried eye may be thus adapted for maximum photosensitivity.

The maximum of spectral sensitivity is found at 500 m μ (Fig. 10), and it agrees with the results obtained by Steven ('54) with behavioral responses. Denton & Warren ('57) reported that several species of fish caught at depths of about 500 meters possessed a visual pigment with the maximum absorption (λ_{\max}) about 480 m μ , and they described its color as golden. Münz ('57) confirmed that deep-sea fish had golden-colored retinæ. Wald *et al.* ('57) described that the maximum absorption (λ_{\max}) of rhodopsin from marine fishes shifted toward shorter wave-length in rough correlation with the depth of the habitat. The author ('62) investigated the spectral sensitivity of the coastal fishes by the size of the ERG. He found that the sensitivity curves had the maxima at the range of longer wave-length (550–650 m μ) in the shallow water fishes, and the maximum sensitivity tended to shift toward shorter wave-length with the depth of habitat of the fishes. Therefore, it is suggestive that the visual pigment of the hagfish is suitable for the life on the bottom or in the mud, with considerable depth of sea water. The shape of the spectral distribution curve obtained in the hagfish, is found to correspond to the spectral curve of light found in deep sea water (Clark '54). Assumably the light sensitivity of the hagfish is advantageous for its habitat on deeper.

The pineal organ is well known in Cyclostomata, and the photosensitivity of the organ is described with regard to color changes in the body (Young '35, Breder & Rasquin '47). In hagfish found in Japan, however, such an organ has not yet been confirmed. The histological structure of the hagfish eye is not in any way suggestive of a pineal organ. But the stratified structure like the retina is found and the feature is similar to the state of neuroblasts observed in course of the development of the human retina (Detwilar '43). And nerve paths are found in the neurological arrangement of the retina through histological analyses of nerve supply to the eye. Moreover, a feature corresponding to *papilla nervi optici* of retina is also observed (the arrow in Fig. 11). It is assumed that perhaps the skin, covering the eye, keeps the eye from being exposed to excessive light. The buried eye, thus, shows appropriate fitness so it should not be regarded as "rudimentary" or inadequate.

The experimental results showing that the reaction time is prolonged in the animals from which the eyes were removed also show the role of the eye in the photo-perceptive function of the animal. Although Newth & Ross ('55) observed that the hagfish continued to respond after destruction of the eye, the reaction interval was not noted. The source of the receptivity was theorized to be in the skin by them.

However, it seems evident from the present work that the eyes also play a role in reaction to light as photo-receptors, though the possibility of the photo-perception in the skin of the hagfish is undeniable.

SUMMARY

ERGs were obtained from the eye buried beneath the skin in the hagfish, *Myxine garmani*. The potential was a slow positive monophasic deflection and the maximum of the potential was about 0.1 mV. The amplitude of the potential augmented with the increase in stimulus intensity, but the potential attained a maximum at an unexpectedly low intensity of stimulus light of 1 lux. Having no off-effect the ERG of the hagfish does not respond to usual flicker stimuli. A long refractory period of the ERG was found in the experiments using slowly repetitive stimuli. The properties of these potentials were found to be similar to those of the ERG obtained from benthonic fish, particularly from Batoidei. Although the potential is considerably suppressed with light adaptation, the eye recovered sensitivity rapidly in darkness. Spectral sensitivity curve investigated in dark adaptation showed the maximum at 500 m μ and the maximum was not shifted by light adaptation. The fact suggests that the eye has only one type of visual pigment. Histological studies of the hagfish eye failed to show any affinities to the pineal organ which is photo-receptive. Moreover, the hagfish eye has a retinal layer, which is believed to generate the observed action potentials. And nerve supply to the eye was neuro-histologically confirmed by the silver impregnation of the retina. Behavioral experiments also supported the view mentioned above. The reaction time of the hagfish in the behavioral response to light was prolonged after removal of the eyes.

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REFERENCES

- BREDER, C.M. & Pr. RASQUIN, 1950: A preliminary report on the role of the pineal body organ in the control of pigment cells and light reactions in recent teleost fishes. *Science*, **111**, 10—12.
- BRETT, J.R., 1957: The eye. The physiology of fishes, ed. Brown, M.E., Vcl. II, 121—154, New York.
- CLARKE, G.E., 1954: Element of ecology. New York.
- DENTON, E.J. & F.J. WARREN, 1957: The photosensitive pigment in the retinae of deep-sea fish. *Jour. Mar. Biol. Assoc.*, **36**(3), 651—662.
- DETWILER, S.R., 1945: Vertebrate photoreceptor. New York.

- GUSTAFSON, G., 1935 : On the biology of *Myxine glutinosa* L. *Ark. Zool.*, **28** A (2), 1—8.
- JORDAN, D.S. & J.O. SNYDER, 1901 : A review of the lancelets, hagfishes, and lampreys of Japan, with description of two new species. *Proc. U. S. Nat. Mus.*, **23**(1233), 725—734.
- KOBAYASHI, H., 1962 : A comparative study on electroretinogram in fish, with special reference to ecological aspects. *This Bull.*, **11**(3), 407—538.
- KOBAYASHI, H., 1963 : Preliminary report on the action potentials recorded from the rudimentary eye of hagfish, *Myxine garmani*. *Zoological Magazine*, **72**(1), 6—12. (in Japanese with English summary)
- MÜNZ, F. W., 1957 : Photosensitive pigments from retinas of deep-sea fishes. *Science*, **125**, 1142—1143.
- NEWTH, D.R. & D.M. ROSS, 1955 : On the reaction to light of *Myxine glutinosa* L. *Jour. Exp. Biol.*, **32**(1), 4—21.
- STEVEN, D.M., 1955 : Experiments on the light sense of the hag, *Myxine glutinosa* L. *Jour. Exp. Biol.*, **32**(1), 22—38.
- WALD, C., P.K. Brown & P.S. Brown, 1957 : Visual pigments and depths of habitat of marine fishes. *Nature*, **180**(4593), 969—971.
- YAMAMOTO, T., 1949 : Dobutsu seiri no zikken. Tokyo. (in Japanese)
- YOUNG, J.Z., 1935 : The photoreceptors of lampreys, II The functions of the pineal complex. *Jour. Exp. Biol.*, **12**(3), 254—270.