

# A Comparative Study on Electroretinogram in Fish, with Special Reference to Ecological Aspects\*

(With 11 Tables and 141 Text-figures)

By

Hiroshi KOBAYASHI

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## Chapter I    Some aspects on the electroretinogram of a dog fish, *Mustelus manazo* BLEEKER

Though there are many investigations on electrical response of fish retina to light (DEWAR *et M*'KENDRICK 1874, DAY 1915, ADRIAN *et al.* '27, GRANIT '47, SVAETICHIN '53, TOMITA '57, TAMURA '57 and others), we have not yet any information on electroretinogram (ERG) of elasmobranch fish. The retina of Elasmobranchii, phylogenetically the lowest among fishes, is known as rod retina and reveals the peculiar structure (BAYLISS *et al.* '36, DETWILAR '43 and others).

In the present paper the general properties of the ERG obtained from *Mustelus manazo* are described and these properties are ecologically considered on the behaviour of the fish.

### Material and method

A dog fish, *Mustelus manazo* BLEEKER, used in this work was caught some five miles off the coast of Yamaguchi Prefecture in the Japan Sea. Before the experiments, the fish was preserved for several days in a crawl near the coast. Body length of these fishes was 80 to 100 cm. Experiments were made during the period of November '58 to January '59.

Experiments were carried out with an eye excised. The eye was excised from the head cut from off the fish body, and the cornea, lens and vitreous humor were removed to expose the retina. This preparation was made under a dim red lamp after about one hour of dark-adaptation of the fish. The optic cup obtained in such a way was placed in a dark chamber which was shielded electrically. The light obtained from the projector lamp stimulated the whole retina through the shutter fitted at the hole on one side of the chamber. The intensity of stimulating light was controlled by neutral filters. The intensity of stimulating light applied directly is referred to as a 100% or unit intensity (500 lux).

As an active electrode was used a slender cotton wick moistened with a saline solution, which was in contact with the inner surface of the retina. The other end of the wick was immersed in one arm of a U-tube containing saline solution. From the opening of another arm of the U-tube the action potential was picked up with Ag-AgCl electrode leading off to the input of an amplifier connected with a cathode ray oscilloscope. Ag-AgCl plate covered with a moistened cotton sheet was used as an indifferent electrode, on which the eye was set. The amplifier was CR-coupling and the time constant was about one second. The shutter-release was controlled by thyatron-relay connected with a single sweeper of the oscilloscope. The method for recording the ERG was diagrammatically shown in Fig. 1.

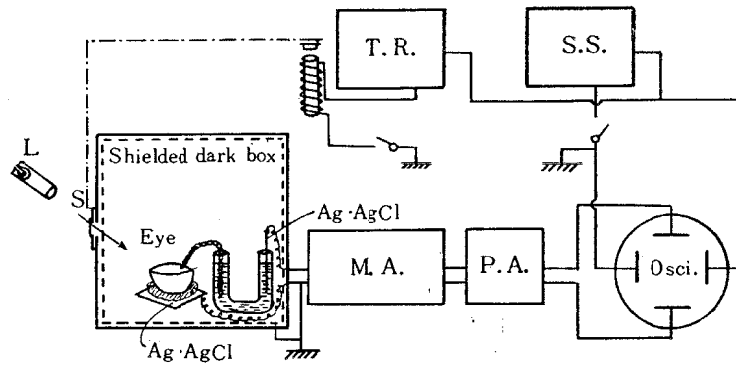


Fig. 1. Diagram showing the method for recording the ERG from the excised fish eye. L, light source; M. A., main amplifier; P. A., post amplifier; S. S., single sweeper; s, shutter; T. R., thyatron relay; Osci., oscilloscope.

The details of the method used for the determination of spectral sensitivity of the retina were described elsewhere (*cf.* Chapter III). Experiments were carried out under a room-temperature ranging from 10°C to 15°C.

## Results

### (1) Structure and dimension of the dog fish retina

The excised retina was fixed with Carnoy's solution for 1 hour, washed by absolute alcohol and then imbedded with paraffin by the usual method. Sections were stained with haematoxylin and eosin.

In the retina of *Mustelus*, the average thickness in the region of the fundus was approximately 120  $\mu$  and that in the *ora serrata* was about 60  $\mu$ . The thickness of pigment epithelium is about 10  $\mu$  and it comprises a single layer of regularly arranged

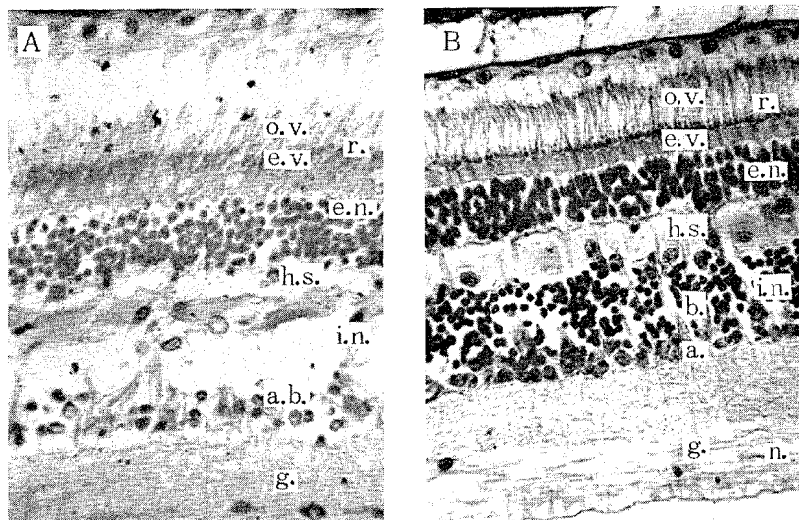


Fig. 2. Photomicrographs of dog fish retina. A, adult fish; B, young fish.

prismatic cell, which contains a spherical nucleus. The receptor layer comprises only one type of visual cell. The average length of their outer segments, ellipsoids and myoids were  $15\mu$ ,  $4\mu$  and  $6\mu$ , respectively, and the average breadth of the ellipsoids and outer segments were  $2.5\mu$  and  $2\mu$ , respectively. The thickness of the external nuclear layer was from  $20\mu$  to  $12\mu$  and that of the internal nuclear layer was about  $12\mu$  (Fig. 2, A). In the young fish (less than 30 cm in body-length) the thickness of the entire retina was about  $450\mu$  in fundus and about  $110\mu$  in *ora serrata*; the thickness

of pigment epithelium was about  $50\mu$ ; in the visual cell the average length of their outer segments, ellipsoids and myoids were  $9.5\mu$ ,  $7.2\mu$  and  $5\mu$ , respectively. The thickness of the external nuclear layer was from  $20\mu$  to  $60\mu$  and that of the internal nuclear layer was  $50\mu$  to  $180\mu$ ; the latter was almost two or three times as thick as the former (Fig. 2, B). In the wave-form of the ERG, however, no differences seemed to be found between the adult and the young fish retina.

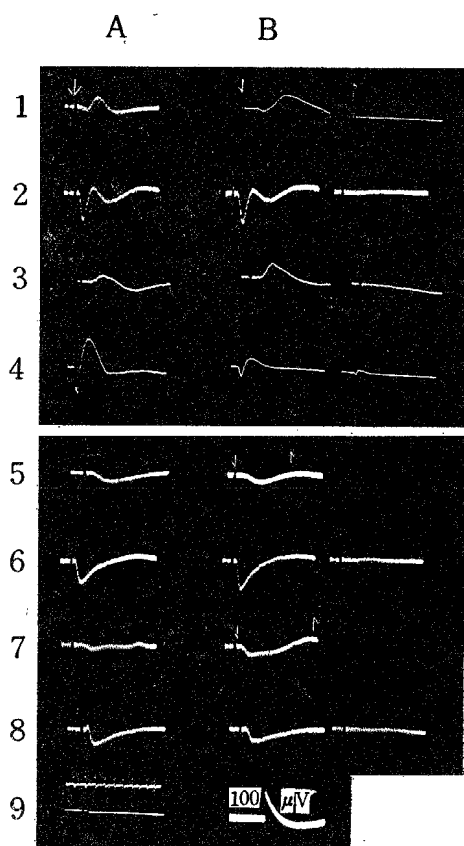


Fig. 3. Typical wave-forms of ERGs in dog-fish. The stimulus duration is 20 msec. in A, and about 3 sec. in B, except B—5,7 in which it is 1 sec. 1, 2, 3 and 4, ERGs of the fast type; 5, 6, 7 and 8, ERGs of the slow type. 1 and 5, responses to weak light stimulus in light-adapted retina. 2 and 6, to intense light stimulus in light adapted retina. 3 and 7, to weak light stimulus in dark-adapted retina. 4 and 8, to intense light-stimulus in dark-adapted retina. 9, signal of stimulus duration. Polarity of the record is upward positive, and calibration voltage is 0.1 mV, through this study, except for the case described in particular. Time marking: 1/6 sec.

## (2) Wave-form and polarity

Two types of the ERG are found in *Mustelus*, the fast type of diphasic form and the slow type of negative monophasic form. The fast type seemed to have a tendency to be led from the ventral region of the retina and on the contrary the slow one seemed to be led from the dorsal side of the retina.

Typical ERGs of the fast type obtained from the light- and the dark-adapted eyes are reproduced in Fig. 3 together with those of the slow type.

The ERG from partly the dark-adapted eye elicited with weak light stimulus of short

duration was a positive monophasic wave with a latency of approximately 50 msec. and about  $60 \mu\text{V}$  in amplitude, but in the completely dark-adapted eye the wave was followed by a small slow negative one, taking as a result the diphasic form as shown in 3-A of Fig. 3. In the case of stimulation with long duration the response was a positive monophasic form, and did not elicit off-response (Fig. 3, 3-B). In the case of stimulation with intense light the positive potential was preceded by a fast negative potential, the amplitude of which was nearly equal to the positive one (Fig. 3, 4-A), and also in this case the stimulation with long duration elicited unnoticeably a diphasic off-potential (Fig. 3, 4-B). In the light-adapted eye a small negative potential preceded the positive one (Fig. 3, 1-A and B), in particular it was distinct in the case of stimulation with intense light (Fig. 3, 2-A and B). And also in this case the response to the long stimulation did not elicit the off-effect (Fig. 3, 2-B), irrespective of light intensity.

On the other hand, the response of the slow type revealed generally the negative deflection, which increased with the intensity of stimulus light. In the thoroughly dark-adapted retina, however, the response to the weak light stimulation had the tendency to show the slow diphasic form (Fig. 3, 7). The off-effect was never observed in this type of response.

### (3) Effect of the stimulus intensity on the amplitude and the latency of the response

The response to various intensity of stimulus light ranging from 0.5 lux to 1,400 lux, were recorded in sequence of five minutes in a dark-adapted eye. The duration of stimulus was 5 msec. As to the slow type response, the amplitude of the negative potential augmented with the increase in stimulus intensity, until it reached plateau at 200 lux (Fig. 4). From the diagram of Fig. 4, the rate of increase in the response amplitude is in linear relation against the logarithm of the stimulus intensity

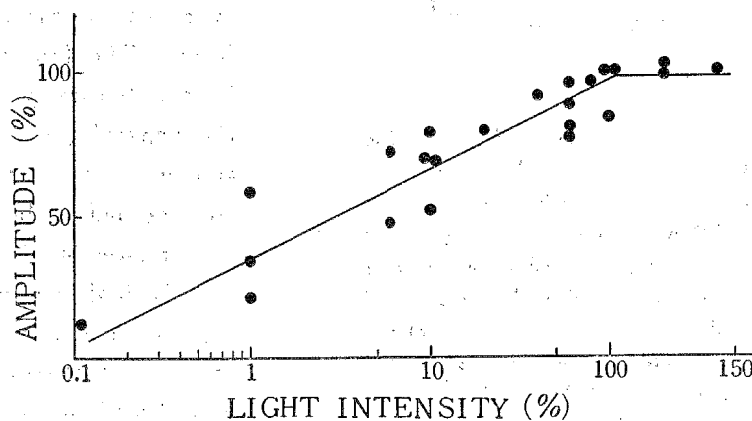


Fig. 4. Relation between the amplitude of negative potential and the stimulus intensity, in the slow type responses. Abscissa, intensity of stimulus light in log scale. Ordinate, the amplitude of the negative potential to maximum one.

in the moderate stimulus range. The latency is shortened and the rising rate of the potential is accelerated with the increase in stimulus intensity.

In the case of the fast type response, the wave-form of the ERG was changed in accordance with the intensity of light stimulus. The form of ERG elicited by weak stimulus was positive monophasic (Fig. 3). In the case of intense light stimulus a fast negative wave appeared preceding to the positive potential which was followed again by a slow negative potential. Amplitude of the initial negative wave and also the positive one following it augmented together with the increase in stimulus intensity. With the further increase in stimulus intensity, however, the positive potential came to be covered by the initial negative potential and it could be detected only as a hump on the falling phase of the negative potential in the case of stimulation with intense light. As shown in Fig. 5, the amplitude of the negative potential (filled circles) increases in linear relation against the logarithm of stimulus

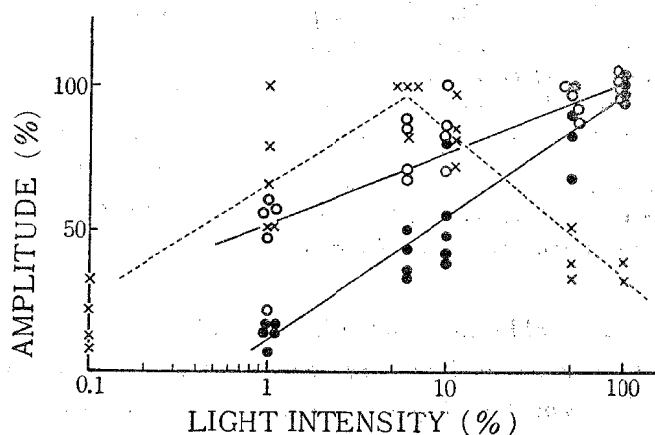


Fig. 5. Relation between the amplitude height and the stimulus intensity, in the fast type responses. Filled circles, the negative potential; open circles, the positive potential; crosses, the magnitude of the positivity over the base line. Other legends are same as in Fig. 4.

intensity in the entire range of the stimulation used. The height of positive deflection, measured from the tip of the negative potential (open circles) increases also with the rise of stimulus intensity. Such positive potential seems to be similar to that obtained from the retinae of batoid fishes (*Holorhinus*, *Urolophus* or *Narke*) and *Scorpaenodes* or *Saurida* (cf. Chapter II and IV), and it may be assumed to be a rod potential. The positive potential of the fast type response can be caused by far lower illumination, in other words the threshold of the positive wave in the fast type response is far lower than that of the negative one in the slow type response.

#### (4) Relation between the response and the duration of stimulus light

Using the stimulus of constant intensity, the changes in shape and amplitude of the response were investigated varying the duration of stimulus light. Rather weak stimulus was used for this experiment, for it was shown in both types of response,

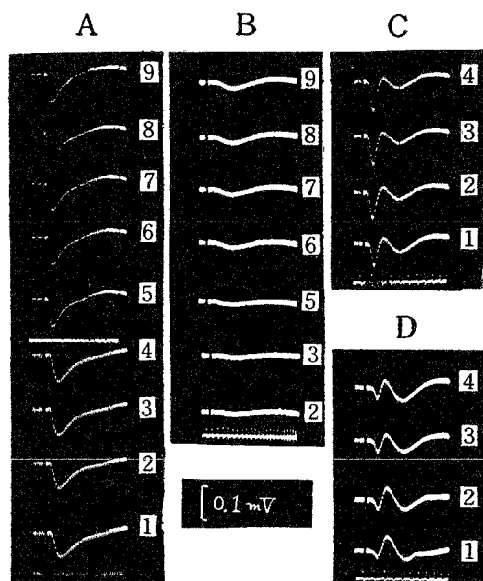


Fig. 6. ERGs for various durations of light stimulus: A and B, the slow responses; the durations of stimulus light with intensity of 500 lux (A) and of 5 lux (B), in 1—9 are 1/300, 1/100, 1/50, 1/25, 1/25, 1/10, 1/5, 1/2 and 1 sec., respectively; C and D, the fast type; the durations of stimulus light with intensity of 50 lux (C) and of 2 lux (D), in 1—4 are 1/100, 1/25, 1/5 and 1 sec., respectively.

that in the intensity range of light stimulus where increase in the light intensity causes no more increase in the magnitude of response, the change in the duration of stimulus light also does not exert any influence on the magnitude or shape of the response (Fig. 6, A and C).

With the increase in duration of stimulus light the amplitude of the response increased slightly in the slow type response (Fig. 6, B), and the positive component decreased and the negative component augmented in the fast type response (Fig. 6, D). These results seem to be interpreted after Bunsen-Roscoe's law.

#### (5) Effect of potassium chloride and ethylalcohol on the ERG

It is well known as a general feature that the potassium ion depresses the positive component of ERG whereas ethylalcohol depresses the negative one. In *Mustelus* also the positive potential disappeared within one minute after the application of a 10% solution of potassium chloride (Fig. 7, A). In this case the latency of the

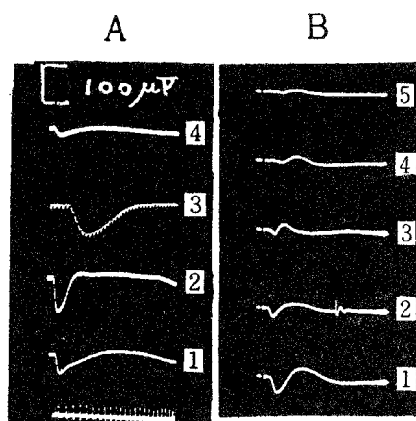


Fig. 7. A, effect of 10% KCl sol. on ERG: 1, control; 2, a few minutes after the application; 3, the same as 2, but in fast sweep; 4, five minutes after the application. B, effect of 10% ethylalcohol sol. on ERG: 1, control; 2—3, immediately after the application; 4—5, three and five minutes after the application. The stimulus light is 50 lux and 20 msec. in duration.



negative wave was not affected in any way, and the falling phase of the potential was shortened. This negative potential, however, disappeared rapidly with the lapse of time (Fig. 7, 4—A).

It was also observed that the application of ethylalcohol caused the selective extinction of the negative potential within a few minutes, and only the positive potential remained. But the positive potential also disappeared soon after that (Fig. 7, B).

#### (6) Light and dark adaptation

Two kinds of index were used for the determination of the grade of dark- or light-adaptation in *Mustelus* retina; i) the amplitude of the ERG elicited by flash stimulation with constant duration and intensity, and ii) threshold intensity of stimulus light for eliciting ERG.

(i) After the exposure of the dark-adapted retina to weak light (about 0.5 lux), the test flash was applied every 2 to 5 minutes to record the fast response, and the amplitudes (filled circles in Fig. 8) and the latencies (crosses in Fig. 8) of the positive potential thus obtained were plotted against the time after the beginning of the light-adaptation. The magnitude of the positive potential decreased rapidly with the progress of the adaptation and it attained the steady state 25 minutes later. The change of the latency of response followed the similar course as the amplitude, but it seemed to reach the plateau about 10 minutes later.

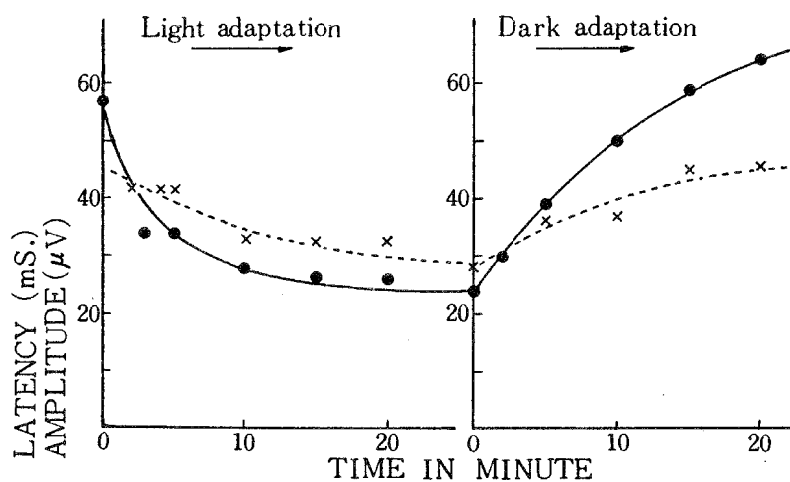


Fig. 8. Changes in amplitude and latency of positive potential in ERGs of the fast type in the course of light- and dark adaptations. Test flash is 0.5 lux and 20 msec. in duration. Filled circles, amplitude ( $\mu V$ ); crosses, latency (msec.).

After the exposure to such weak light for 30 minutes, the illumination was put out, and the change of the response to the test flash during the course of the dark-adaptation was investigated. The result is shown in Fig. 8. The amplitude of the

positive potential of the response increased with the progress of the dark-adaptation, and after about 20 minutes it reached the plateau. The latency of the response was prolonged in the dark-adaptation.

In the experiments of the light-adaptation with intense light (about 30 lux), the test flash was given after a provisional cessation of the adaptation light. The result is shown in Fig. 9. The transient increase in amplitude of the negative potential is seen at the beginning of the light-adaptation, but thereafter the amplitude begins to decrease and reaches a steady state 30 minutes later. However, the amplitude of the negative potential in the final state was slightly larger as compared with the initial value. The latency of the negative potential tended to prolong a little in the light-adaptation (Fig. 9, left).

After the exposure to such intense light for 30 minutes, the illumination was put out, and the change of the response to the test flash during the course of the dark-adaptation was investigated. The amplitude of the negative potential decreased gradually a little within 10 minutes, and thereafter it was left unchanged. Though the change in the latency of the response was not noticeable, it was somewhat short in the dark-adaptation (Fig. 9, right). Moreover the rising and falling phases of the response were clearly prolonged with the progress of the dark-adaptation.

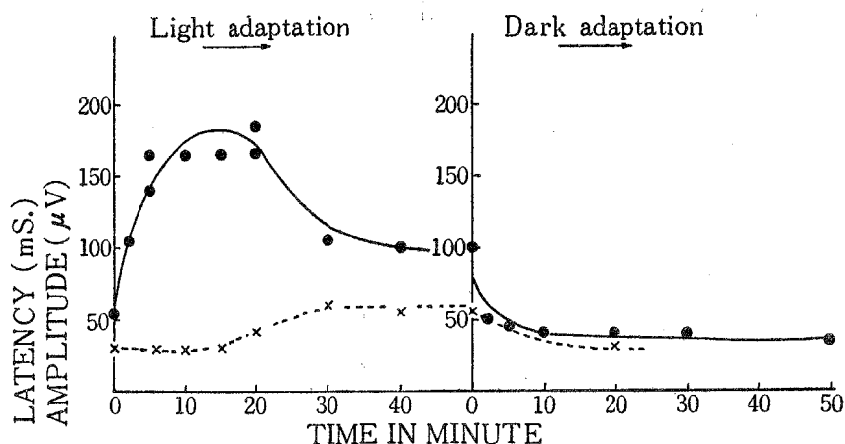


Fig. 9. Changes in amplitude and latency of negative potential in ERGs of the slow type in the course of light- and dark-adaptations. The intensity of test flash is 250 lux. Other legends are same as in Fig. 8.

(ii) In this case the course of the light- and the dark-adaptation was investigated using the threshold intensity of light stimulus eliciting ERG as the indices. The response of the fast type was used for the investigation.

When the retina was exposed to a weak light (0.2 lux) the threshold of the retina rose rapidly at first, thereafter slowly and attained the steady state 10 minutes later (filled circles in Fig. 10). If the retina was exposed to extremely weak light (0.05 lux) no remarkable change in the threshold was seen (open circles in Fig. 10).

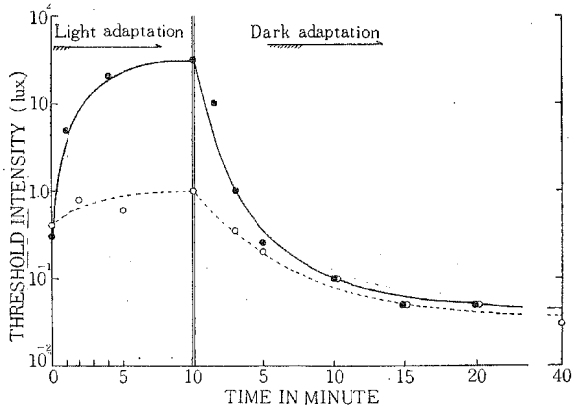


Fig. 10. Change in threshold intensity of stimulus light for eliciting the fast type ERG, in the course of light- and dark-adaptations. Filled circles, light intensity of adaptation light is 0.5 lux; open circles, 0.05 lux.

and attained the steady state about 20 minutes later. Even if the intensity of the preadaptation is 0.05 lux, the course of the following dark-adaptation was not noticeably different from that of the previous case, although the rapid decrease in the threshold was not seen in the initial phase of the dark-adaptation.

From these results mentioned above, it is found that the process of dark-adaptation consists of the initial rapid process and the secondary slow one. Therefore, it is assumed that *Mustelus* retina contains two kinds of photo-labile pigment.

(7) Effect of intermittent light stimulation

It was observed that the intermittent light projected on the retina elicited the response which was different from that in teleost fish. A part of the results obtained in the response of the fast type is shown in the records of Fig. 11. In the intermittent stimulation of weak light, the response

After the exposure to such a weak light, the illumination was put out, and the change in the threshold of ERG was investigated during the course of the dark-adaptation (Fig. 10). The threshold decreased rapidly within 3 minutes in the initial phase of the dark-adaptation, and thereafter it decreased slowly

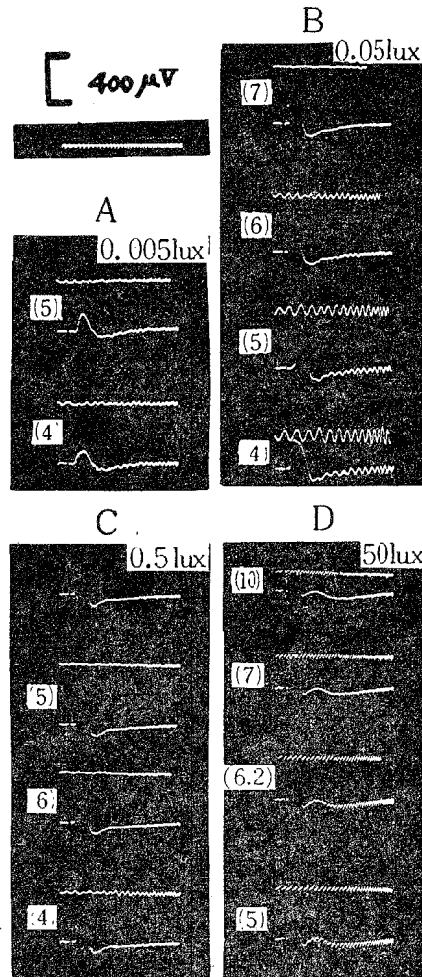


Fig. 11. The fast type responses to intermittent light stimuli. Intensity of stimulus light in A—D are 0.005 lux, 0.05 lux, 0.5 lux and 50 lux, respectively. Numeral on each series of records represents the frequency (c/s) of the intermittent stimuli.

showed the large positive potential followed by a succession of positive ripples (Fig. 11, A and B). And the fusion of the ripples occurred at relatively low frequency of the intermittent stimuli, for example, at about 7 c/s in the stimulus intensity of 0.05 lux. With the increase in the light intensity of the intermittent stimuli, the amplitude of the initial positive potential was augmented, its duration was prolonged, and the amplitudes of the following ripples decreased (Fig. 11, C). In this case the fusion frequency of the ripples seemed to be decreased. With the further increase in the stimulus intensity, the initial response showed a large diphasic deflection, namely a small negative potential preceded a large positive potential followed by a succession of negative ripples (Fig. 11, D), the fusion frequency of which was above 10 c/s in the stimulus intensity of 50 lux. Therefore, it follows that the ERG elicited by the intermittent stimuli of weak light is a succession of b-wave whereas that elicited by intense light is a succession of a-wave. And this may be also assumed from the records shown in Fig. 12, where in A) the retina is subjected to the intermittent stimuli of different intensity with a

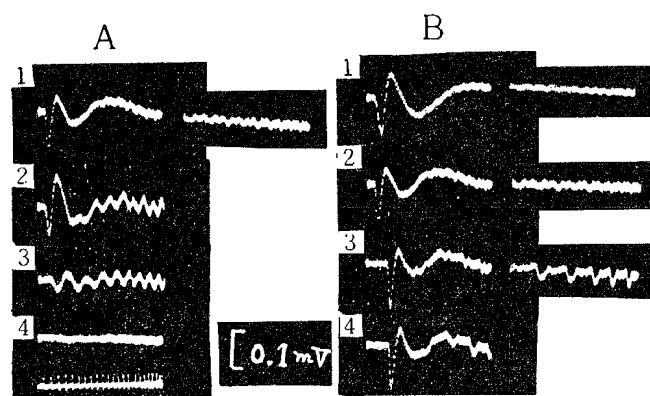


Fig. 12. A, the fast type responses to intermittent light (2 c/s) with various intensity; the intensity in 1-4 are 270, 50, 5 and 0.5 lux, respectively. B, responses to intermittent light of various frequencies with constant intensity (270 lux); the frequencies in 1-4 are 6, 3, 1.8 and 1.5 c/s, respectively.

constant frequency (about 3 c/s), and in B) to that of different frequency with constant intensity (270 lux). Further investigations are necessary regarding to the effects of the light intensity, temperature, state of light-adaptation and others on the flicker fusion frequency of ERG.

The slow type ERG responded only to very slowly repetitive stimuli with the intense light. The responses to the repetitive stimuli with various frequency were examined. The results shown in Fig. 13 suggest the existence of a long refractory period in the response. The absolute refractory period may be assumed to be about one second. The relative refractory period was very long, changing with the intensity of the stimuli and also with the frequency of the repetition of the stimuli.

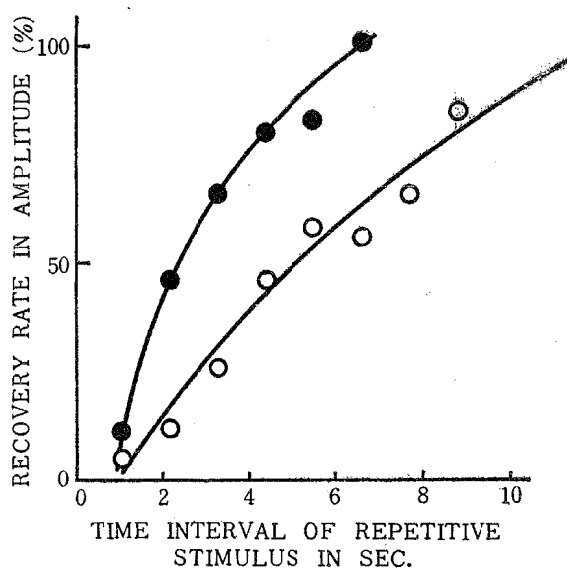


Fig. 13. Suppression of second response in repetitive stimulation. Abscissa, the time interval of repetitive stimuli in sec. Ordinate, the rate of the height of the second response to that of the first one. Filled circles, intensity of stimulus light is 50 lux; open circles, 500 lux. Duration of stimulus light is about 100 msec.

#### (8) Spectral sensitivity

The spectral sensitivity curves were obtained as to the response of both types. The results are shown in Fig. 14. The solid line of Fig. 14 is the sensitivity curve which was determined with regard to the positive potential in the response of the fast type, and the broken line is that concerning the negative potential of the slow type. Both curves had the maximum at 500  $m\mu$  of the spectrum as well and fall down rather steeply on both sides. Both curves were similar to each other in the shape though the curve for the fast type showed somewhat expansion in the range from 500 to 580  $m\mu$  of the spectrum, and the sensitivity in the response of the fast type was about 10 times as high as the slow type. And from the diagram the retina was almost insensitive to the light of above 650  $m\mu$ . The maxima of the sensitivity curves mentioned just above, seem to correspond to the maximum (505  $m\mu$ ) in the absorption curve of visual pigment that BAYLISS *et al.* ('36) obtained in the retina of *Scyllium canicula*.

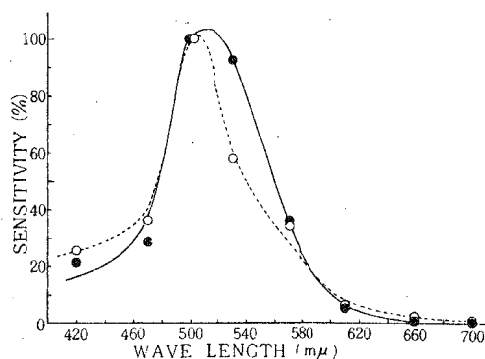


Fig. 14. Spectral sensitivity curve of a dog fish retina. Filled circles represent the sensitivity curve investigated in the fast type response; open circles, that in the slow type response.

### Discussion

Two different types of ERG, fast and slow type, were recorded in *Mustelus*. Judging from the following facts the slow type response can not be assumed to be degenerated one of the fast type; i) the fast type response is apt to be recorded from the dorsal side of the retina, ii) the slow response can be obtained even in the fresh preparation, iii) the fast response is observed to be transformed easily into the slow response by the slight displacement of the electrode on the retina, *vice versa*. According to the histological observation, however, any differences in the retinal structure were not found among all the regions of the retina. Different types of ERG such as found in *Mustelus* retina have not hitherto been observed in the retinae of other fishes, although it seems that there is unnoticeably a tendency to show such different types of ERG in some batoid fishes or plaice (*cf.* Chapter II and IV).

The nature of the fast type response in *Mustelus* is similar to ERG of batoid fish (*cf.* Chapter II), and it seems to belong to Group-II according to the classification of the ERG in teleost fishes (*cf.* Chapter IV). ERG of Group-II in the classification is suggesting the benthonic life, and in fact, the fish is known to be caught from the deep sea of about 200 fathoms, though it swims actively at surface water in the daytime.

On the other hand, the threshold of the positive potential of the fast type response was considerably lower than that of the negative potential in the response of the slow type. In the dark-adapted retina, the response to extremely weak light was the positive potential followed by the negative potential (Fig. 3, 3-A), and then such potential seemed to correspond to rod potential that was obtained by SVAETICHIN ('53b) from the retina of *Perca* by the aid of a micro-electrode. Therefore such positive potential obtained from the *Mustelus* retina will be assumed to have resulted in either rod or rod-paths. The fact that such positive potential is suppressed by light-adaptation, supports this assumption. It is interesting that these results match well the vision of a dog fish from its behaviour described above. According to GRANIT ('57) the ERG of rod retina is a succession of b-wave in the response to flicker stimulation, hence the *Mustelus* retina is considered to reveal the character of rod-retina in the case of stimulation by weak light at least. The response of the positive potential to the intermittent stimulation of weak light was a succession of b-wave and showed low fusion frequency (6—7 c/s). While, the response to the stimulation by intense light showed a succession of a-wave following the initial diphasic response. Such peculiar response to intermittent stimuli was not yet found in the retinae of other fishes, because the ERG of cone retina was a succession of a-, b-, a-, b-wave ..... In future, the response to intermittent stimuli must be investigated in detail in this species.

In the response of the slow type, the threshold of light intensity eliciting the negative potential was about 10 times as high as that of the positive potential in the fast type. With the increase in stimulus intensity the negative potential also increased,

and the steady state was attained by a relatively higher stimulus intensity as compared with that for positive potential in the fast type. This result seems to suggest the great capacity of the fish in adapting itself to intense light. SVAETICHIN ('53) and MITARAI *et al.* ('56) have described that there is no adaptation to darkness or illumination in the cone of fish retinae. Almost no influence of the adaptation on the negative potential was found in the response of the slow type, although the potential brought the transitory augment of the amplitude in the initial course of light-adaptation (Fig. 9). These facts seem to suggest the photopic character of the slow type response except the curious fact that the negative potential in the response does not respond to intermittent stimuli at all.

If it is assumed that the slow type response is photopic while the fast type is scotopic, it is presumable that the spectral sensitivity curve obtained in the responses of each type should be different from each other in the position of their maxima. In the present experiment, however, no difference has been observed between them, and the spectral sensitivity curves of both types show the maxima together at about 500 m $\mu$ . Position of the maximum of spectral sensitivity at such a shorter wave-length is suggesting that it is suitable for oceanic or deeper sea behavioural life, because the light which most effectively penetrate clear oceanic water is that of 480 m $\mu$  to 500 m $\mu$  in wave-length, while in coastal waters the light of 530 m $\mu$  or longer wave-length penetrates most easily (UTTERBACK '36). This assumption is also supported by the relation, which is described elsewhere, between the maximum of spectral sensitivity curve and the depth of the habitat of fish (*cf.* Chapter III).

BRETT ('57) has described the existence of cone together with rod in *Mustelus* retina, and TAKATSUJI ('34) reported that there might be no differentiation toward rod and cone in the development of visual cells in elasmobranch fish, and the author could recognize only one type of visual cell in the histological investigation of the retina (Fig. 2) as well as DETWILER ('43). However, the physiological results obtained in the present work seem to suggest the existence of some components showing the photopic character which may be assumed to be caused from the cell like cone. Therefore, it may be regarded as appropriate that the receptor cells in *Mustelus* retina should be differentiated into rod and cone physiologically, even though there are no morphological differences between them.

### Summary

(1) The electroretinogram (ERG) of a dog fish, *Mustelus manazo*, was investigated. ERG was taken from the exposed retina directly, and the results were considered ecologically.

(2) In the ERG of *Mustelus* two types of the response were found; the fast type showing a fast initial negative potential followed by a positive potential, and the slow type of monophasic negative potential. And the threshold of stimulus light was one log unit lower in the former than in the latter.

(3) In the fast type response, the response to weak light (below 0.5 lux) was positive monophasic, and that to intense light was negative potential followed by positive potential. With the increase in stimulus intensity the amplitude of both positive and negative potentials increased in the linear relation to stimulus intensity, but the negative potential had a tendency to cover the positive potential in the case of stimulation of extremely intense light.

(4) In the response of the slow type, the amplitude of the negative potential augmented with the increase in stimulus intensity, and it attained the steady state only in relatively high intensity.

(5) The ERGs of both types were not almost affected by the stimulus duration, therefore the ERG of the dark-adapted retina did not elicit the off-response.

(6) Although the amplitude of negative potential increased temporarily by light-adaptation in the response of the slow type, the potential was not almost affected by the adaptation.

(7) In the response of the fast type, the time-course of light- or dark-adaptation was investigated by the threshold intensity and the size of ERG, respectively. The light-adaptation with weak light (0.5 lux) was completed in about 10 minutes and the following dark-adaptation was accomplished in about 20 minutes.

(8) In the fast type the ERG to intermittent stimuli of weak light (below 0.5 lux) was a succession of b-wave, and the maximum value of the fusion frequency was 6—7 c/s. The ERG to that of intense light was a succession of a-wave, and the fusion frequency was above about 10 c/s.

(9) The response of the slow type did not follow the intermittent stimuli, and the refractory period of the potential was found to be considerably long.

(10) The spectral sensitivity curves obtained from the ERGs of both types had the maxima at about 500 m $\mu$ , coincidentally.

(11) It was considered that the response of the slow type revealed the photopic characters and that of the fast type, scotopic ones dominantly. In addition, the consideration on the retinal structure was described.

(12) From the characters of the ERG mentioned above, it is assumed that a dog fish has the retina applied to photopic and scotopic vision, and the fish is behavioural in deeper sea and has such noticeable disposition as the vertical movement.



## Chapter II    Electoretinogram of rays and skates, Batoidei, with reference to ecological aspects

The study of the electroretinogram (ERG) in vertebrate has not yet been thoroughly extended to the Elasmobranchii. In Chapter I the author described that a dog-fish, *Mustelus manazo*, showed the ERG consisting of negative and positive deflections in spite of its rod retina, and he further considered the ecological significance of the character of the ERG in connection with the behaviour of the fish. Comparative studies on the eye of Batoidei were carried out in this chapter. The batoid fishes are usually known to have pure rod retina (BAYLISS *et al.* '36, DETWILER '43), although the eagle ray, *Myliobatis*, has a few cones in its retina (BRETT '57). As regards the habits of these fishes, it is also known that the rays and skates are typical benthonic fishes and live on the bottom of the sea most of the time, sometimes are buried in the sand or mud of the bottom, and in the case of a certain species the depth of the habitat extends down to 1,000 fathoms (BIGELOW and SNYDER '53).

It is worthwhile not only from the comparative physiology of vision but also from the ecological view point to investigate the ERG of the fishes having such a peculiar habit.

### Material and method

Rays and skates covering 6 species were used as materials in this work, and their names were shown in Table 1, together with their disc-lengths and the period during which they were used. The fishes were caught either with a small trawl net or with a set-net, off the coast of Yamaguchi Prefecture facing the Japan Sea. Before the experiment, the fishes were preserved for a few days in the aquarium of our laboratory.

Table 1. Species of fishes used in the experiment of this chapter, its disc-length and period through which it is used.

Family	Species	Common name	Disc-length (cm)	Experimental period (month)
Torpedinidae	<i>Narke japonica</i> (TEMMINCK et SCHLEGEL)	electric ray	25—30	XI, VI
Myliobatidae	<i>Holorhinus tobije</i> (BLEEKER)	eagle ray	25—35	VIII
Dasyatidae	<i>Dasyatis akaje</i> (MÜLLER et HENLE)	sting ray	15—25	V, VI
"	<i>Urolophus fuscus</i> GARMAN	—	25—30	VIII
Platyrrhinidae	<i>Platyrrhina sinensis</i> (BLOCH et SCHNEIDER)	fan ray	35—40	VI
Rajidae	<i>Raja porosa</i> (GÜNTHER)	skate	45	XI

Experiments were carried out with an eye excised. Active electrode, Ag-AgCl type, was in contact with the inner surface of the opened eye from which cornea, lens and vitreous humor had been removed. Indifferent electrode was the Ag-AgCl type and it was put to the outside of sclera through the moistened cotton sheet. The arrangement for recording the ERG was principally the same as that described in Chapter I. Intensity of stimulus light was controlled by neutral filters, and narrow bands of spectral light were obtained by the interference filters (*cf.* Chapter III), although in the experiments of *Narke* and *Raja*, Erma's monochromatic filters were used. The details of the methods used for the determination of spectral sensitivity of the retina were described in Chapter III.

The method used for the investigation of threshold intensity in the course of light- and dark-adaptation was as follows. The measurements of the threshold intensity in the course of light-adaptation were carried out with interval as near to 2 minutes as possible until the light-adaptation was judged to be completed. To make the measurement, the neutral filter was initially set at a level of illumination estimated to be below the threshold intensity for the retina, and then the stimulus light was projected through the filter. If no action potential was elicited, the stimulus intensity was increased by about 0.5 log unit, and the stimulus was again given at an interval of about a quarter of a minute. This procedure was repeated until discernible action potential was elicited, when the total concentration of the neutral filter and the time that had elapsed since the beginning of the adaptation were recorded. In this way, the change of the threshold intensity was obtained in progress of the light-adaptation with a definite illumination. After that the retina was brought in darkness, and the change of the threshold intensity of the retina was obtained in the course of the dark-adaptation, in the same manner mentioned above until the retina recovered the sensitivity of the initial level in dark-adaptation. Therefore, the retina had been left in darkness as it was for least several minutes before starting the measurement under illumination with the next level.

## Results

### (1) Wave-form of ERG

*Narke japonica* The shape of eyeball (*bulbus oculi*) is semi-cylindrical, and the outside of the eyeball is extremely calcified and it shows the so-called cartilaginous sclera.

In dark-adapted retina the ERG elicited with flash stimulus was a small slow negative potential followed by a large positive one, and moreover few fast deflections were superimposed on the rising phase of the large positive potential (Fig. 15, A). The fast component seemed to be prominent in the experiments made in winter. With the increase in stimulus intensity the amplitude of the slow response augmented slightly, while the latent period of the response was shortened considerably. In this case the latent period of the fast deflection was also shortened, but the amplitude

of that was not affected noticeably. The response of dark-adapted retina to the stimulus of long duration (one second) was the same in wave-form with that to flash stimulus; the off-effect did not appear.

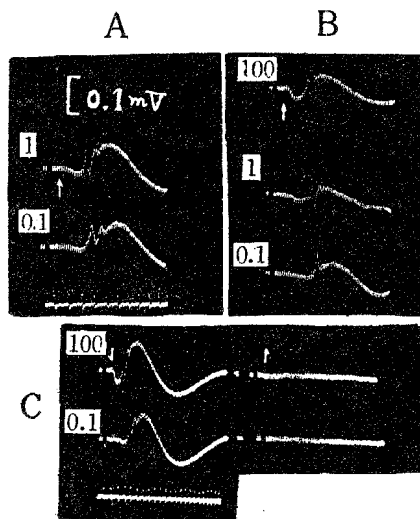


Fig. 15. ERGs recorded from *Narke*. A, obtained from the dark-adapted retina; B and C, from the light-adapted. Arrows indicate the stimulus signal, which is 1/50 sec. in A, B and about 3 secs. in C. Numeral on each series of the records represents the stimulus intensity in percentage of unit intensity, 500 lux. Time mark: 1/6 sec.

In the retina light-adapted with about 30 lux illumination, the ERG elicited with stimulus flash was shown in the records of Fig. 15, B; the slow positive potential was remarkably suppressed, whereas the fast deflection superimposed on the slow potential was not affected noticeably. The latent period of the whole response underwent remarkably prolongation. In the case of stimulation of the duration of about 3 secs., the response did not cause off-effect even if any intense light was projected. In this case the change in wave-form of on-response was not found over the wide range of stimulus intensity, although the latent period and the amplitude of the initial negative potential changed remarkably with the stimulus intensity (Fig. 15, C).

In order to know the relation between the slow and the fast responses, the dark-adapted retina was subjected to a series of stimulus flash with different degrees of the intensity (Fig. 16). The amplitude of the slow response decreased with the lowering of stimulus intensity as mentioned above, while the amplitude of the fast response was seen to increase with the decrease in stimulus intensity. The increase in amplitude of the fast response may probably be due to the release from being balanced by the slow response. When the stimulus intensity was extremely brought down (below 0.1 % of unit intensity), the negative potential of the slow response disappeared, while the fast response remained still, being followed by the slow positive potential. The fast response remained took diphasic form at first, but with the further lowering in stimulus intensity the response became a positive monophasic wave (0.006 % in Fig. 16). In this case the time duration of the fast response prolonged remarkably. As the lowering in stimulus intensity proceeded further, the

positive wave mentioned above came to be followed by a slow negative wave, and

with the further lowering all the response disappeared. From these results it may be assumed that the slow response represents photopic components, whereas the fast deflection scotopic ones, because the threshold of the fast deflection is lower than that of the slow response.

The fast potential superimposed on the slow response, was not so striking in the ERG obtained in the summer experiments. A few types of these ERGs was shown in Fig. 17. In the response to intense stimulus, the negative potential was dominant, and a notch was observed to be superimposed on the response, instead of the remarkable fast deflection obtained in the above-mentioned winter experiment. The site of the notch on the response was different as the case may be, and in some occasions the notch preceded the negative slow response (Fig. 17, A and B), and also the notch became distinct with the decrease in stimulus intensity, as in winter experiment. Moreover, the latency of the notch was reduced noticeably with the increase in stimulus intensity in any case. From

these results it is assumed that the notch is homologous with the fast deflection in the winter experiment.

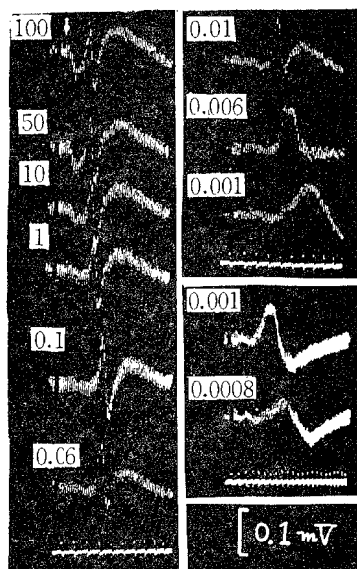


Fig. 16. Change in wave-form of ERG in *Narke*, according to the variation in stimulus intensity. Test flash is 1/50 sec. Other legends are same as in Fig. 15.

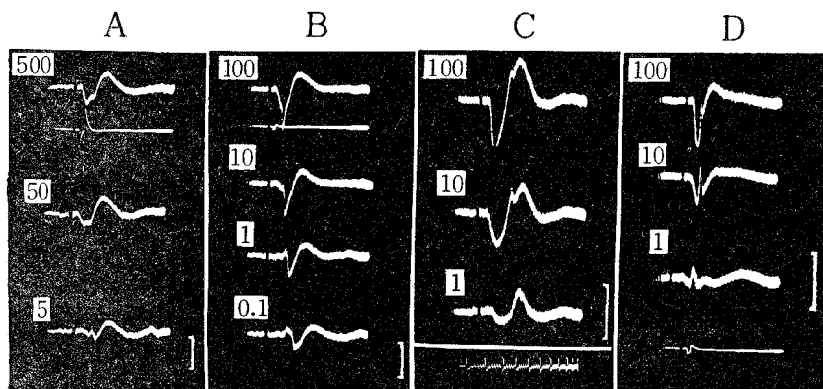


Fig. 17. Different types of ERG in *Narke*, observed in summer experiments. A, C, obtained from the dark-adapted retina; B, D, from the light-adapted one. Numeral on each series of the records represents the stimulus intensity in lux. Test flash, 1/50 sec. Other legends are same as in Fig. 15.

In the application of 10% KCl solution on the retina, the positive potential of the slow response disappeared within about 5 minutes, whereas the negative potential

appeared remarkably. Even in the case where the stimulus intensity was so low that it could not cause the negative potential of the slow response in normal retina, a negative potential appeared soon after the application of KCl. The fast deflection superimposed on the slow response seemed to have been not at all affected by the application of the KCl solution.

*Holorhinus tobijeii* Typical wave-forms of the ERG are shown in Fig. 18. In the dark-adapted retina, the stimulus flash with extremely weak light elicited a small positive monophasic potential with the latent period of about 150 msec. (Fig. 18, A-3). If the strength of the stimulus flash increased 10 times, a small negative potential preceded the positive one (Fig. 18, A-2). The response to the flash with intense light was the negative potential followed by the slow positive potential, and a hump occurred on the falling phase of the negative potential, and also the length of time-duration of the response became longer (Fig. 18, A-1). In stimulus of comparatively long time (about one second), the response was the same as that of the flash stimulation, except for the increase in amplitude of the slow potential (Fig. 18, B). In this case the stimulus with intense light did not cause the off-effect, while that with weak light seemed to cause the unnoticeable positive off-potential (Fig. 18, B- 2, 3).

When the retina was light-adapted under about 3 lux illumination, the sensitivity of the retina decreased and many wave-lets appeared on the base line even if the test flash was not projected (Fig. 18, A-7). Under such illumination, the wave-form of the ERG elicited by stimulus flash was essentially the same with that of the dark-adapted retina, except that the time-course of the response became short (Fig. 18, A- 4-7). In the stimulus of long duration, off-effect appeared remarkably; in the response to intense stimulus light on-effect was the negative potential whereas off-effect was the positive potential followed by the negative one, and also in the response to weak light both on- and off-effects were positive potential (Fig. 18, B-4-7).

As shown in Fig. 19, the threshold of the protrudent potential occurring on the rising phase of the slow positive potential in the dark-adapted eye is lower than that

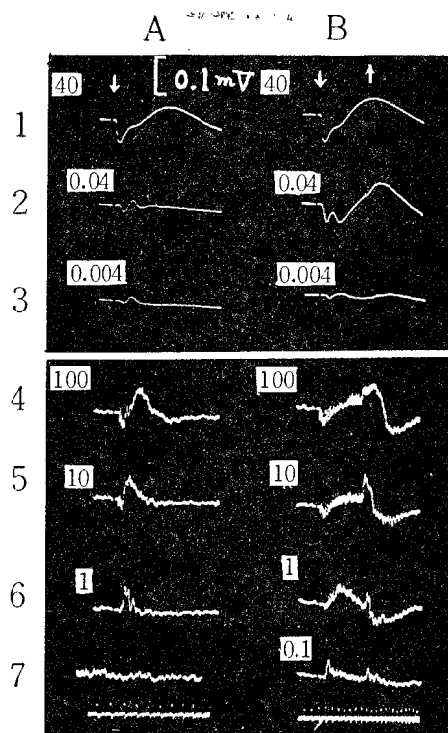


Fig. 18. Typical wave-forms of the ERG recorded from *Holorhinus*. The stimulus duration is 1/300 sec. in A and 1 sec. in B; 1-3, obtained from the dark-adapted retina; 4-7, from the light-adapted. Other legends are same as in Fig. 15.

of the slow positive potential, and therefore it is assumed that the response to stimulus with extremely weak light may be constructed of another potential, of which the origin is different from that of the large slow positive potential elicited by intense light. With the increase in stimulus intensity the amplitude of the protrudent potential increases, too, while the potential seems to be balanced by the negative potential which develops at the same time. Hence, the response to the stimulus with intense light seems to be a simple negative potential followed by a positive potential (Figs. 19, 40 and 110).

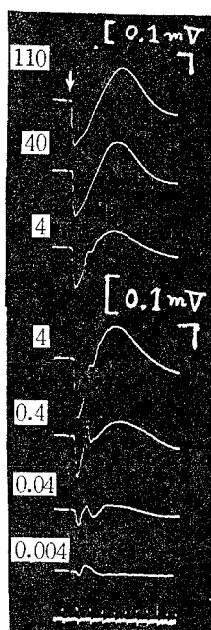


Fig. 19. Change in wave-form of the ERG in *Holorhinus*, according to the variation in stimulus intensity. Test flash is 1/50 sec. Other legends are same as in Fig. 15.

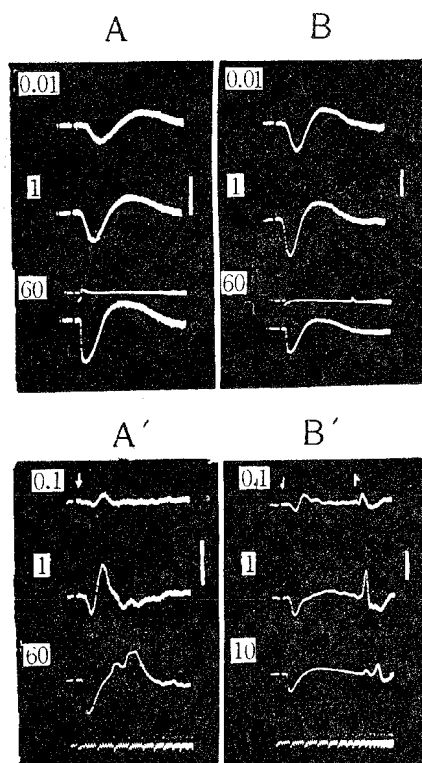


Fig. 20. ERGs recorded in *Dasyatis*. A, B, obtained from the dark-adapted retina; A', B', from the light-adapted. The stimulus duration is 1/300 sec. in A, A' and 1 sec. in B, B'. Other legends are same as in Fig. 15.

*Dasyatis akajei* The typical wave-forms of the ERG obtained in the fresh preparation are shown in Fig. 20. In dark-adapted retina, the response to stimulus flash was a large negative potential followed by a positive potential, and both negative and positive potentials increased the amplitude with the increase in stimulus intensity (Fig. 20, A). The response to stimulus light of long duration was almost the same as that to the flash; the off-effect was never caused by the long lasted stimulation, irrespective of the strength of the stimulus light (Fig. 20, B). Moreover, as observed in the ERGs of *Holorhinus* or *Narke*, the notch or the fast deflection superimposed on the response was not found in this species. Thus, the negative

potential was dominant in the ERG of dark-adapted retina of *Dasyatis*. However, on some occasions the positive potential was apt to occur noticeably in fresh material.

In the retina light-adapted with about 3 lux illumination, the response to stimulus flash with weak light was a small positive monophasic potential, and with the increase in stimulus intensity a negative potential came to precede the positive one, and also the positive potential came to be followed by some slow deflections (Fig. 20, A'). And the response to intense stimulus light decreased the rising rate of the positive potential and it elicited few crests on the rising phase of the positive deflection (Fig. 20, A'-60).

In the case of stimulation of long duration the response of the light-adapted retina showed remarkable off-effects (Fig. 20, B'); the off-effect was large positive potential followed by the slow negative potential, and also the positive potential was divided into two positive potentials by the stimulus with intense light. In this case on-response was the negative potential and the positive on-potential was suppressed.

*Urolophus fuscus* The wave-forms of the ERG seem to be essentially the same as those of *Holorhinus* and *Narke*. Typical forms of the ERG are shown in Fig. 21.

In the dark-adapted retina the response to stimulus flash with weak light was a positive monophasic wave-form (Fig. 21, A-3), though that to the extreme weak light was a slow positive-negative diphasic wave-form as shown in Fig. 22. With the light stimulus of moderate intensity a small negative potential preceded the positive potential and further, the latter was followed by a further slow positive potential (Fig. 21, A-2). With the increase in stimulus intensity, the amplitude of the negative potential augmented considerably. Thus the positive potential which occurred noticeably in the stimulation by weak light, came to be balanced by the increasing negative potential and became the hump appearing on the falling phase of the negative potential which occurred in the stimulation by intense light (Fig. 21, A-1 and Fig. 22). The response to long lasted stimulation by weak light was the positive potential followed by the negative potential and did not cause the off-effect (Fig. 21, B-3). And also the response to intense light was the remarkable negative potential followed by the slow positive potential alone (Fig. 21, B-1). In this

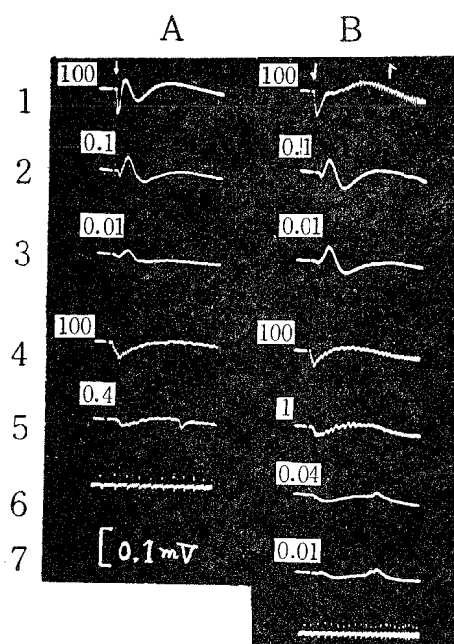


Fig. 21 ERGs recorded in *Urolophus*. 1—3, obtained from the dark-adapted retina; 4—7, from the light-adapted (3 lux). The stimulus duration is 1/100 sec. in A and 1 sec. in B. Other legends are same as in Fig. 15.

case it was noticed that many wave-lets occurred on the phase of the slow positive potential (Fig. 21, B-1).

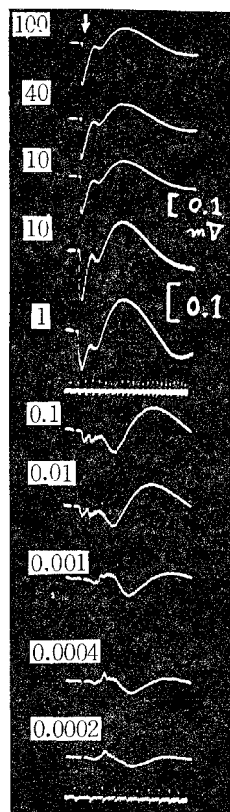


Fig. 22. Change in wave-form of the ERG in *Urolophus*, according to the variation in stimulus intensity. Test flash is 1/100 sec. Other legends are same as in Fig. 15.

in weak stimulus light is assumed to be suppressed with the application of ethylalcohol, and it may be considered to be different from the positive potential occurring in intense stimulus light.

*Platyrrhina sinensis* In the dark-adapted retina the response to stimulus flash with weak light (about 0.5 lux) was positive potential and the time-duration of the response was relatively long (Fig. 24, 0.1). With the increase in stimulus intensity, the amplitude of the positive potential decreased. With the further increase in stimulus intensity the rather fast negative potential occurred preceding the positive potential, which increased in its amplitude with the increase in the low range of stimulus intensity. The slow positive potential which occurred noticeably in the stimulation by weak light decreased with the increase in stimulus intensity and it almost disappeared in the stimulus of a unit intensity (500 lux).

In the retina light-adapted with about 3 lux illumination the positive potential which was remarkable in the dark-adapted retina, was suppressed. In the stimulation of long duration, the positive off-potential occurred in the response to weak light (Fig. 21, B- 6, 7), and the response to intense light seemed to be the same as that obtained in the short flash, except for the remarkable wave-let which appeared on the rising phase of the slow positive potential (Fig. 21, B- 4, 5).

The change in wave-form of the ERGs of the dark-adapted retina according to the various stimulus intensity is shown in Fig. 22. The records indicate that the positive potential which is remarkable in the response to weak light is clearly found to be canceled by the large negative potential followed in the stimulation by intense light.

As shown in Fig. 23, the negative potential of the response soon disappeared by the application of 10 % ethylalcohol on the inner surface of the retina and then only positive potential remained. The positive potential remained did not change in the long lasting stimulation of the same intensity (Fig. 23, 3). With the decrease in stimulus intensity, the positive potential disappeared and any other positive potential was not found in the stimulation by weak light (Fig. 23, A- 4-6, B). From these results, the positive potential evoked remarkably



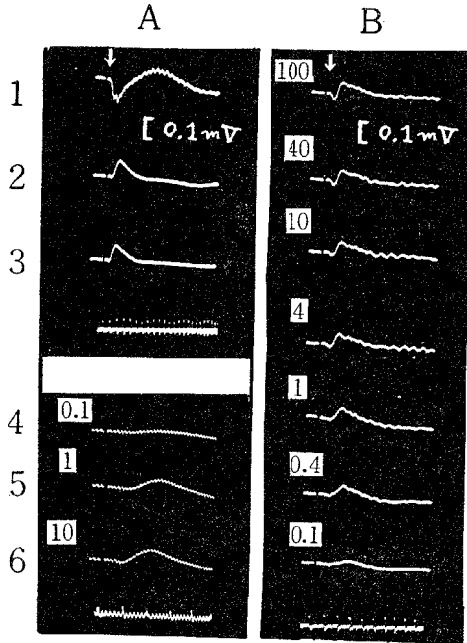


Fig. 23. Effect of application of ethylalcohol sol. on ERG of *Urolophus*. Test flash is 10 lux and 1/100 sec. in duration. A : 1, before the application; 2, 2 minutes after the application; 3, duration of stimulus light is prolonged to 1 sec.; 4, 5, 6, the response of the remained positive potential according to variation in stimulus intensity. B : Change in the remained positive potential according to light stimuli of various intensity. Other legends are same as in Fig. 15.

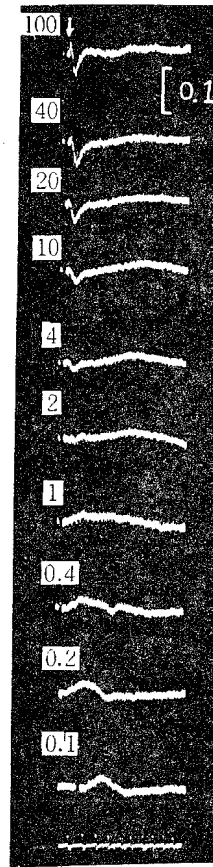


Fig. 24. ERGs recorded in *Platyrhina*. Change in wave-form of the ERG according to the variation in stimulus intensity. Test flash is 1/50 sec. Other legends are same as in Fig. 15.

ERG for the stimulation of long duration is shown in Fig. 25. In the weak light stimulation (about 1 lux), the retina evoked dominantly the positive potential regardless of the duration of stimulus (Fig. 25, A). The positive potential tended to be followed by the slow negative potential, and some small crests were found on the falling phase of the positive potential. With the prolongation of stimulus duration the initial positive crest became noticeable (Fig. 25, A- 4-7) and further, the crest seemed to change into slow positive plateau potential. Thus, in the stimulation of long duration, the response was the fast positive potential followed by the slow positive plateau potential. The amplitude of the fast positive potential was almost the same irrespective of the duration of the stimulus, but the time-course of the slow plateau potential was prolonged with the increase in stimulus duration (Fig. 25, A- 1-4).

On the other hand, the response to the stimulus with intense light was always initiated with the negative potential (Fig. 25, B), the amplitude of which was almost

the same regardless of the stimulus duration, except in the case of stimulation of extremely short duration. The off-effect did not occur even in the stimulation of long duration of one second. From the records shown in Fig. 25, it may be assumed that the magnitude of the response is determined by the stimulus intensity in the main, and the positive potential is scotopic and the negative potential photopic in character.

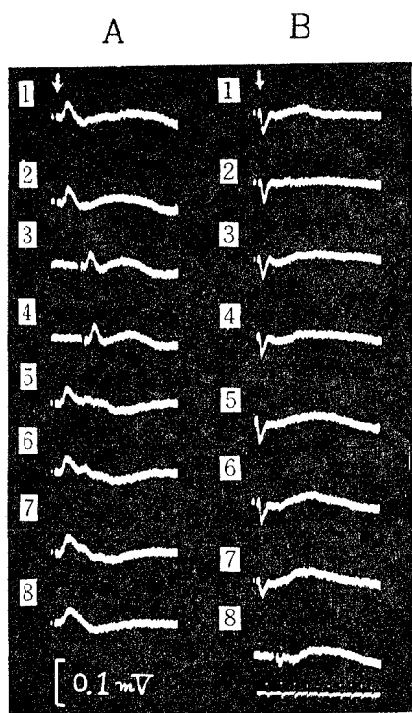


Fig. 25. Influence of stimulus duration with a constant intensity on the ERG of *Platyrrhina*. The stimulus intensity is 0.2% of unit intensity in A and 20% in B, respectively. The durations of stimulus in 1-8 are 1, 1/2, 1/5, 1/10, 1/25, 1/50, 1/100 and 1/300 sec., respectively.

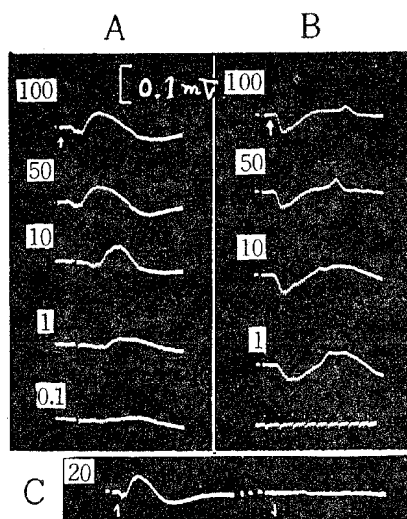


Fig. 26. ERGs recorded in *Raja*. A, obtained from the dark-adapted retina; B, from the light-adapted. Stimulus duration is 1/50 sec., except for the record of C, in which it is about 5 sec. Other legends are same as in Fig. 15.

*Raja porosa* In the dark-adapted retina, the response to stimulus flash with weak light was also a slow positive monophasic potential, having rather long time-course (Fig. 26). With the increase in stimulus intensity, the amplitude of the positive potential augmented, and a small negative potential came to precede the positive one. In the stimulus light over 50 lux, however, the amplitude of the positive potential remained in constant height, although the magnitude of the negative potential and the latency of the response still changed obviously (Fig. 26, A). In the case of stimulation of long duration (about 5 seconds) on-response was the same with that of the flash stimulus, and any off-response did not occur even by the intense light stimulus (Fig. 26, C).

In the light-adapted retina (Fig. 26, B), the response to stimulus flash with weak light was the negative potential followed by the positive potential. With the increase in stimulus intensity, the falling rate of the negative potential came to be larger, and the positive potential was suppressed. In the stimulation with intense light, the large slow positive potential changed to a small fast one.

## (2) Relation between the magnitude of the response and the stimulus intensity

In *Narke*, the relation between the amplitude of the slow response and the intensity of stimulus light (A—I relation) was investigated in the dark-adapted retina. The amplitudes of the positive component (filled circles in Fig. 27) and negative one (open circles in Fig. 27) were measured, respectively. The result is shown in Fig. 27, in which the abscissa represents the stimulus intensity (percentage of unit intensity) in logarithmic scale, and the ordinate represents the height of each amplitude in the per cent of the maximum one. From the diagram, it is understood that the amplitude of the positive potential, height of b-wave, increases in linear relation to the stimulus intensity, but the increase attains the steady state in relatively low stimulus intensity. And also the amplitude of the negative potential, a-wave, changes noticeably with the increase in stimulus intensity, but the amplitude does not attain the steady state within the stimulus intensity used in the present experiment.

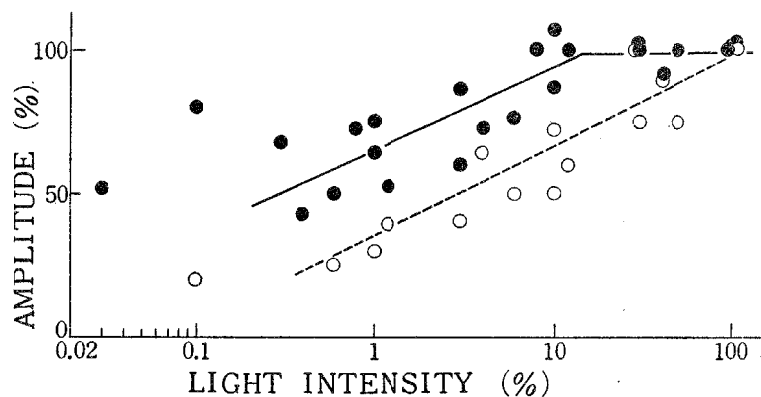


Fig. 27. Relation between the amplitude height and the stimulus intensity in ERGs of *Narke*. Abscissa, the stimulus intensity graded in log scale; ordinate, the height of the amplitude against the maximum one. Filled circles, height of b-wave; open circles, of a-wave. Test flash is 1/100 sec. in duration.

In *Holorhinus*, the A—I relation is also linear over the wide range of the stimulus intensity as is shown in Fig. 28, and the amplitude height does not attain the final steady state within the range of the intensity used in the present experiment, being different from the relation in other species of Batoidei. Moreover, it was found in the same figure that the increasing rate of the positive potential (b-wave) investigated in complete dark-adaptation (filled circles in Fig. 28), was smaller than that

investigated in the semi-dark-adaptation following the preliminary faint illumination (semi-filled circles, in Fig. 28., and *cf.* p. 110 in Chapter IV).

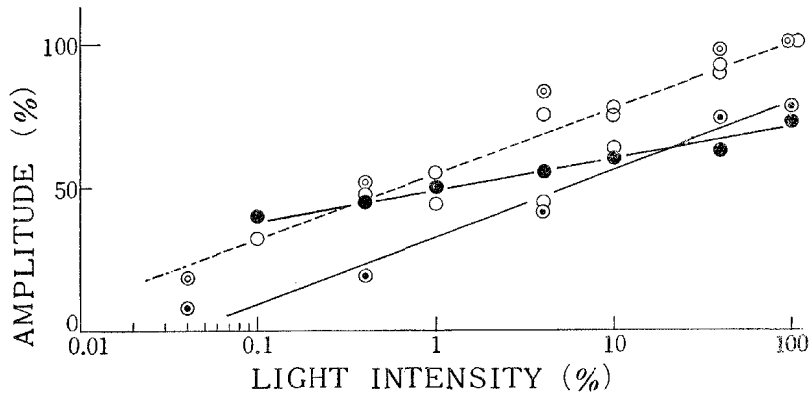


Fig. 28. Relation between the amplitude height and the stimulus intensity, in ERGs of *Holorhinus*. Filled and partially filled circles, height of b-wave; open and double circles, of a-wave. Other legends are same as in Fig. 27.

In *Dasyatis*, the same relation as mentioned above was obtained in the fresh and dark-adapted retina. The result is shown in Fig. 29. The change in the negative potential (open circles) was more remarkable than that of the positive one, and it attained the steady state at 50 lux. On the contrary, the amplitude of the positive potential (filled circles) augmented very slowly with the increase in stimulus intensity and it attained a final steady state at the stimulus light of about 20 lux.

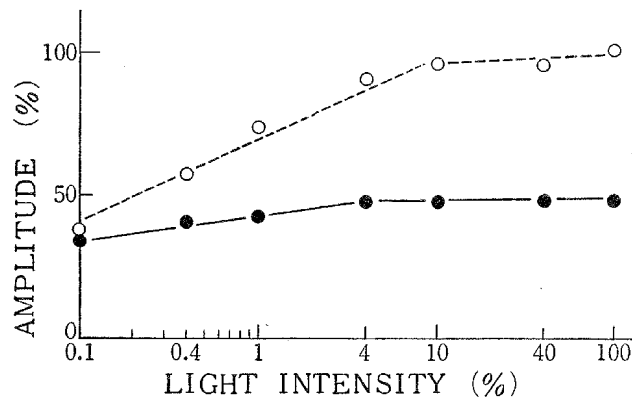


Fig. 29. Relation between the amplitude height and the stimulus intensity, in ERGs of *Dasyatis*. Other legends are same as in Fig. 27.

In *Urolophus*, the A—I relation is shown in Fig. 30. Though some individual varieties were observed, the amplitude of the negative potential increased in linear relation, and that of the slow positive one increased also but it attained a steady state in relatively low stimulus intensity, for example, in about one lux in the present experiment. The amplitude of the potential that occurred noticeably in the stimulus with weak light, tended to increase also by a linear relation, too (dotted-broken line in Fig. 30).

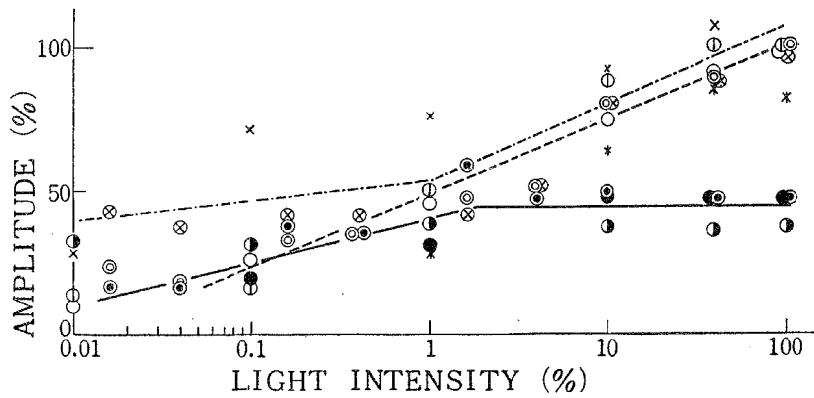


Fig. 30. Relation between the amplitude height and the stimulus intensity, in ERGs of *Urolophus*. ●, ⊙, ⊕, the height of slow positive potential (b-wave); ○, ⊖, ⊗, the height of negative potential (a-wave); ×, ⊗, \*, the magnitude measured from the tip of initial negative potential to base of the notch on the rising phase of positive potential. Test flash is 1/100 sec. In abscissa, stimulus intensity in % of unit intensity (110 lux).

In *Raja*, the A—I relation is also found to be similar to that of other batoid fish, as is shown in Fig. 31. The amplitude of the positive potential attained the final steady state in relatively low intensity, for example, in the light intensity of about 5 lux. However, the amplitude of the negative potential increased in linear relation to logarithm of the stimulus light, and also the latent period of the potential seemed to shorten in the range of such stimulus intensity as that in which the amplitude of positive potential increased no more with the increase in stimulus intensity.

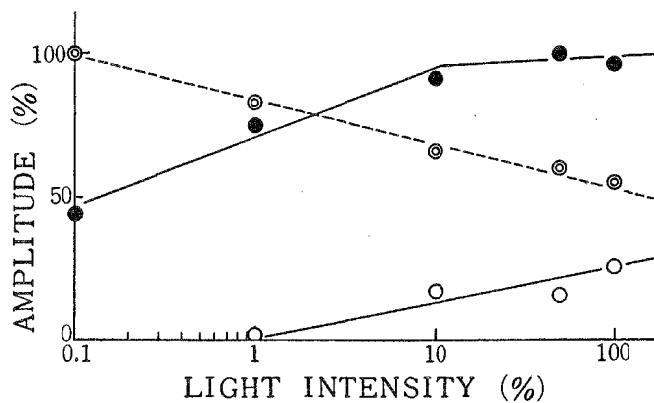


Fig. 31. Relations between the amplitude height, the latency of the response and the stimulus intensity, in ERGs of *Raja*. Double circles, latency of the response. Other legends are same as in Fig. 27.

### (3) Response to colour light and spectral sensitivity of retina

*Narke japonica* As the retina was exposed to equi-energy monochromatic light of various wave-lengths, latent period and wave-form of the response changed in accordance with the wave-length (Fig. 32). Monochromatic light was obtained with Erma's monochromatic filter and light energy was controlled with neutral filter, although the energy of the light for wave-lengths of 420 m $\mu$  and 700 m $\mu$  was not controlled in this case. The change in wave-form was similar to that caused by the different stimulus intensity (Figs. 15 and 16). These changes in the response caused by the monochromatic light are assumed to be due to the spectral sensitivity of the retina.

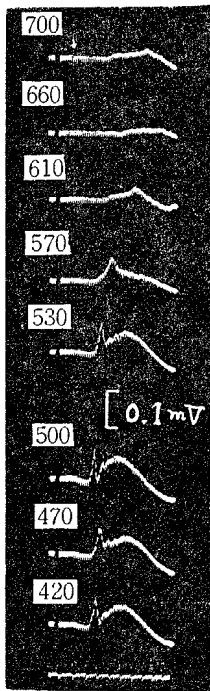


Fig. 32. ERGs of *Narke*, elicited by the stimulus light of various wave-lengths. The intensity of each flash is equi-energy in the stimulus light of the wave-length except 420 m $\mu$  and 700 m $\mu$ . Test flash is 1/50 sec. Time mark: 1/6 sec.

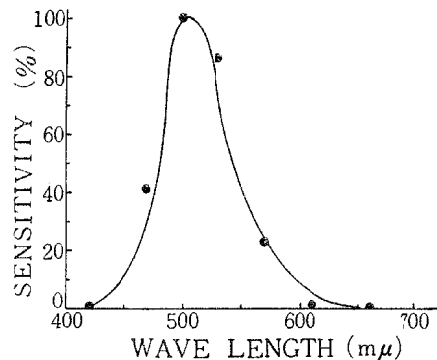


Fig. 33. Spectral sensitivity curve of *Narke*, in the dark-adapted retina. The sensitivity is represented as per cent of the maximum value.

The spectral sensitivity curve of *Narke* is shown in Fig. 33. The spectral sensitivity was represented as the reciprocal of minimum light intensity required to cause the response in the stimulation of monochromatic light. The curve has the maximum at 500 m $\mu$  of the spectrum, and falls away steeply on both sides of the spectrum. The retina is almost insensitive to the light of the wave-length longer than 650 m $\mu$ . The shift of the maximum of spectral sensitivity by light adaptation could scarcely be found.

*Holorhinus tobijeji* As was previously stated, with the decrease in stimulus intensity the retinal response to the illumination lost the initial negative potential at first, and with the further decrease in the intensity the positive potential disappeared finally (Figs. 18 and 19). Therefore, the spectral sensitivity of negative and positive

components in the ERG was investigated, respectively. Both scotopic spectral sensitivity curves mentioned above are shown in Fig. 34, A. Although the sensitivity of the positive component (filled circles in Fig. 34, A) was higher than that of the negative one generally (open circles in Fig. 34, A), both components were almost insensitive to the light of the wave-length longer than 650  $m\mu$ . The spectral sensitivity curve of the positive component had the maximum at 500  $m\mu$ . The sensitivity curve of the negative component showed the maximum at 525  $m\mu$  and seemed to have a hump at 575  $m\mu$ .

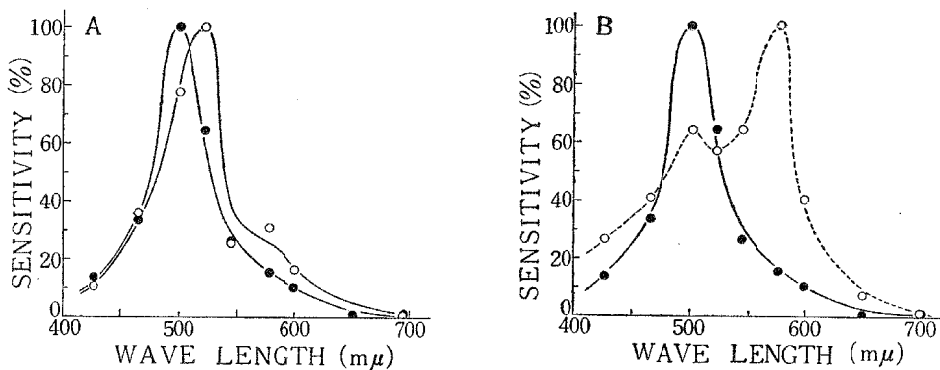


Fig. 34. Spectral sensitivity curve of *Holorhinus* retina. A, the sensitivity curve of the dark-adapted retina, investigated by positive (filled circle) and negative (open circle) components as indices, respectively. B, effect of light-adaptation on the sensitivity curve investigated by positive component as index. Filled circle, in dark-adaptation; open circle, in light-adaptation.

When the retina is exposed to the light of about 3 lux, the positive potential is dominant in the threshold response (Fig. 18, A-6). Therefore, the spectral sensitivity in the light-adaptation was investigated with the positive potential as index (open circles and broken line in Fig. 34, B). Such photopic spectral sensitivity curve shifted the maximum to 575  $m\mu$  of the spectrum and had the submaximum at 500  $m\mu$ , and further the sensitivity to the light of longer wave-length was higher than that of scotopic one.

It is moreover noticed that the wave-length of the maximum in the photopic curve well corresponds to that of the hump in scotopic curve of the negative component, and that of the submaximum corresponds also to that of the maximum in scotopic curve of the positive component. Therefore, it may be assumed that the negative potential in the response of the dark-adapted retina shows a photopic character and the positive potential shows a scotopic character. It may be also inferred from the results mentioned above that the retina of *Holorhinus* retina has two visual pigments.

*Dasyatis akajei* As shown in Fig. 35, the response showed different wave-forms for different wave-lengths of the stimulus light. It may be assumed from the record that various kinds of component are involved in the response and each component is dominant for a certain definite wave-length of the illumination. But the

components could not be analyzed from the present experiment. It is necessary to analyze them by the aid of a micro-electrode, and the experiment will be made further on.

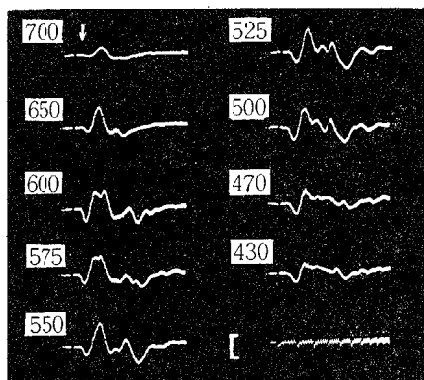


Fig. 35. ERGs of *Dasyatis*, elicited by the stimulus light of various wave-lengths. Other legends are same as in Fig. 32.

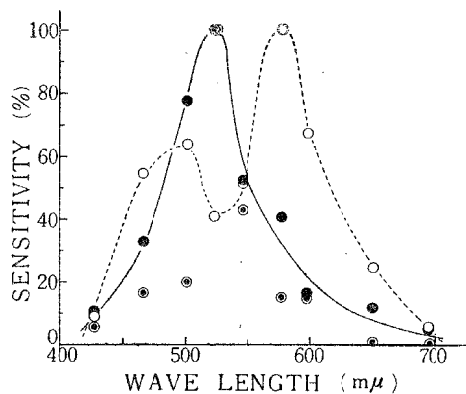


Fig. 36. Spectral sensitivity curve of *Dasyatis* retina. Solid line, scotopic; broken line, photopic; partially filled circles, the sensitivity determined in completely dark-adapted retina; filled circles in semi-dark-adapted; open circles, in light-adaptation of 3 lux.

Since it can be assumed from the former experiment that the various components involved in the response have different spectral sensitivities, it is necessary first to investigate the sensitivity of the whole retina. Using the ERG as index, the scotopic sensitivity curves were obtained from both complete dark- and semi-dark-adapted retinæ (partially-filled and filled circles in Fig. 36), and the photopic sensitivity curve was obtained under the illumination of 5 lux (open circles in Fig. 36). The scotopic spectral sensitivity curve had the maximum at the blue-green, being close upon 525  $m\mu$  of the spectrum, and fell away steeply toward both sides of the spectrum. The steepness of the curve was remarkable in that obtained from the complete dark-adapted retina. According to the sharpness of the curve, the sensitivity was about 1–5% of the maximum at the wave-length of 430  $m\mu$  and at 650  $m\mu$  of the spectrum, respectively. In the photopic sensitivity curve the maximum shifted to 575  $m\mu$  of the spectrum, and a submaximum appeared in 500  $m\mu$ . And also the curve fell away comparatively slowly toward the short end of the spectrum as compared with the scotopic one. It was 6% of the maximum at 430  $m\mu$  and 4% at 700  $m\mu$ . Since it is supposed that the component showing the submaximum of the photopic sensitivity curve will probably correspond to that having the maximum shown in the scotopic sensitivity curve, the duplicity theory may be applied to the retina of *Dasyatis*, too.

*Urolophus fuscus* The spectral sensitivity curves of the retina are shown in Fig. 37. As was the case with the retina of *Holorhinus*, the spectral sensitivity of negative and positive component in the ERG was investigated, respectively (Fig. 37, A). Both curves peaked sharply at 500  $m\mu$  of the spectrum and the curves rapidly fell toward both sides of the spectrum, and the retina was almost insensitive to the



light of the wave-length longer than  $650\text{ m}\mu$ .

The photopic spectral sensitivity curve of the retina light-adapted with 3 lux light, shifted the maximum around  $525\text{ m}\mu$  of the spectrum (Fig. 37, B), and the sensitivity for the light of longer wave-lengths increased greater than that of scotopic one. The response in this species caused a negative potential with the decrease in stimulus intensity, under the light-adaptation, and therefore the curve measured with the negative potential in dark-adaptation was used for the comparison with the scotopic one. From the above findings it seems that the "Shift of Purkinje" exists slightly in the retina of *Urolophus* species.

As the vitreous humor of the retina was imperfectly removed, the response was dominant in negative potential, and positive potential could hardly be found. Using such negative potential as index, the spectral sensitivity of the retina was investigated (Fig. 37, C). In both of them the spectral sensitivity curve hardly changed with the adaptation and the curve had the maximum in  $525\text{ m}\mu$ . This result agreed well with that of the photopic curve in Fig. 37, B. Therefore, the negative component is assumed to represent the photopic character.

*Raja porosa* As it is shown in Fig. 38, the negative potential elicited in the dark-adapted retina does not suffer any remarkable influence by different wave-lengths of the stimulus light, but the amplitude of positive potential changes with

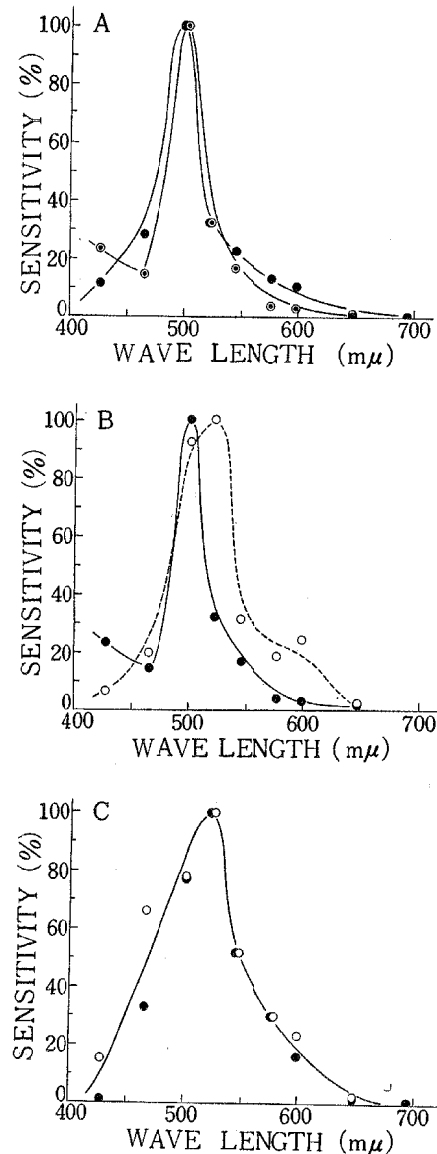


Fig. 37. Spectral sensitivity curve of *Urolophus* retina. A, scotopic spectral sensitivity curve: partially filled circles, the sensitivity determined by negative component of the ERG; filled circles, by positive component. B, effect of light-adaptation on the sensitivity curve: broken line and open circles, photopic curve determined by negative component; solid line and filled circles, scotopic curve, by negative component. C, the sensitivity curve investigated in negative monophasic response. Legends are same as in B.

the wave-lengths, and it shows the maximum height at 500 m $\mu$ , and the retina is almost insensitive to the light of the wave-length longer than 600 m $\mu$  (Fig. 38, B). In the light-adapted retina the negative potential was elicited most distinctly with the stimulus light of 500 m $\mu$  (Fig. 38, A), and the splitting of the positive potential occurred in the wave-lengths of the range from 500 to 530 m $\mu$ , too. These records suggest the existence of some components specifically sensitive to a certain definite wave-length of the spectrum. However, the analysis of the components must be deferred to further experiments by the aid of a micro-electrode.

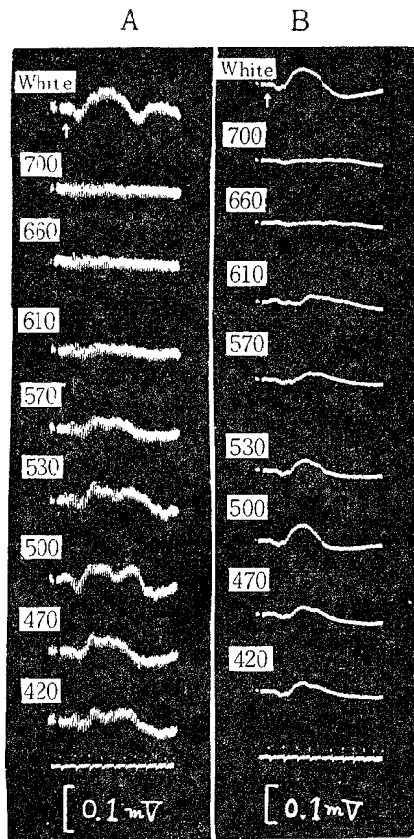


Fig. 38. ERGs of *Raja*, elicited by the stimulus light of various wave-lengths. A, recorded in the light-adaptation; B, in the dark-adaptation. Other legends are same as in Fig. 32.

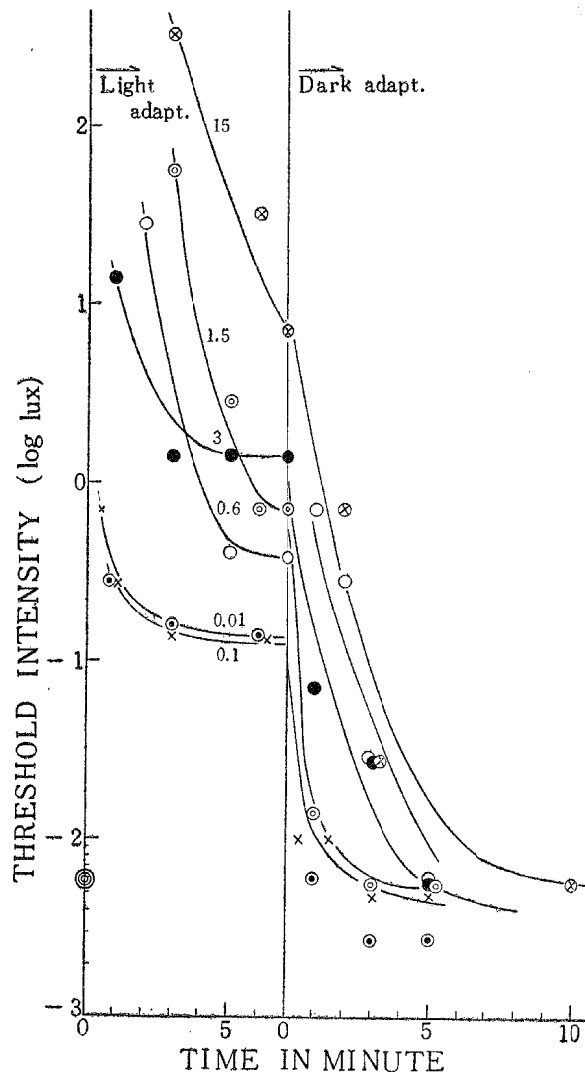


Fig. 39. Change in threshold intensity (lux) of stimulus light for eliciting ERG of *Narke*, in the course of light- and dark-adaptations. Numeral on each curve indicates the light intensity (lux) of adaptation light. Ordinate, log threshold intensity. Abscissa, time elapsed in the adaptation. Tricircles, the initial level of the threshold intensity.

#### (4) Light and dark adaptation

*Narke japonica* The change in sensitivity of the retina was investigated in the

course of light- and dark-adaptation, using the threshold intensity of the retina. The complete dark-adapted retina was at first exposed to the illumination with a certain level of the intensity, and the change in the threshold intensity of the retina was recorded in the course of the light-adaptation. After several minutes, the retina was removed to darkness and the change in threshold intensity of the retina was again recorded in the progress of the dark-adaptation. Such test was repeated in several levels of the intensity of adaptation light. As soon as the retina was exposed to the illumination, the threshold intensity of the retina rose rapidly, and of course, the stronger the illumination that was applied, the more remarkable was the rising of the threshold. However, the rapidly rising threshold which occurred transiently in the initial period of the light-adaptation, began to fall down within a few minutes after the beginning of the light-adaptation, till it attained a definite threshold intensity in several minutes later. The intensity of the adaptation light used in this experiment was the range from 0.01 to 15 lux, because the retina exposed to the illumination exceeding 15 lux did not recover the sensitivity in the following dark-adaptation. These results are shown in the left of Fig. 39.

The retina subjected to the preliminary light-adaptation for several minutes recovered the sensitivity rapidly in darkness, and the change in the sensitivity in the course of dark-adaptation proved to be a simple process, and was completed in 3–7 minutes. Although the recovery from the weak light-adaptation was more rapid, the dark-adaptation was established comparatively fast as compared with that of the human eye. Thus, it was difficult to find any sign of a break, or sudden change in the slope of the dark-adaptation curve. These results are shown in the right of Fig. 39.

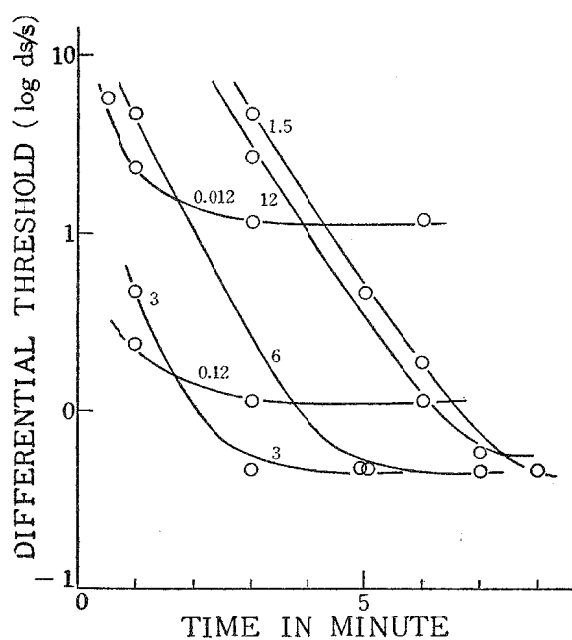


Fig. 40. Change in differential threshold of stimulus light for eliciting ERG of *Narke*, in the course of light-adaptation. Numeral on each curve shows the light intensity (lux) of adaptation light.

The increase in sensitivity was small as compared with the range of dark-adaptation of the human eye, being only about 3 log unit.

In the retina of *Narke* the differential threshold,  $\log ds/s$ , was plotted with the lapse of time in the light-adaptation, in which the intensity of seven levels was used for adaptation light (Fig. 40). It is found from the figure that the values of the differential threshold in each level are all large in the initial stage of the adaptation regardless of the strength of the adaptation light, but the value attains a constant level in a few minutes after the beginning of the adaptation. And the constant level settles to the same extent in the case of the moderate intensity of adaptation light, for example, at about 0.1 to 10 lux. The relation mentioned above was also clearly shown in Fig. 41, in which the value of  $ds/s$  was plotted to the logarithm of the intensity of adaptation light. The relation seems to be confirmed closely to the Weber-Fächner Law,  $\log ds/s = \text{constant}$  at moderate intensity of the adaptation light.

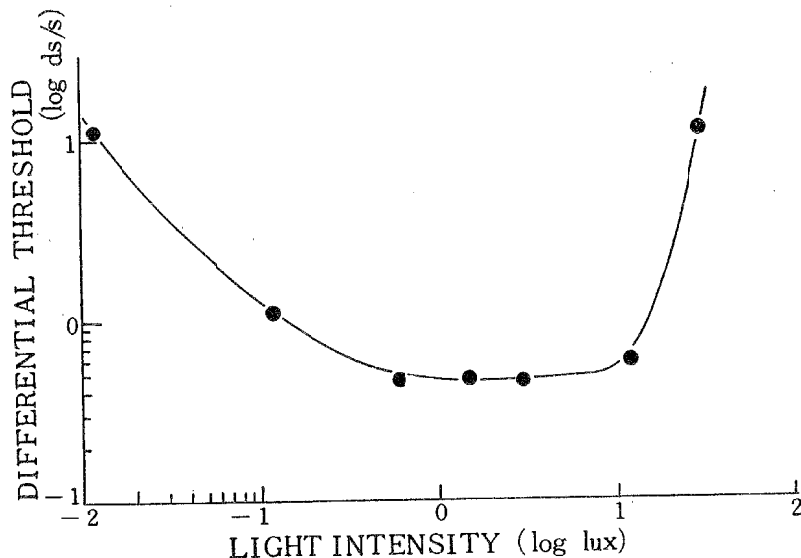


Fig. 41. Relation between the differential threshold ( $ds/s$ ) and light intensity of adaptation light, in the retina of *Narke*. Ordinate,  $\log ds/s$ ; abscissa, light intensity in log lux.

*Holorhinus tobijeii* The results obtained in the retina of *Holorhinus* are shown in Figs. 42, 43 and 44. The investigation was made in the same manner as in the case of *Narke*, but the light intensity of the range from 0.6 to 12 lux was applied to the adaptation light in this case. Although the result was similar to that of *Narke*, the changing range of the sensitivity in light-adaptation was small as compared with those in other species of Batoidei, being about 2 log units of intensity (Fig. 42). And also the differential threshold was found to be almost constant when the illumination of moderate intensity was applied as the adaptation light, for example, in the range from 10 lux to 1 lux, which was small as compared with that of other species. In this case the value of  $ds/s$  was 0.006 (Figs. 43 and 44).

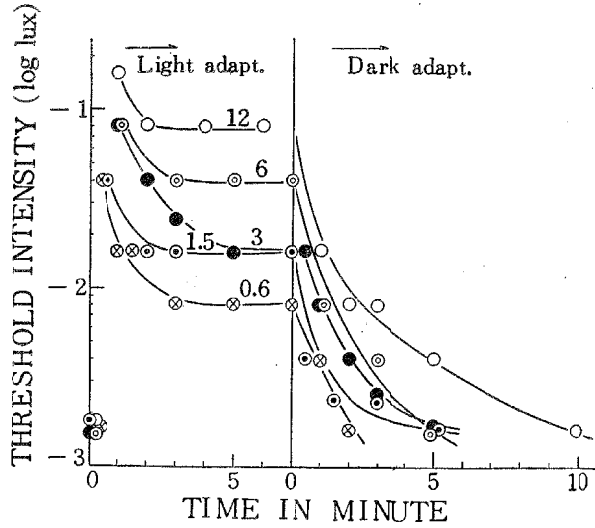


Fig. 42. Change in threshold intensity of stimulus light for eliciting ERG of *Holorhinus*, in the course of the light- and dark-adaptation. Other legends are same as in Fig. 39.

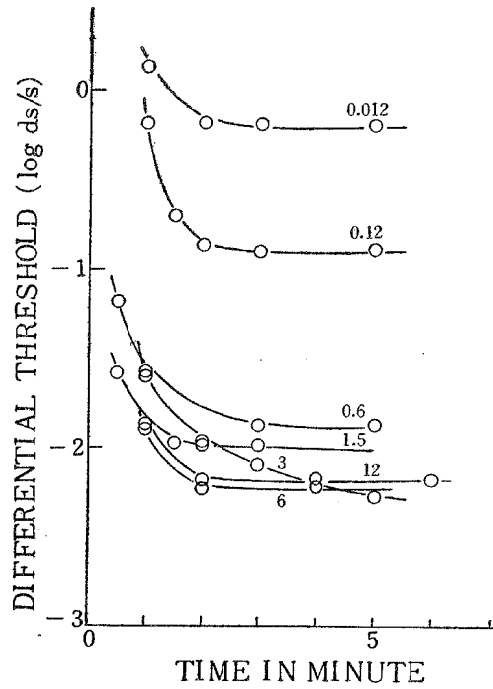


Fig. 43. Change in differential threshold of stimulus light for eliciting ERG of *Holorhinus*, in the course of the light-adaptation. Other legends are same as in Fig. 40.

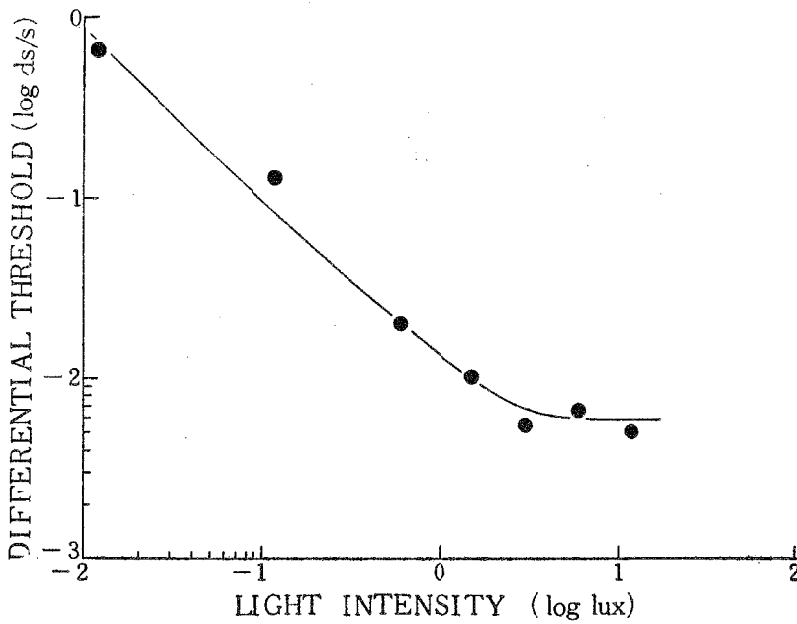


Fig. 44. Relation between the differential threshold ( $ds/s$ ) and the light intensity of adaptation light, in *Holorhinus*. Other legends are same as in Fig. 41.

*Dasyatis akajei* The process of the adaptation was investigated from the change in wave-form. The results are shown in the records of Fig. 45. The record of Fig.

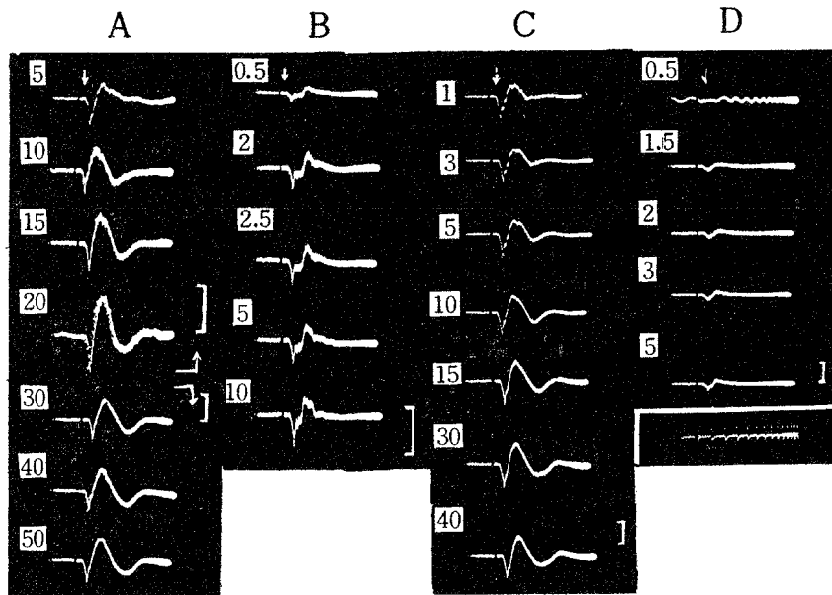


Fig. 45. Change in wave-form of the ERG of *Dasyatis*, in the course of the dark- and light-adaptations. Test flash is 10 lux and 1/50 sec. in duration. A, ERG recorded in the course of the dark-adaptation after the light-adaptation of 200 lux. B, recorded in the course of light-adaptation of about 3 lux. C, recorded in the course of dark-adaptation after the B-records. D, recorded in the course of light-adaptation of 25 lux. Numeral on each record represents the time in minute after the beginning of the adaptation, respectively. Time mark: 1/6 sec. Calibrate voltage: 0.1 mV.

45, A is the course of dark-adaptation following the preliminary light-adaptation with the light intensity of about 200 lux, in which the preparation of the retina was made. With the progress of the dark-adaptation, the increase in amplitude and the prolongation in time duration of the response were found and those attained a steady state in 20 minutes later, although the falling rate of the positive potential increased, and the positive potential was followed by the slow negative potential, within several minutes. The record of Fig. 45, B is the change in response caused by the light-adaptation to 4 lux. In the initial stage the magnitude of the response decreased rapidly and thereafter it again increased, and thus the light-adaptation was completed in 2 to 2.5 minutes. The dark-adaptation following the preliminary light-adaptation in the records of Fig. 45, B was completed in 15 minutes, and the change in wave-form was the same as that of the records of Fig. 45, A (Fig. 45, C). When the retina was again exposed to the light of about 25 lux (Fig. 45, D), the response was not obtained in the initial stage of the adaptation on account of the rapid decrease of the sensitivity. However, the oscillatory potential was found in that stage. After 1.5 minutes the oscillatory potential disappeared and the response to the test flash was a small negative potential followed by a positive potential. And the response settled to

the steady state in 3 to 5 minutes. The records mentioned seemed not to represent the process of the adaptation plainly, because the amplitude of the response attained the maximum rapidly.

On the other hand the sensitivity of the retina in the course of the adaptation was investigated using the threshold intensity. The results are shown in Fig. 46. At first, in this experiment the complete dark-adapted retina was illuminated with extremely weak light (about 0.03 lux). In a few minutes after the beginning of the adaptation, the threshold of the retina rose to the level of about 100 times higher; however, it decreased upon the level of about 20 times of the initial stage in five minutes later, when the differential threshold,  $ds/s$ , was 0.66. Thereafter the retina

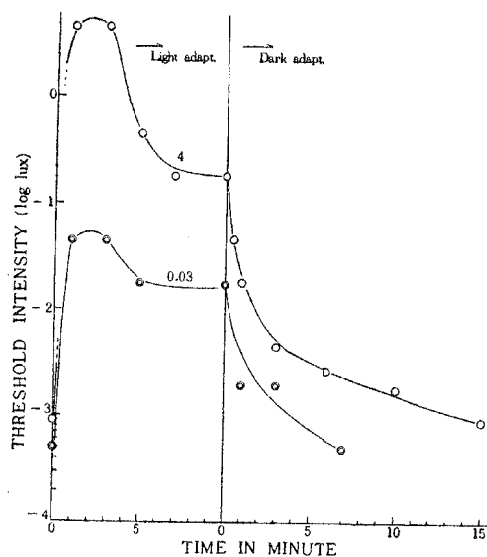


Fig. 46. Change in threshold intensity of stimulus light for eliciting ERG of *Dasyatis*, in the course of the light- and dark-adaptations. Other legends are same as in Fig. 39.

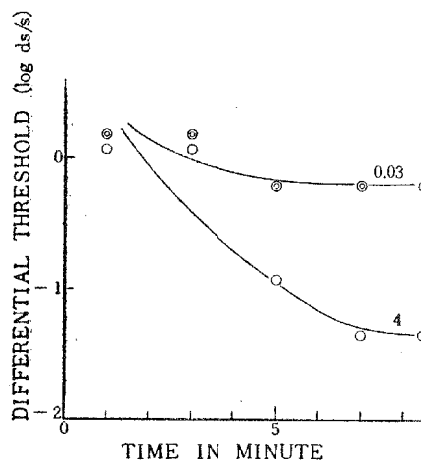


Fig. 47. Change in differential threshold of stimulus light for eliciting ERG of *Dasyatis*, in the course of the light-adaptation. Other legends are same as in Fig. 40.

was returned to darkness, and the change in the threshold was again recorded with the lapse of time until the initial threshold was obtained. The course of dark-adaptation following the such preliminary light-adaptation for 10 minutes showed a simple process and it was completed in 7 minutes after the cessation of the illumination.

Next, the same retina was again exposed to the illumination of about 4 lux, and then the course of the light-adaptation was completed in about 7 minutes, and in this case the differential threshold,  $ds/s$ , was 0.045. The dark-adaptation following the preliminary such light-adaptation, was completed in above 15 minutes. In Fig. 47, the change in the retinal sensitivity in the light-adaptation is represented with the differential threshold.

*Urolophus fuscus* The results of *Urolophus*, which is obtained in the same

manner as that of *Narke* or *Holorhinus*, are shown in Fig. 48. Although the result is similar to that of *Narke*, the rising and falling rates in the threshold intensity in the initial stage of the light-adaptation are larger than those of *Holorhinus*, and the decrease in sensitivity caused by the light-adaptation extends to a wider range, being 3–4 log units, changing with the intensity of the adaptation.

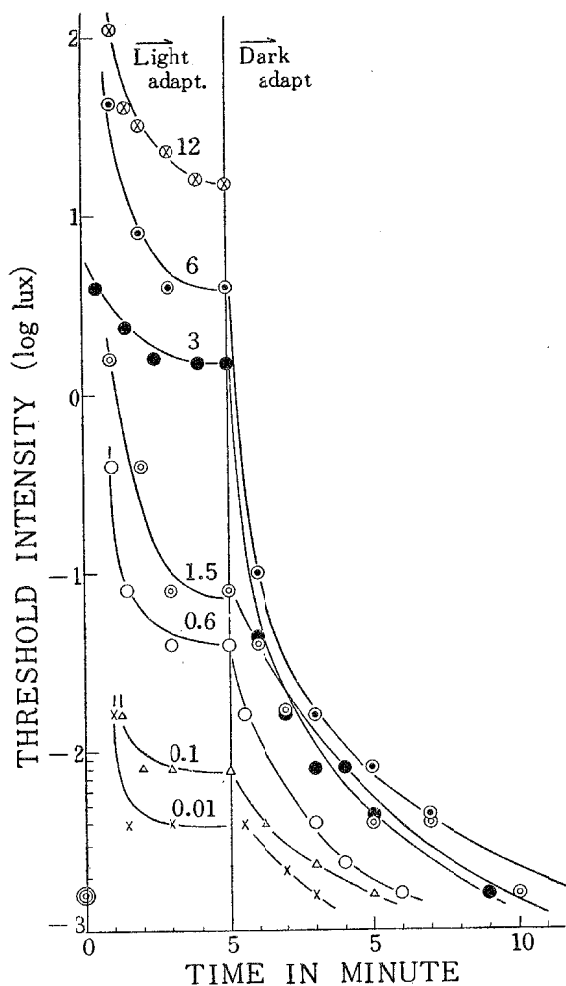


Fig. 48. Change in threshold intensity of stimulus light eliciting ERG of *Urolophus*, in the course of the light- and the following dark-adaptation. Other legends are same as in Fig. 39.

The dark-adaptation of the retina after the preliminary illumination for several minutes shows a simple process. Dark-adaptation is completed in about 5 to 10 minutes in the present experiment.

Moreover the differential threshold,  $ds/s$ , was computed in the experimental results of the light-adaptation and it was plotted to the intensity of adaptation light (Fig. 49). The value of the differential threshold was about 0.07 and it was kept nearly constant in the range between 1 and 0.1 lux of the adaptation light.



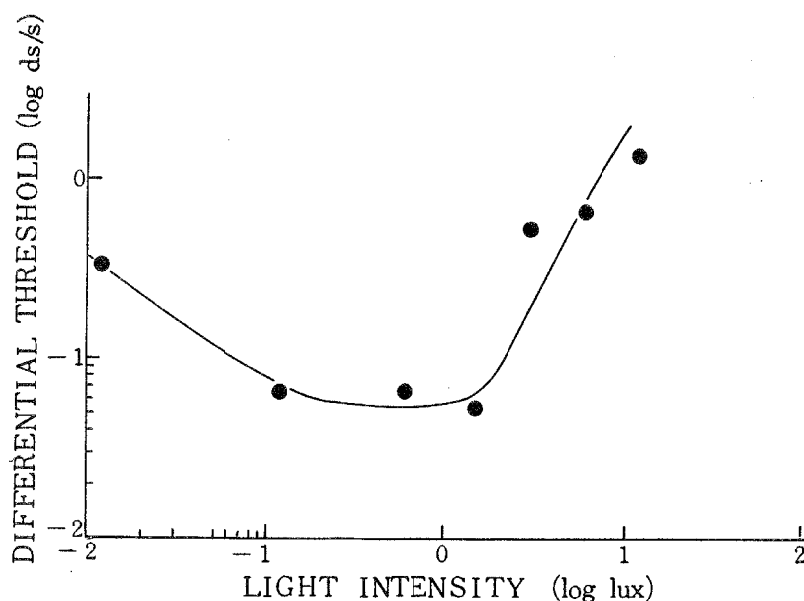


Fig. 49. Relation between the differential threshold ( $ds/s$ ) and the light intensity of adaptation light in the retina of *Urolophus*. Other legends are same as in Fig. 41.

#### (5) Response to flicker stimulation

When the flicker stimulation with equal durations of light and dark phase were given on the eye of *Narke*, the response differed much according to intensity of the flicker light: In the intensity of about 0.015 lux, the response was a succession of small positive potential corresponding to the flicker light, and in moderate intensity of the light the response showed initially the large diphasic deflections (negative-positive) and then about 10 sec. later, it began to elicit a succession of ripples. The ripple consisted of both positive and negative deflections and had a small notch on the tip of the positive deflection. The ripple showed the maximum amplitude in the flicker light of about 1.5 lux. In the flicker light of about 9 lux the initial response showed the largest deflection and about 20 sec. later, it began to cause a succession of ripple, of which the amplitude was rather lower and the notch disappeared. In the flicker light above 15 lux the response was the initial deflections only and did not cause any following ripples even in the flicker light of any frequency and any intensity. Of course, the fusion of the ripple occurred with the increase in flicker

Table 2. Flicker fusion frequency determined in the retina of *Narke japonica*. Explanation in text.

Intensity (lux)	0.015	0.15	1.5	9	15
Fusion frequency (c/s)	4	4	6	3	no flicker response

frequency. The relation between the frequency of the fusion and the intensity of the flicker light is shown in Table 2.

Thus, it becomes obvious that the retina of *Narke* responds only to the flicker light of low intensity and have relatively low flicker fusion frequency.

The flicker response of *Urolophus*' retina was investigated in the same manner as that used in *Narke*, and the result was similar to that of *Narke*. Part of the result is shown in the records of Fig. 50, in which the flicker stimulations of the

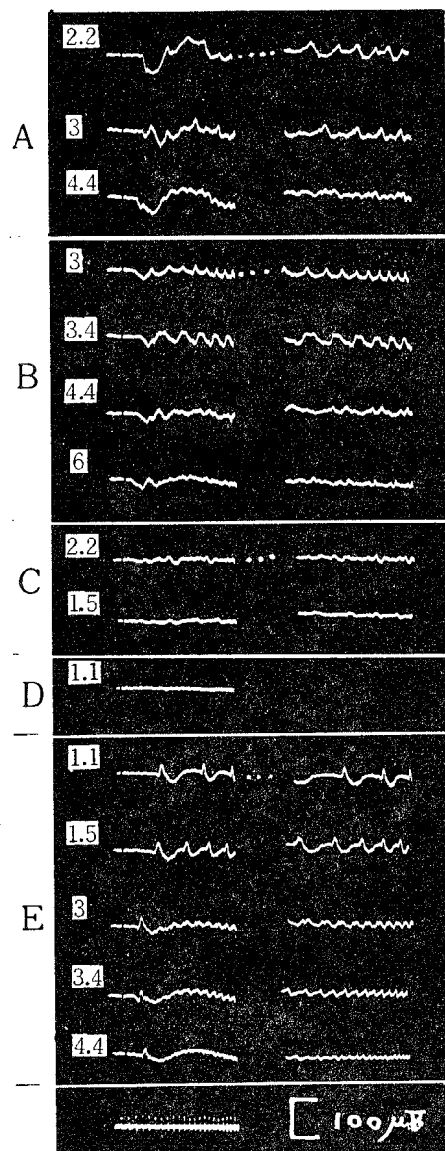


Fig. 50. The response to flicker stimulus light with equal duration of light and dark phase, in the retina of *Urolophus*. Numeral on each record indicates frequency of the flicker light (c/s). Intensity of the flicker light in A, B, C and D are 100, 10, 1 and 0.1 lux, respectively. In E-group the duration of the light-phase is decreased to about 1/10 of the dark-phase, though the intensity of the light source is the same with that of D. Time mark : 1/6 sec.

various frequency with different intensity were used. As the flicker response reveals the complicated wave-form, the details will be described elsewhere. In brief the response seems from the Fig. 50 to be a succession of the positive potentials and the fusion of the response is apt to occur in the frequency with 4.4 to 6 c/s, varying with the stimulus intensity. Moreover, it is noticed that the retina seems to elicit both on- and off-responses in each flash of the flicker light with low intensity, whereas it seems to cause the response attributing to either on- or off- effect in that of high intensity. Consequently the fusion frequency tends to rather increase in the low intensity of the flicker light. It is necessary in future to investigate in detail the change in the wave-form in relation to the intensity and the frequency of the flicker light.

The retina did not respond to the flicker stimulus light with the intensity of about 0.5 lux, and it was assumed that the refractory period of the preceding response was long. Then the duration of "light" in the flicker stimulation was shortened for that of "darkness", namely the flash with the duration of about 10 msec. was repetitively projected. In this case, the retina responded remarkably to the flicker light as shown in Fig. 50, E; however, the fusion frequency of the response was found in about 6 c/s. From the fact it may be assumed that the flicker response in this species occurs noticeably in the low intensity of the flicker light, and one of the factors eliciting the flicker response would be related to the duration of the flash in the flicker stimulation.

### Discussion

Generally the wave-form of the ERG in Batoidei changes markedly in accordance with the change in the intensity of stimulus light. In the low stimulus intensity slow positive monophasic wave was observed distinctly in all species except for *Dasyatis*, and with the further lowering of the intensity the potential was followed by slow negative potential, as may be seen in the examples given in Figs. 16 and 22. The positive potential obtained in the extreme weak stimulus showed a latent period of 70 to 100 msec. and it seemed to be corresponding to the rod potential that was obtained by SVAETICHIN ('53<sub>b</sub>) with the aid of a micro-electrode in the enough dark-adapted retina of *Perca*, with a very low stimulus intensity. And the fact that such potential is easily recorded without the aid of micro-electrode, may be due to the peculiar structure in the retina of elasmobranch fish that is known as rod retina or rod dominant retina (BAYLISS *et al.* '36, BRETT '57).

Though the amplitude of such rod-potential appears to augment with the increase in stimulus intensity, the potential is canceled by the negative potential increasing at the same time. Consequently it is assumed that the hump that is observed on the falling phase of the initial negative potential in the ERG obtained in the stimulus light of moderate intensity, is also related to rods. Such rod potential was most vividly observed in the retina of electric rays, *Narke*, as shown in Fig. 16. It is known that electric rays usually bury themselves in mud or sand at the bottom of

the sea and can swim only sluggishly (BIGELOW and SCHROEDER '53), and so the assumption mentioned above is also ecologically supported by their behaviour. Since such a hump was also observed on the rising phase of b-wave in the ERGs of hagfish, eel, plaice and others (KOBAYASHI '62, *cf.* Chapter IV), the occurrence of such potential may be characteristic of the ERG in the fishes living benthonic or nocturnal lives, in which rods play an important role.

The relation between the amplitude of the response and the logarithm of the stimulus intensity was found to be linear, and the graded response of the retina was also confirmed, as described by many investigators. However, it was found in the retina of these fishes that the amplitude height, particularly of the positive potential (b-wave), attained its final steady state in relatively low intensity of stimulus light. As pointed out by TAMURA *et al.* ('57) in S-potential of teleost fish, if the intensity that makes the amplitude of the potential attain its final steady state is useful as a measure of the environmental illumination which is suitable for the fish to live in, the skates and rays are inferred to have a retina well fitted to a dim illumination. The lowest value of the intensity was found in *Urolophus*. In *Holorhinus*, the amplitude has not attained the steady state even with the highest intensity of stimulus light used in the present experiment. It may perhaps be an adaptation to their lives, for it is well known that the fish often swims actively at middle layers or near the surface of the sea, occasionally leaping clear out of the water.

On the other hand, the positive potential or the notch which occurred in extremely low stimulus intensity was evidently covered by another large positive potential in the stimulation with intense light, and so it is assumed that the large positive potential (b-wave) caused by the intense light represents photopic character in the species such as *Narke*, *Urolophus* and *Holorhinus*, at least. This will support the assumption that the component of the cone is also included in b-wave of ERG (GRANIT '37, GOTO and TOIDA '54). Therefore, the measurement with the amplitude of the large positive potential will have an ecological meaning that the lowest stimulus intensity attaining the steady state of the amplitude serves to indicate the upper limit of the intensity of the suitable environmental illumination of the fish.

Retina has the ability of discriminating the intensity of the illumination only when the stimulation is made within the range of the intensity eliciting the graded response, that is, in the final steady state of the response all visual receptors may be fully excited, because there is no additional increase in the amplitude, even though with the further increase in stimulus intensity. When the retina is illuminated with an intensity greater than that in which the amplitude of the response shows the steady state, it is assumed that the fish can hardly discriminate objects within its visual field. This assumption is supported from the results obtained in the experiment of brightness discrimination; the differential threshold ( $ds/s$ ) increases distinctly with the intensity (about 20—30 lux) of adaptation light, in which the amplitude of the response attains the steady state (Figs. 41, 44 and 49). In nature, the regulation of incident light will be probably made in the bright scene, because the contraction of the pupil has been noticed in batoid fish (BRETT '57).

In the ERG of batoid fish the off-effect did not occur usually with the stimulus of about one sec., even though it was observed in the stimulation of longer period or in the stale preparation. Therefore, if the retina evokes the duplication of the image, the visual accuracy of the retina decreases; consequently it may be considered that the batoid fish is inferior to pelagic or shallow water fish in visual accuracy. On the other hand, however, it has been described that the effect of image duplication acting reversely compared with that above-mentioned occurs through the component of inhibition in the retina (GRANIT '47), and so the fact that the negative potential, inhibitory component in ERG, occurs noticeably in these fishes, will be considered to show no lowering of visual accuracy. Then, it is inferred that the batoid fish is able to have usual visual perceptive function even in very dim light intensity as at the bottom of the sea.

The transitory rapid decrease of retinal sensitivity which was observed in the initial stages of the light-adaptation, was mostly distinct in *Narke*, and this seems to be an adaptation to its habits. They lie flat in the bottom of the sea or buried in the mud and perceive usually only dim light in nature. On the contrary, the decrease of the sensitivity by light-adaptation, is found to be small in *Holorhinus* (Fig. 42), and this seems to be advantageous for the life swimming often near the surface of the sea.

Among species of batoid fishes there is a little difference in ability of brightness discrimination. In *Narke*, the value of  $ds/s$  is about 0.6, whereas in *Holorhinus* 0.006. The latter is the same order as that of the human eye (ca. 1/100—1/120, KATO '53). If the range of light intensity, in which the value of the differential threshold ( $ds/s$ ) is constant, is considered to be a suitable brightness of the habitat, the depth of the habitat may be inferred; for example in the present result the depth of the habitat increases in the following orders; *Holorhinus*, *Narke* and *Urolophus*, and the inference seems to be supported by the knowledge from the practice of fishery, according to the private communications from Drs. Takai and Ochiai.

Spectral sensitivity curves of dark-adapted retinae peaked sharply in all species and had the maxima at 525  $m\mu$  of the spectrum in *Dasyatis* and *Holorhinus*, and also 500  $m\mu$  in *Narke* and *Urolophus*. In *Raja*, the maximum was estimated at 500  $m\mu$  from the action spectrum (Fig. 38), and it agreed approximately with the absorption maximum (510  $m\mu$ ) in the visual pigment of *Raja clavata* (BAYLISS *et al.* '36). The relation between the maximum of spectral sensitivity and the habitat of fish will be described in Chapter III. From the result it is assumed that *Raja*, *Narke* and *Urolophus* live in deeper bottoms than the others. This assumption is consistent with the observations of many authors (BIGELOW and SCHROEDER '53, and others) and also with the assumption from the results of experiments on brightness discrimination (Figs. 41, 44, 47 and 49).

In light-adapted retina, the peak of the sensitivity curve shifted toward long wave-lengths; 575  $m\mu$  in *Holorhinus* and *Dasyatis*, and 525  $m\mu$  in *Urolophus*. The Purkinje's phenomenon was confirmed in these fishes, too. In *Narke* and *Raja*, however, the shift was not clearly found. The fact suggests that these fishes have

a retinal luminosity mechanism only, and this supports the assumption described by SVAETICHIN and MACNICHOL ('58), that fish inhabiting deep places (30—70 metres) is colour-blindness or cone monochromats. On the other hand, the complex wave-form eliciting the splitting was quite noticeably observed in the ERG of *Dasyatis* (Fig. 32), and then in this species the existence of some components dominant in the definite wave-lengths may be suggested from the consideration described by GOTO and TOIDA ('58). It is necessary, however, to analyze in detail the colour component by the aid of a micro-electrode, in future.

On some species, the spectral sensitivity of dark adapted retina was investigated with negative component and positive component respectively, and it was observed that the sensitivity curve of the negative potential was similar to that of the light-adapted retina. The negative potential of the ERG was of the character of photopic response, as was described by SVAETICHIN ('53<sub>b</sub>). The sensitivity of the retina was very low to the light of wave-lengths longer than 650 m $\mu$  in all species, and it was quite insensitive to the light above 700 m $\mu$  in the species except *Dasyatis* and *Holorhinus*. These facts are also understood from the consideration as to the depth of their habitats.

Flicker fusion frequency was measured in *Narke* and *Urolophus*. It became clear in these fishes that the flicker response occurred in only the low intensity of stimulus light, and the fusion occurred in low frequency; it was about 6 c/s as compared with 30 to 55 c/s in teleost fish (SVAETICHIN '53<sub>a</sub>, TAMURA *et al.* '59, WOLF '36 and others), and also it was smaller than about 10 in a dog fish (*cf.* Chapter I). This is again significant in connection with their sluggish habits.

The retina of elasmobranch fish excepting some species has been known to consist of rod only (DETWILER '43, BRETT '57). And also TAKATSUJI ('38) described that in the receptor cell of *Raja koini*, rod and cone did not become differentiated from the original cell. The author also investigated the retinal structure of the species used in the present work and identified receptor cells as rod cell like in all species. However, the present physiological results suggested the existence of some components showing the photopic character, which may be assumed to be caused by the cell like a cone cell. Therefore, it may be regarded as appropriate that the receptor cells should be differentiated into rod and cone physiologically, even though they are indiscernible from each other morphologically.

### Summary and conclusions

(1) Electroretinogram (ERG) of rays and skates, Batoidei, was investigated. The ERG was taken from the opened eye excised, and the results were considered ecologically.

(2) In dark-adapted retina of *Holorhinus*, *Urolophus* and *Narke* the ERG showed a slow positive potential followed by a negative one in the response to extremely weak light, and the positive potential was covered with another large positive po-

tential (b-wave) in the stimulation with the light over moderate intensity and it appeared as a notch on the rising phase of b-wave. The notch is assumed to look rod potential. In *Platyrrhina* the slow positive potential was quite suppressed by the intense stimulus light. In *Dasyatis* and *Raja* the notch was not clearly found.

(3) In all species, dark-adapted retina did not cause off-effect in the stimulation of the duration less than several seconds.

(4) In light-adapted retina the negative potential was dominant generally in the stimulation with intense light. In *Holorhinus* and *Dasyatis*, the light-adapted retina elicited the remarkable off-effect with the stimulus light of long duration. In the other species the off-effect was not clearly found.

(5) The change in amplitude height of the ERG according to variation of stimulus intensity showed the linear relation against the logarithm of stimulus intensity. However, the increase in amplitude height of positive potential (b-wave) attained to a plateau state by relatively low stimulus intensity in the species except *Holorhinus*, in which the plateau state of amplitude height was not yet attained within the range of stimulus intensity used in the present experiment.

(6) The time-course of light- and dark-adaptations was investigated by the threshold intensity of stimulus light eliciting the ERG.

In the initial period of light-adaptation the threshold intensity rose rapidly, however it began to decrease immediately in the progress of the light-adaptation and attained a steady state within a few minutes. The extent of the change in the threshold tended to become broader in the species inhabiting deeper seas than those inhabiting shallow seas. The dark-adaptation was completed in relatively short time (within about 10 minutes) in most cases.

(7) The ability of brightness discrimination was investigated at the several levels of light intensity of adaptation light. The values of differential threshold,  $ds/s$ , were as follows; 0.006 in *Holorhinus*, 0.6 in *Narke*, 0.045 in *Dasyatis*, 0.07 in *Urolophus*. The intensity of adaptation light at which the value of  $ds/s$  was constant, was considered ecologically. From the results it is suggested that the light sense of batoid fish is functional value.

(8) Spectral sensitivity curves of dark-adapted retina had the maxima at 500  $m\mu$  in *Narke*, *Urolophus* and *Raja*, and at 525  $m\mu$  in *Dasyatis* and *Holorhinus*, respectively. The retina was almost insensitive against the light of wave-lengths longer than 650  $m\mu$  in the species except for *Dasyatis* and *Holorhinus*.

(9) By light-adaptation, the curve shifted the maximum toward 575  $m\mu$  in *Holorhinus* and *Dasyatis*, and toward 525  $m\mu$  in *Urolophus*. In *Narke* and *Raja*, the shift was not found clearly.

(10) Above-mentioned results assume that the retina of *Narke* and *Raja* may have only luminosity mechanism for the vision of their fishes, while in other species the retina may have some components dominant for the definite wave-length of the spectrum.

(11) The flicker response occurred in only low intensity of the stimulus light, and the fusion frequency ranged from 4.4 to 6.0 c/s, varying with the stimulus intensity,

in *Narke* and *Urolophus*.

(12) From the experimental results obtained in this chapter, it is concluded that the retina of batoid fish is well developed in spite of the dim illumination of the environment in which the fish lives, and the particular responses found in the ERG are considered to be adaptive to their behaviour having a deeper or benthonic habitat and conducting sluggish locomotion. And moreover, it is evidently assumed that the depth of the habitats in *Holorhinus* and *Dasyatis* is shallower than that of the rest.



### Chapter III Spectral sensitivity of fish retina and its ecological aspects

GRANT ('41) investigated spectral sensitivity in *Carpio*, *Tinca* and *Anguilla* depending upon the impulses of single optic fibre and found that the maximum of the sensitivity curve coincided with that of the absorption curve of the visual pigments. SVAETICHIN and MACNICHOL ('58) determined the action spectrum of the so-called S-potential in several kinds of marine fishes and found that the maximum of action spectrum in shallow water fishes did not coincide with that of fishes living in deeper sea.

The distribution of solar energy in the sea water shows a noticeable difference according to its depth; blue-green light penetrates into the sea water distinctly, while red light reveals the marked reductoin in strength even in a depth of 10 metres. Therefore, it is significant and interesting not only from the physiological but also from the ecological view point to study comparatively the spectral sensitivity of fishes inhabiting various depths.

#### Material and method

Involving the species having no rod in their retinae, 20 species of fish were used as materials in this work. Most of them were caught with a small trawl net about 5 miles off the coast of Yamaguchi Prefecture along the Sea of Japan. The rest about 10 miles caught by angling about 2 miles off the coast or with a hand-net by the sea side or in ponds and rivers.

These are listed in Table 3, with their body-lengths, the period during which the fishes were used in the experiments and the method of the induction of ERG.

Previously to the experiment, the fishes were preserved for several days in the refrigerator.

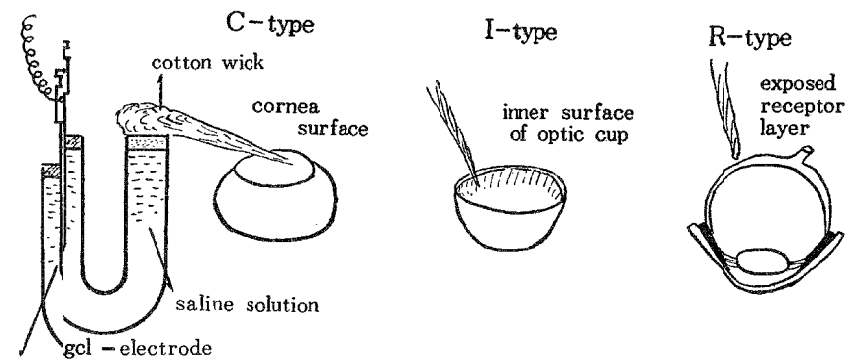


Fig. 51. Various sites of the active electrode in the induction of ERG.

Table 3. Species of fishes used in the experiment of this chapter, its body-length, period during which it is used, and the method of induction of ERG. R, action potential is induced from the receptor side of the retina; C, from the cornea surface; I, from the inner surface of the opened eye.

Species	Body-length (cm)	Experimental period (month)	Method of induction
1. <i>Acanthogobius flavimanus</i>	15—20	X	R
2. <i>Anguilla japonica</i>	30	X	C
3. <i>Carassius auratus</i>	20	VIII	I
4. <i>Chrysophrys major</i>	15—20	X	R
5. <i>Cyprinus carpio</i>	30—40	XI	R
6. <i>Dictyosoma burgeri</i>	20—25	XI	R
7. <i>Gymnothorax reticularis</i>	60—70	VIII	C
8. <i>Halichoeres poecilopterus</i>	15—20	X	R
9. <i>Hoplognathus fasciata</i>	17	X	R
10. <i>Inegocia crocodila</i>	20	X	R
11. <i>Lagocephalus lunaris</i>	25	X	R
12. <i>Misgurnus anguillicaudatus</i>	10—15	XI	C
13. <i>Mustelus manazo</i>	80—100	XII	I
14. <i>Narke japonica</i>	25	XI	I
15. <i>Pseudorhombus cinnamomeus</i>	15—20	XI	I
16. <i>Scorpaenodes guamensis</i>	20—25	XI	R
17. <i>Stephanolepis cirrhifer</i>	15—20	XI	R
18. <i>Trachurus japonicus</i>	10—15	VI	I
19. <i>Argyrosomus argentatus</i>	20	V	R
20. <i>Chelidonichthys kumu</i>	15—20	V	R

aquarium of the laboratory. Experiments were made with the eyes excised from the head cut off from the body under dim red light after about one hour of dark-adaptation. The arrangement for recording ERG was principally the same as that described

Table 4. Characteristics of interference filters used in the present experiment and ratio of radiant energy (%) of tungsten filament lamp transmitted through the filters.

No. of filter	Central wave-length (m $\mu$ )	Transmission ratio (%)	$\lambda_{1/2}$ (m $\mu$ )	Energy ratio through the filter (%)
430	428	43.0	20	30.0
470	466	40.0	12	24.5
500	501	43.0	11	20.9
525	523	41.0	11	32.3
550	545	38.0	9	31.0
575	577	41.0	10	26.5
600	598	41.0	14	19.7
650	651	41.0	15	26.9
700	695	42.0	18	27.6

in Chapter I. But the site of the active electrode was slightly modified with the different species (Fig. 51). The active electrode was in contact with the retina in the area of the fundus and the measurement of the action potential was made under dark-adaptation. Interference filters (made in Shonan Optical Thin Film Co., Ltd.) were used for getting monochromatic light, characteristics of which are listed in Table 4.

The reciprocals of the threshold intensity of different monochromatic light were used for the determination of spectral sensitivity. The intensity of stimulating light was controlled with the neutral filters. The relative energy output through a given filter was determined by means of thermocouple and the value was shown in Table 4. The threshold intensity of stimulating light were determined as follows.

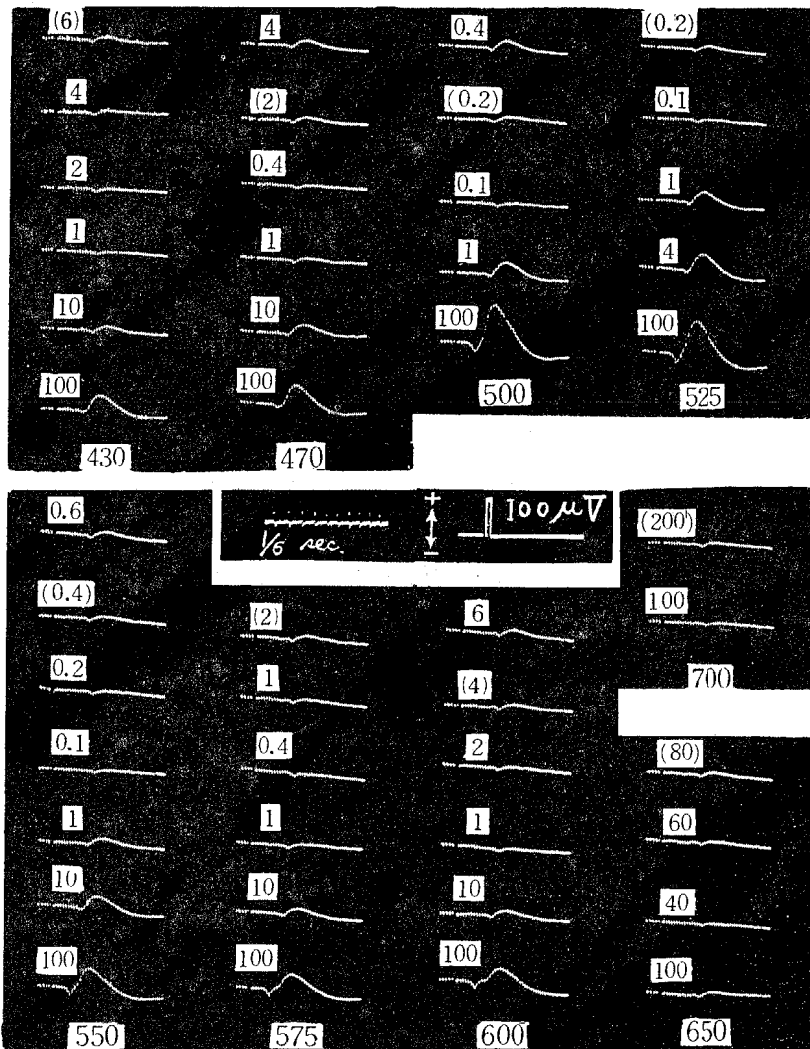


Fig. 52a. ERGs recorded in the investigation of the spectral sensitivity of *Anguilla* retina. Each series shows the change in size of ERGs caused by various intensity of monochromatic light. The numerals on each record indicate the relative intensity of the light and the parentheses indicate the threshold intensity. ERG was induced from the cornea surface.

The retina was first stimulated only through the interference filter, then the intensity was decreased in steps of one logarithmic unit until the response diminished. Next the light stimulus was increased in steps of 0.2 logarithmic unit until the response appeared. Each test was done after about three minutes of dark-adaptation. The examples of the recording are shown in Figs. 52a, b and c. Each value of the threshold intensity thus obtained was corrected in reference to the relative value of energy transmitted through the interference filter. The threshold intensity obtained in three retinæ were averaged, and the sensitivity curve was constructed as shown in Figs. 53 and 54.

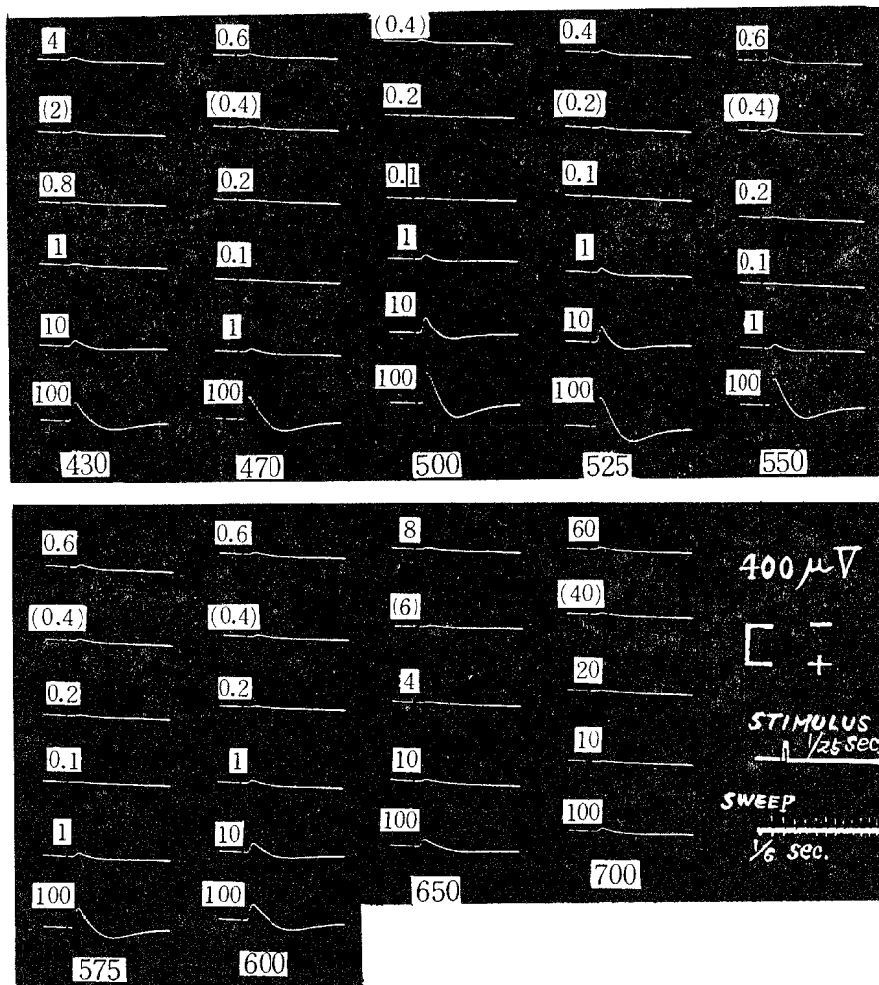


Fig. 52b. ERGs recorded in the investigation of the spectral sensitivity of *Stephanolepis cirrhifer*. ERG was induced from the receptor side of the retina. Legends are same as in Fig. 52a.

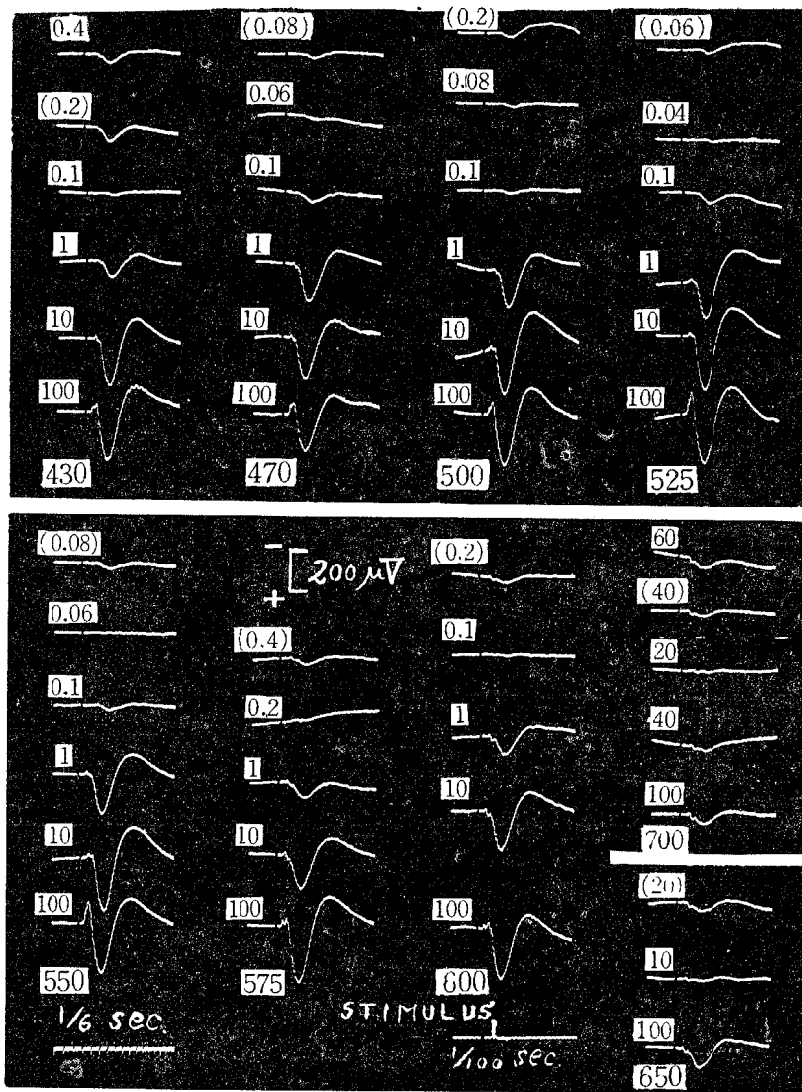


Fig. 52c. ERGs recorded in the investigation of the spectral sensitivity of *Scorpaenodes guamensis*. ERG was induced from the receptor side of the retina. Legends are same as in Fig. 52a.

### Results

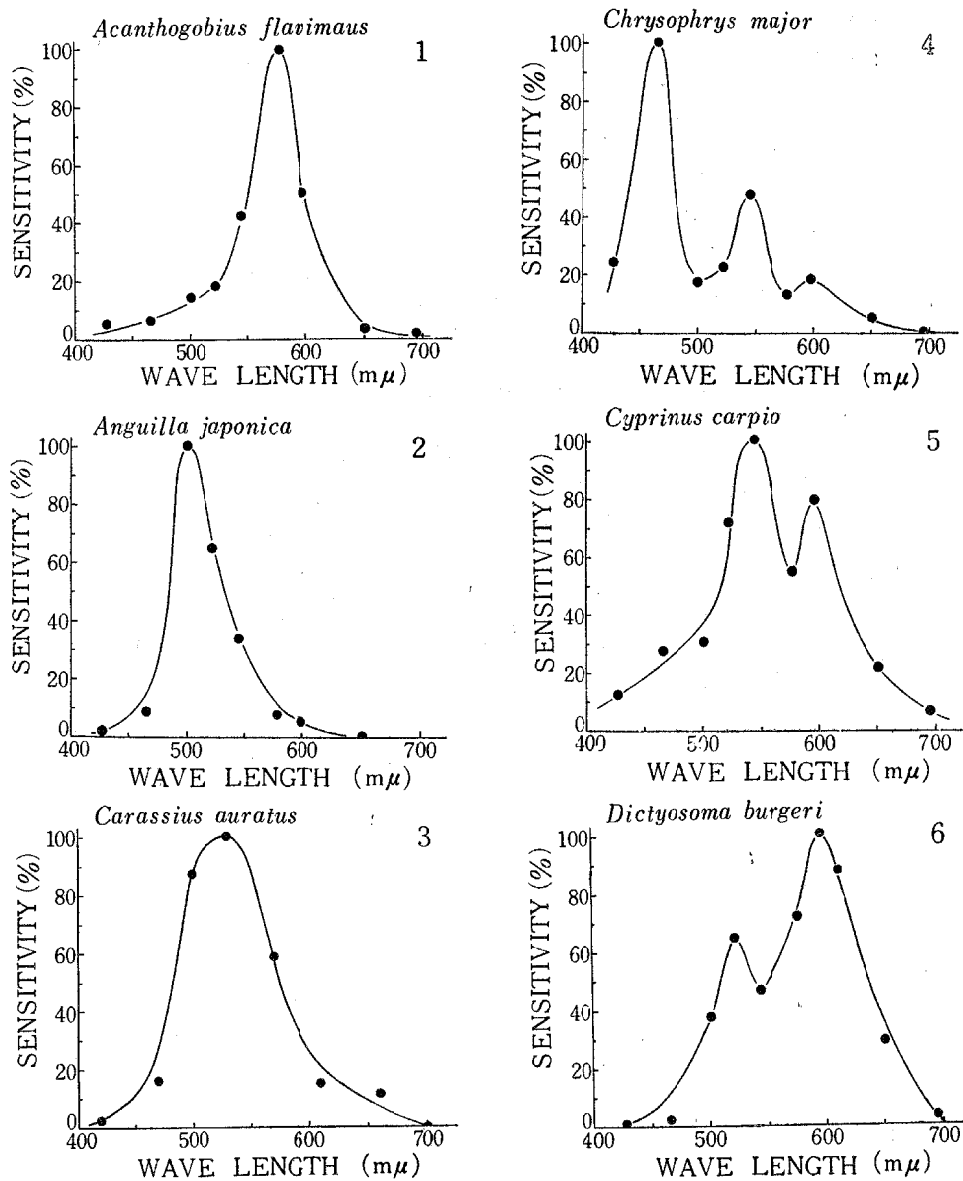
#### (1) Spectral sensitivity curve in each species

1. *Acanthogobius flavimanus* Action potential was directly induced from the receptor side of the retina, though it was rather difficult to remove the pigment layer even in the state of dark-adaptation. Negative potential was used for the determination of the sensitivity.

The spectral sensitivity curve is shown in Fig. 53a—1. The maximum is at 575 mμ and the sensitivity decreases rather sharply toward both sides of the spectrum. MÜN Z reported recently that the visual pigment of *Gillichthy mirabili*, which

belonged to the same family with and had similar habits to the species used in the present work, showed the maximum absorption at 512  $m\mu$ , whereas in the present experiment the ERG showed no maximum in the spectrum range between 500  $m\mu$  and 525  $m\mu$ .

2. *Anguilla japonica* The potential was induced from the cornea surface of the eyeball, and positive potential, b-wave in ERG, was used for the investigation. The spectral sensitivity curve is shown in Fig. 53a-2, in which the maximum lies at 500  $m\mu$  and the curve is steep in shape. It may be assumed that the fish is almost insensitive to the light of the wave-lengths longer than 650  $m\mu$ .



Figs. 53a. Showing the spectral sensitivity curve of scotopic retina. The name of each species is entered in the upward corner of each graph.

BAYLISS, LYTHGOE and TANSLEY ('36) showed that the eye of *Anguilla vulgaris* had absorption maximum at 505 m $\mu$ . WALD ('37) also showed that *Anguilla anguilla* had the maximum at the same wave-lengths. These results seem to coincide with those of the present work. GRANIT ('41) described that it was very difficult to obtain the photopic sensitivity curve in a light-adapted eel, although he found that the curves tended to give secondary maximum between 500 m $\mu$  and 560 m $\mu$ . In the present experiment the sensitivity curve with light-adaptation could not be obtained, since the adaptation of the eye tended to depress the sensitivity below the threshold of the energy available in the spectrum.

3. *Carassius auratus* Action potential was induced from the inner surface of the opened eye and positive potential, b-wave in ERG, was used for the determination of the sensitivity.

The spectral sensitivity curve is shown in Fig. 53a—3. The curve has a maximum at 525 m $\mu$ . The maximum of the sensitivity agrees well with the absorption maximum of porphyropsin. The sensitivity slowly decreases toward both ends of the spectrum; its reduction is greater in a short wave-length than in a long one.

4. *Chrysophrys major* Action potential was directly induced from the receptor side of the retina, but the polarity of ERG differed in various wave-lengths of stimulus light. Irrespective of the polarity, however, the sensitivity was determined by the threshold intensity which elicited the response.

The spectral sensitivity curve is shown in Fig. 53a—4. The curve has a maximum at 470 m $\mu$  and has submaxima at 550 m $\mu$  and 600 m $\mu$ . It is assumed that the so-called blue-sensitive elements are strikingly dominant in this species. The sensitivity curve having its peak at 525 m $\mu$ , as shown in Fig. 54—3, was obtained from the eye adapted to weak light (about 3 lux). Moreover, the retina was almost insensitive to the light of the wave-lengths longer than 650 m $\mu$  in this species.

5. *Cyprinus carpio* The potential was induced from the receptor side of the retina and the negative potential was used for the determination of the sensitivity curve.

The spectral sensitivity curve is shown in Fig. 53a—5, in which the curve has a maximum at 550 m $\mu$  and a submaximum at 600 m $\mu$ . With the light-adaptation the maximum of the curve decreases gradually, and the submaximum comes to increase. And so it seems that the element which shows a submaximum in the dark-adapted retina becomes the most sensitive one in the light-adaptation. These results agree well with the sensitivity curves obtained in carp and tench by GRANIT ('41). It may be assumed that the red sensitive element showing the increasing sensitivity with light-adaptation has photopic character, and this supported by the fact that the fish is known as a diurnal animal and has relatively many cones in the retina (WUNDER '25 and others).

6. *Dictyosoma burgeri* The potential was picked up from the receptor side of the retina, though it was rather difficult to remove the pigment layer. The negative

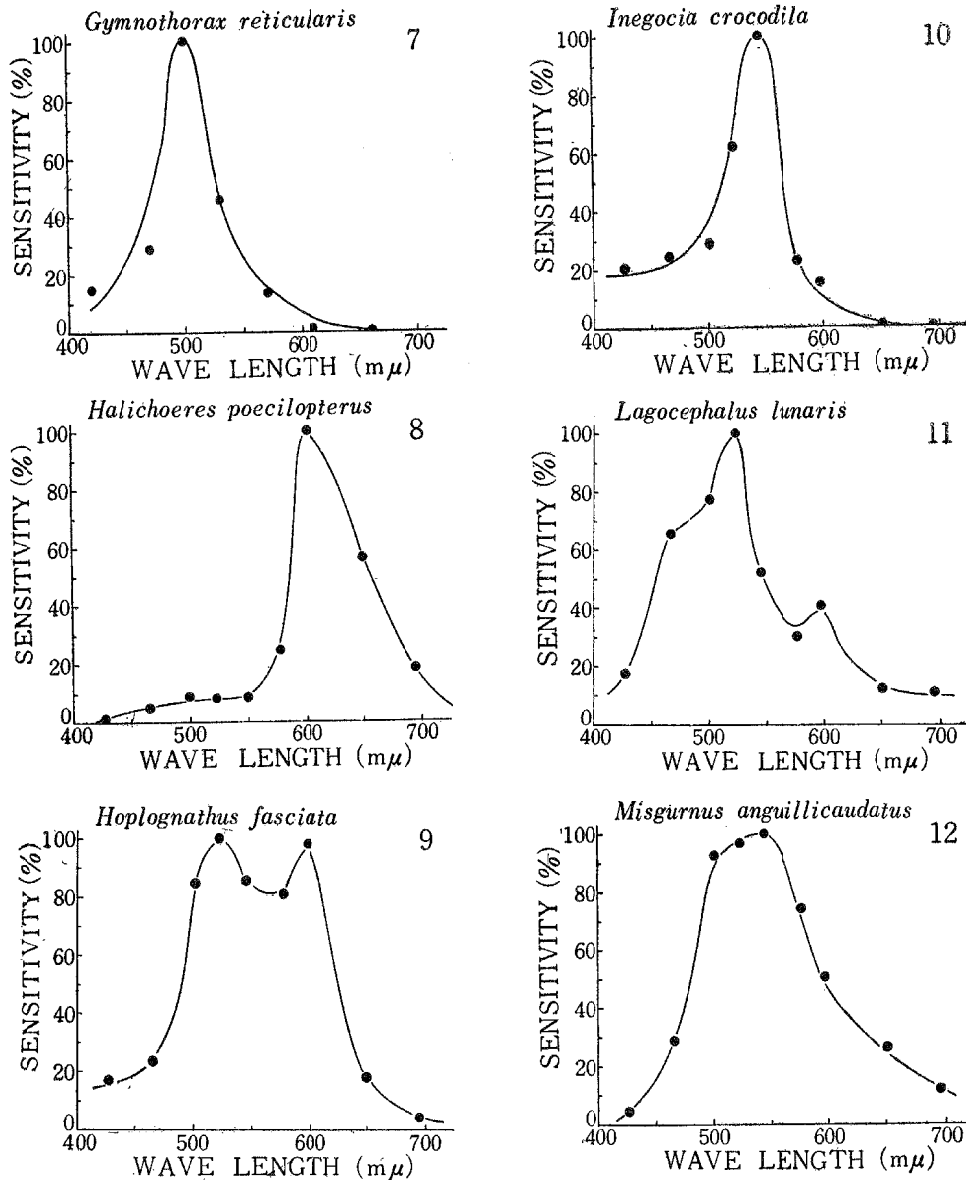


Fig. 53b. Showing the spectral sensitivity curve of scotopic retina. The name of each species is entered in the upward corner of each graph.

potential was used for the determination of the sensitivity curve.

The spectral sensitivity curve is shown in Fig. 53a—6. The facts that the sensitivity curve has the maximum at 600 mμ and that the curve covers a relatively broad range in the spectrum, are interesting from the ecological view point, since the fish is usually found among rocks near the sea shore. The sensitivity curve has submaximum at 525 mμ.

7. *Gymnothorax reticularis* The potential was induced from the cornea surface of the eyeball, and positive potential, b-wave in ERG, was used for the determination of the sensitivity curve. The fish is known as a typical nocturnal animal.

The spectral sensitivity curve is shown in Fig. 53b—7. The curve has the maxi-



mum at 500  $m\mu$  and falls away rapidly toward both sides of the spectrum. With light-adaptation, however, the maximum shifts a little toward long wave-lengths (about 530  $m\mu$ ), as shown in Fig. 54—4. The fish was almost insensitive to the light of the wave-lengths longer than 650  $m\mu$ .

8. *Halichoeres poccilopterus* The potential was picked up from the receptor side of the retina, although it was rather difficult to remove the pigment layer. Negative potential was used for the determination of the sensitivity curve. The curve is shown in Fig. 53<sub>b</sub>—8. The maximum sensitivity is at 600  $m\mu$  of the spectrum and the small submaximum, at 500  $m\mu$ . The retina has the extremely low sensitivity to light of the wave-lengths shorter than 550  $m\mu$ . And also it is found that this curve is considerably similar to the curve obtained from the light-adapted retina in *Cyprinus* (broken line in Fig. 54—1). Such a type of spectral sensitivity curve as was obtained in the retina of *Halichoeres* has not yet been recorded from the retina of any other fish. It may be assumed that the red sensitive elements such as seen in the retina of tortoise (GRANIT '41), are dominant in the retina of *Halichoeres*. This is very interesting in relation to the facts that the fish living near the sea shore has red coloured retina as described by WALD ('53), and also that the *Halichoeres* retina is constituted of cone only (TAKATSUZI '39).

By light-adaptation the sensitivity curve does not show the shift of the maximum, but the curve expands slightly toward the shorter wave-length, and it has the tendency to show the submaximum at 525  $m\mu$  (Fig. 54—5).

9. *Hoplognathus fasciata* The potential was induced from the receptor side of the retina, and negative potential was used for the investigation.

This fish is also an inhabitant near the sea shore. The spectral sensitivity curve is shown in Fig. 53<sub>b</sub>—9. The curve has the maximum at 525  $m\mu$  and moreover the secondary maximum at 600  $m\mu$ , and then the curve shows the almost equal sensitivity in the range from 500  $m\mu$  to 600  $m\mu$  of the spectrum. Therefore, the spectral sensitivity diminishes rapidly both in the wave-lengths shorter than 500  $m\mu$  and in those longer than 600  $m\mu$ .

10. *Inegocia crocodila* The potential was picked up from the receptor side of the retina and negative potential was used for the investigation.

This fish is benthonic near the coast and is not an active swimmer. The spectral sensitivity curve is shown in Fig. 53<sub>b</sub>—10. The curve is steep in shape and has the maximum at 550  $m\mu$ . It also shows low sensitivity both in the wave-lengths shorter than 500  $m\mu$  and in those longer than 575  $m\mu$ . When the retina was adapted to weak light of about 10 lux, the maximum of the sensitivity curve did not change, though the curve expanded somewhat on both sides of the spectrum.

11. *Lagocephalus lunaris* The potential was induced from the receptor side of the retina. The response was diphasic (negative-positive) in the case of stimulation with monochromatic light of the wave-lengths shorter than 525  $m\mu$ , while it was

negative monophasic in the case of the wave-lengths longer than that. Irrespective of the polarity, however, the sensitivity was determined by the threshold intensity which elicited the response.

The spectral sensitivity curve is shown in Fig. 53<sub>b</sub>—11. The curve has the maximum at 525 m $\mu$  and two submaxima at 600 m $\mu$  and 470 m $\mu$ . The sensitivity decreases more slowly in the longer wave-length side than in the shorter one.

12. *Misgurnus anguillicaudatus* The potential was induced from the cornea surface of the eyeball and b-wave in ERG was used.

The fish has a habit to hide itself in the mud. The spectral sensitivity curve is shown in Fig. 53<sub>b</sub>—12. The curve has the maximum at 550 m $\mu$  and shows the almost equal sensitivity in the range from 500 m $\mu$  to 550 m $\mu$ , and also shows a rather slow fall toward both ends of the spectrum.

13. *Mustelus manazo* The potential was induced from the inner surface of the opened eye and positive potential, b-wave in ERG, was used.

The spectral sensitivity curve is shown in Fig. 53<sub>c</sub>—13. The maximum sensitivity is at 500 m $\mu$  and the decrement of the sensitivity is more remarkable in longer wave-lengths than in shorter ones. The peak of the sensitivity curve seems to coincide with the peak (505 m $\mu$ ) of the absorption curve of visual pigment obtained by BAYLISS *et al.* ('36) in *Scyllium canicula*. With the adaptation of weak light (about 3 lux) the threshold of the eye response rose higher than the light intensity level available in the spectrum. Therefore, the investigation of the sensitivity in light-adaptation was not made in the present work.

Two types of the ERG have been found in the retina of *Mustelus* (*cf.* Chapter I); one is the fast type of scotopic character, the other is the slow type of photopic one and it has been observed that the mode of spectral sensitivity of both types is almost the same.

14. *Narke japonica* The potential was induced from the inner surface of the opened eye and positive potential was used. The spectral sensitivity is shown in Fig. 53<sub>c</sub>—14. The curve has the maximum at about 500 m $\mu$ , and the curve falls away more steeply on either side of the maximum than that obtained in shallow water fish. Moreover, the retina was insensitive to the light longer than 700 m $\mu$  in wave-length. The light-adaptation caused no change in the spectral sensitivity curve.

15. *Pseudorhombus cinnamoneus* Action potential was induced from the inner surface of the opened eye and negative potential, a-wave in ERG, was used for the determination of spectral sensitivity.

The fish is known to inhabit rather deeper sea near the coast. The spectral sensitivity curve is shown in Fig. 53<sub>c</sub>—15. The curve has its maximum at 500 m $\mu$  and it falls away rather steeply on both sides of the maximum. The sensitivity to the light of the wave-length longer than 650 m $\mu$  diminishes distinctly.

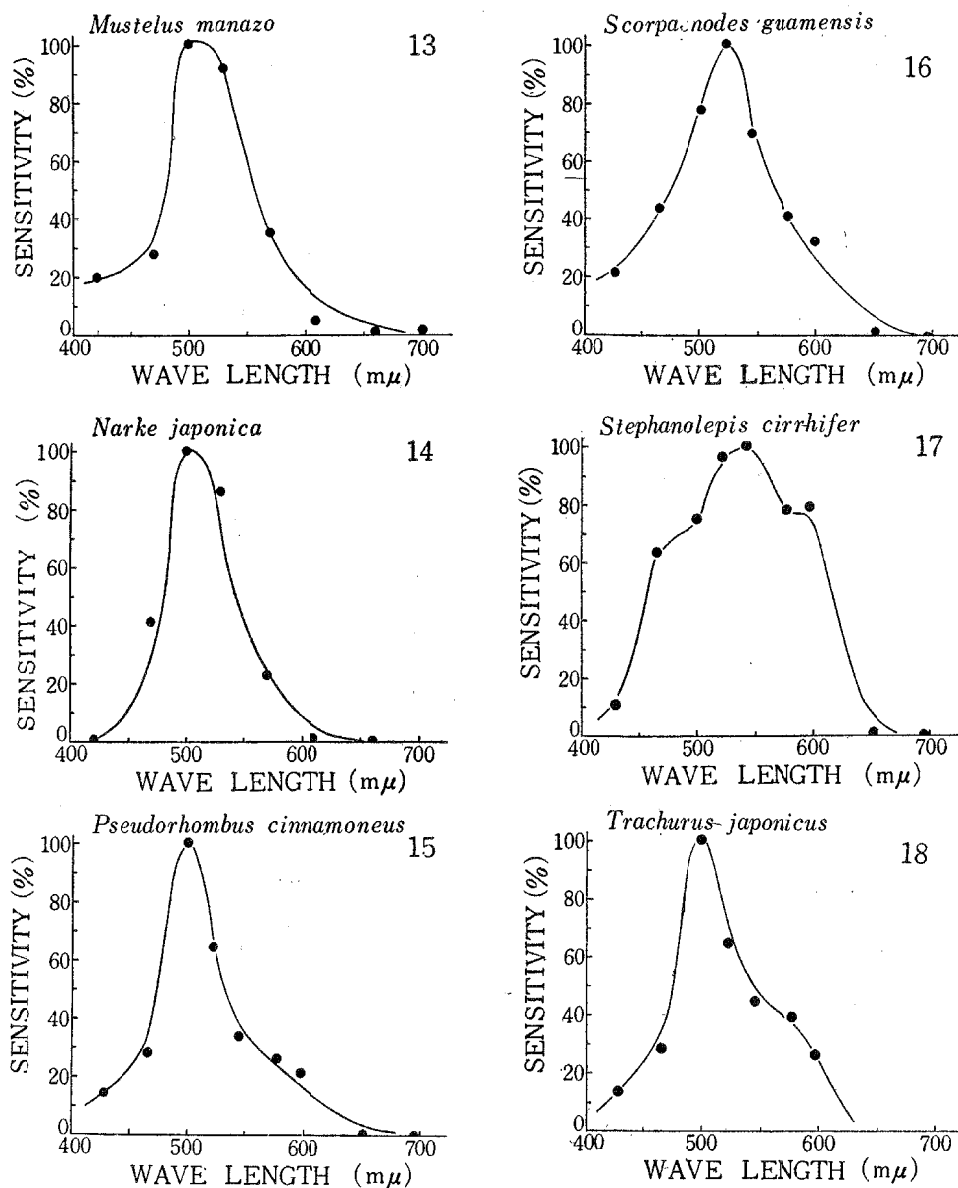


Fig. 53c. Showing the spectral sensitivity curve of scotopic retina. The name of each species is entered in the upward corner of each graph.

16. *Scorpaenodes guamensis* Action potential was induced from the receptor side of the retina. The positive potential was investigated in the dark-adapted retina, while the negative one in the light-adapted retina, since the positive one was suppressed by the light-adaptation. The spectral sensitivity curve is shown in Fig. 53c—16. The maximum sensitivity is at 525 mμ and the sensitivity to the light of the wave-lengths longer than 650 mμ is remarkably low. By the light-adaptation, the maximum shifted to 575 mμ and the sensitivity to the light of 600 mμ of the spectrum rose considerably (Fig. 54—2).

17. *Stephanolepis cirrhifer* The potential was induced from the receptor side

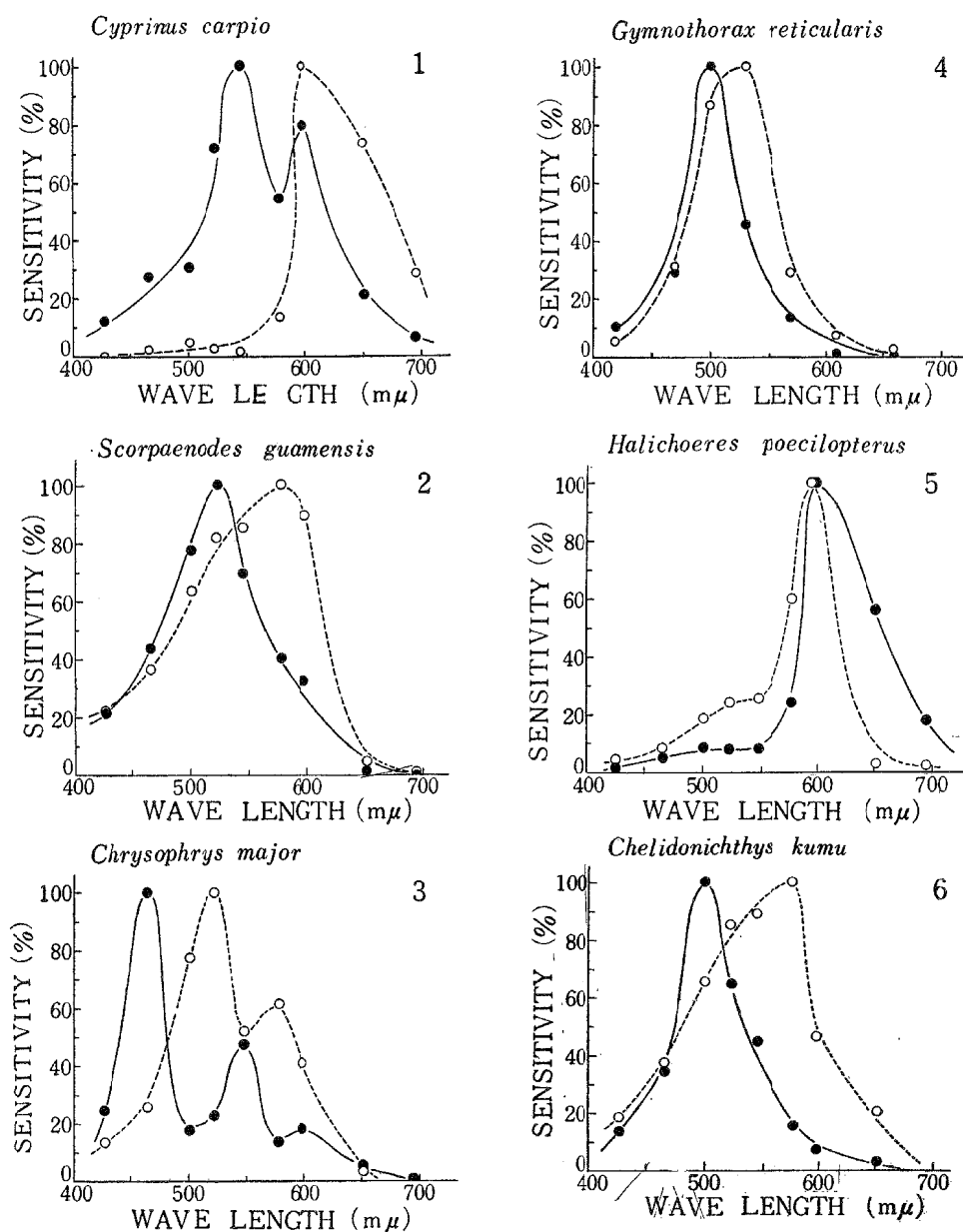


Fig. 54. Effect of light adaptation on the spectral sensitivity curve. Filled circles, spectral sensitivity investigated in scotopic retina; open circles, in photopic retina. Species name is entered in the upward corner of each graph.

of the retina and negative potential was used for the determination of spectral sensitivity.

The fish is known as an inhabitant near the sea shore and is also known to have the retina without rods (TAKATSUZI '39). The spectral sensitivity curve is shown in Fig. 53c—17. The curve has a maximum at 550  $m\mu$  and submaxima at 470  $m\mu$  and 600  $m\mu$ . Therefore, the curve is broad in shape, but the sensitivity to the light of the wave-lengths longer than 650  $m\mu$  and shorter than 430  $m\mu$  is considerably low.

18. *Trachurus japonica* Action potential was induced from the inner surface of the opened eye, and negative potential, a-wave in ERG, was used for the investigation. This fish is a swimmer in the middle water and never comes up to the surface of the water.

The spectral sensitivity curve is shown in Fig. 53<sub>c</sub>—18. The curve has a maximum at 500 m $\mu$  and has a submaximum at 575 m $\mu$ . It was noticed that the retina was insensitive to the light of the wave-lengths longer than 650 m $\mu$ . In the light-adaptation the sensitivity of the retina considerably decreased so that the investigation of the spectral sensitivity was impossible.

19. *Argyrosomus argentatus* Action potential was induced from the receptor side of the retina and positive potential was used.

The fish is known to live on the bottom of the sea near the coast and is also known as a bottom feeder and a nocturnal animal. The spectral sensitivity curve has a maximum at 500 m $\mu$  and the sensitivity decreases rather sharply toward both ends of the spectrum. The retina was almost insensitive to the light of the wave-lengths longer than 650 m $\mu$ .

20. *Chelidonichthys kumu* Action potential was induced from the receptor side of the retina and positive potential was used.

The fish is known as an inhabitant in deeper sea, but it has remarkable vertical movement in its swimming. The spectral sensitivity curves are shown in Fig. 54—6. The curve has a maximum at 500 m $\mu$  and it falls down rather sharply toward both ends of the spectrum. In the light-adaptation, the maximum of the curve is shifted to 575 m $\mu$ , and the fall of the sensitivity becomes more sharp in the longer wave-lengths than the shorter ones. The retina was insensitive to the light longer than 700 m $\mu$  in wave-length.

## (2) Ecological aspects on the spectral sensitivity of fish retina

As the spectral distribution of the solar energy penetrated into sea water changes according to the depth of the sea (CLARKE '54), it is necessary to consider the characteristics of spectral sensitivity of fish vision with regard to the depth of their habitat. The characteristics of the spectral sensitivity described in the part (1) were drawn from the maximum depth in which the fishes were found (Fig. 55). The data of the depth were obtained from "The investigation of benthos around Hyuganada in 1950" made by the Nankai Regional Fisheries Laboratory and also from the author's own experience. As shown in Fig. 55, an obvious correlation was found between the maximum of the sensitivity (filled circles in Fig. 55) and the depth, except in the case of a few species. Namely, the maximum of the sensitivity tended to shift toward the shorter wave-length with the increase of the depth. The fish having the maximum at 500 m $\mu$  of the spectrum, absorption maximum of rhodopsin, can be assumed to be the species able to live in a depth deeper some 100 metres. Fishes usually found near the sea shore have the maximum of sensitivity in the wave-

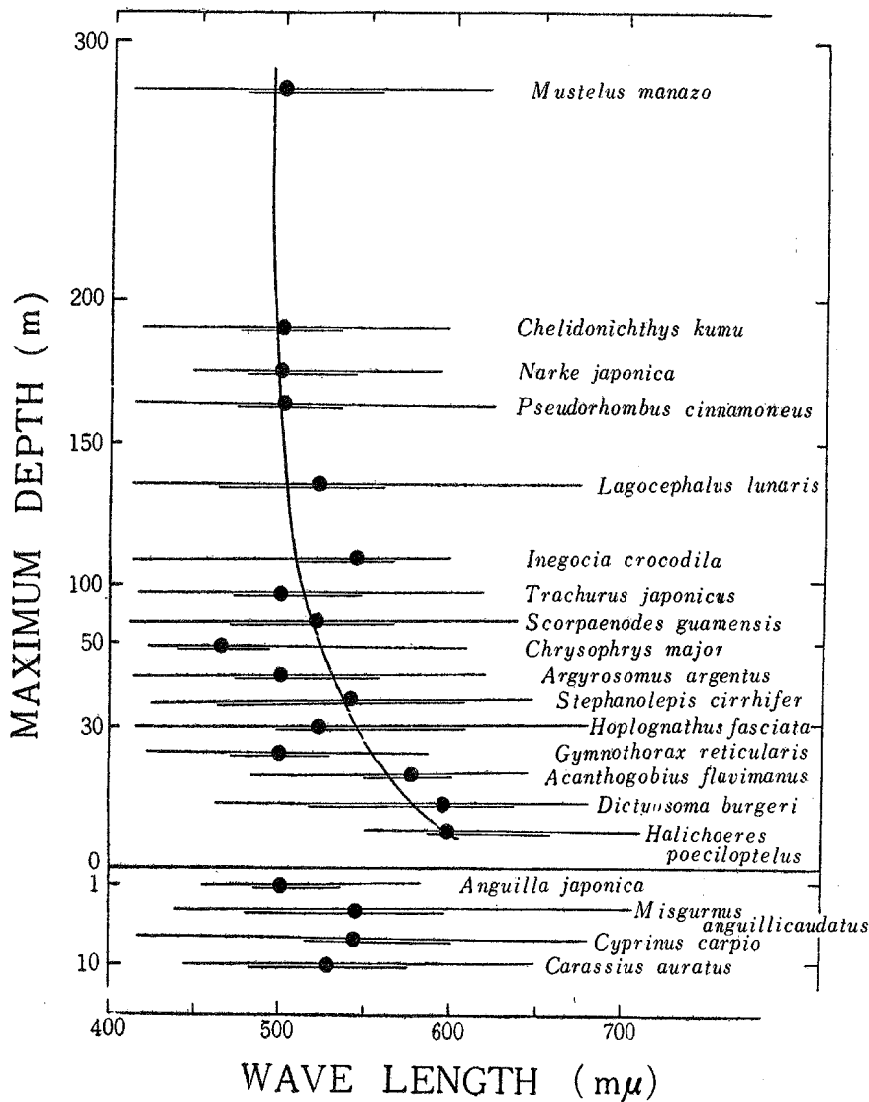


Fig. 55. Showing the relation between the characteristics of spectral sensitivity curve of fish and the maximum depth of their habitats. Explanation in text.

lengths longer than 550  $m\mu$  and the coastal fishes have it between 500 and 550  $m\mu$ . The fact that DENTON *et al.* ('57) and others found several deep-sea fishes having the visual pigment with absorption maximum at 480  $m\mu$ , supports the tendency shown in Fig. 55. And also the present results showed the same tendency as the maximum sensitivity of L-response obtained by SVAETICHIN and MACNICHOL ('58), that the maximum of the fish caught at depths of 30 to 70 metres was at about 500 to 530  $m\mu$  of the spectrum and that of shallow water fishes was in the region from 550 to 600  $m\mu$ .

The maxima of fresh water fishes were all found in the spectrum ranging from 525 to 550  $m\mu$  and the maxima coincided with the absorption maximum of porphyropsin. If the wave-length of the maximum is considered with regard to the depth of

their habitats, it seems to be independent to the relation obtained in marine fishes.

In Fig. 55, horizontal lines drawn across the filled circles indicate the spectrum region in which the sensitivity is kept higher than 10 and 50 per cent of the maximum, respectively. In the length of these lines under comparison it is clear that the sensitivity curve is broad in shallow water fishes, while it is narrow in the fishes found in the sea deeper than 100 metres and in typical nocturnal fishes such as *Anguilla* and *Gymnothorax*. Moreover, the sensitivity curve does not show the shift of its maximum in light-adaptation in the fishes found in deeper sea, but in the case of shallow water fishes the maximum of the curve is shifted noticeably in light-adaptation and the curve tends to have the submaximum.

(3) Threshold intensity of the retina in each species

The threshold intensity of the retina obtained through another series of experiment is shown in Fig. 56 together with those of other batoid fishes. The threshold intensity is represented by the percentage of unit intensity (500 lux) required to elicit the response by the white light stimulation with the duration of 10 msec. In the same diagram the open circle indicates the average of the threshold intensity obtained in several individuals and the straight line across the circle shows the extent of standard deviation. The names of the species are arranged in the order of the depths of their habitats.

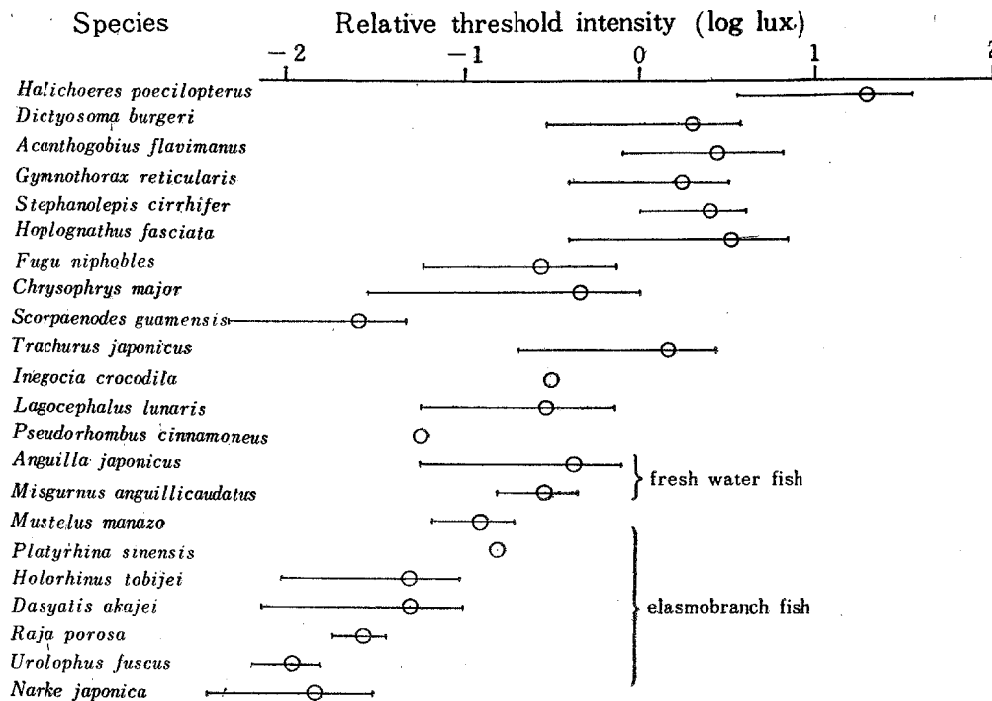


Fig. 56. Showing the threshold intensity of the retina of the fishes used in the present work. Open circles show the average value, and the length of the straight lines through the circles shows the standard deviation in the determination of the threshold intensity.

As shown in Fig. 56, the threshold intensity of shallow water fishes is higher than that of the fishes inhabiting the sea deeper than 100 metres, and the threshold intensity obtained from the former fishes was about ten times as high as that in the latter fishes. Among the shallow water fishes, except in the case of *Halichoeres*, no remarkable differences are seen in threshold, while in the deeper sea fishes the threshold intensity tends to become lower with the increase of the depth of their habitats.

### Discussion

It is well known that the visual pigment of marine fish is rhodopsin with its wave-length of maximum absorption at about 500 m $\mu$  and that of the fresh water fish is porphyropsin with the maximum at about 520—530 m $\mu$ , although the cysopsin with the maximum at 480 m $\mu$  has recently been found in deepsea fish (DENTON and WARREN '55, MÜNZ '57). On the other hand, it has been known that the maximum of the spectral sensitivity of dark-adapted retina coincides with that of the absorption of the visual pigment in the case of frogs, carps and others, though the expansion of the sensitivity curve exists in the short wave-lengths (GRANIT '41, '47).

In the present work the results in *Carpio* and *Anguilla* agreed with those obtained by GRANIT ('41). But the marine fishes caught in the water shallower than 30 metres, such as *Halichoeres* and *Dictyosoma*, showed the maximum sensitivity in longer wave-lengths of the spectrum than in the case of the investigation by GRANIT. According to GRANIT ('41) the photopic sensitivity curve is composite consisting of narrow and broad elementary sensitivity bands. And maxima of those elements seem to be found over the wide range of the spectrum. Therefore it is assumed that the maximum and the submaximum of the sensitivity curve appeared in the present work suggest the existence of several kinds of elements. From above consideration, red sensitive elements which are assumed to be photopic elements, are dominant in the retina of shallow water fishes. It may also be assumed that the spectral sensitivity curve having the maximum in the range of long wave-lengths, has some correlation with the characteristics of the fishes having life-long habitat in shallow water or having the typical diurnal nature. In fact, it is said by many fishermen that *Halichoeres* sleeps at night, and also the observation in the aquarium shows that the fish lies hidden motionless under the sand of the bottom at night.

Here, fears are not to be entertained that the element of scotopic character may have been removed by the experimental procedure in the case in which the photopic character seemed to be dominant, since the experiment was done under dark-adaptation and the pigment layer of the retina had been removed with careful procedure to avoid loss or destruction of rods. The retina which showed clearly photopic character in this experiment, will be the cone retina in some cases. In fact no existence of rods has been histologically found in the retina of *Halichoeres* and *Stephanolepis* (TAKATSUJI '39).



WALD *et al.* ('57) have investigated the absorption curve of the visual pigment in six species of marine fishes involving lancet fish, *Alepisaurus ferox*, and have described that the maximum spectral absorption of the visual pigment of the fishes varied with the depth of their habitats. Although the spectral sensitivity of so-called deepsea fishes ordinarily found below 200 fathoms could not be investigated in the present work, the tendency pointed out by WALD *et al.* was confirmed by the present result obtained from the retinal sensitivity investigated in many species of fishes inhabiting the continental shelf of the sea, as shown in Fig. 55. And also the fact that the result obtained in Fig. 55 is corresponding to the change in the spectral distribution of solar energy penetrating the sea water, is important in regard to the fish vision.

Of the species which slip out remarkably from the relation curve shown in Fig. 55, considerations as follows may be given. In *Gymnothorax*, the reason may be found in the fact that the fish is a typically nocturnal animal and is well caught by a trawl net at night, although the fish conceals itself in the cave or among rocks near the sea shore in the daytime. In *Chrysophrys*, it is difficult from ecological view points to understand the fact that the sensitivity curve has its maximum at about 470 m $\mu$  of the spectrum, since the fish is usually found in shallow water and even the deepest place in which the fish is found, is only about 60–70 metres. And the facts that submaxima are found in 550 m $\mu$  and 600 m $\mu$  (Fig. 52a—4) and that sensitivity for wave-length of 470 m $\mu$  decreases distinctly with light-adaptation (Fig. 54—3), suggest the complicated construction of its retinal elements. However, it is interesting that the blue-sensitive element responds dominantly to light in dark-adapted retina in this species. In *Anguilla*, it is a well-known fact that the fish migrates to the extraordinarily deep sea for spawning, and so the spectral sensitivity of *Anguilla* having the maximum in 500 m $\mu$ , is understood to the point from the ecological views mentioned above.

In fishes found in the sea deeper than some 100 metres, the spectral sensitivity curve has a narrow spread against the spectrum. It is probably due to the fact that the spectral distribution curve of solar energy penetrating the sea water becomes narrower at the deep places of the sea, since the most effective wave-length in terms of their stimulating capacity is just that in which the sea water is most transparent.

The results that the shift of the maximum by light-adaptation usually appears in most of the shallow water fishes and that the submaximum appears in their sensitivity curve, while not in fishes found in the sea deeper than some 100 metres, suggest the chromatic vision of the former and the achromatic vision of the latter. SVAETICHIN and MACNICHOL ('58) described that the fishes caught at the depths of from 30 to 70 metres might possibly be cone monochromats, since both R-G and Y-B responses were not found in those fishes. Then, the results obtained from the present work support the assumption made by SVAETICHIN *et al.*, putting aside the strict limit of the depths. On the other hand the threshold intensity of the retina in fishes inhabiting the sea deeper than some 100 metres, was found to be obviously low as compared with that of shallow water fishes (Fig. 56). In consequence, it is assumed

that in these fishes the retina may be so designed as to strengthen the sensation of the brightness, instead of the development of chromatic vision. From the present experiments, however, the problem of the colour perception of fishes cannot be solved. The thorough research of colour-sensitive components is necessary to be made by the aid of micro-electrode technique.

### Summary

The spectral sensitivity of the retina was investigated in 20 species of fishes, using the size of electroretinogram (ERG) as index and it was ecologically considered from their behaviour, particularly at the depth in which the fishes live. It was found that the maximum sensitivity tended to shift toward shorter wave-lengths with the depth of habitat of fishes.

In shallow water fishes the spectral sensitivity curve had the maximum in the range of longer wave-length, and had submaximum in the curve. Particularly, in *Halichoeres*, *Dictyosoma* and *Acanthogobius*, red sensitive element or photopic element was found to be dominant. The shift of the maximum was usually found noticeably by light-adaptation.

In fishes inhabiting a sea deeper than some 100 metres the sensitivity curve had the maximum in shorter wave-length and had generally no submaximum. Moreover, in these fishes the sensitivity curve had a narrow spread against the spectrum and showed slightly or not the shift of the maximum by light-adaptation.

From these results it may be assumed that the fishes inhabiting deeper seas have achromatic vision, and shallow water fishes have chromatic vision.

The tendency was obviously shown that the threshold intensity of the retina obtained in shallow water fishes was higher than that of the fishes inhabiting deeper places.

## Chapter IV Electroretinogram in teleost fish, with reference to ecological aspects

Recently, the retina of fish has been often used as material for the investigation of sensory mechanism of vision. However, the fishes used for those studies seem to be restricted to some species of Teleostei, particularly of fresh water fish. Various types of behaviour are found in fishes; diurnal or nocturnal, pelagic or benthonic, deeper habitable or on-shore habitable and others. The characteristics of the light penetrated through sea water are different according to various conditions, especially to the depth. It is significant not only from physiological but also from ecological view point to investigate comparatively the electroretinogram (ERG) of various species including marine and fresh water fishes.

In Chapter I and Chapter II the author described the characters of the ERGs of a dog fish, *Mustelus manazo*, and rays and skates, Batoidei, and considered them with relation to ecological meaning of the fish-vision. In the present chapter the general properties of the ERG obtained from various species of teleost fish are described.

### Material and method

Through this work, 25 species of teleost fish used as the material: Most of the materials were caught with a small trawl net about 5 to 10 miles off the coast of Yamaguchi Prefecture along the Sea of Japan; some fishes with angling about 1 mile off the coast; some fishes were caught by the set-net about 0.5 miles off the coast; some others were caught with a hand net by the seashore, or in ponds and rivers. The species of these fishes are listed in Table 5, with their body-lengths.

Before the experiment, fishes were preserved for several days in the aquarium of the laboratory or in a crawl near the coast. Experiments were made with the eyes excised from the head, cut off from the body under dim red light after dark-adaptation of some hour or so.

The eye prepared in such a way was situated in a dark chamber which was shielded electrically. The light obtained from the projector lamp stimulated the whole retina through the shutter fitted on one side of the chamber. The intensity of stimulus light was controlled by the neutral filters and the intensity of stimulus light without the filters was referred to as a 100 per cent or unit intensity. Spectral light was made by the interference filter.

As an active electrode was used a slender cotton wick moistened with the saline solution, which was in contact with the eye, although the site of the electrode was a little modified with the difference of the species (*cf.* Chapter III). The other end of the wick was immersed in one arm of a U-tube containing the saline solution.

Table 5. Species of fishes used in the experiment of this chapter and its body-length.

Species	Body-length (cm)
<i>Saurida undosquamis</i>	25—30
<i>Cyprinus carpio</i>	30—40
<i>Carassius auratus</i>	15—20
<i>Misgurnus anguillicaudatus</i>	10—15
<i>Anguilla japonica</i>	30
<i>Gymnothorax reticularis</i>	60—70
<i>Trachurus japonicus</i>	15—20
<i>Sillago japonica</i>	15—20
<i>Chrysophrys major</i>	15—20
<i>Hoplognathus fasciata</i>	15—20
<i>Halichoeres poecilopterus</i>	15—20
<i>Argyrosomus argentatus</i>	15—20
<i>Dictyosoma burgeri</i>	20—25
<i>Epinephelus septemfasciatus</i>	10
<i>Lagocephalus lunaris</i>	25—30
<i>Fugu niphobles</i>	15—20
<i>Stephanolepis cirrhifer</i>	15—20
<i>Scorpaenodes guamensis</i>	20—25
<i>Inegocia crocodila</i>	20
<i>Chelidonichthys kumu</i>	15
<i>Acanthogobius flavimanus</i>	15—20
<i>Pseudorhombus cinnamomeus</i>	15—20
<i>Kareius bicoloratus</i>	15—20

From the other arm of the U-tube, Ag-AgCl electrode led to the input of an amplifier connected with a cathode-ray oscilloscope. Ag-AgCl plate covered with moistened cotton sheet was used as an indifferent electrode, on which the eye was set. The amplifier was CR-coupling and the time constant was about one second. The shutter-release was controlled by a thyatron relay connected with a single sweeper of the oscilloscope. In the case of the experiment by the micro-electrode, a capillary glass electrode filled with 3 Mol KCl with a tip diameter of *ca.* 1 $\mu$  was positioned on the inverted retina by the special micromanipulator. The retinal action potential caused by stimulus light was picked up by a 6AK5 type tube served as a preamplifier (Fig. 57), which was followed by a D.C. amplifier. The other apparatus was the same as that described previously.

The method measuring the threshold intensity of light stimulus eliciting the ERG during the course of dark- or light-adaptation was also the same as that described in Chapter II.

The experiments were carried out at room temperature ranging from 15 °C to 20 °C, except for the case described in particular. Under these experimental conditions

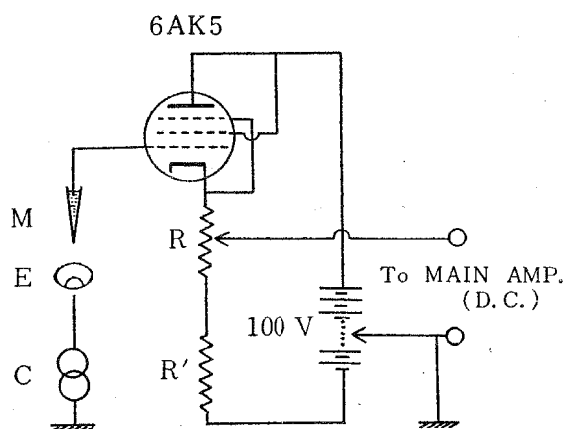


Fig. 57. Schematic representation of the method for recording the ERG by micro-electrode. M, micro-electrode; E, inverted retina; C, calibrator; 6AK5, R, R', circuit of pre-amplifier.

the preparation showed normal response (*i.e.* an ERG showing the typical wave-form) during the period of about 2 hours.

Histological preparation was made from the eye used in the physiological experiments. The eye was fixed with Carnoy's solution for 30 minutes. Thereafter, the eye had a good wash in absolute alcohol, and then lens, cornea and vitreous humor were removed from the eye. The retina prepared by such manner was imbedded in paraffin according to the usual method. Then sections were cut at the thickness of 8  $\mu$ . The section was stained with haematoxylin and eosin.

## Results

### (1) Wave-form and polarity in ERG

#### *Saurida undosquamis*

Action potential was induced from the receptor layer of the retina. Typical ERGs in *Saurida* are shown in Fig. 58,A; the ERG from dark-adapted eye elicited by weak light stimulus (about 0.02 lux) of short duration is a positive monophasic wave, and with the increase in stimulus intensity the potential comes to be followed by slow negative potential. With further increase in stimulus intensity (more than 1 lux) the positive potential decreased and it is preceded by fast negative potential. The amplitude of the initial negative potential augments with the further increase in stimulus intensity. But sometimes the fast initial negative potential was not observed in the ERG elicited with intense light stimulus as shown in Fig. 58, B and C.

In this case the time-course of the response prolonged and the amplitude of the response decreased a little with the increase in stimulus intensity (Fig. 58,B). In a certain case, only slow negative potential was observed, and the time-course of the potential prolonged also with the increase in stimulus intensity. The amplitude of

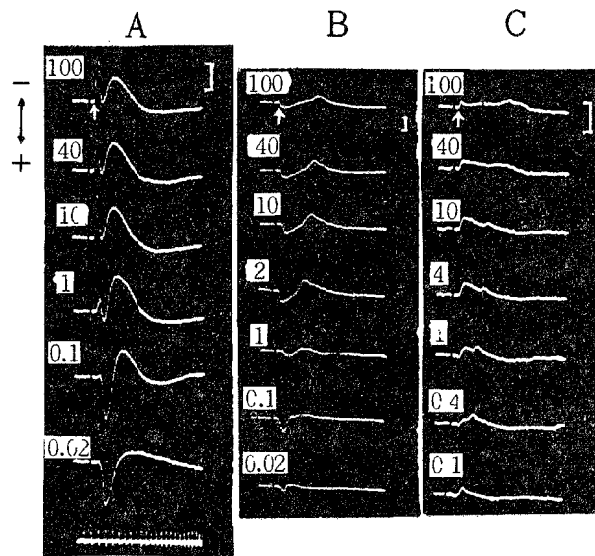


Fig. 58. ERGs recorded from the receptor layer of *Saurida* eye. Test flash is  $1/25$  sec. Numeral on each series of records represents the stimulus intensity in the percentage of unit intensity (500 lux). Time mark:  $1/6$  sec. Calibration voltage: 0.1 mV. Polarity of the record is upward negative.

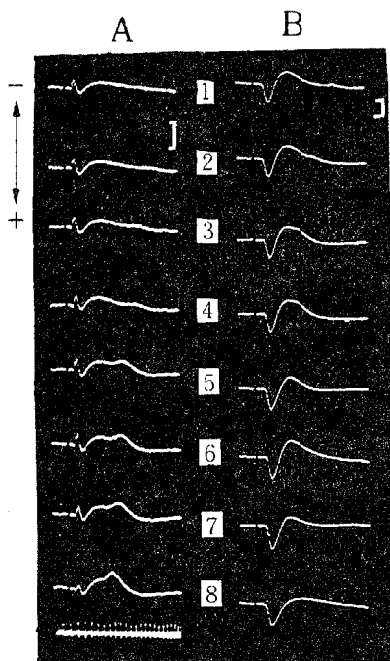


Fig. 59. ERGs from the receptor layer of *Saurida* eye. Stimulus intensity is 10% of unit intensity in A and 0.1% in B. The durations of stimulus light in 1—8 are 1,  $1/2$ ,  $1/5$ ,  $1/10$ ,  $1/25$ ,  $1/50$ ,  $1/100$  and  $1/300$ , respectively. Other legends are same as in Fig. 58.

the response was the largest in the case of the stimulation with moderate intensity (Fig. 58, C).

The ERGs elicited by stimulus light of various durations with constant intensity are shown in Fig. 59. In the case of stimulation with intense light (50 lux) the amplitude of the initial fast negative potential was unchanged irrespective of the various durations of stimulus light except for the stimulation of extremely short duration of 3.3 msec. But the amplitude of the slow negative potential which followed to the positive potential, decreased with the increase of stimulus duration, and finally the slow potential was suppressed completely at the duration longer than  $1/5$  sec. Off-response did not occur in the stimulation of about 1 sec., at least (Fig. 59, A).

In the case of stimulation with weak light (about 0.5 lux) the amplitude of positive potential was almost unchanged regardless of the duration, but the amplitude of the slow negative potential following to the positive potential, increased with the prolongation of the stimulus duration and it attained a plateau at the duration above 1/50 sec. In the case of stimulation of the duration above 1/2 sec. a small negative off-response was found unnoticeably.

*Cyprinus carpio*

The active electrode was in contact with the receptor layer of the retina. Typical wave-forms of the ERG are shown in Fig. 60. The records shown in Fig. 60,A were obtained from the light-adapted retina, and the polarity of the ERG showed to be cornea negative. Oscillation of the resting potential was noticeably observed from the ERGs recorded. Even in the case of stimulation with weak light, remarkable positive monophasic wave was not elicited. With the increase in stimulus intensity, however, the negative potential was followed by slow positive potential. In dark-adapted retina (Fig. 60, B) the response was positive potential followed by negative one in the stimulation with weak light. While, with intense light, the positive potential was preceded by a small negative potential and the positive potential was remarkably suppressed. In the case of stimulation of long duration (Fig. 60,C), the response to weak light was almost the same with that of short duration in the dark-adapted retina, but the response to intense light showed the decrease of the positive potential. In this case the response seemed to elicit the slow positive potential as off-effect.

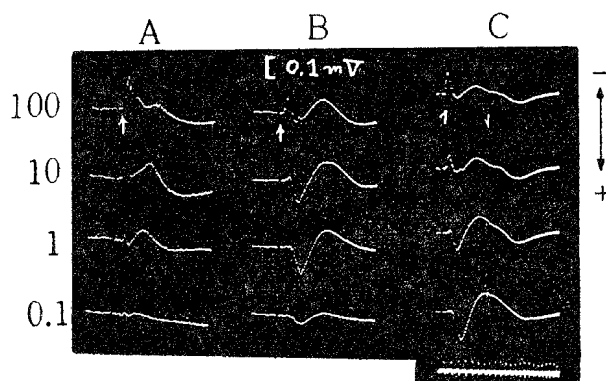


Fig. 60. ERGs recorded from the receptor layer of *Cyprinus* eye. A, from light-adapted eye; test flash, 1/25 sec. B and C, from dark-adapted eye; test flash, 1/25 sec. in B and 1 sec. in C. Other legends are same as in Fig. 58.

The records shown in Fig. 61,A were the ERGs elicited by a series of light stimuli of various intensity. From the records it is assumed obviously that the ERG contains two kinds of component, each of which occurs dominantly in the stimulations with intense and weak light respectively, and then two components are canceled each other in the stimulation with moderate intensity. And from the records it is

clearly seen that the notch on the rising phase of the positive potential shifts to negative potential with the increase in stimulus intensity. In Fig. 61, B the same ERGs with that of A were recorded in fast sweep.

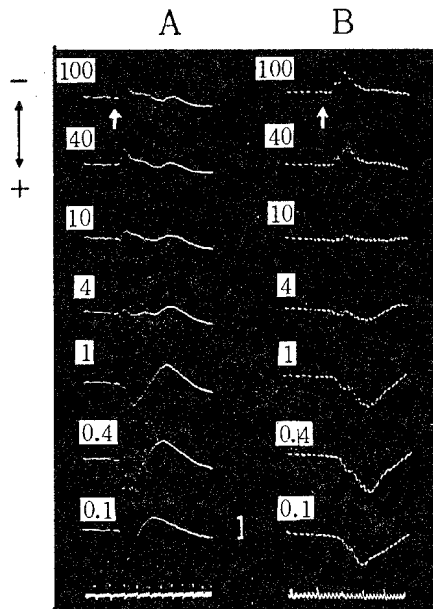


Fig. 61. ERGs from the receptor layer of *Cyprinus* eye. A, showing the change in wave-form according to the variation in stimulus intensity. B, ERGs recorded by fast sweep. Other legends are same as in Fig. 58.

#### *Misgurnus anguillicaudatus*

Action potential was led from the corneal surface of the eyeball. Typical wave-forms of the ERG are shown in Fig. 62. The ERG elicited from the dark-adapted eye was a small negative potential followed by a large positive potential in stimulation of short duration, but it was positive potential in the case of stimulation with extremely

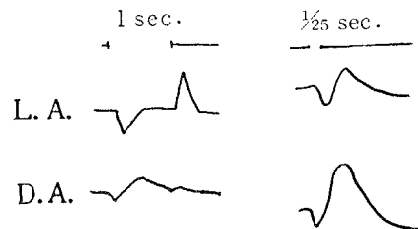


Fig. 62. Typical wave-forms of ERGs recorded from the cornea surface of *Misgurnus* eye. Upper line of the records represents the stimulus signal. Stimulus intensity is 300 lux. L. A., in light-adapted eye; D. A., in dark-adapted.

weak light as shown in Fig. 63, A and B. In the stimulation of long duration, off-response showed a small positive potential unnoticeably (Fig. 62).

In the light-adapted eye, negative potential became remarkable, and positive potential was suppressed slightly (Figs. 62 and 63, C). In the stimulation of long duration the response showed remarkable negative on- and positive off-effects, and positive potential of on-effect was completely suppressed in thoroughly light-adapted eye. The ERGs of dark- (A) and light- (B and C) adapted eyes elicited by a series of light stimuli of various intensity are shown in Fig. 63.

#### *Anguilla japonica*

Action potential was induced from the corneal surface of the eyeball. The typical ERGs recorded in various light conditions are shown in Fig. 64. In dark-adapted eye the ERG elicited by weak light stimulus of short duration showed a positive monophasic wave, but by intense light a small negative wave preceded to the positive one (Fig. 64, A). Sometimes a notch was found on the rising phase of the positive potential. In the case of stimulation of long duration the off-response was hardly



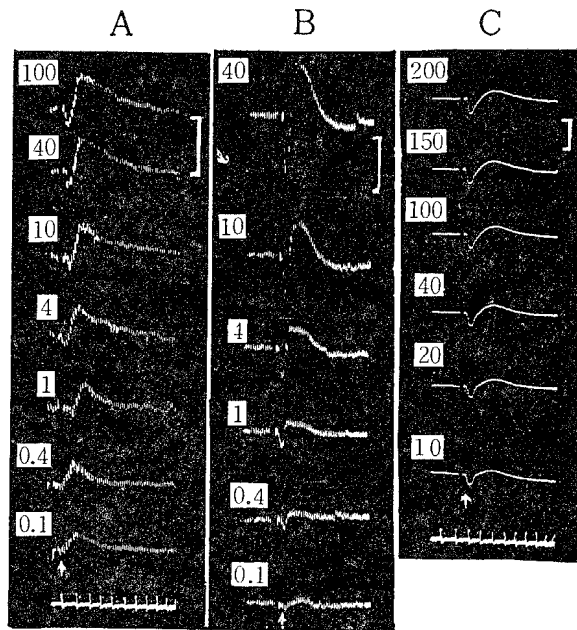


Fig. 63. ERGs recorded from the cornea surface of *Misgurnus*, according to the variation in stimulus intensity. A, ERG recorded from dark-adapted eye; B, from slightly light-adapted eye; C, from light-adapted eye. Duration of test flash is 1/50 sec. Time mark: 1/6 sec. Calibration voltage: 0.1 mV. Polarity of the record is upward positive.

found irrespective of the stimulus intensity (Fig. 64,B).

The ERG from light-adapted eye showed negative potential remarkably, and also showed a positive off-response unnoticeably in the stimulation with intense light of long duration, although the retinal sensitivity decreased remarkably (Fig. 64, L).

Effects of various reagents on the ERG: In order to investigate the effect of potassium ion, 10% solution of KCl was applied to the cotton sheet contacting with the sclera of the eye. After the application of the solution the latency of the positive potential of the ERG prolonged immediately. Thereafter, the amplitude of the positive potential decreased rapidly, and only a negative monophasic wave was left to occur 5 minutes later (Fig. 65).

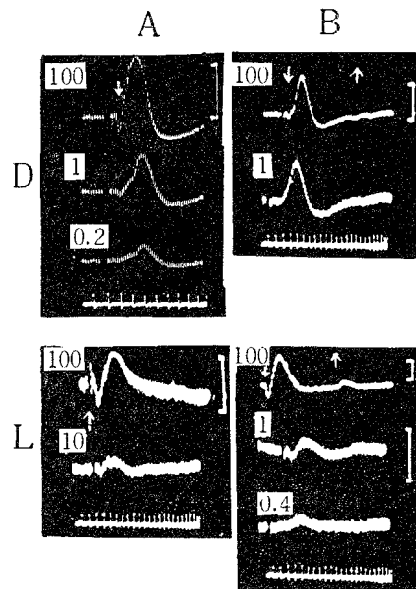


Fig. 64. Typical wave-forms of ERGs recorded from the cornea surface of *Anguilla*. Stimulus duration is 1/50 sec. in A and 1 sec. in B. D, from dark-adapted eye; L, from light-adapted eye. Other legends are same as in Fig. 63.

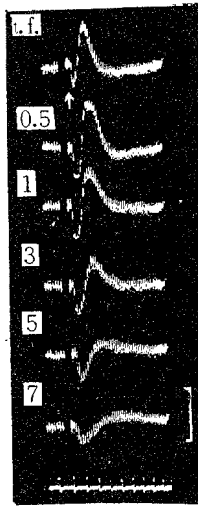


Fig. 65. Effect of KCl on the ERG of *Anguilla*. Test flash is unit intensity and 1/50 sec. in duration. Numerals show the time elapsed after the application of KCl. t.f., before the application. Other legends are same as in Fig. 63.

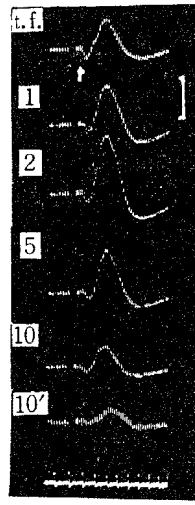


Fig. 66. Effect of 10% alc. Ringer sol. on the ERG of *Anguilla*. Test flash is 60% of unit intensity and 1/50 sec. in duration. 10', stimulus intensity was lowered in one sixth. Other legends are same as in Fig. 65.

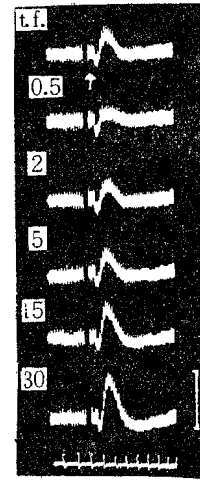


Fig. 67. Effect of 0.01% strychnine on the ERG of *Anguilla*. Test flash is unit intensity and 1/50 sec. in duration. Other legends are same as in Fig. 65.

As 10% Ringer alcohol was applied to the eye by the same manner as in the case of KCl, the positive potential of the ERG increased transitorily 2 minutes later, but it began to decrease soon after that. The suppression of negative potential of the ERG was not complete in 5 minutes after the application, except in the case of stimulation with weak light (Fig. 66).

In the application of 0.01% strychnine the responses caused by the test flash were as follows; the positive potential of the ERG was transitorily suppressed, immediately after the application. Two minutes later, however, the positive potential recovered the amplitude, and thereafter increased slowly and attained to the value of about 2 times as high as the initial amplitude. On the contrary, the negative potential was not affected at all (Fig. 67). One per cent strychnine suppressed the response at all.

As the solution of urethan was often used for the anesthesia of fish, the effect of urethan was investigated. When 5% solution of urethan was applied to the retina by the same manner as in the case of KCl, the response to the test flash was as follows; the amplitude of the response decreased a little immediately after the application, thereafter the hump on the rising phase of b-wave became remarkable. Five minutes later, however, the amplitude of b-wave began to increase and came to involve the hump several minutes later. Finally, the response returned to the initial magnitude 20 minutes later. The negative potential transitorily decreased but it

seemed to recover the normal response in half hour after the application (Fig. 68,A). On the contrary, in application of 20 % urethan the response began to decrease immediately after the application and it disappeared 15 minutes later (Fig. 68, B).

From the results it is concluded that the ERG is suppressed in high concentration of urethan but is augmented transiently in low one, presumably of less than 5 %.

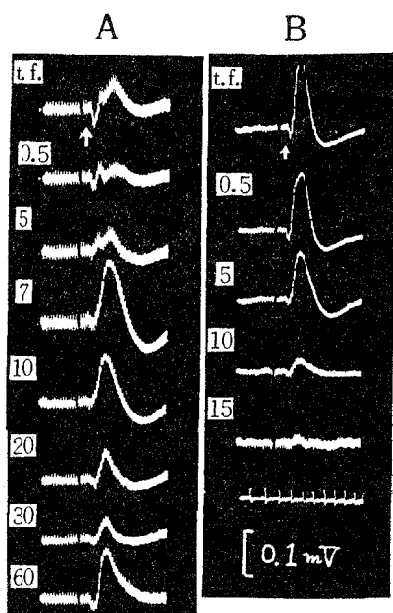


Fig. 68. Effects of 5 % (A) and 20 % (B) urethan sol. on the ERG of *Anguilla*. Other legends are same as in Fig. 65.

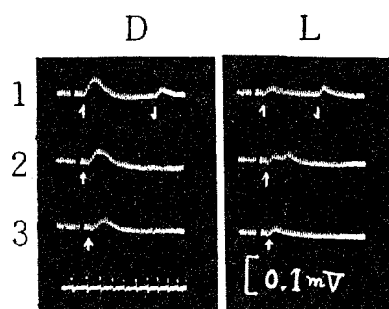


Fig. 69. Typical wave-forms of ERGs recorded from the cornea surface of dark- (D) and light- (L) adapted eye, in *Gymnothorax*. The durations of test flash in 1—3 are 1 sec., 1/10 sec. and 1/100 sec., respectively. The intensity of test flash is 1 % of unit intensity. Other legends are same as in Fig. 63.

### *Gymnothorax reticularis*

Action potential was induced from the corneal surface of the eyeball. The ERG was composed of only positive wave, irrespective of the stimulus intensity.

As illustrated in Fig. 69 the ERG from the dark-adapted eye was a positive monophasic wave and it was only on-effect in the case of stimulation of the duration less than 1/10 sec. In the case of stimulation of longer duration than 1/5 sec., the ERG showed positive off-potential, and the amplitude of the off-potential was always lower than that of on-potential. In the light-adapted eye the sensitivity of the retina decreased, and positive off-response was caused by the stimulus light of shorter duration than that in the case of the dark-adapted eye. In this case the amplitude of off-potential was always larger than that of on-potential.

The ERGs for a series of various stimulus intensity are shown in Fig. 70. Though the amplitude of the positive potential increased with the intense stimulus light, rate of the increase in amplitude was relatively small. The rising and falling rates of the potential became large with the increase in stimulus intensity, but no remarkable changes in latency were found. The effect of potassium ion on the ERG is shown in Fig. 71. As illustrated in the figure, a small negative potential came to occur preceding to the positive one with latent time of about 30 msec. as soon as 10 % solution of KCl was applied to the sclera. Thereafter, the positive potential

disappeared within a few minutes after the application of the solution. And moreover the negative potential elicited by KCl solution was observed to be apt to augment with the increase in stimulus intensity. Consequently, the ERG of *Gymnothorax*, positive monophasic wave, is inferred to involve negative component.

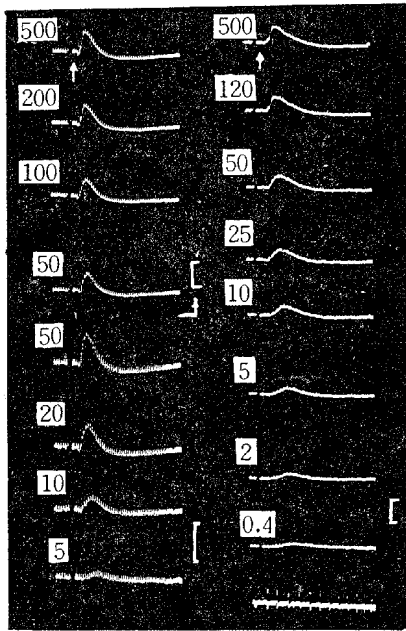


Fig. 70. ERGs recorded from dark-adapted eye of *Gymnothorax* according to varying the strength in stimulus light. Test flash is 1/50 sec. in duration. Other legends are same as in Fig. 63.

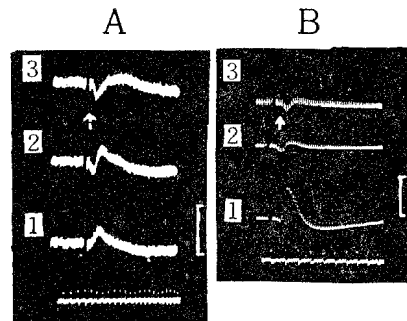


Fig. 71. Effects of KCl sol. on the ERG of *Gymnothorax*. Test flash is 10% and 1/50 sec. in A, and 60% and 1/25 sec. in B. 1, before the application; 2 immediately after that; 3, 3 minutes after that. Other legends are same as in Fig. 63.

### *Trachurus japonicus*

Action potential was induced in two ways, from the inner surface and from the receptor side of the retina.

In the case of the induction from the inner surface of the retina, the ERG from the dark-adapted eye elicited by the stimulus light of short duration was a negative potential followed by a slow positive potential, and the amplitude of the positive potential was considerably low. With the increase in stimulus intensity, the amplitude and the rising rate of the negative potential were increased. In the case of stimulation of long duration the ERG showed a negative on-potential and a small positive off-potential. In the light-adapted eye, the ERG showed a positive off-potential noticeably although the sensitivity of the retina decreased remarkably.

In the case of the induction from the receptor side of the retina, various types of the ERG were obtained as shown in Fig. 72. The ERG of the dark-adapted eye in the response to weak stimulus light of short duration was a negative potential dominantly. But, with the increase in stimulus intensity another potential came to occur, and so that the ERG showed two humps on the negative wave. With the

intense stimulus light the splitting of the negative potential became remarkable, and the amplitude of the initial negative potential became larger than that of the secondary potential (Fig. 72, A and B). In the case of stimulation of long duration, however, the ERG showed negative on-response and negative-positive off-response. Namely, the remarkable off-response appeared. With the intense stimulus light the amplitude of off-response augmented noticeably, while that of on-response did not show any change in wave-form, irrespective of the increase in stimulus intensity (Fig. 72,C).

On rare occasions, the positive potential preceded to the negative one, and the splitting of both potentials occurred in the intense stimulus (Fig. 72,D).

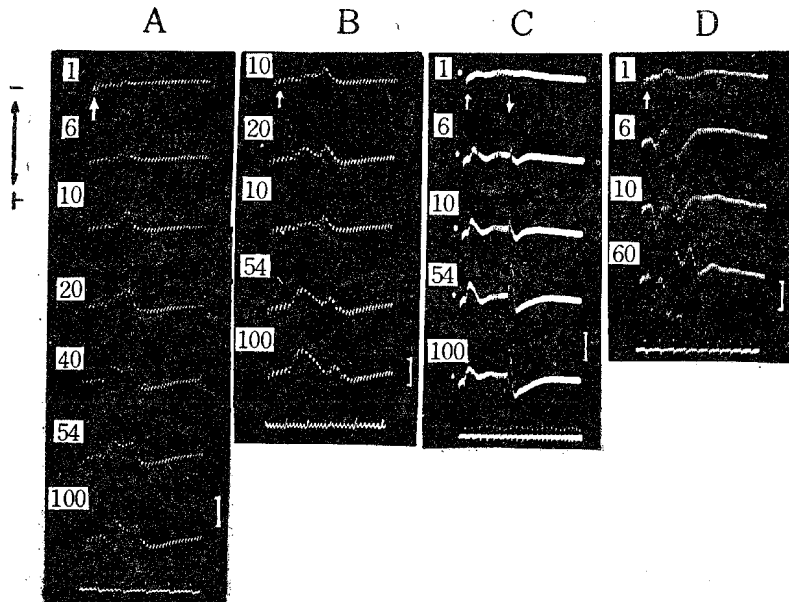


Fig. 72. Various types of ERG recorded from the receptor layer of dark-adapted retina of *Trachurus*. Stimulus duration is 1/50 sec. in A, B and D, and 1 sec. in C. Other legends are same as in Fig. 58.

In the case of stimulation of various durations two types of the response were found; one was the same response with that mentioned above (Figs. 72, D and 73, B), the other was the response consisting of negative plateau on-response and remarkable off-response (Fig. 73, A). And the off-response was negative potential followed by positive potential, and the response to stimulus light of short duration showed the same wave-form as that of off-effect occurred in the response to the stimulus of long duration.

There were no effects of the state of dark- or light-adaptation on the response induced by such way, as shown in Fig. 74.

#### *Sillago japonica*

The ERG was recorded by the aid of micro-electrode. The electrode was inserted into the inverted retina until the maximum amplitude of the response to stimulus

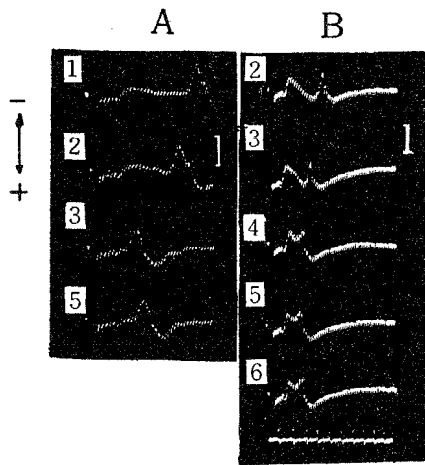


Fig. 73. ERGs recorded from the receptor layer of the retina of *Trachurus*. The stimulus durations in 1—6 are 1/2, 1/5, 1/10, 1/50, 1/100 and 1/250 sec., respectively. Stimulus intensity is 50% of unit intensity. Other legends are same as in Fig. 58.

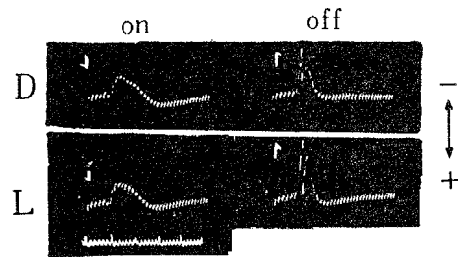


Fig. 74. On- and off-responses in the ERG of *Trachurus*. The stimulus duration is about 5 secs. and the intensity is 50% of unit intensity. The ERG was induced from the receptor layer of dark-(D) and light-(L) adapted eyes. Other legends are same as in Fig. 58.

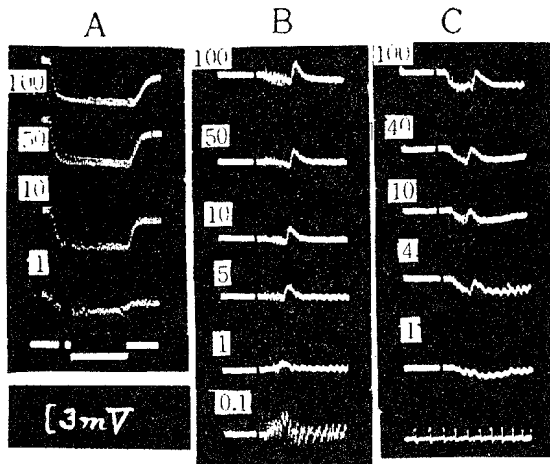


Fig. 75. Various types of S-potential recorded in *Sillago*. Stimulus duration is 1 sec. in A, 1/10 sec. in B, C. Numeral on each series of records represents stimulus intensity in % of unit intensity. Calibration voltage: 3 mV. Time mark: 1/6 sec.

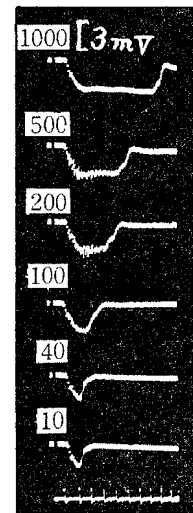


Fig. 76. S-potential in *Sillago*, according to variation in stimulus duration. Numeral on each record shows the stimulus duration in msec. Time mark: 1/6 sec.

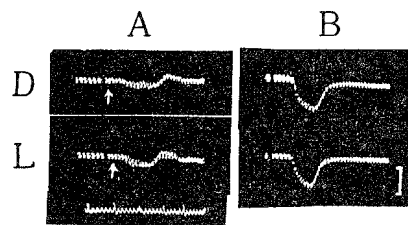


Fig. 77. Effect of the state of dark-(D) and light-(L) adaptations on S-potential, in *Sillago*. Stimulus duration is short (1/250 sec.) in A and is long (1/10 sec.) in B. Stimulus intensity is unit intensity. Time mark: 1/6 sec.

light was obtained. The ERG elicited from the dark-adapted retina showed typical S-potential as shown in Fig. 75,A. On the other hand, peculiar forms of the ERG as mentioned below in *Chrysophrys* were also observed in this species. In the records of Fig. 75,B, the ERG showed positive off-potential remarkably. With the increase in stimulus intensity the latency of off-potential prolonged, and the amplitude of off-potential augmented somewhat. In the case of stimulation with low intensity light the response was positive slow potential, while with high intensity, negative slow potential superimposed with spike potential appeared, preceding the positive potential. In the records of Fig. 75, C the ERG seemed to be composed of the ERGs of A and B in the same figure. And also in the case of stimulation with weak light slow spontaneous deflections are superimposed on, or followed to the response (Fig. 75, B and C). In varying duration of light stimulus with constant intensity, change in shape of negative potential was obtained as illustrated in Fig. 76. A spike-like response was recorded in stimulus light of the duration than about 100 msec. After the exposure of the retina to light of about 10 lux, no influences of the light adaptation on the ERG were found, except for the reduction of off-latency (Fig. 77).

#### *Chrysophrys major*

i) In the case of the induction of ERG from the inner-surface of the retina, the ERG from the dark-adapted eye elicited by stimulus light of short duration was a large negative potential followed by a slow positive potential, as shown in Fig. 78,A. With the increase in stimulus intensity, the ERG showed the similar change to that described on the former species. In this species the amplitude of the slow positive potential was extremely low. In the case of stimulation of long duration on-response was negative, and off-response was noticeable positive potential (Fig. 78, B). With the increase in stimulus intensity the amplitude of on-response (negative potential) augmented, while the amplitude of off-response was the largest in moderate intensity of stimulus light (Fig. 78, B).

ii) In the case of the induction from the receptor side of the eye, several types of the response were obtained as shown in Fig. 79. The records represented in A of the figure were the ERGs elicited by stimulus flash in the dark-adapted eye, and they were just the mirror image of Fig. 78,A. These were most common responses. The records of B were the ERGs obtained from the light-adapted retina, and the time-

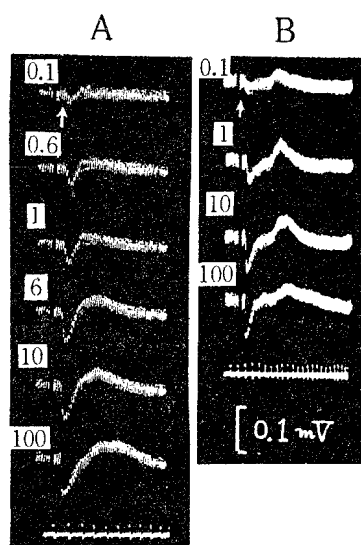


Fig. 78. ERGs recorded from the inner surface of the retina of *Chrysophrys* according to light stimuli of various intensity. Stimulus duration is short (1/50 sec.) in A and is long (1 sec.) in B. Other legends are same as in Fig. 63.

course of the response was found to prolong noticeably with the increase in stimulus intensity, though the remarkable change in amplitude of the ERG was not found. In the records of C, the positive potential preceded the negative potential, and the amplitude of both potentials decreased with the increase in stimulus intensity. In these cases, moreover, the fluctuation (about 8 c/s) of potential was found to be superimposed on the ERG elicited by stimulus light with very low intensity.

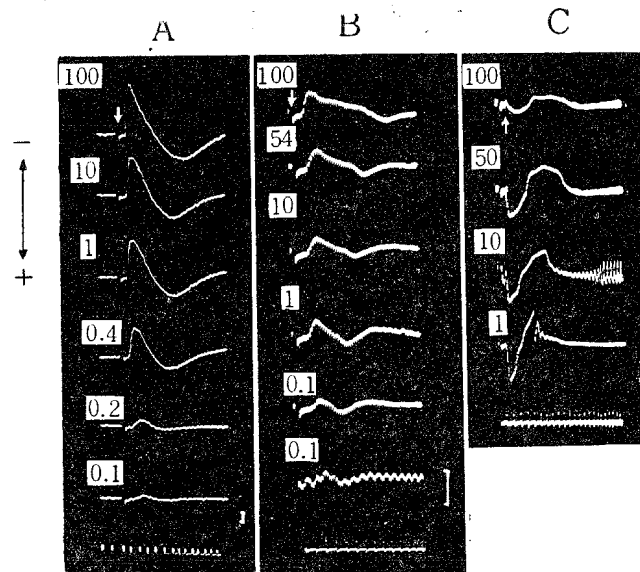


Fig. 79. Various types of the ERG recorded from the receptor layer of the retina of *Chrysophrys*. A, C, from dark-adapted eye; B, from light-adapted eye. Stimulus duration is 1/250 sec. Other legends are same as in Fig. 58.

In the case of stimulation of long duration (about 5 seconds) on- and off-effects on the ERG were investigated under various intensity of stimulus light and under dark- or light-state of the adaptation. The records shown in Fig. 80 were obtained from the dark-adapted eye.

On-response was negative potential and the amplitude of the negative potential increased with the increase in light intensity. The off-response was negative potential followed by slow positive one, and the amplitude of the negative potential was not almost changed with regard to stimulus intensity, although the negative off-potential was decreased by the extremely intense light.

The records shown in Fig. 81 were obtained from the light-adapted eye. On-response was negative potential, too and the rising and falling rates of the potential were small relatively. Off-response was only negative potential and it appeared remarkably as compared with that of the dark-adapted eye, although the off-response to stimulus light with very low intensity was positive monophasic wave. In this case, moreover, the fluctuation of the potential was found superimposing or following the off-response.



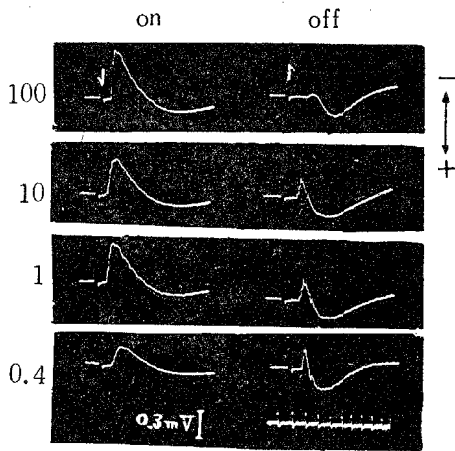


Fig. 80. Effect of stimulus intensity on the on- and off-responses of dark-adapted retina of *Crisophrys*. Stimulus duration is about 5 secs. The response was recorded from the receptor layer. Other legends are same as in Fig. 58.

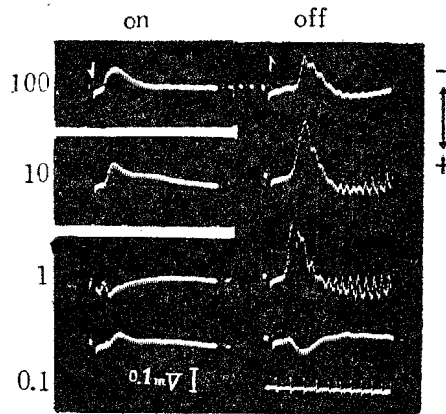


Fig. 81. Effect of stimulus intensity on the on- and off-responses of light-adapted retina of *Crisophrys*. Stimulus duration is about 5 secs. The response was recorded from the receptor layer. Other legends are same as in Fig. 58.

iii) By the aid of micro-electrode, action potential was induced from the inverted retina. The ERG elicited by the stimulus light of unit intensity (about 200 lux) and duration of 100 msec. showed the various forms as shown in Fig. 82 ; 1) the ERG

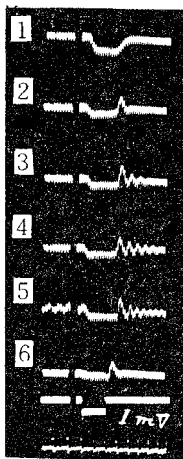


Fig. 82. Various types of S-potential recorded from *Crisophrys*. The lowest line shows stimulus duration. Time mark : 1/6 sec.

was negative potential and the maximum level of the potential remained unchanged as long as the stimulus lasted. The amplitude of the potential was about 1.5 mV; 2) at the cessation of the stimulus light, the negative steady potential was followed by positive off-response which ran over the resting level; 3) the off-response mentioned above was still followed by few wave-lets of spontaneous electrode negative deflections; 4) the spontaneous response continued for several minutes after the cessation of the stimulus light; 5) such repeated slow spontaneous deflections were blocked during the period of the stimulus light; 6) only positive off-response rarely occurred irrespective of the stimulus duration. Among these responses the form 1) seems to be similar to that of the S-potential (SVAETICHIN '53).

The records shown in Fig. 83 were the ERGs elicited by stimulus light with various intensity of constant duration from the dark- and light-adapted retinae. Though there was no influence of the the state of light-adaptation on the amplitude of the negative steady potential, off-latency of the potential prolonged remarkably in light-

adaptation. And also positive off-deflection appeared noticeably, and the rising rate of the negative potential became great in the dark-adapted retina. The time-course of the response prolonged remarkably with the increase in stimulus intensity (Figs. 83 and 84).

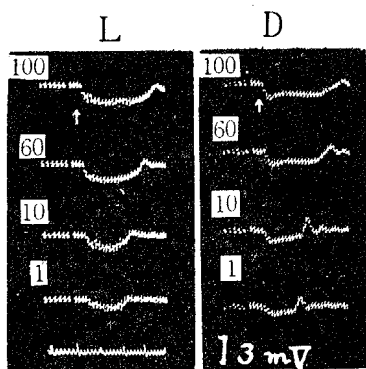


Fig. 83. Influence of the state of light- (L) or dark- (D) adaptation on S-potential in *Chrysophrys*. Other legends are same as in Fig. 75.

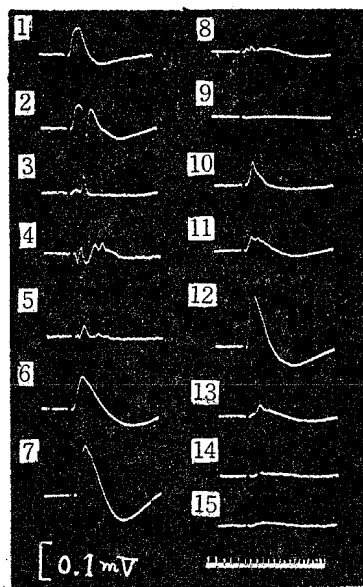


Fig. 85. Showing the various wave-forms of the ERG recorded from different localities of *Chrysophrys* retina. Numeral at each record represents the location of the point at which the tip of the electrode is set, as shown in lower schema. The response is recorded from receptor layer of the retina. Test flash is 1/50 sec. and unit intensity.

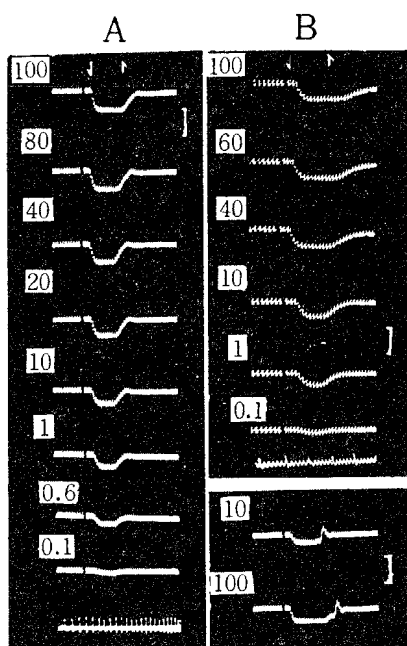


Fig. 84. S-potential recorded from *Chrysophrys* according to variation in stimulus intensity. A, normal form; B, unusual form. Other legends are same as in Fig. 75.

Topographic variation of the ERG: It is well known in fish that the retinal structure is different according to the localities of the retina. Therefore, it was

investigated whether the ERG elicited in one region of the retina differed or not from that in the other region. The results are shown in Fig. 85. In *ora serrata* the ERG is generally small potential, while in the anterior part of the retina it is the large potential of relatively simple form (6, 7 and 12 in Fig. 85). On the other hand in the posterior and ventral regions of the retina the response shows the complicated wave-form. It is known generally that fish has many twin cones or great density of the receptor cells in the ventral and posterior regions of the retina (WUNDER '25, DETWILER '43, TAMURA '57, *et al.*). So that it is supposed that the complicated wave-form of ERG mentioned above will be related to the local differences of the retinal structure, and it is assumed also that the regional differences observed in the electrical response from the visual center (KONISHI '59) concern with in any way with such local differences of peripheral responses found in retina.

#### *Halichoeres poecilopterus*

Action potential was induced from the receptor side of the retina. The ERG from the dark-adapted eye elicited by stimulus light of short duration was a negative potential followed by a positive one. The change in wave-form with the increase in stimulus intensity is shown in Fig. 86,A. In the case of stimulation of long duration the ERG showed negative on-potential, and no off-potential. In the stimulation with extremely intense light, however, the off-potential appeared as a small negative one (Fig. 86,B).

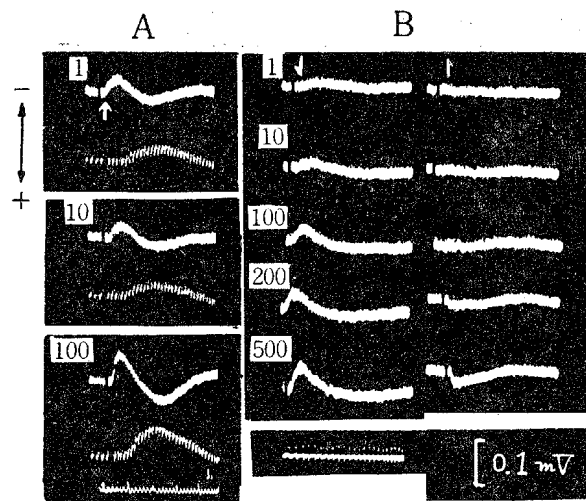


Fig. 86. ERGs of *Halichoeres*. ERG is recorded from the receptor side of the retina. Stimulus duration is short (1/50 sec.) in A and is long (about 3 secs.) in B. Other legends are same as in Fig. 58.

There was no remarkable effect of light- or dark-adaptation on the wave-form of the ERG, although the amplitude of the ERG slightly increased, and the time-course of the ERG was apt to shorten a little by light-adaptation (Fig. 87).

*Lagocephalus lunaris*

i) Action potential was induced from the receptor side of the retina. The ERG from the dark-adapted eye elicited with stimulus light of short duration (40 msec.) was a negative potential followed by a positive potential, as shown in Fig. 88. Although the ERG showed the negative monophasic wave in the stimulation with weak light, it came to be followed by the positive one with the increase in stimulus intensity, and it was seen in the stimulation with the high intensity light that both potentials were almost the same in amplitude. Other changes in the wave-form caused by the increase in stimulus intensity were observed as usual. In the stimulation of longer duration than 1/10 sec. the marked positive off-response appeared, and the positive on-potential came to be suppressed with the prolongation of stimulus duration thereafter (Fig. 89).

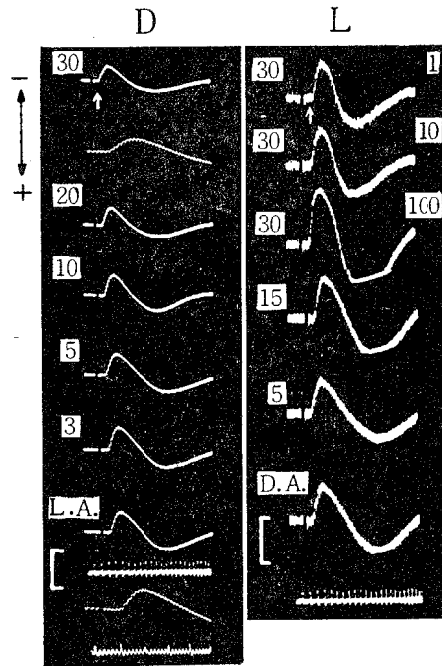


Fig. 87. Showing the ERGs of *Halichoeres* obtained in the course of light-(L) and dark-(D) adaptations. The ERG was recorded from the receptor layer of the retina. Test flash is 1/250 sec. of unit intensity. Numerals in left side of the records indicate the time elapsed in the adaptation. Other legends are same as in Fig. 58.

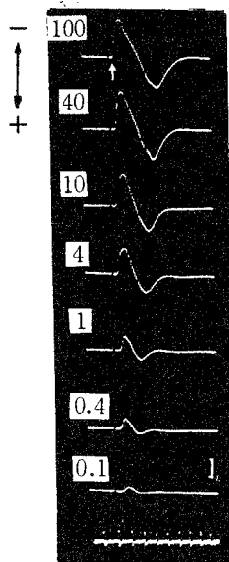


Fig. 88. ERGs recorded from the receptor layer of the retina in *Lagocephalus* in accordance with variation in stimulus intensity. Test flash is 1/25 sec. Other legends are same as in Fig. 58.

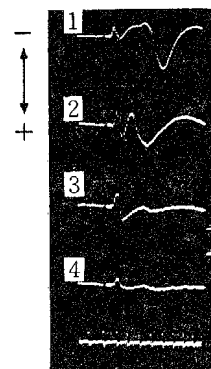


Fig. 89. ERGs recorded from the receptor layer of the retina in *Lagocephalus*. Stimulus durations in 1-4 are 1/2, 1/10, 1/50 and 1/300 sec., respectively. Stimulus intensity is 0.1% of unit intensity. Calibration voltage: 0.1 mV.

ii) Action potential was induced from the receptor side of the retina by the aid of micro-electrode. Though the ERG from the dark-adapted eye elicited by stimulus light was a negative steady potential, typical S-potential was not obtained in this species. In general, the rising and falling rates of the negative potential were variable with the change in stimulus intensity. It is found in the records of Fig. 90 that the change of stimulus intensity affects the time-course of the ERG as well as the amplitude of the ERG. The ERG was apt to show the square shaped potential in the stimulation with intense light. In the records of B and C of the figure the impulse superimposed on the ERG was seen. In this case it was observed that the impulse was easily recorded by low stimulus intensity.

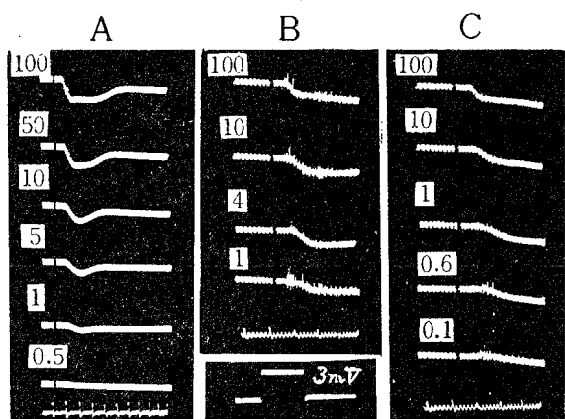


Fig. 90. S-potential in *Lagocephalus*. Stimulus duration is 1/10 sec. in A, B and 1 sec. in C. Other legends are same as in Fig. 75.

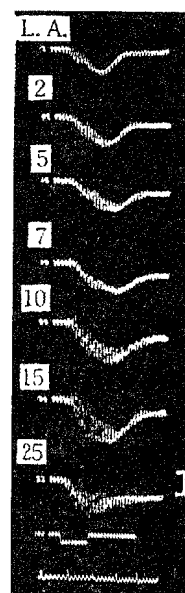


Fig. 91. Change in wave-form of S-potential recorded in the course of dark-adaptation. Numeral on each record represents the time elapsed in the adaptation. Square wave of lower record shows the stimulus duration. Calibration voltage : 3 mV. Time mark : 1/6 sec.

With the progress of dark-adaptation the falling rate of the negative potential decreased. This phenomenon was remarkable during the first 5 to 7.5 minutes of the dark-adaptation. The response showed a slight increase in amplitude during 15 to 20 minutes after the beginning of the dark-adaptation (Fig. 91).

#### *Fugu niphobles*

i) Action potential was induced from the receptor side of the retina. In the dark-adapted retina (Fig. 92,D), the ERG elicited by stimulus light of short duration was positive potential followed by negative potential, and both potentials were almost same in amplitude. And with intense stimulus light, a small negative potential preceded the positive potential. In the case of stimulation of long duration the ERG elicited by weak light showed positive on- and negative off-potential, while in the ERG elicited by intense light on-potential was a fast small negative one and moreover no off-potential was seen.

In the light-adapted retina (Fig. 92,L), the wave-form of the ERG was almost similar to that from the dark-adapted one, but the time-course of the response was usually shorter, and in the case of stimulation of long duration the noticeable positive off-potential was seen.

ii) ERGs were recorded by the aid of micro-electrode from the inverted retina.

Magnitude of the ERG showed no change according to the depth of the electrode tip, within a range about 50 $\mu$  from the surface.

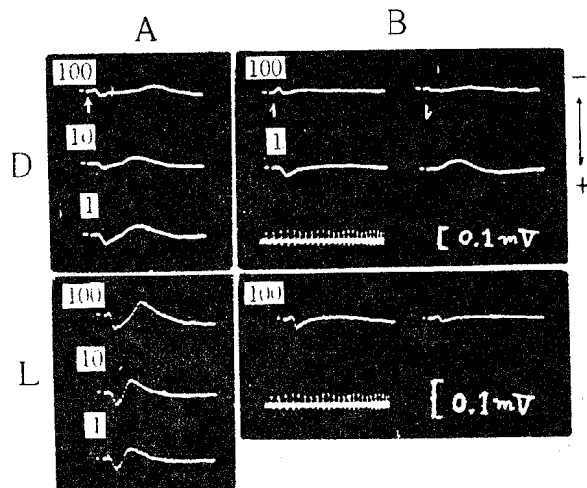


Fig. 92. ERGs recorded from the receptor layer of dark- (D) and light- (L) adapted retinæ in *Fugu*. Stimulus duration is 1/250 sec. in A and about 3 secs. in B. Other legends are same as in Fig. 58.

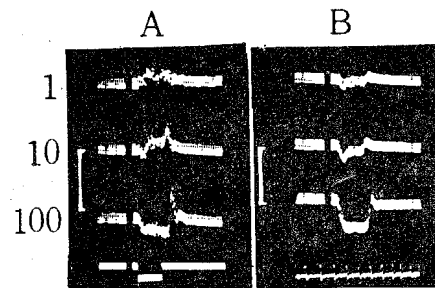


Fig. 93. S-potential recorded in dark- (A) and light- (B) adapted retinæ in *Fugu*. Numerals show stimulus intensity (100 = 500 lux), and the square wave shows stimulus duration. Time mark : 1/6 sec. Calibration voltage : 1 mV.

The ERG elicited by the stimulation with unit intensity was quite similar to the typical S-potential. The potential was frequently followed by a small positive off-deflection, which ran over the resting level in the dark-adapted retina, while not in the light-adapted one (Fig. 93).

With the increase in stimulus intensity the following changes were seen in the ERG. In the dark-adapted retina, the ERG was positive monophasic wave when the stimulus light was weak, negative on- and positive off-responses appeared in moderate light stimulus, and in intense light stimulus the ERG was negative square shaped potential followed by positive off-deflection (Fig. 93,A).

The similar changes were also observed in the light-adapted retina (Fig. 93,B), however the positive off-deflections were not so remarkable. From these records it is suggested that the ERG in *Fugu* is composed of positive and negative monophasic components and the negative potential occurs dominantly in intense stimulus and the positive potential is dominant in weak stimulus. In moderate intensity both potentials are canceled each other, and spike like on- and off-deflections occur according to the difference in the latency of both components. Positive component is inhibited by light-adaptation.

As the stimulus duration decreased, the square shaped response became a spike

like deflection in the stimulus duration of less than about 10 msec. and the amplitude of the response decreased in that case (Fig. 94).

*Stephanolepis cirrhifer*

i) The ERG induced from the inner surface of the retina was large negative potential (a-wave) followed by slow positive potential (b-wave), as illustrated in Fig. 95. With the decrease in stimulus intensity, the amplitude of the response decreased, and in the stimulus of weak light (below 20 lux) the positive component did not appear. In the case of stimulation of long duration, the ERG showed a small positive off-potential.

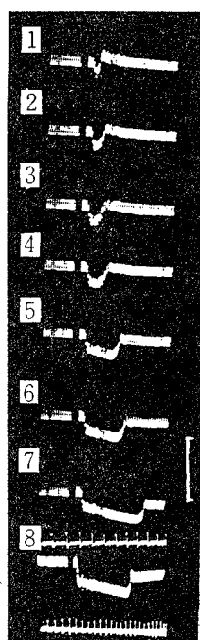


Fig. 94. S-potential recorded from *Fugu* according to light stimuli of various durations. The duration in 1—8 is 1/300, 1/100, 1/50, 1/25, 1/10, 1/5, 1/2 and 1 sec., respectively. Calibration voltage : 1 mV. Time mark : 1/6 sec.

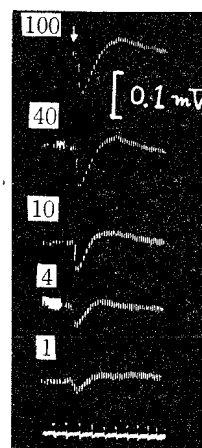


Fig. 95. ERGs recorded from the inner surface of the retina in *Stephanolepis*. Stimulus duration is 1/25 sec. Other legends are same as in Fig. 63.

The ERG induced from the receptor side of the retina was just the mirror image of that induced from the inner surface of the retina as shown in Fig. 96,A, and the negative potential and the time-course of the response increased with the increase in stimulus intensity.

In the case of stimulation of long duration, negative on- and positive off-potential appeared in the ERG, and the amplitude of the off-potential was the largest in the stimulus of moderate intensity (Fig. 96,B).

ii) ERGs were induced from the inverted retina by the aid of micro-electrode. The ERG elicited by stimulus flash with unit intensity was the typical S-potential (Figs. 97 and 98). No peculiar forms of the responses shown in *Chrysophrys* and *Sillago* were found in this species. ERGs for a series of light stimuli with various intensity were illustrated in Fig. 97. In this case the latency of off-response did not change

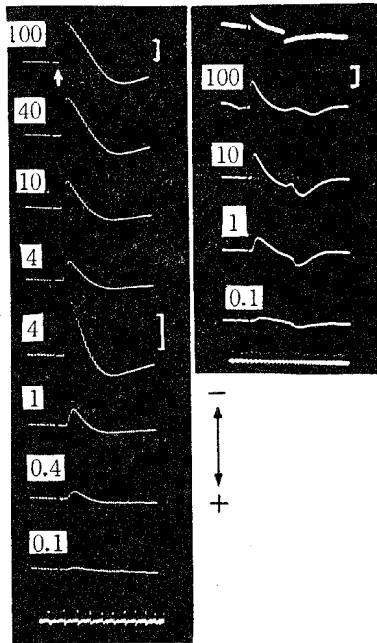


Fig. 96. ERGs recorded from the receptor layer of the retina in *Stephanolepis*. Stimulus duration is 1/300 sec. in A and 1 sec. in B. Other legends are same as in Fig. 58.

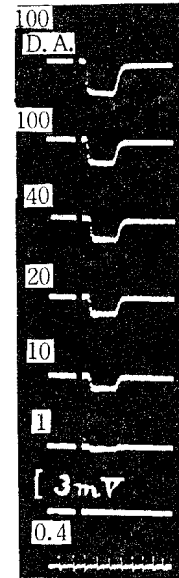


Fig. 97. S-potential in *Stephanolepis* in accordance with variation in strength of stimulus light. Other legends are same as in Fig. 75.

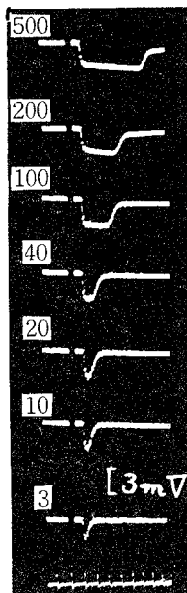


Fig. 98. S-potential in *Stephanolepis* in accordance with the variation of stimulus duration. Numeral on each record shows stimulus duration in msec. Stimulus intensity is unit intensity.

with the increase in stimulus intensity, although the increase in amplitude was found remarkably. In the records of the same figure, the retina kept in darkness for 10 minutes showed a slight increase in amplitude as compared with that light-adapted with the illumination of the test flashes (100 and 100 D. A., in Fig. 97).

The ERGs for a series of various durations of stimulus with constant intensity are shown in Fig. 98. In the case of stimulation of duration less than 20 msec., the ERG shows a negative spike like response.

*Scorpaenodes guamensis*

Action potential was induced from the receptor side of the eye. Typical wave-forms of the ERG are shown in Fig. 99. In the ERG from the dark-



adapted eye the response elicited by weak light stimulus of short duration was a positive potential followed by a negative potential, although it was only positive potential in stimulus of extremely weak light. By the stimulation of intense light the fast negative potential preceded the positive potential (Fig. 99, A-D and Fig. 100, A). In the response to illumination of long duration the wave-form of the ERG was the same with that of short flash stimulus; off-response did not occur in the stimulation of about 1 sec. (Fig. 99, B-D).

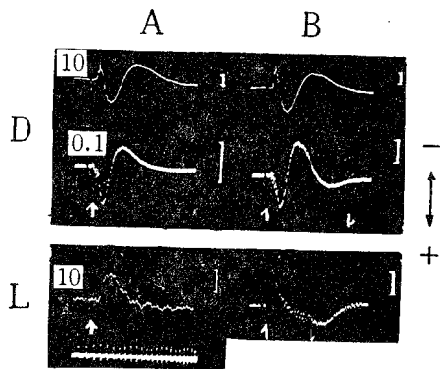


Fig. 99. Typical wave-forms of the ERG recorded from the receptor layer of dark- (D) and light- (L) adapted retinæ of *Scorpaenodes*. Stimulus duration is 1/100 sec. in A and 1 sec. in B. Other legends are same as in Fig. 58.

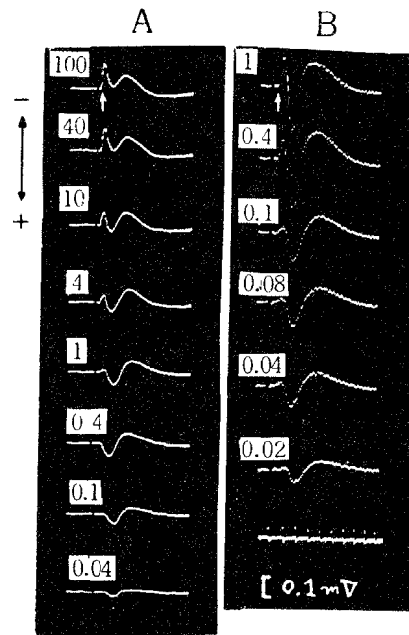


Fig. 100. ERGs recorded from the receptor layer of the semi-dark- (A) and complete dark- (B) adapted eyes of *Scorpaenodes*. Stimulus duration is 1/100 sec. Other legends are same as in Fig. 58.

In the light-adapted retina, the response to stimulus flash is simple negative potential and that to stimulus of long duration (1 sec.) is shown to consist of negative on- and positive off-potentials (Fig. 99,L). It is found, moreover, in Fig. 99 that a number of noticeable small deflections are superimposed on the ERG obtained from the light-adapted eye.

ERGs for varying the intensity of stimulus light of constant duration are shown in Fig. 100. The positive component is dominant in the response to weak light, and it develops with the increase in stimulus intensity, but with the further increase in stimulus intensity it is assumable that the positive component is balanced with the development of the negative component.

In application of 10% solution of KCl, positive component of the response disappeared and negative wave was remained only. The latency of the potential was the same with that of the initial negative potential of the response before the application of the reagent (Fig. 101). The remained negative potential responded to stimulus intensity independently.

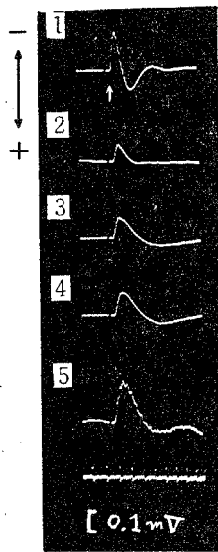


Fig. 101. Effect of KCl sol. on the ERG induced from the receptor layer of the retina of *Scorpaenodes*. Test flash is 1/100 sec. in duration and 10% of unit intensity. 1, before the application; 2, immediately after the application; 3, 1 minute later; 4, 3 minutes later; 5, induced from another position 3 minutes later.

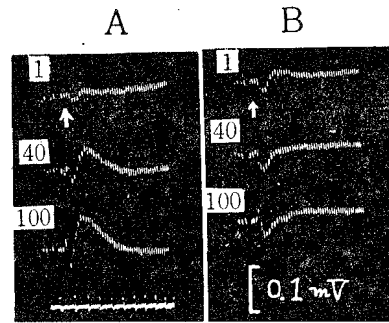


Fig. 102. ERGs recorded from the cornea surface of intact eye in *Acanthogobius* anesthetized. A, recorded immediately after the anesthesia; B, about 5 minutes later. Other legends are same as in Fig. 63.

*Acanthogobius flavimanus*

Induction of ERG was most difficult in this species among those used through the present work. It was almost impossible to induce the action potential from the cornea surface or from the inner surface of the retina of excised eyeball. The ERG shown in Fig. 102 was induced from the intact eye.

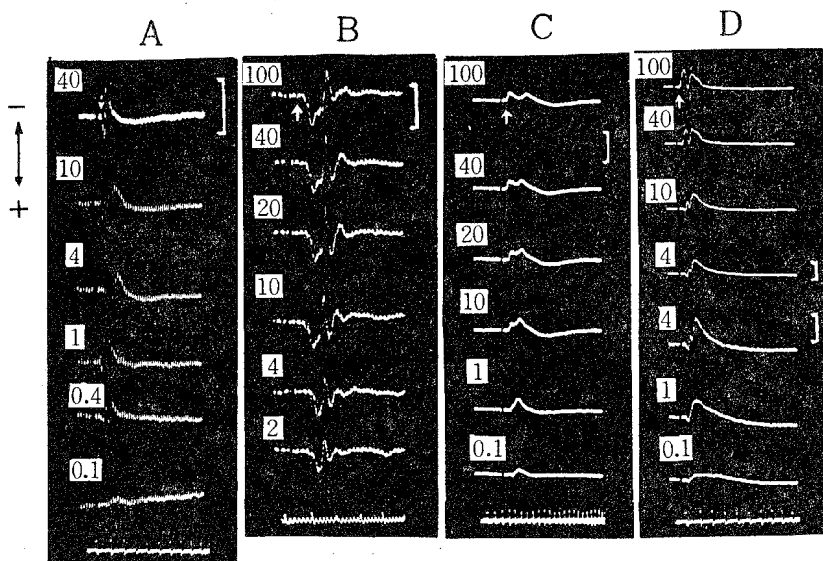


Fig. 103. Various types of the ERG induced from the receptor layer in the retina of *Acanthogobius*. Stimulus duration is 1/25 sec. Other legends are same as in Fig. 58.

The fish was anesthetized with urethan, and active electrode was made contact with the cornea surface and indifferent electrode was placed at the site of the removed orbit of another eye. The ERG obtained thus was a negative potential (a-wave) followed by large positive potential (b-wave). The decrease in the potential was remarkable with the lowering of the stimulus intensity. After about 10 to 15 minutes anesthesia of the fish, the positive potential of the ERG was suppressed remarkably (Fig. 102,B). The time-course of the ERG obtained in such way was relatively short in general (Fig. 102).

Although it was rather difficult to remove the pigment layer, various types of the ERG were induced from the receptor side of the eye as shown in Fig. 103. These ERGs were recorded under the same conditions.

In Fig. 103, A: The response to stimulus flash with moderate intensity is positive potential followed by negative potential and that of low intensity is negative potential. B: The response is positive potential, splitted with negative component. With the increase in stimulus intensity, the amplitude and the time-course of the response increase. C and D: The response is negative potential, which is splitted with negative component in stimulation with high intensity light. With the increase in stimulus intensity, the time-course of the response shortens in the records of D whereas prolongs in those of C.

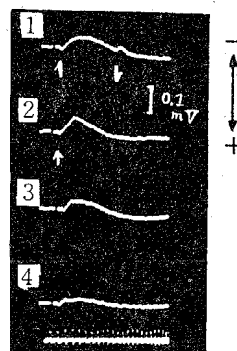


Fig. 104. ERGs recorded from the retinal layer of *Acanthogobius* in accordance with various stimulus durations, in 1—4 which is 1, 1/10, 1/25 and 1/100 sec., respectively. Stimulus intensity is 1% of unit intensity.

In the stimulation of long duration the response elicited a small negative off-response as shown in Fig. 104. Owing to the remarkable decrease in sensitivity of the retina, the ERG could not be picked up under light-adaptation.

#### *Pseudorhombus cinnamoneus*

Action potential was induced from the inner-surface of the retina. In the ERG elicited by stimulus light of short duration the negative potential was dominant. The ERGs for varying stimulus intensity are shown in Fig. 105. The response to weak light was a negative potential followed by a slow positive potential, but that to intense light often consisted of negative potential only (Fig. 105,A). In the case of stimulation of long duration the ERG was only a negative on-potential (Fig. 105,B).

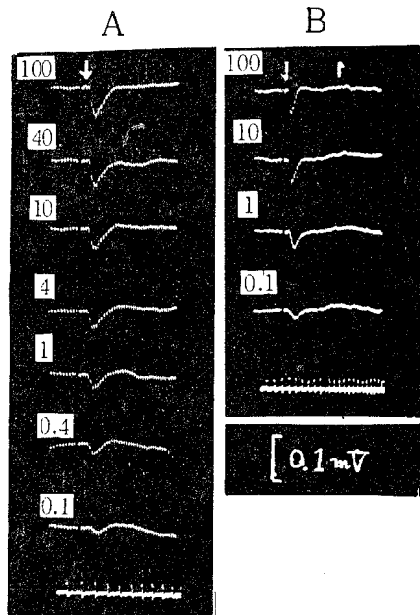


Fig. 105. ERGs recorded from the inner surface of the retina according to light stimuli of various intensity, in *Pseudorhombus*. Stimulus duration is 1/50 sec. in A and 1 sec. in B.

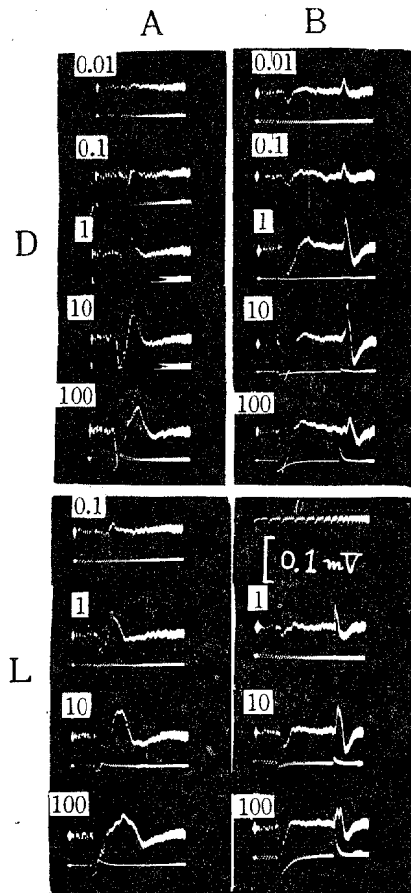


Fig. 106. ERGs recorded from the inner surface of the retina of *Kareius* according to stimulus light of various intensity. Stimulus duration is 1/50 sec. in A and 1 sec. in B. Other legends are same as in fig. 63.

### *Kareius bicoloratus*

Action potentials were induced from the inner surface of the retina. The ERG from the dark-adapted eye elicited by stimulus light (about 40 msec.) was negative potential followed by positive potential. Both potentials were same in amplitude (Fig. 106, A—D). In the response to stimulus light of long duration (about 1 sec.) on-response was negative, and the amplitude of the negative potential increased considerably with the increase in stimulus intensity. And the positive potential of on-response was not almost found irrespective of the change in stimulus intensity. Off-response was positive potential followed by negative one and showed the largest amplitude of positive potential in the stimulation with moderate intensity light, although the off-response was only positive potential in the stimulation with low intensity (Fig. 106, B—D). In the eye light-adapted with 30 lux light for 10 minutes, no remarkable change in wave-form was observed as compared with that of the dark-adapted one, although in the response to stimulus flash of high intensity

it was seen the prolongation of time-course of the positive potential on which few knobs were often found (Fig. 106, A—L), and the response to stimulus light of long duration did not show a positive on-potential at all (Fig. 106, B—L).

On the other hand it was observed that the negative potential was dominant in the ERG, irrespective of any intensity and duration of the stimuli. The ERG of such type tended to be usually recorded from *ora serrata* (Fig. 107).

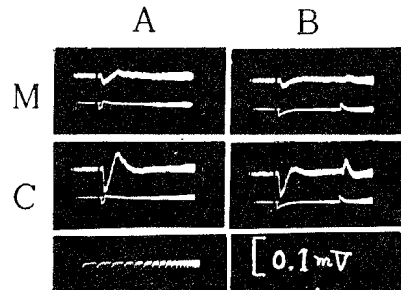


Fig. 107. ERGs recorded from the inner surface of marginal region (M) and fundus (C) of the retina in *Kareius*. Underline indicating the duration of stimulus light which is 1/25 sec. in A, and 1 sec. in B.

(2) Relation between the magnitude of response and the stimulus intensity

The relation between the amplitude of b-wave in ERG and the stimulus intensity was investigated on various species. The responses to stimulus light with various intensity ranging from 0.01 lux to 500 lux were recorded in sequence of five minutes in a dark-adapted eye. A few series of the records which are made by three kinds of the induction is shown in Fig. 108.

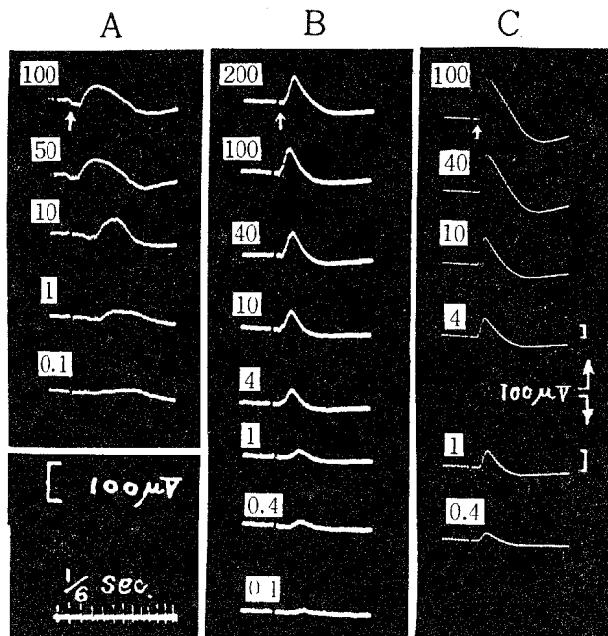


Fig. 108. Showing some records of the ERGs used for the investigation of the relation between the amplitude height of the ERG and the stimulus intensity. A, the ERG induced from the inner surface of the optic cup in *Raja*; B, induced from the cornea surface of the eyeball in *Gymnothorax*; C, induced from the receptor layer of the retina in *Stephanolepis*. In only C, polarity of the record is upward negative. Other legends are same as in Fig. 63.

The magnitude of the positive potential (b-wave) was plotted against the logarithm of stimulus intensity. The magnitude was represented with the percentage to the maximum amplitude. As shown in Figs. 109 and 110, the linear relation was generally obtained between the amplitude of the positive potential and the logarithm of stimulus intensity. This linear relation did not seem to change at all with the differences in the way of the induction of ERG.

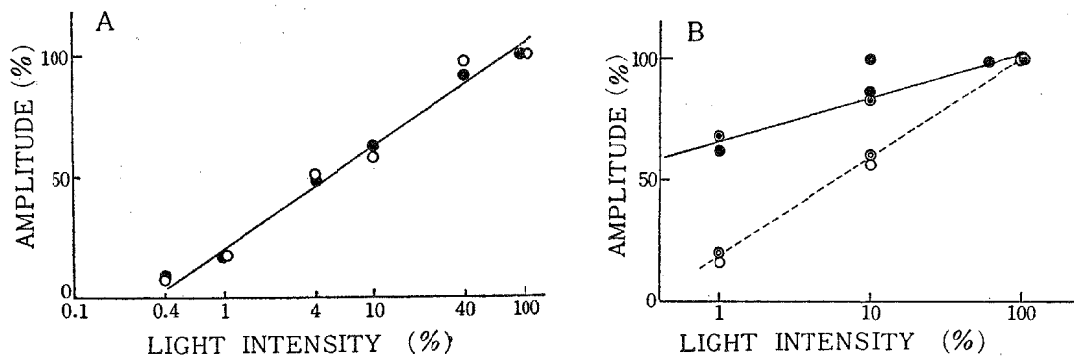


Fig. 109. A : Influence of the distinction of method in the induction of ERG on the relation between amplitude height of ERG and the stimulus intensity, in *Stephanolepis*. Filled circles, induced from the inner surface of the retina. Open circles, from the receptor layer. Ordinate, amplitude height of b-wave in % of maximum one. Abscissa, intensity of stimulus light in log scale.

B : Influence of the state of light- and dark-adaptations on the relation between the amplitude height of ERG and the stimulus intensity, in *Anguilla*. Filled circles and solid line, relation obtained in dark-adaptation; open circles and broken line, in light-adaptation; Single circle, stimulus duration in 1/50 sec.; double circles, in 1 sec. Other legends are same as in A.

For example, in the case of *Stephanolepis cirrhifer* (Fig. 109,A) the relation obtained in the induction from the inner surface of the retina showed the same result as that obtained directly from the receptor side of the retina. On the other hand, the relation seemed to be affected by the state of dark- or light-adaptation of the retina (Fig. 109,B); in the linear relation obtained from the dark-adapted retina the gradient of the straight line against the abscissa was rather small, while in that from the light-adapted one it was large, although the sensitivity of the retina decreased noticeably by the light-adaptation. Therefore, the relation shown in Fig. 110 were all obtained from the dark-adapted retina in order to make comparison.

In some species such as *Halichoeres* and *Epinephelus*, the magnitude of the positive potential increased in the exponential relation to the logarithm of stimulus intensity. Moreover, in *Chrysophrys*, *Trachurus*, *Hoplognathus* and *Kareius*, the amplitude of the response augmented with the increase in stimulus intensity, but the increase of the potential attained to a plateau state within the limit of the stimulus intensity used in the present experiments. The fishes showing this phenomenon are assumed to have a benthonic life, because it is well-known facts that *Trachurus*

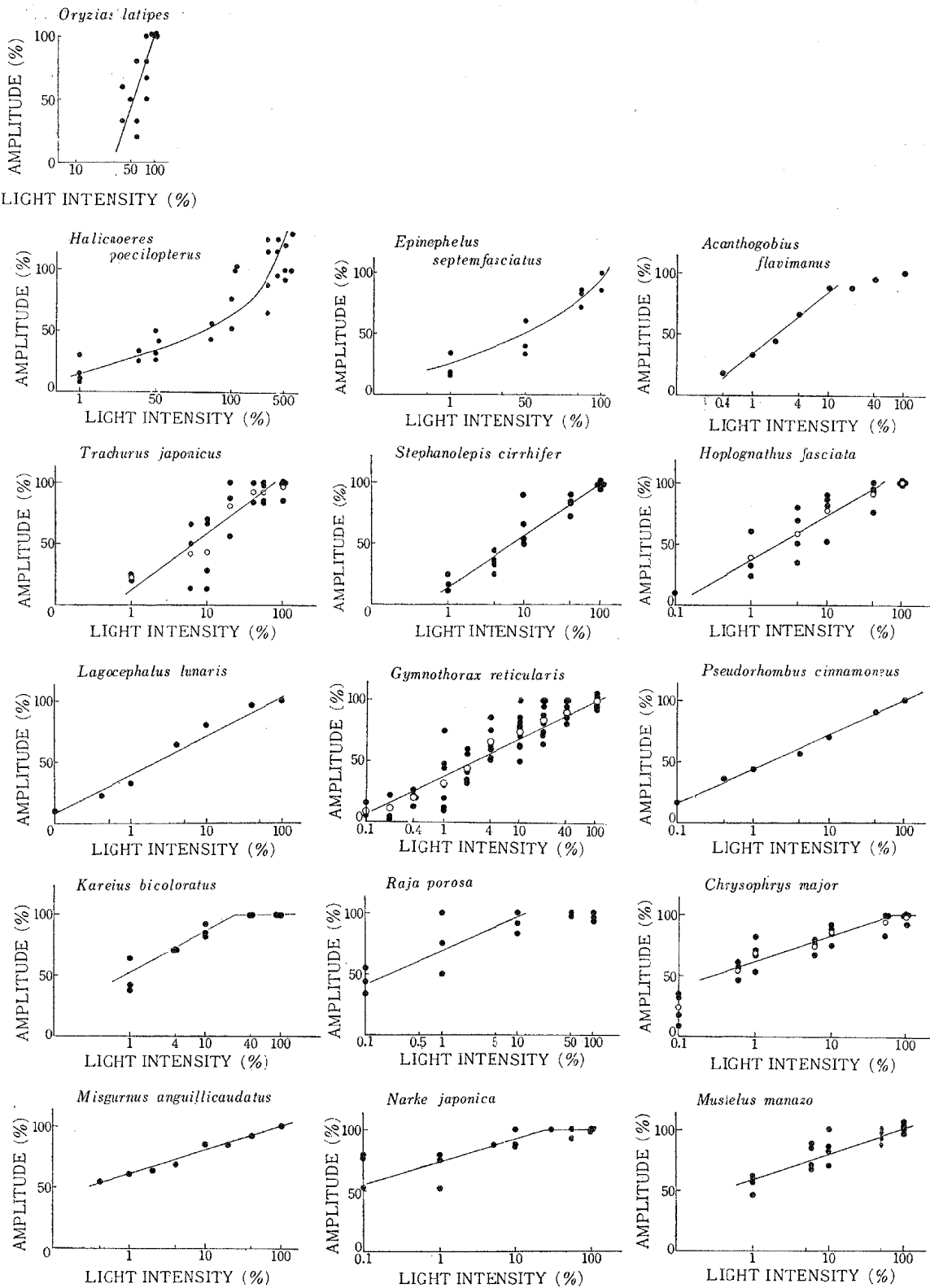


Fig. 110. Relation between the amplitude height of b-wave in ERG and the stimulus intensity, in various species examined. Open circles, the average. Other legends are same as in Fig. 109.

never comes to the surface, scups take their food on bottom of the sea and *Kareius* lies flatways on the bottom in nature.

In the results obtained in Fig. 110, the changing rate in amplitude of positive potential caused by the increase in stimulus intensity seems to be different from species to species, even if the experiment is made under the same condition. If  $L_{10}$  represents the percentage of the increase in amplitude caused by ten times increase of stimulus intensity, it is found that the value of  $L_{10}$  is generally large in diurnal fishes and small in nocturnal ones. And also the value seems to have the correlation with the index of the nuclear layer of the retina which, will be described later on (Table 11).

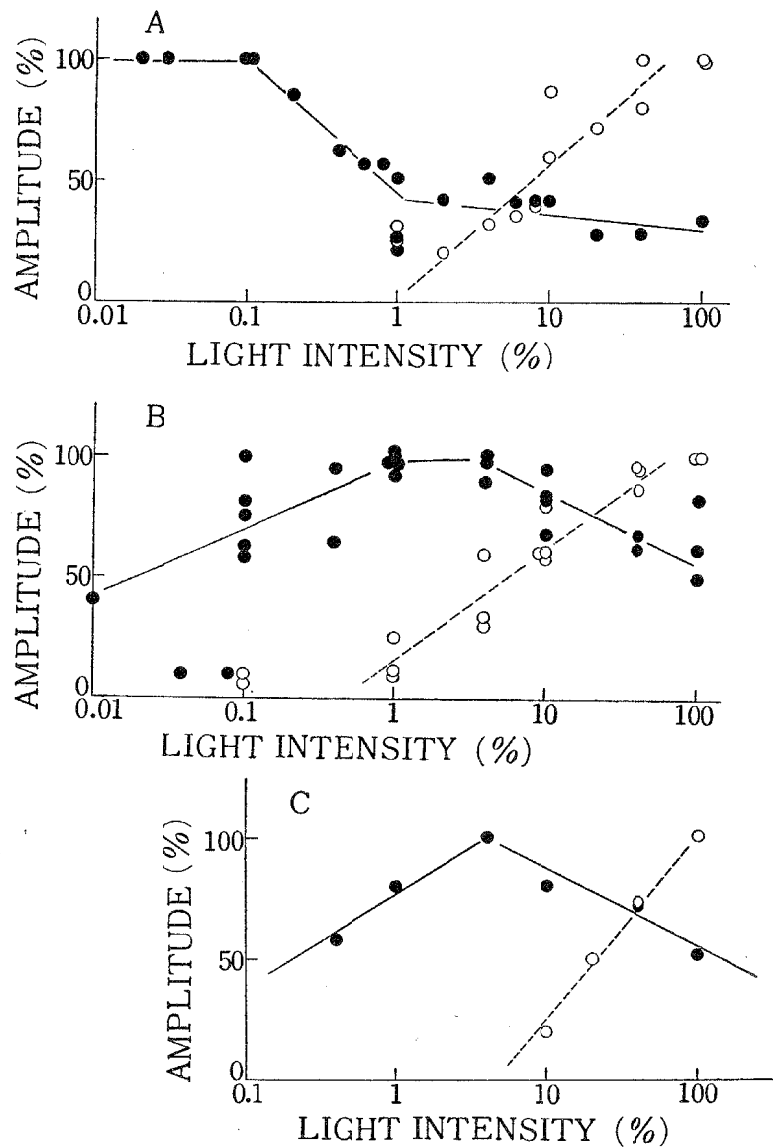


Fig. 111. Relation between the amplitude height of ERG and the stimulus intensity. Filled circles, amplitude of b-wave; open circles, that of a-wave. A, *Saurida*; B, *Scorpaenodes*; C, *Cyprinus*. Other legends are same as in Fig. 109.



On the other hand, in *Scorpaenodes*, *Saurida* and *Cyprinus* the wave-form of the ERGs changed in complicated manner with the increase in the strength of stimulus light, and so the relation between the magnitude of the response and the stimulus intensity was hard to obtain all over the wide range of the intensity. In these fishes the response to low stimulus intensity consists of positive potential only, but negative potential appears with the increase in stimulus intensity, and the negative potential becomes dominant in the stimulation of relatively high intensity.

Therefore, for the investigation of the relation of the magnitude of the response with stimulus intensity, the positive potential was used in the case of response to low stimulus intensity as the index, whereas the negative potential was used in the case of high intensity. The results are shown in Fig. 111. The changing rate of the negative potential (open circles in Fig. 111) was the largest in *Cyprinus* and was about the same in both *Scorpaenodes* and *Saurida*. And also the intensity at

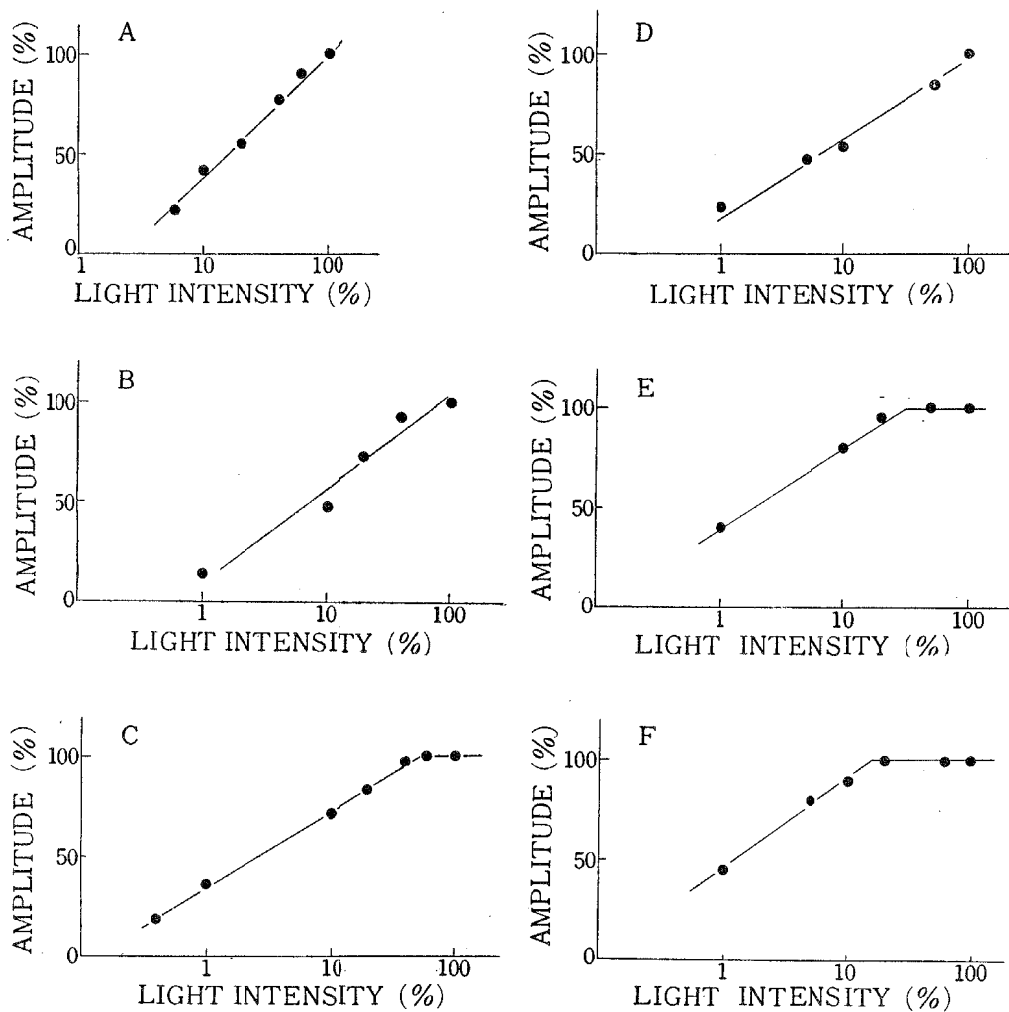


Fig. 112. Relation between the amplitude height of S-potential and the stimulus intensity. Filled circle is average value of three individuals. A, *Sojori*; B, *Stephanolepis*; C, *Chrysophrys*; D, *Lagocephalus*; E, *Sillago*; F, *Fugu*. Other legends are same as in Fig. 109.

which the magnitude of positive potential attained to a plateau state, increased in the following order of species; *Saurida*, *Scorpaenodes* and *Cyprinus*. These results seem to have correlation with the depth of the habitats of the fishes.

Using the amplitude of S-potential as an index the magnitude of the response was investigated in relation to the stimulus intensity in several kinds of fishes. As shown in Fig. 112, the same linear relation was obtained between them as that of the previous experiments (Fig. 110). And further, in benthonic fishes such as *Chrysophrys* and *Fugu* the amplitude of S-potential was found to attain to a plateau state in relatively low stimulus intensity, whereas in other fishes which are known as shallow water or surface fishes in the sea, the amplitude of S-potential did not attain the plateau within the limit of stimulus intensity used in the present experiment. It is also observed from the figure that the gradient of the straight line in the linear relation, the changing rate of S-potential in relation to the variation of stimulus intensity, is large in the latter fishes. Therefore, it seems that these results obtained by S-potential are the same with the relation obtained from the amplitude of ERG.

### (3) Light and dark adaptation

It is a well-known fact that the retinal sensitivity decreases with the light-adaptation and increases with the dark-adaptation. The change of the retinal sensitivity in the course of the adaptation was investigated on number of fish species by measuring the size of ERG or the threshold intensity of stimulus light for eliciting ERG.

#### *Misgurnus anguillicaudatus*

As shown in Fig. 113, in the experiment of light-adaptation with about 3 lux, the retina rose the threshold intensity to 0.3 log unit of the value obtained in the dark-adapted eye, within a few minutes after the beginning of the adaptation and remained in this value thereafter. The retina exposed to such preliminary illumination for 15 minutes was then returned to darkness, and the threshold intensity returned to the initial level at once.

In the light-adaptation with about 30 lux, the threshold intensity rose considerably immediately after the beginning of the adaptation and then it continued to increase slowly until it attained to a plateau state several minutes later. The increment of the threshold intensity was about 3 log units. The retina thus light-adapted showed the rapid recovery of the sensitivity within a few minutes in the dark. In the light-adaptation with 200 lux, the change in the threshold showed the same tendency as that in the former experiment, and the threshold intensity was the highest of the three. In the following dark-adaptation the threshold returned to the initial level within about 10 minutes.

When the change in the threshold intensity caused by the light-adaptation (30 lux) is compared with that of the wave-form shown in Fig. 114 in which the records

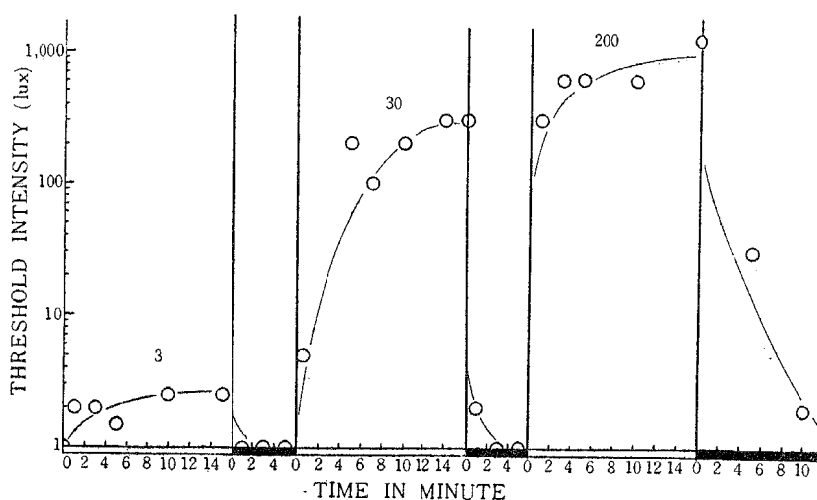


Fig. 113. Change in threshold intensity of stimulus light eliciting ERG of *Misgurnus* during light- and dark-adaptations. Numeral on each curve, intensity (lux) of the adaptation light. Empty and filled underlines show the duration of light- or dark-adaptation, respectively. Test flash, 1/100 sec. in duration.

were obtained in the course of light-adaptation with the same intensity, the time at which the rapid rising of the threshold is caused by the light-adaptation seems to correspond to the time at which the maximum amplitude of off-response is attained, and also the time at which the threshold attains a maximum state seems to correspond to the time at which the positive on-response (b-wave) disappears.

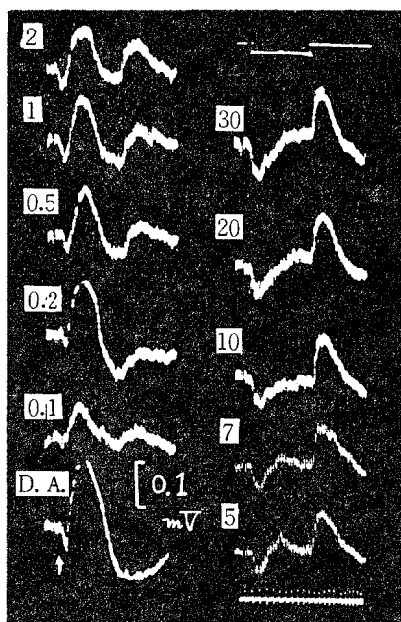


Fig. 114. ERGs recorded from the cornea surface of *Misgurnus* in progress of the light-adaptation (30 lux). Numeral on each record, the time elapsed in the adaptation. Time mark : 1/6 sec.

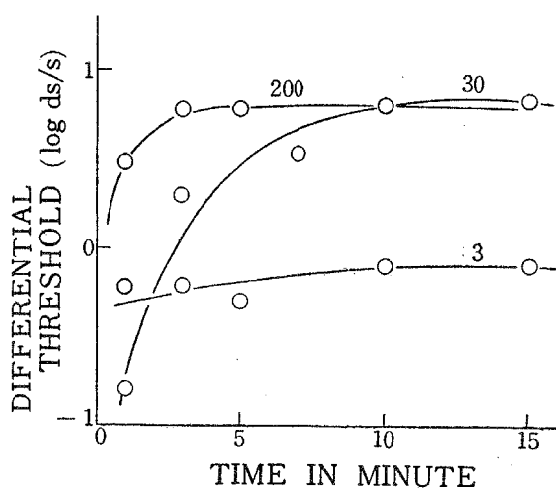


Fig. 115. Change in differential threshold of stimulus light eliciting ERG in the course of light-adaptation, in the retina of *Misgurnus*. Numeral on each curve, intensity (lux) of adaptation light.

In Fig. 115 the change in the sensitivity caused by the light-adaptation was represented in the differential threshold ( $ds/s$ ). When the light intensity of the adaptation was about 3 lux, the minimum value of  $ds/s$  was 0.8.

### *Anguilla japonica*

The response to test flash (1/50 sec., unit intensity) was recorded every few minutes in the course of dark-adaptation (Fig. 116). Aside from the question of the change in wave-form, the magnitude of the positive potential increased slowly but clearly in the progress of the dark-adaptation and it attained to a plateau state in 40 minutes after the beginning of the dark-adaptation.

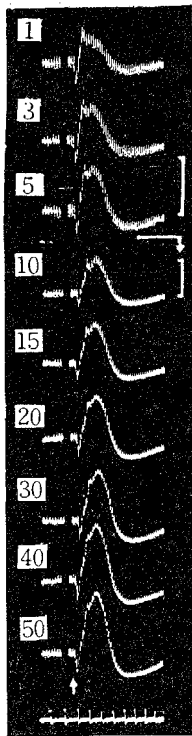


Fig. 116. Change in wave-form of ERG during the dark-adaptation, in *Anguilla*. Numeral on each record, the time elapsed in the dark-adaptation. Test flash is unit intensity, 1/50 sec. in duration.

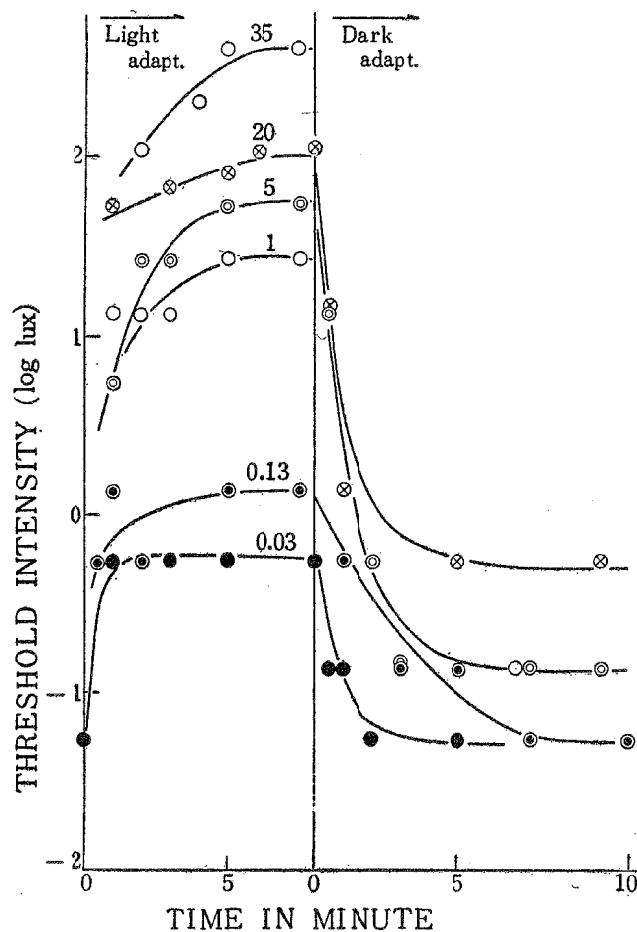


Fig. 117. Change in threshold intensity of stimulus light eliciting ERG in *Anguilla*, in the course of light- and dark-adaptations. Numeral on each curve, intensity (lux) of adaptation light. Test flash is 1/100 sec. in duration.

On the other hand, the course of dark- or light-adaptation was investigated by using a threshold intensity of the stimulus light eliciting ERG, the results of which were shown in Fig. 117. In this case, the change in the threshold intensity caused by light-adaptation showed the same tendency as that in *Misgurnus*, and it seemed

to become constant within about 5 minutes even in the adaptation with high intensity. When the retina exposed to high intensity light was returned to darkness, the retina recovered the sensitivity within a few minutes.

The change in retinal sensitivity caused by the light-adaptation was also represented with the differential threshold (ds/s). The relation between the value of ds/s and the light intensity of adaptation light was shown in Fig. 118, in which the value was found to be nearly unchanging over the wide range of the intensity of adaptation light.

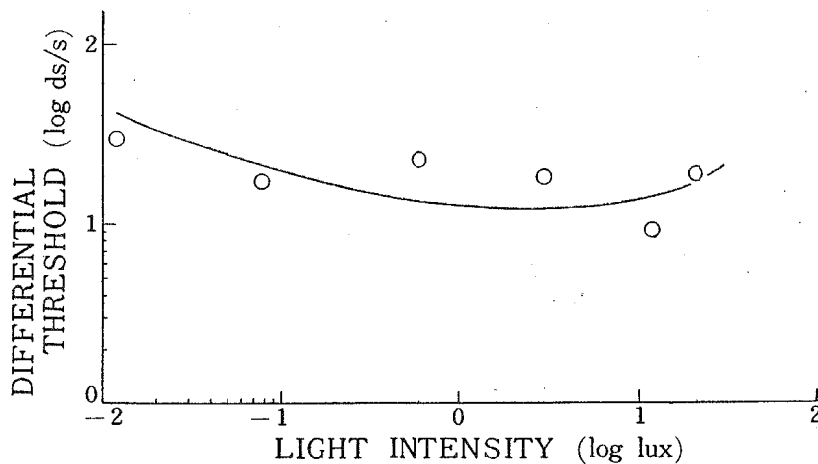


Fig. 118. Relation between the differential threshold (log ds/s) and the intensity (log lux) of adaptation light, in the retina of *Anguilla*.

#### *Gymnothorax reticularis*

The amplitude of the ERG for the constant flash light was used for the determination of the grade of dark- or light-adaptation. Even when the retina was illuminated with a weak light (about 3 lux), the sensitivity decreased noticeably, and the positive potential was almost indistinguishable. Therefore, the test flash (about 50 lux, 1/10 sec.) was given after a provisional cessation of the background illumination. Although the prolongation of time-course of the ERG was marked in the progress of the light-adaptation, the magnitude of the response remained almost unchanged (Fig. 119, A).

The retina exposed to such preliminary light for 30 minutes, was brought back into darkness. Then, the retina recovered the sensitivity noticeably within 1 minute after the beginning of the adaptation, and thereafter the amplitude increased slowly and then attained to a plateau state 20 minutes later (Fig. 119, B). The retina adapted by the intense light (about 300 lux) for 10 minutes required a longer time (about 80 minutes) in the recovery of the sensitivity in the following dark-adaptation, in which the amplitude of the ERG increased slowly.

#### *Chrysophrys major*

After the exposure to preliminary light of about 10 lux for 7 minutes, the optic

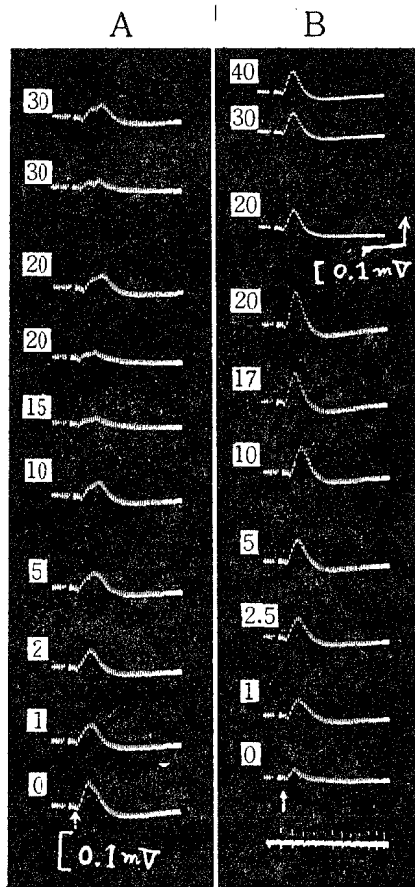


Fig. 119. ERGs of *Gymnothorax* recorded in the course of light-(A) and dark-(B) adaptations. Intensity of adaptation light is about 3 lux. Numeral on each record, the time elapsed in the adaptation. Test flash is 5 lux and 1/10 sec. in duration. In A, the test flash is given after the provisional cessation of the background illumination, except for the case of the parenthesized numeral.

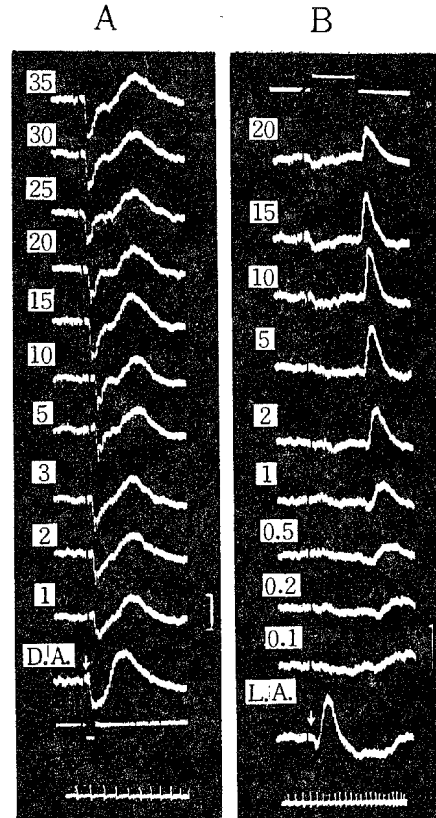


Fig. 120. Change in wave-form of the ERG in *Chrysophrys* in the course of dark-(A) and light-(B) adaptations. Intensity of adaptation light is about 3 lux. Numeral on each record, the time elapsed in the adaptation. Test flash is 50 lux, 1/50 sec. (A) and 1 sec. (B) in duration. Calibration voltage: 0.1 mV. Time mark: 1/6 sec.

cup was brought into darkness, and the response to test flash (50 lux, 1/10 sec.) was recorded in the progress of dark-adaptation, during which the amplitude of the response scarcely changed, but the falling rate of the negative potential increased, and the latent period of the positive potential prolonged. These changes began to occur in 10 minutes after the beginning of the adaptation and attained to a plateau state in 30 minutes (Fig. 120, A). As the dark-adapted optic-cup was exposed to light of 10 lux, the response to test flash (50 lux, 1 sec.) was a small negative on-potential and a large positive off-potential, and the positive off-response became noticeable in one minute after the exposure and attained to the maximum amplitude 10 minutes later (Fig. 120, B).

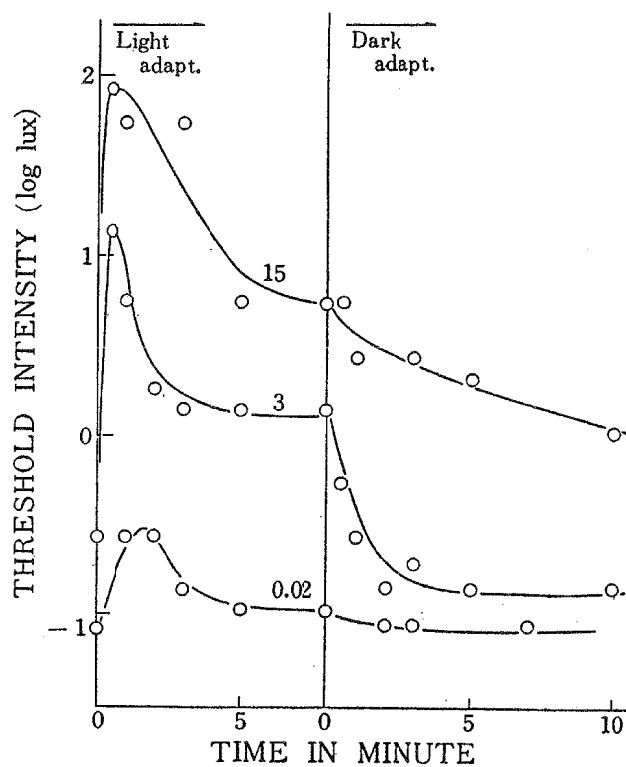


Fig. 121. Change in threshold intensity of stimulus light eliciting ERG of *Chrysophrys* in the course of light- and dark-adaptations. Other legends are same as in Fig. 117.

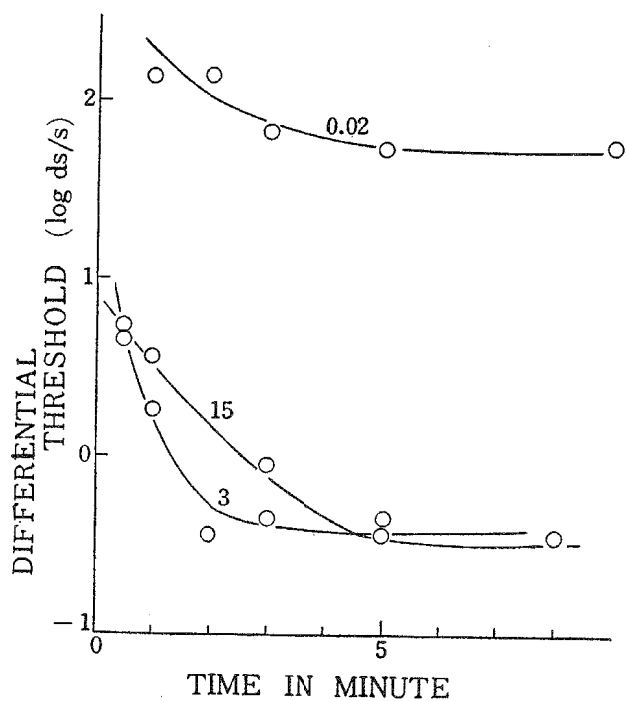


Fig. 122. Change in differential threshold (log ds/s) in the course of light-adaptation, in the retina of *Chrysophrys*. Other legends are same as in Fig. 115.

Result obtained by using threshold intensity is shown in Fig. 121. In this case, the action potential was directly induced from the receptor side of the retina. The threshold rose rapidly with the on-set of the light, and thereafter began to decrease slowly and then attained to a plateau state within several minutes. Intense light-adaptation caused more remarkable rise of the threshold and the threshold in the final state was also high in intense light-adaptation.

In Fig. 122, the change in the sensitivity in the light-adaptation was represented with the differential threshold ( $ds/s$ ). The value of  $ds/s$  attained to the plateau within about five minutes and it seemed to be constant at the range from 3 to 15 lux of the adaptation light. The minimum value of  $ds/s$  was 0.36.

### *Trachurus japonicus*

The threshold intensity of stimulus light eliciting ERG was used for the investigation. In this case action potential was induced from the inner surface of the retina. As shown in Fig. 123, the change in the sensitivity through the light-adaptation with a weak light (0.02 lux) was the same as that in *Misgurnus*. As the retina was exposed to the light of about 3 lux, the recording of the retinal sensitivity became impossible because of the rapid decrease of the sensitivity. As soon as the retina was brought into darkness, however, the recovery process began immediately and attained the sensitivity of the initial level 5 minutes later. Even in the retina so intensely light-adapted that no more response could be obtained, the sensitivity was recovered completely in the following dark-adaptation.

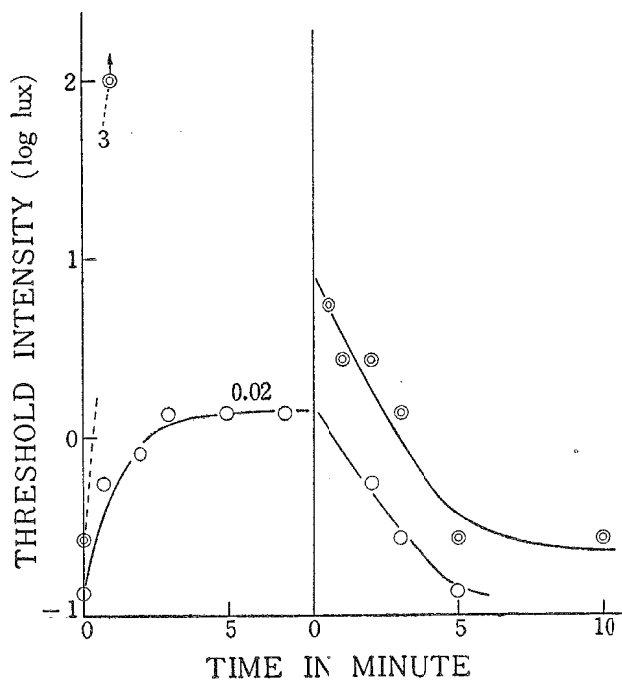


Fig. 123. Changed in threshold intensity of stimulus light eliciting ERG of *Trachurus* in the course of light- and dark-adaptations. Other legends are same as in Fig. 117.



*Chelidonichthys kumu*

Threshold intensity of stimulus light eliciting ERG was used for the investigation. In the adaptation with light of about 0.02 lux the threshold rose slowly about 0.5 log unit. In the adaptation with light of about 3 lux, the threshold rose rapidly within first 1 minute and thereafter it continued to increase slowly until it attained a plateau state several minutes later. These changes were represented with the differential threshold in Fig. 124.

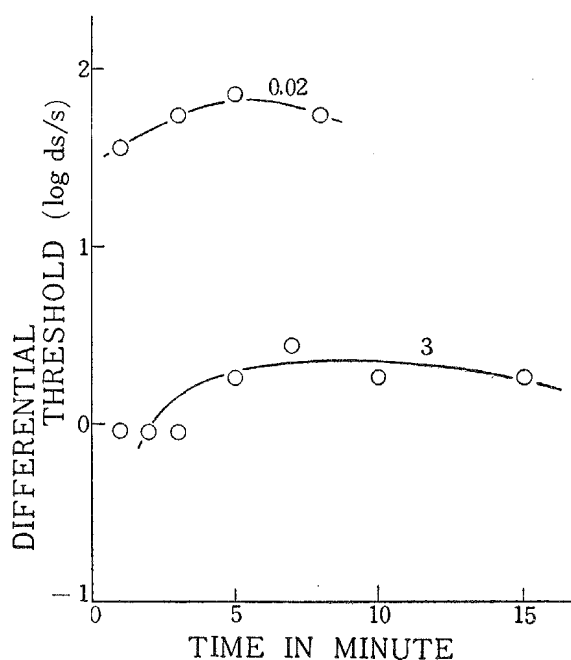


Fig. 124. Change in differential threshold ( $\log ds/s$ ) in the course of light-adaptation, in the retina of *Chelidonichthys*. Numeral on each curve, intensity (lux) of adaptation light.

*Stephanolepis cirrhifer*

The threshold intensity was used for the investigation. In the course of the light-adaptation, the change in the threshold showed the same process as that of *Chrysophrys*, but the time for attaining a plateau state was shorter in this species. The degree of the rising of the threshold was rather small (about 1 log unit). These changes were represented with differential threshold ( $ds/s$ ) in Fig. 125, in which it was found that the value of  $ds/s$  in the plateau was closely similar irrespective of the intensity of background-illumination, and showed the range from about 1.8 to about 0.9. The result implies that the ability of brightness discrimination is constant within relatively wide range of the illumination.

*Kareius bicoloratus*

The threshold intensity of stimulus light eliciting ERG was used for the determination of the grade of light-adaptation. The process of the light-adaptation was shown in Fig. 126, in which the change in the retinal sensitivity was represented

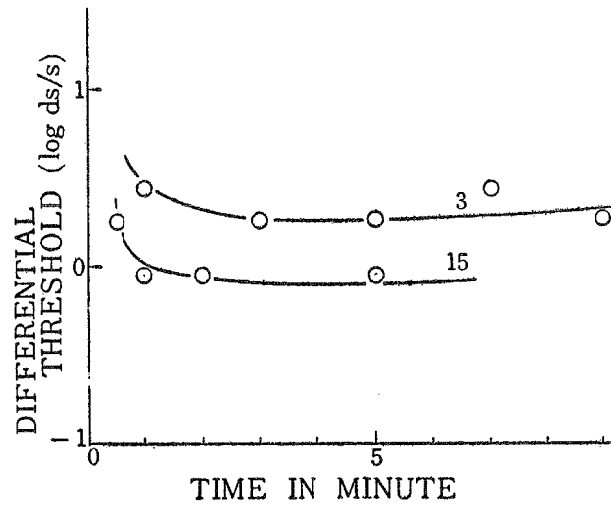


Fig. 125. Change in differential threshold ( $\log ds/s$ ) in the course of light-adaptation, in the retina of *Stephanolepis*. Other legends are same as in Fig. 115.

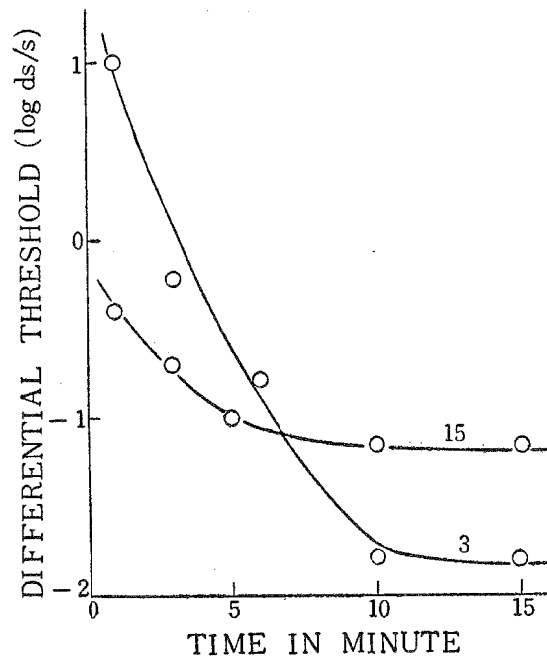


Fig. 126. Change in differential threshold ( $\log ds/s$ ) in the course of light-adaptation, in the retina of *Kareius*. Other legends are same as in Fig. 115.

with the differential threshold. The threshold intensity rose rapidly at first and thereafter it decreased a little slowly and then it attained to a plateau state about 10 minutes later. Although the change in the sensitivity in *Kareius* shows the same tendency as that in *Chrysophrys*, the range of the change is wider and the time for attaining the plateau is longer than those of the latter. The value of the differential threshold was smaller in the adaptation with light of 3 lux than that of 15 lux, and the value was about 0.02.

Judging from the results mentioned above, it can be safely said that the recovery of the amplitude of ERG during the dark-adaptation requires a long time in general, while the recovery of the threshold intensity is completed more rapidly. No distinctions were found in the process of the change in retinal sensitivity brought about by the dark-adaptation among the various species examined. The retinae of nocturnal fish such as *Gymnothorax* and *Anguilla* showed the wide range of the change in threshold intensity, in spite of the indistinguishable increase in amplitude of the ERG.

On the other hand, in process of the light-adaptation two types of response were seen; in one type the threshold intensity increased with the progress of the adaptation until it attained a plateau state, and in another type the rapid increase of threshold intensity occurred transitorily at the initial phase of the adaptation and then the threshold decreased slowly until it attained a plateau state. Threshold in the plateau state is higher than that in the initial state, not to mention. The former type was found in *Misgurnus*, *Anguilla* and *Chelidonichthys*, which were usually known as nocturnal, burrowing fishes. The latter type was found in *Kareius*, *Chrysophrys* and *Stephanolepis*, which were usually known as diurnal fishes and shallow water ones.

With regard to the species examined, the smallest value of the differential thresholds obtained from various intensity of the adaptation light, was listed in Table 6 together with the corresponding intensity of the adaptation light. The values of differential threshold obtained from some other animals are added for the comparison, in the lower part of Table 6. It is assumed from Table 6 that the benthonic fishes generally have small values of  $ds/s$ , some of which can be close to that in human at least.

Table 6. Differential threshold of stimulus light for eliciting the ERG in the light-adaptation and the intensity of the adaptation light in which the smallest value of the differential threshold is determined, in various species of fish examined.

Species	$ds/s$	Intensity (lux)	Species	$ds/s$	Intensity (lux)
<i>Mustelus</i>	14	0.2	<i>Narke</i>	0.6	0.2—10
<i>Anguilla</i>	5.4	0.2—15	<i>Chrysophrys</i>	0.36	3 —15
<i>Trachurus</i>	3.2	10	<i>Dasyatis</i>	0.05	4
<i>Chelidonichthys</i>	1.8	3	<i>Urolophus</i>	0.07	0.1— 1.2
<i>Stephanolepis</i>	0.9	15	<i>Kareius</i>	0.02	3
<i>Misgurnus</i>	0.4	3	<i>Holorhinus</i>	0.006	1 —12
Soft clam ( <i>Mya</i> )	10	HECHT ('36)			
Lamprey	4	STEVEN ('50)			
Human	0.01—0.008	KATO ('53)			

#### (4) Response to flicker stimulus light

The criterion of the flicker fusion frequency (f.f.f.) was the disappearance of responses to flicker light in the ERG, and in the case of experiments with the micro-

electrode it was disappearance of fluctuation following flicker light in the negative potential. All f. f. f. determinations were made with a light: dark ratio of 1, at a temperature of 20°C—21°C, except for the cases described in particular. In all species except for *Gymnothorax*, the action potential was induced from the inverted retina by the micro-electrode.

#### *Gymnothorax reticularis*

In the response to flicker light, the retina elicited the initial large positive deflection (b-wave) followed by a succession of ripples as shown in Fig. 127. In the stimulation with flicker light of low intensity (about 3 lux), the response of the dark-adapted retina showed a succession of b-wave; with that of high intensity (about 300 lux), the response showed a succession of b-, d-, d-, .....d-wave (Fig. 127,A). When the retina was adapted with the light of about 3 lux, b-wave in the response to the intense flicker light was obviously suppressed by the light-adaptation, and the amplitude of the ripple increased (Fig. 127,B). The tendency mentioned above was more obvious in the records of Fig. 127,C, in which the light intensity of the adaptation was about 30 lux. In this case the response to the intense flicker light is considered to be a succession of the d-wave.

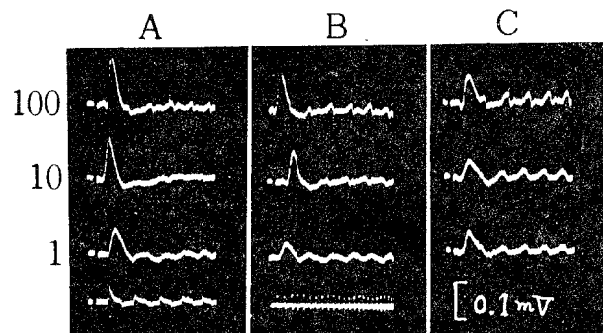


Fig. 127. ERGs of *Gymnothorax* in the response to flicker light with frequency of 4.4 c/s. A, dark-adapted retina; B, light-adapted (3 lux) retina; C, light-adapted (15 lux) retina. Numerals on left side, intensity (lux) of the flicker light. Time mark: 1/12 sec.

The fusion frequency of the ripples was determined at the various intensity of the flicker light (Fig. 128). As shown in Fig. 129, the fusion frequency increased with the linear relation to the logarithm of flicker light intensity in the range of stimulus intensity of less than about 25 lux and it was almost unchanged (16—17 c/s) in the range of higher intensity than 25 lux.

#### *Carassius auratus*

As it was shown in Fig. 130, the regular fluctuation of the negative potential was obtained in the response to the flicker stimulation of low frequency. With the increase in stimulus frequency, the fluctuation of the negative potential fused in

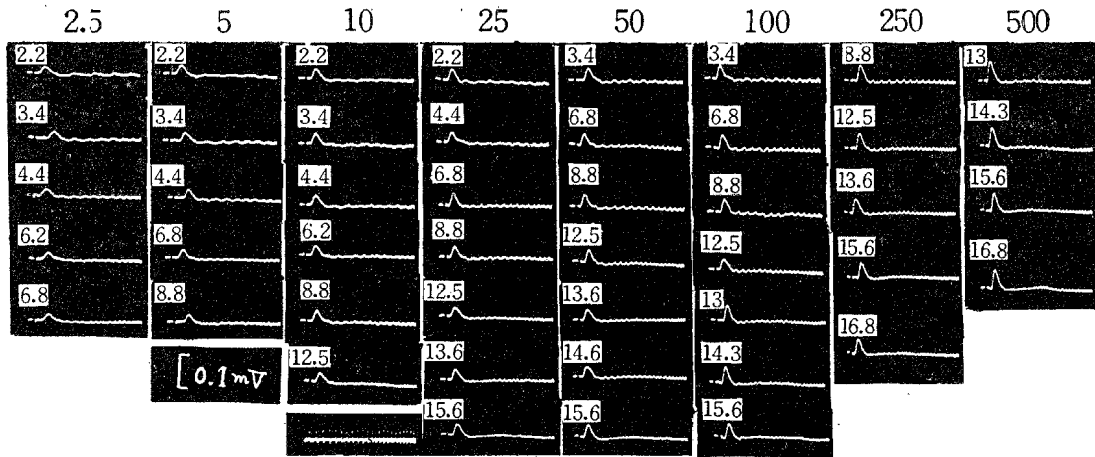


Fig. 128. ERGs of *Gymnothorax* in response to flicker stimulus light with various intensity. Numeral on each record, frequency of the flicker light and that on upper side, intensity of the flicker light. Time mark : 1/6 sec.

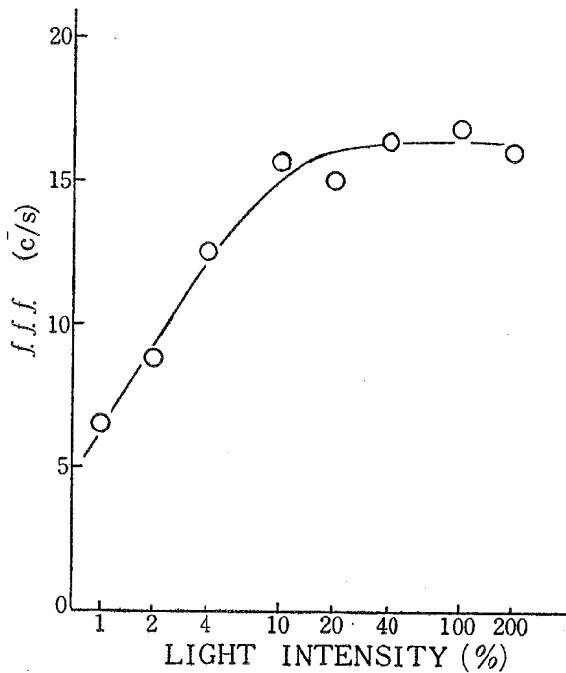


Fig. 129. Relation between f.f.f. and intensity of flicker stimulus light, in *Gymnothorax*. Temperature, 20 °C.

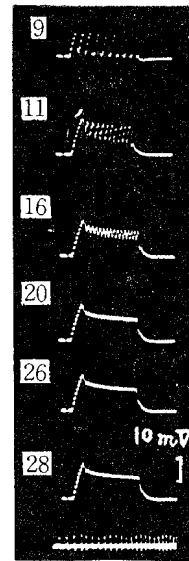


Fig. 130. Records of S-potential used for f.f.f.—determination in *Carassius*. Numeral on each record, frequency of the flicker light. Temperature, 10 °C. Time mark : 1/6 sec.

certain frequency of the stimulus light. Of course, the fusion frequency is affected by various experimental conditions such as are mentioned below.

The dark-adapted retina was exposed for few seconds to the flicker light with constant intensity every 1 minute in the dark, and the fusion frequency of the response was successively determined at each exposure.

Table 7. The increase in f.f.f. in the case of stimulation in which *Carassius* retina is exposed for few seconds to the flicker light with constant intensity every one minute in the dark-adaptation (D.A.) and in the light-adaptation of about 3 lux (L.A.). Temperature, 10°C.

Order of the experiment elapsed in the adaptation		1	2	3	4	5	6
f.f.f.	D. A.	10	10	15	18.3	10	19
	L. A.	14.3	13.2	21.0	24.0	23.6	28.0

As is shown in Table 7, the increase in the fusion frequency is obviously found in the succession of the flicker stimulation. In order to investigate the influence of the state of dark- or light-adaptation on the flicker fusion frequency, it was determined in the retina adapted directly to the background illumination (about 10 lux) for 10 minutes.

Table 8. Influences of the stimulus intensity and the state of dark- or light-adaptation on the f.f.f. determined by means of S-potential in the retinae of *Carassius* and *Trachurus*. Temperature, 10°C.

Intensity of flicker light (lux)	f.f.f. in <i>Carassius</i>		f.f.f. in <i>Trachurus</i>	
	D. A. *	L. A. **	D. A.	L. A.
500	9.3	13	9.3	14.3
250	9.3	14.3	13	17
120	11	13	9	13
50	7	9.3	9	10

\*D. A., dark-adapted retina. \*\*L. A., light-adapted retina.

From the result shown in Table 8 it was evident that the f.f.f. increased in the state of light-adaptation and the influence of the light-adaptation was independent of the intensity of flicker stimulus light. In order to avoid the effect of the light-adaptation owing to the flicker light, the flicker light of one second duration was given with few minutes intervals in the determination of f.f.f.

The relation between the fusion frequency and the stimulus intensity of the flicker light was investigated in the dark-adapted retina, as shown in Fig. 131. The f.f.f. (open and filled circles in Fig. 131.) increased with the increase in stimulus intensity, reaching the maximum (20 c/s on the average) in the intensity of above 200 lux. Another group of the receptors (double circles in Fig. 131.) was found, however, in this retina in which the fusion frequency decreased in the range of high intensity of the flicker stimulus light. This is not to be considered as the injury of the cells, since the result is reversibly obtained.

#### *Trachurus japonicus*

In the same manner as with *Carassius*, the flicker fusion frequency was determined at a temperature of 10°C. A series of the records is shown in Fig. 132, in

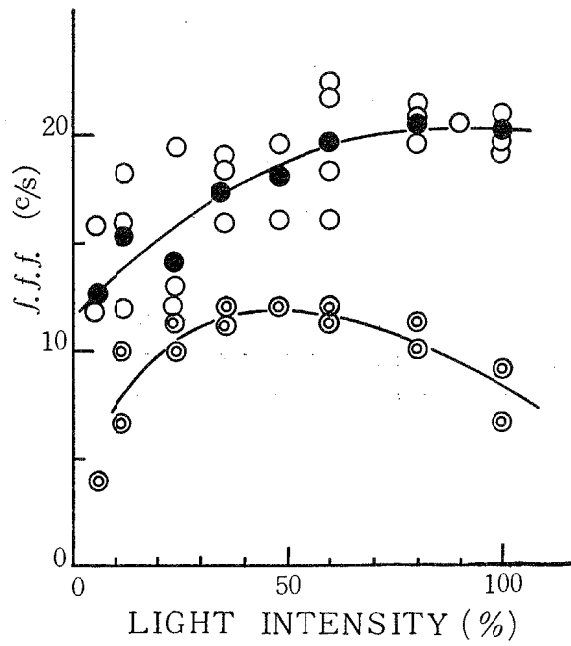


Fig. 131. Relation between f.f.f. and intensity of flicker stimulus light, in *Carassius*. Explanation in text.

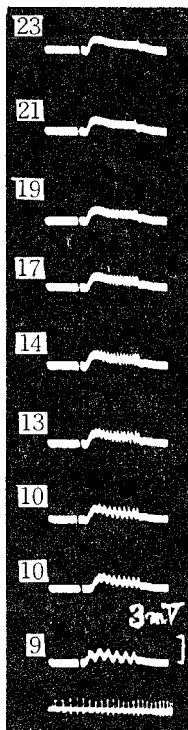


Fig. 132. Records of S-potential used for f.f.f.—determination in *Trachurus*. Other legends are same as in Fig. 130.

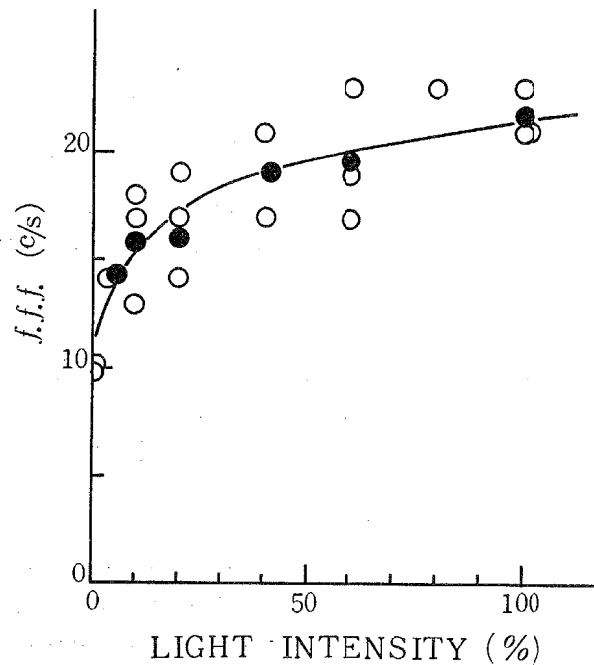


Fig. 133. Relation between f.f.f. and intensity of flicker stimulus light, in *Trachurus*. Filled circles, the average.

which the fusion appears in the flicker light of 23 c/s. The relation between the fusion frequency and the stimulus intensity is shown in Fig. 133. The fusion frequency increased with the intensity of stimulus light, but did not attain the maximum level within the limit of the intensity used in the present experiment.

The group of the receptors showing no increase in frequency with high stimulus intensity, was also observed in this species as well as in *Carassius*.

#### *Halichoeres poecilopterus*

The f.f.f. determination was made at the temperature of 10°C. The relation between the fusion frequency and the intensity of flicker light, was shown in Fig. 134. The value of f.f.f. was 13 c/s in the flicker stimulus of unit intensity.

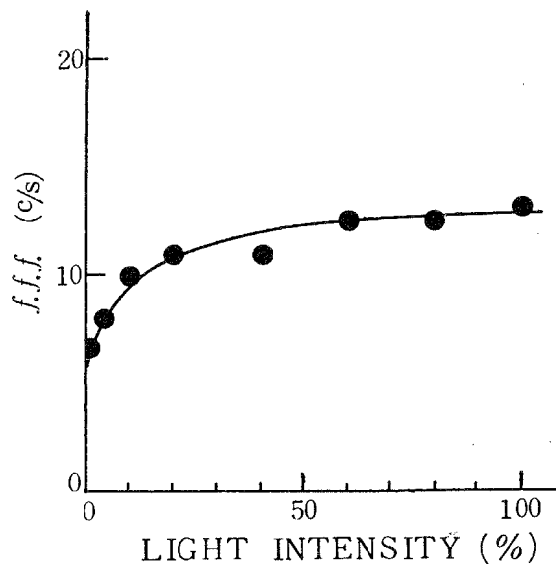


Fig. 134. Relation between f.f.f. and intensity of flicker stimulus light, in *Halichoeres*.

#### *Sillago japonica*

The response to the flicker stimulus light was the same as in the case of *Carassius*. One series of the records is shown in Fig. 135. In this species the highest value of the fusion frequency was 22 c/s in the flicker stimulus light of unit intensity.

#### *Chrysophrys major*

Some of the records obtained in the determination of fusion frequency are shown in Fig. 136. The increase in fusion frequency with light-adaptation was observed to be the same as that of *Carassius*, as shown in the records of the same figure and Table 9, although the amplitude of the response decreased a little in the light-adaptation. From the relation between the fusion frequency and the intensity of the flicker light, it was seen that the highest value of f.f.f. in the dark-adapted retina was determined in about 18 c/s.



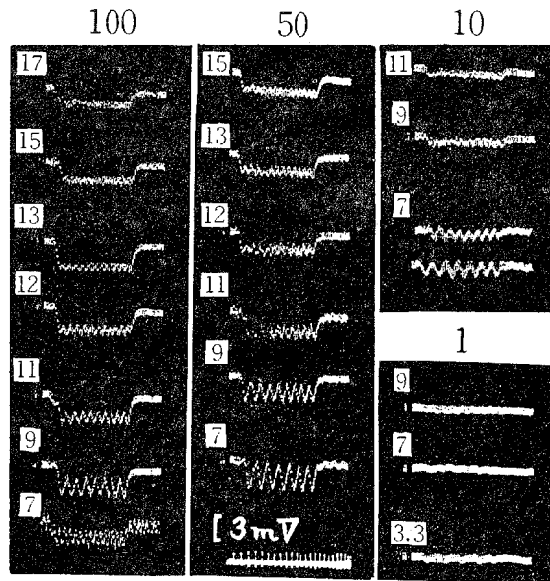


Fig. 135. Records of S-potential used for f.f.f.—determination in *Sillago*. Numerals on each record, frequency of the flicker stimulus light and that on upper side, intensity of the flicker stimulus light. Time mark : 1/6 sec.

Table 9. Influences of the stimulus intensity and the state of adaptation on the f.f.f. determined by means of S-potential in *Chrysophrys* retina. The potential is induced from the same position on the retina. Temperature, 20°C.

		Intensity of flicker light (%)					
			1	5	10	54	100
f.f.f.	No. 1	D. A. *	12	—	13	5	3
		L. A. **	—	—	15	13	12
	No. 2	D. A.	15	20	—	6	5
		L. A.	—	—	11	13	13
	No. 3	D. A.	5.5	9	11	9	—
		L. A.	—	—	11	15	16

\*D. A., dark-adapted retina. \*\*L. A., light-adapted retina.

*Lagocephalus lunaris*

A series of the records used for the determination of the f.f.f. is shown in Fig. 137. In moderate intensity (300 lux) of the flicker light, the highest fusion frequency was 20 c/s. In this species the increase in value of the f.f.f. seemed unlikely to be found in the light-adaptation.

*Fugu niphobles*

Series of the records used for the determination of the fusion frequency is shown

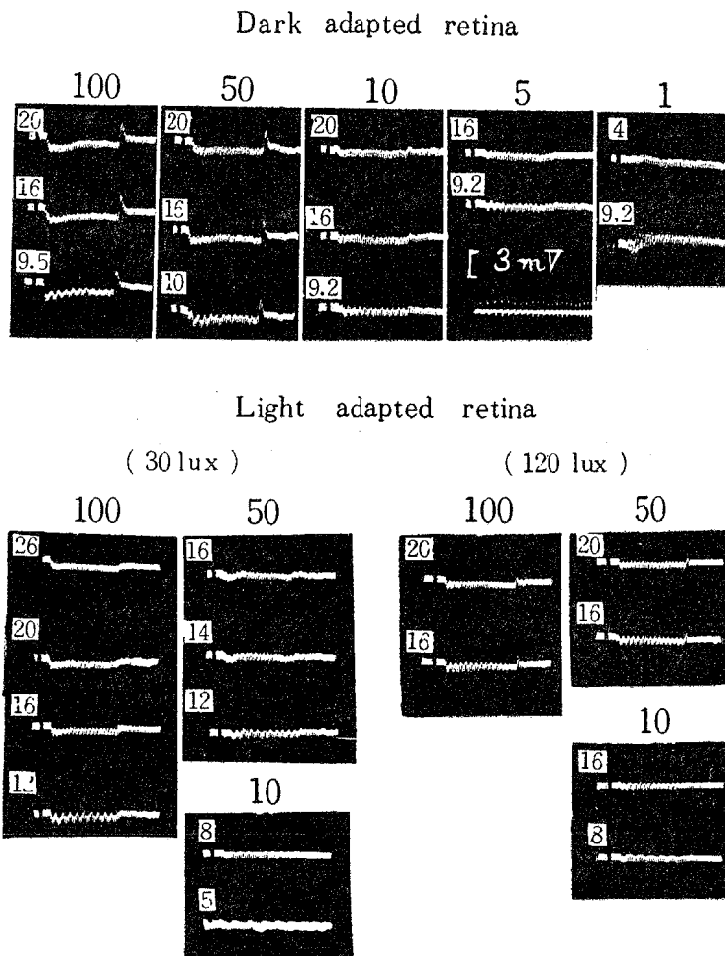


Fig. 136. Records of S-potential used for f.f.f.—determination in dark- and light-adapted retinae of *Chrysophrys*. Other legends are same as in Fig. 135.

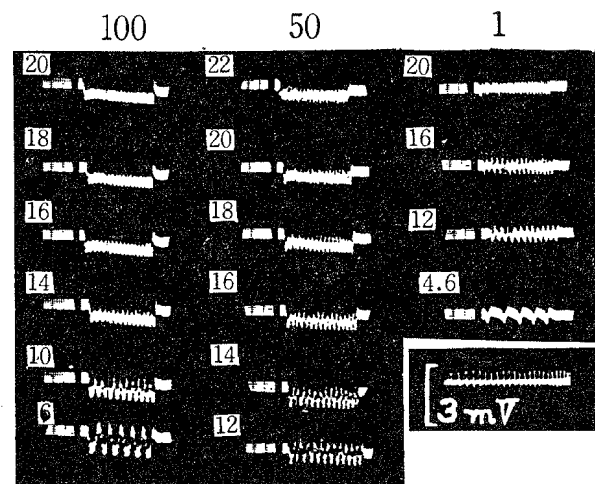


Fig. 137. Records of S-potential used for f.f.f.—determination in dark-adapted retina of *Lagocephalus*. Other legends are same as in Fig. 135.

in Fig. 138. The response to flicker stimulus light was remarkably suppressed in the stimulation of high intensity. The highest value (about 16 c/s) of the fusion frequency was obtained in the stimulus intensity of about 25 lux.

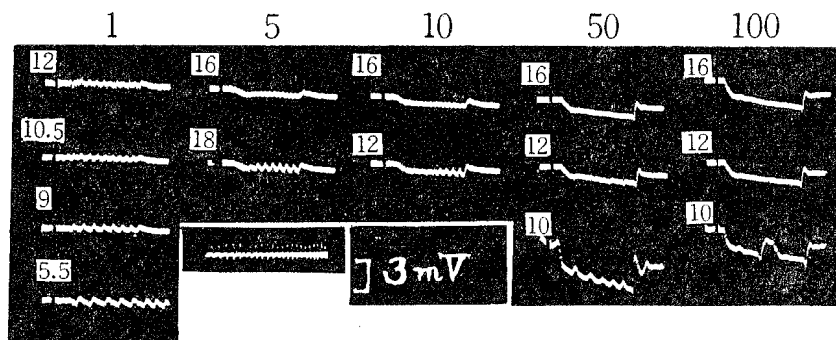


Fig. 138. Records of S-potential used for f.f.f.—determination in *Fugu*. Other legends are same as in Fig. 135.

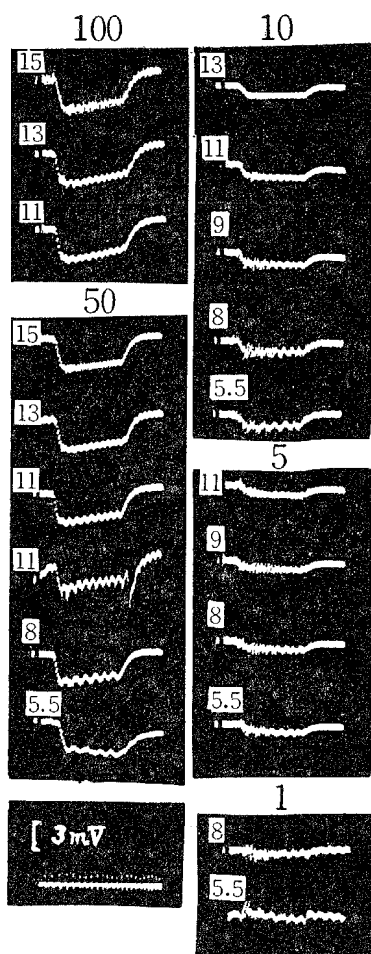


Fig. 139. Records of S-potential used for f.f.f.—determination in *Stephanolepis*. Other legends are same as in Fig. 135.

*Stephanolepis cirrhifer*

The response to the flicker light of various intensity is shown in Fig. 139. From the records, it was seen that the highest value of the fusion frequency was 15 c/s, but it became greater with the increase in stimulus intensity.

The highest f.f.f. determined in each species of fish was listed in Table 10. In this case, the f.f.f. which was determined at 10°C was converted into the value of 20°C in order to make comparison under the definite condition, according to the relation between temperature and f.f.f., which was obtained from TAMURA's experiments (TAMURA *et al.* '59). From Table 10 it was found that the f.f.f. of benthonic and nocturnal fishes showed generally the low value of less than about 20 c/s, whereas that of active swimming fish such as *Trachurus* showed clearly the higher value.

Table 10. Flicker fusion frequency in fish retina. Temperature, 20°C.

Species	f.f.f. (c/s)	Light intensity (lux)
<i>Trachurus</i>	32	600
<i>Carassius</i>	28	"
<i>Sillago</i>	22	"
<i>Lagocephalus</i>	20	250
<i>Halichoeres</i>	18	600
<i>Chrysophrys</i>	18	250
<i>Stephanolepis</i>	15	600
<i>Fugu</i>	16	25
<i>Gymnothorax</i>	15	50
<i>Mustelus</i>	above 10*	50
<i>Narke</i>	6	1.5
<i>Urolophus</i>	4.4—6	"

\*Experimental temperature, 10°C.

#### (5) Some aspects of the retinal structure

Although number of studies on retinal structure have been made in fish, most of them seem to have been restricted to the receptor layer, such as the ratio of cone to rod, size of the receptor cell, and their density or arrangement. Recently the neuro-layer of the retina has been known as the origin of ERG (TOMITA '57 and others). Therefore, some characteristics of the retinal structure involving the neuro-layer were investigated comparatively through this study.

i) Thickness of the entire retina: The distance from the base of the pigment layer to the inner limiting membrane was measured in the three regions of retina, fundus, *ora serrata* and the region located midway between them. The results are shown in Table 11.

The thickness of the retina was investigated in relation to the depth of the habitat of the fishes. In most of the fishes, the thickness of the retina varied considerably according to the locality of the retina, and so the comparison among the species was made by the thickness in the region near the midway between the fundus and *ora serrata* in the retina. The data of the depth were obtained from the report of "The investigation of benthonic fish" made by Nankai Regional Research Laboratory and through the private communications from Drs. R. ISHIYAMA and T. TAKAI. The result was shown in Fig. 140. Thin retina tended to be found in the fishes inhabiting deep sea and having a nocturnal or burrowing behaviour, whereas thick retina in the fishes inhabiting shallow water near the sea coast.

ii) External nuclear layer and internal nuclear layer: WALLS ('42) observed that the external nuclear layer was much thicker than the internal nuclear layer in

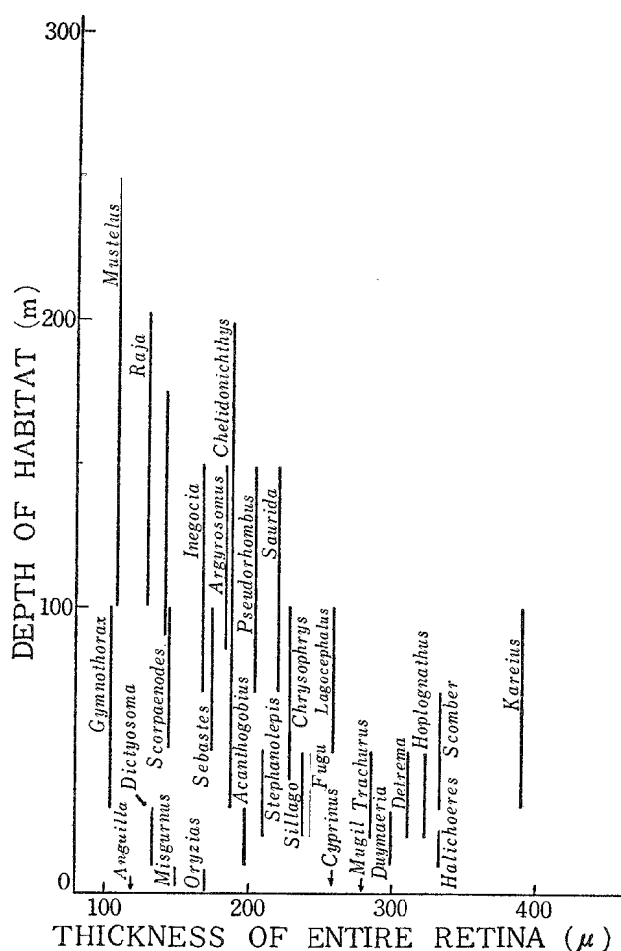


Fig. 140. Relation between the thickness of entire retina and the depth of the habitat, in various species of fish. Explanation in text.

nocturnal animals while the relation was reversed in diurnal ones. In order to research the characters of both layers in various species of fish, the ratio in thickness of both layers ( $T_{int}/T_{ext}$ ), which were measured in the region near the midway between the fundus of the retina and the *ora serrata*, was computed. And also the ratio was compared with the value of  $L_{10}$ , mentioned previously as the indicator of the changing rate in the amplitude of ERG. From the results shown in Table 11, it was found that the ratio ( $T_{int}/T_{ext}$ ) was larger than 1 in the fishes having large value of  $L_{10}$ , which naturally led to the conclusion that the ratio was larger in the diurnal fishes having their habitats in shallow water or having the pelagic behaviour, whereas in benthonic and nocturnal fishes the ratio was generally observed to be less than 1. Particularly in nocturnal fish such as *Gymnothorax* the ratio was the smallest (0.2). In *Fugu*, *Kareius* and *Rhina*, however, the ratio was found to be more than 1, in spite of their benthonic lives.

The fish showing a larger ratio than 1, tended to have compactly arranged nuclei of small size in the nuclear layer of their retina.

Table 11. The changing rate ( $L_{10}$ ) obtained in amplitude of ERG according to various intensity of stimulus light, and various characteristics in the retinal structure of fish. Explanation in text.

Species	$L_{10}$ (%)	Thickness of entire retina in $\mu$			$T_{int}/T_{ext}$			Number of ganglion cells found in the range of 30 $\mu$ in the section	Approximate diameter of the largest ganglion cell in $\mu$ *
		F	M	O **	F	M	O		
<i>Oryzias</i>	180	172			1.1			15	3
<i>Halichoeres</i>	30-65	150-204-336			1.8-1.2-1.3			7	3
<i>Epinephelus</i>	22-55	204-144-108			0.6-1.0-2.0			3.5	3
<i>Acanthogobius</i>	50	211-198-135			1.1-1.6-1.4			4.5	8-5
<i>Trachurus</i>	46	288-234-120			0.7-1.7-1.3			2.5	14-9
<i>Stephanolepis</i>	44	211-179-122			0.9-1.1-2.2			3.5	9-7
<i>Hoplognathus</i>	38	348-324-252			2.1-1.4-1.5			4.5	10
<i>Sillago</i>	35	264-240-144			0.6			5	4
<i>Lagocephalus</i>	32	396-348-147			0.9-0.8-0.5			3.5	7
<i>Gymnothorax</i>	30	105			0.5-0.2			2	3
<i>Pseudorhombus</i>	28	204			1.1-1.0			2	6
<i>Chrysophrys</i>	20	283-228-115			0.2-0.7-1.4			5	9-6
<i>Misgurnus</i>	20	150-120			3.3-1.3			2.5	5
<i>Anguilla</i>	18	117-109-93			0.7-0.6-1.1			3	4
<i>Saurida</i>		276-220-144			0.5-1.2				6
<i>Cyprinus</i>		288 -240			1.1 -1.1			4	6
<i>Mugil</i>		350-296-300			0.8-1.1-1.7			2	6
<i>Scomber</i>		348-336-300			1.0-1.0-1.5			3.4	8
<i>Argyrosomus</i>		225-187-157			0.5-0.8-1.0			4.5	6
<i>Dictyosoma</i>		135-120			2.2-3.3			3.5	6
<i>Detrema</i>		355-314-180			1.1-0.8-1.5			6	3
<i>Scorpaenodes</i>		160-144-120			1.1-0.2-0.2			2	9-6
<i>Inegocia</i>		168-140-84			1.8-4.3			5-12	5
<i>Chelidonichthys</i>		265-188-123			1.1-0.8-0.8			3.5	6
<i>Fugu</i>		289-244-132			2.2-3.1-2.6			7	6
<i>Kareius</i>		396-312-119			2.0-2.4-1.3			22	2
<i>Rhina</i> (young)		265-188-123			1.9-1.6-1.6			1.8	9
<i>Raja</i> ( " )		160-140-120			1.5-3.3			1.2	8
<i>Holorhinus</i>		132-96-84			0.7			1.3	9
<i>Urolophus</i>		120-96-60			0.5			0.3	9
<i>Mustelus</i>		84-70			0.4			0.5	7

\*The range of the variation is represented only in the noticeable variation of size.

\*\*F, M, O; Fundus, midway between fundus and *ora serrata*, *ora serrata*, in where the measurements were made, respectively.

iii) Character of the tertiarily neuron layer: In order to know the density and the size of the ganglion cell, the number of the ganglion cells arranged in single layer was counted in the range of 30  $\mu$  in the ganglion cell layer of the retina, and the approximate diameter of the cell was measured. The ganglion cell of the retina

was usually observed in the arrangement of one layer, and the density of the ganglion cells did not seem to differ according to the locality of the retina in most fishes except for *Kareius*, in which the density in the ventral side of the retina was found to be higher than that in the dorsal side of the retina. Measurements of the ganglion cell density were made in three parts of the retina, and the size of the ten cells in each part was averaged. The density of ganglion cells in the region of fundus was adopted to give the comparison among various species. The results were also shown in Table 11. The obvious correlation was not found between the character of the ganglion cell layer and the characteristics found in the ERG, although the fishes having the large ganglion cell in their retinae showed the tendency that the negative on-potential occurred remarkably in their ERGs.

iv) Size and shape of the receptor cell: Although the size of the receptor cell in various fish has been already described in detail by WUNDER ('36), TAKATSUJI ('39) and others, but the description concerning the outer segment of the cell was insufficient because it was covered with the enlarged retinal pigment. The author obtained the histological preparation of the retina from a semi-dark-adapted eye or depigmented retina and measured the size and shape of the receptor cell, mainly of the cone cell. For the measurement, he employed the large cell as carefully as possible in the regions midway between fundus and *ora serrata* of the retina in a horizontal section of the eye. These schemata are shown in Fig. 141, in which those of tuna, *Neothunnus albacora*, and elasmobranch fishes are added for comparison.

From the illustration, the receptor cell is roughly classified into the following types:

Type A, the outer segment of the cone is long; *Kareius*, *Scomber*, *Fugu*, *Lagocephalus*, and *Argyrosomus*.

Type B, the outer segment is not long, but the ellipsoid is rather wide, and then the receptor cell seems to be a stocky cylinder.

Type B-1, the ellipsoid is very large; *Scorpaenodes*, *Saurida*.

Type B-2, the ellipsoid is very small; *Anguilla*, *Gymnothorax*.

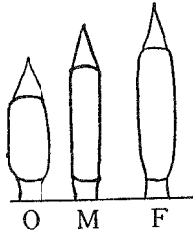
Type B-3, the ellipsoid is middle sized; *Acanthogobius*, *Sillago*, *Cyprinus*, *Epinephelus* and *Carassius*.

Type C, the ellipsoid is comparatively long, and the width of the ellipsoid is small; *Halichoeres*, *Dictyosoma*, *Hoplognathus*, *Stephanolepis*, *Chrysophrys*, and *Pseudorhombus*.

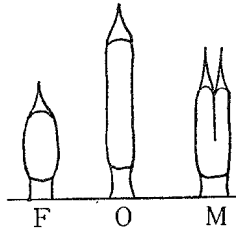
The fishes of type A have been known as benthonic fishes and their motions are known to be rather active. It is also well considered that most of them migrate from deep place to shallow one for spawning. The fishes of type B-2 are typical nocturnal fishes. The fishes of type B-3 are known to have both diurnal and nocturnal behaviours. It is noticed that the retina is usually thin in the fishes belonging to types B-1 and 2 (Fig. 140). Most of fishes of type-C are usually found in diurnal fishes living in shallow water. In these fishes the retina is thick and usually has high threshold intensity of stimulus light for eliciting the ERG.



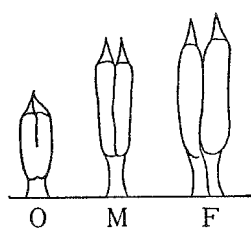
*Halichoeres poecilopterus*



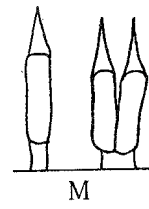
*Dictyosoma burgeri*



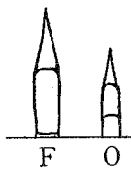
*Stephanolepis cirrhifer*



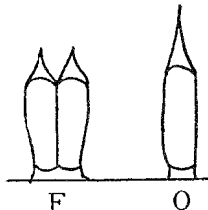
*Hoplognathus fasciata*



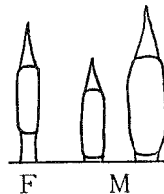
*Inegocia crocodila*



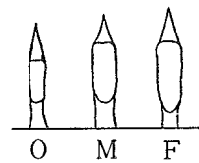
*Pseudorhombus cinnamomeus*



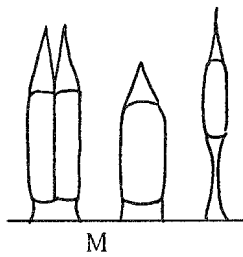
*Chrysophrys major*



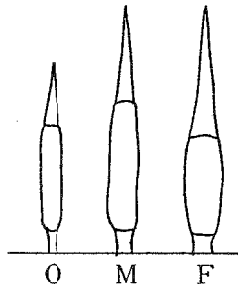
*Detrema temminki*



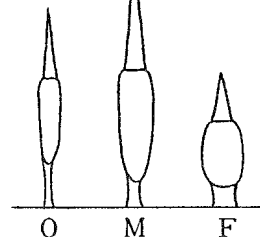
*Trachurus japonicus*



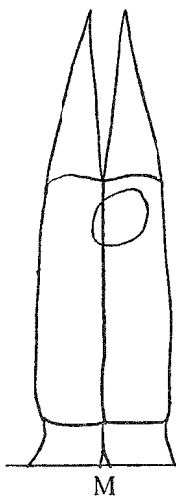
*Scomber scomburus*



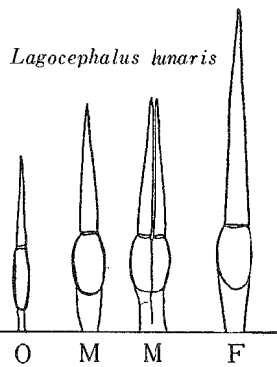
*Kareius bicoloratus*



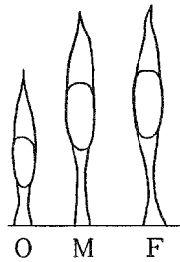
*Neothunnus albacora*



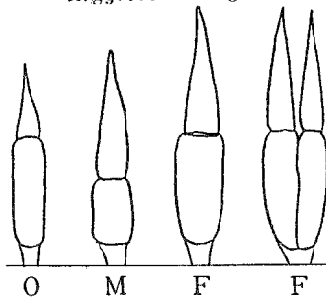
*Lagocephalus lunaris*



*Fugu niphobles*



*Argyrosomus argentatus*





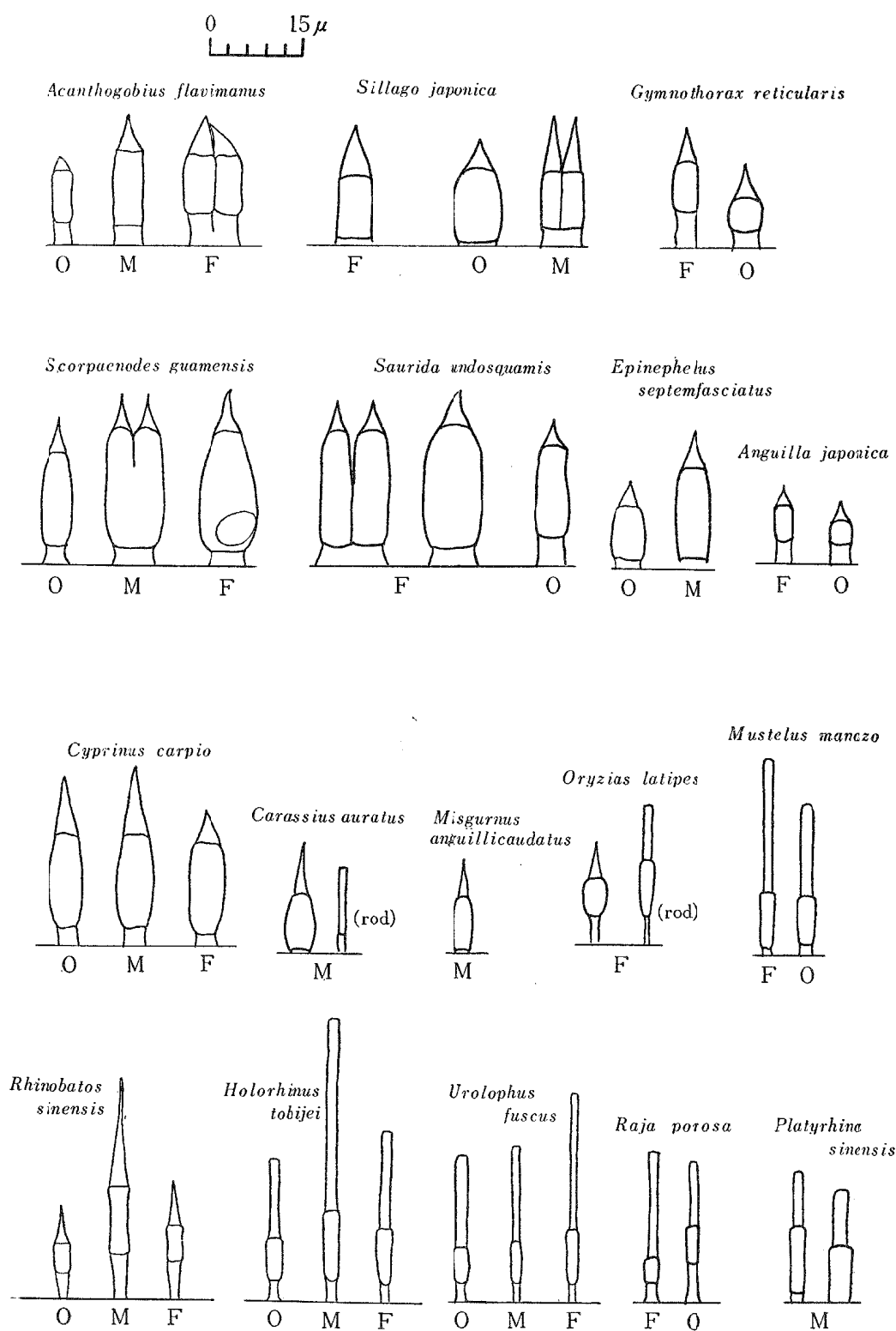


Fig. 141. Size and shape of the receptor cell in the retina of various species of fish. F, fundus; O, ora serrata; M, midway region between F and O, in where the measurements of the receptor cell are made, respectively.

### Discussion

GRANIT ('47) described that the fish retina was an unsuitable material for the investigation of ERG. In fact, it was difficult and unstable to induce the ERG from marine fishes. By the modified method, however, the author has found it possible to record the ERG from many species including marine and fresh water fishes.

Although the characters of the ERG in fish were not essentially different from those in other higher vertebrate eye, particularly of the frog's eye, it was found in general that the ERG of fish showed in light-adaptation, noticeable negative potential (a-wave), off-response and the suppression of positive potential (b-wave), and also the form of off-response was found to be specific according to the various species, differing from one another in their behaviour. It is well known that the ERGs of vertebrate eye are classified into E-type and I-type, and the ERG of fish is subjected to the latter (GRANIT '47). The ERGs recorded in the present work showed, of course, the characters of I-type, but it was possible to subdivide according to the characteristics of off-response. The classification based on these characteristics was found to be related to the behaviours of the species.

#### Classification of the fish depending upon wave-form of ERG and its relation to ecological aspects

As described previously, various forms of the ERG were obtained from various species of fish. Two kinds of classification of species were made according to various characters found in the wave-form of the ERG, and some considerations were made from the ecological view points.

(A) Classification according to off-response of the ERG obtained in dark-adapted eye.

Group I The response to the stimulus light of long duration (1—3 seconds) does not elicit the off-response remarkably.

*Anguilla, Halichoeres, Misgurnus, Pseudorhombus, Dictyosoma.*

Moreover also *Kareius* shows the response of this type occasionally. Among these species the retina of *Misgurnus* shows the remarkable off-response in light-adaptation, and *Anguilla* also shows a small off-response in light-adaptation only by intense stimulus light.

Group II The response to the stimulus light of long duration (1—3 seconds) elicits a small off-response.

*Saurida, Scorpaenodes, Cyprinus, Gymnothorax, Fugu and Argyrosomus.*

Group III The response to the stimulus light of long duration (1 second) shows the remarkable off-response.

*Trachurus, Chrysophrys, Stephanolepis, Lagocephalus, Hoplognathus, Kareius.*

The fishes subjected to Group I seem to have benthonic habit in general and burrow into sand or mud of the bottom. It is well known that in the culture pond *Anguilla* burrows under bottom-ground except in the feeding times, and *Halichoeres* hides itself motionless in the sand of the bottom at night, and also *Misgurnus* burrows into mud in winter. *Pseudorhombus* and *Kareius* are well found covered with the sand in the aquarium. It is noteworthy that these fishes have small eyes in general.

The ERGs obtained in the fishes of Group I, may be considered to be closely related to the E-type. Though the ERG of *Anguilla* shows an off-effect as the case may be, the behaviour of *Anguilla* is typically nocturnal, and the retina consists almost of rods (BAYLISS *et al.* '39). On the other hand, in *Halichoeres* the off-response is not found either, but the retina is known to consist of a cone cell only (TAKATSUJI '39). Therefore both rod-dominant and rod-free retinæ may be hard to elicit off-response.

The fishes subjected to Group II have the habitat in deeper sea or in turbid water. They are known as the fishes which feed at night rather than in daytime, in general (UCHIHASHI '53), except a few species. Further, in these fishes except for *Gymnothorax*, the negative potential of the ERG was particularly remarkable in the stimulation with intense light, although off-response was similar to that in the fishes of Group I. By light-adaptation, the positive potential of the ERG was considerably suppressed in these fishes. In most species of this group the retina showed noticeably low threshold. Therefore, these fishes will be assumed to be able to adapt their eyes to the dim-illuminated environment. This assumption coincides with the ecological situation that the fishes are easily caught in the sea at a depth of 100 metres or so.

The fishes subjected to Group III are typical coastal fishes and are mostly known as diurnal fishes. These fishes are relatively active in their swimming, and usually found in shallow water. Further, in these fishes off-response was elicited remarkably, particularly in the light-adapted eye, and also even in the dark-adapted eye it was caused evidently by the stimulus light of short duration, for example of 1/10 second. And in most species of this group, too, negative potential was dominant without regard to stimulus intensity. These facts mean that the component parts having relation to the inhibition play the role of visual mechanism. Therefore, the ERG obtained in the fishes of Group III is considered to be a typical "inhibitory-form", I-type. Besides, these fishes generally have the high threshold of the retina as compared with that of the fishes in other groups (*cf.* Chapter III). Furthermore, the chromatic vision has been suggested in the fishes of this group (*cf.* Chapter III), and so the ERG accompanying the remarkable off-response may involve the complicated sensory mechanism in relation to colour vision.

(B) Classification according to the special characters found in the wave-form of the ERG

(i) In the response to intense light, the fast negative potential (a-wave) occurs initially, and the succeeding positive potential is remarkably suppressed.

*Scorpaenodes*, *Saurida*, *Fugu* and *Cyprinus*.

The most of these fishes belong to Group II in classification in (A) and are assumed to have the habitat in relatively deeper sea or turbid water. And also these fishes show a low threshold intensity (*cf.* Chapter III).

(ii) ERG consists of positive potential only.

*Gymnothorax.*

This fish is well known as a typical nocturnal animal, then the characteristic of the ERG seems to suggest the rod-retina, although cone cells are obviously found in the histological observation of the retina.

(iii) The negative potential is dominant in the ERG.

*Halichoeres, Stephanolepis and Pseudorhombus.*

These fishes are known as typical diurnal animals, and the characteristic of the ERG will suggest cone retina. Lack of rod is known in the retina in some of them (TAKATSUJI '39).

(iv) The positive potential (b-wave) is remarkably suppressed by the light-adaptation.

*Trachurus, Chrysophrys, Scorpaenodes, Cyprinus and Kareius.*

These fishes are known to be active in both daytime, and night and to perform their feeding action depending upon vision. These fishes are assumed to have the sharpness of visual accuracy.

#### Relation between the magnitude of the response and the stimulus intensity

It was found that the magnitude of the response attained a plateau state in certain definite stimulus intensity. TAMURA *et al.* ('57) assumed that *Sparidae* had a deeper habitat than *Serranidae*, since the minimum intensity which showed the maximum amplitude of cone action potential (S-potential) was lower in *Sparidae* than in *Serranidae*. And they pointed out that this minimum intensity might indicate the environmental illumination that was suitable for the fish to live in. In the present work, the amplitude of positive potential of the ERG (b-wave) was used for the investigation, since b-wave was considered to represent the excitation of the retina in dark-adaptation (GRANIT '47). Consequently, the significance of the present investigation may be essentially different from that of TAMURA's experiment. As shown in Fig. 10, however, the intensity at which the magnitude of positive potential attained to the maximum one, was found to exist within the range of stimulus intensity used in the present work, in some species such as *Trachurus*, *Chrysophrys*, *Raja* and *Narke*. From the ecological situation of the fishes, it is evident that the brightness of the environment in which these fishes live or seek bait, is lower than that in the case of the other fishes investigated, and furthermore among those 4 species *Raja* and *Narke*, in which the plateau of b-wave height is attained in especially low intensity of stimulus light, live in environment of lower brightness than the rest. Consequently, the assumption advanced by TAMURA *et al.* ('57) concerning the character of cone action potential seems to have been applicable for the result obtained by b-wave of the ERG in this work.

In *Scorpaenodes*, *Saurida* and *Cyprinus* the remarkable suppression of positive potential of the ERG was found in the stimulation of the intense light, but the intensity that caused the decrease of positive potential was extremely low (Fig. 111). Therefore, the environmental illumination in which the fishes live or move about, may be also assumed to be noticeably low. This assumption seems not to be inconsistent with the depth of the habitats of these fishes. Moreover, the results obtained by means of S-potential in this work supports also the opinion described by TAMURA *et al.* ('57). However, the present results obtained with regard to many species will show from the ecological situation that the minimum intensity producing the maximum potential is considered to be the brightness of the feeding place of the fish rather than the environment where the fish live.

On the other hand, it was found that ERG showed the linear relation to the logarithm of the stimulus intensity in the range of adequate stimulus (Fig. 110), and the gradient of the straight line against abscissa in the relation was found to be different among the species. The fact that  $L_{10}$  appears to correlate with the depth of the habitats of the fishes, is showing the possibility that  $L_{10}$  value is available as the indicator of the environmental illumination. In fact, the fishes showing large value of  $L_{10}$  such as *Acanthogobius*, *Sajori* and *Oryzias* are typical diurnal fishes and have generally high threshold intensity of the stimulus light, whereas the fishes showing small value of  $L_{10}$ , such as *Anguilla*, *Misgurnus*, *Mustelus* and Batoidae are known as nocturnal or burrowing fishes and generally have the threshold in relatively low intensity.

#### Light adaptation and brightness discrimination

Of the two types found in the course of light-adaptation, one type showing only the rise of the threshold intensity during the adaptation, is encountered in those fishes as *Anguilla*, *Misgurnus* and *Chelidonichthys*, and it is assumed from the ecological view point that those fishes may have an inferior capacity to adapt themselves to light. As the value of differential threshold ( $ds/s$ ) is considered to express the ability of brightness discrimination, it is significant to compare the values obtained from various species of fish, from the ecological view point of the fish vision. In the fishes showing above-mentioned type, the value of  $ds/s$  is not found to be so large as those of shallow water fishes or batoid fishes (Table 6), and it seems to suggest that those fishes do not depend so much on vision. In fact, it is assumed by UCHIHASHI ('53) from the external structures of the brain that those fishes search the bait by smell or taste.

Another type found in the course of light-adaptation seems to permit greater activities in light environment. This suggestion is supported ecologically by the fact that the fishes, showing this type, such as *Chrysophrys*, *Stephanolepis* and *Kareius* are known to be diurnal or sight-feeding fishes. Moreover, the ability of brightness discrimination that is found to be rather superior in these fishes (Table 6),

may be assumed to be advantageous for their behaviours. Furthermore, it seems to be noticeable that change in the threshold of the retina by light-adaptation, is generally large in the former type, while it is small in the latter type.

### Flicker fusion frequency

High flicker fusion frequency (f.f.f.) indicates high "temporal resolving power." This assumption has been studied in detail in terrestrial animals for example in insects (AUTRUM '52), and it has been confirmed that significantly high f.f.f. is characteristic of diurnal or active moving animals, while low f.f.f. is that of nocturnal and sluggish animals. In the case of fish retina, a few of f.f.f. determinations have been investigated through the electro-physiological technique, although f.f.f. of some fishes has been studied by means of behavioural experiments (BENIUC '33, WOLF *et al.* '36). SVAETICHIN ('53) reported the f.f.f. of the perch being 33 c/s determined by S-potential, and TAMURA and HANYU ('59) investigated the relationships between f.f.f. (in terms of half of the maximum amplitude) with temperature or light intensity in the intact eye of *Cyprinus*. In the present work the comparative studies were made on f.f.f. of fish, and from the results summarized in Table 10, it was also found that the f.f.f. of fish tended to correlate with the ecological situation described above. And the results seem to coincide with the opinion represented by AUTRUM ('52) that the sensitivity of insect retina is generally lower in the eyes showing high f.f.f. than in those low f.f.f. Because, it has been evidently confirmed that the fishes showing low f.f.f. value have generally high sensitivity in their retinae (*cf.* Chapter III). High f.f.f. was seen not only in active swimmers but also in benthonic ones such as *Halichoeres*, *Chrysophrys*, *Fugu* etc. High f.f.f. may be useful also for benthonic fish in avoiding a predatory enemy, for example, sharks.

The value of f.f.f. was clearly found to increase by light-adaptation, despite the decrease in amplitude of the potential. The results may suggest that the fish can follow the moving object better in light environment.

### Retinal structure

The individual retina in teleosts is known to vary in thickness from about 100  $\mu$  to more than 500  $\mu$  (WALLS '42). The schemata drawn in Fig. 140 show a tendency that the variation in thickness of the retina has some relations to the fish habit, particularly to the depth of the habitat. The same tendency was obtained also in the relation of the threshold of the retina of various species with fish behaviour (*cf.* Chapter III). In future, it is necessary to investigate the sensory mechanism in the relation between the threshold and the thickness of the retina.

Most of the teleost fish have the dual retina, and the ratio in number of cones and rods varies according to species (WUNDER '23). A part of the differences in specific

characters found in the ERG is assumed to be due to the difference in this ratio as well as to the difference in the cytological structure of the receptor cell (GRANIT '56). In the measurement of this ratio, however, there is technically difficulty in calculating the number of rods. Being, however the nucleus of a cone connects with one bipolar cell whereas the nuclei of some rods correspond to one bipolar cell, the ratio in thickness of the internal nuclear layer and the external nuclear layer ( $T_{int}/T_{ext}$ ) is considered to represent the ratio in number of cone to rod. Therefore, this ratio may have a significance for interpreting diagnostically the fish behaviour and the fish ERG. In fact, the ratio thus obtained well correlated with  $L_{10}$ . In most fishes, this ratio varied slightly according to the locality of the retina, but in some fishes it varied considerably, being smaller or larger than 1. The latter was the case that the fishes might be of both diurnal and nocturnal characters. The fact seems to agree with the ecological situation of the fish to some extent, as was observed in *Chrysophrys*, *Trachurus* and others.

From Table 11, it is seen that the fishes in which the number of the ganglion cells is large or the ganglion cells are moderate in size in their retinae, tend to have the following behaviours; they are shallow water inhabitant and are diurnal in activity, *Halichoeres*, *Hoplognathus*, *Acanthogobius*, *Stephanolepis* and *Chrysophrys* being examples. In these fishes the ability of colour discrimination has been suggested from the investigation of the spectral sensitivity curve (*cf.* Chapter III), in spite of the low sensitivity of their retinae.

It has been well known that the light projected on the retina is absorbed at the surface of the outer segment of the receptor cell. Therefore, it is assumed that the receptor having a long outer segment can utilize a faint light very well. The assumption is supported by the fact that the remarkable elongation of outer segment of the receptor cell is found in the retina of so called deepsea fish inhabiting down to about 2,000 metres (MARSHAL '57). Hence, the fishes belonging to Type A of the author's classification may show the possibility that they can behave in the bottom of a rather deep sea. Moreover, the result that a number of twin cones and rather many ganglion cells are found in the retinae of these fishes, suggests that they develop a vision suited to the life in shallow water as well. Consequently, these fishes may be assumed to be active in movement in shallow waters as well as in the deeper sea.

The fishes inhabiting shallow waters not more than 50 metres or the diurnal fishes have the external nuclear layer thinner than the internal one, and also these fishes have a high density of ganglion cells. On the other hand, the fishes inhabiting greater depths, nocturnal or burrowing, fishes, and the fishes conducting remarkably vertical movement show entirely opposite characters to those mentioned above. And further, the thickness of entire retina tends to be smaller in the latter fishes. As is described above, the fact that there is difference in the retinal structure between the fishes inhabiting deeper sea (more than 50—100 metres) and the shallow water fishes, is assumed to be one of the morphological ground accounting for the physiological finding that the spectral response of the retina in the former fishes differs

from that in the latter.

### Summary and conclusions

1) Various inductions were made in order to record the electroretinogram (ERG) of teleost fish including 24 species of marine and fresh water fishes, and the wave-form of the ERG and the visual functions investigated by use of the ERG were considered on the basis of the ecological situation of the fish species.

2) The wave-form of the ERGs were classified into three groups according to the character of off-potential in the dark-adapted eye: Group I, the ERG showed positive potential dominantly and was hard to elicit the off-response. This form was found in nocturnal fishes, such as *Anguilla*, *Misgurnus* and others. The ERG of this group seemed to resemble the excitatory form of vertebrate ERG. Group II, the ERG showed positive potential dominantly in the case of stimulation with weak light, but the remarkable negative potential in that with intense light. Off-response was also unremarkable. This form was found in *Saurida*, *Scorpaenodes* and *Cyprinus*. These fishes are usually known to inhabit deeper sea or turbid water. Group III, the ERG showed negative potential dominantly, and also showed the remarkable off-potential. This type was found in *Chrysophrys*, *Stephanolepis*, *Lagocephalus*, *Hoplognathus*, *Kareius* and others. These fishes are usually known as inhabitants in shallow water and diurnal fishes. Above-mentioned classification seemed to be supported by the visual functions investigated by the ERG.

3) In some species, the effects of potassium chloride, alcohol, urethan and strychnine on the ERG were investigated, results of which were the same with those of the past investigations.

4) By the aid of micro-electrode the ERGs were recorded from the inverted retina in several kinds of fish. Typical S-potential was obtained in *Chrysophrys* and *Stephanolepis*, but some unusual forms of S-potential were found, too. Moreover, it was seen that the impulse superimposed on the S-potential was easily recorded in *Fugu* and *Lagocephalus*.

5) Relations between the magnitude of the ERG response and the stimulus intensity were investigated. In benthonic and deeper sea inhabiting fishes, with the increase in stimulus intensity the amplitude height of the ERG attained a plateau state at rather low stimulus intensity. In particular, in the fishes belonging to Group II such as *Saurida* and *Scorpaenodes*, the intensity which caused the suppression of the positive potential was found to be considerably low. Therefore, such intensity as is mentioned above will be considered as an ecological indicator representing the depth of the habitat.

6) A distinction was found in the gradient of the straight line obtained in the linear relation between the logarithm of stimulus intensity and the amplitude height of the ERG, in various species. The gradient of the line (called temporarily  $L_{10}$ ) usually showed a larger rate in diurnal fishes or shallow water inhabitants and tended to show a smaller rate in accordance with the increase in the depth of the habitat.



7) By using the threshold intensity of stimulus light for eliciting ERG or a size of ERG as index of the retinal sensitivity, the process of light- or dark-adaptation was investigated.

Two types of the process of light-adaptation were found: In one type the threshold intensity increased in progress of the adaptation and finally attained to a plateau state. This type was found in nocturnal fishes such as *Anguilla*, *Misgurnus* and *Chelidomichthys*. In another type the threshold intensity increased rapidly transiently at the initial phase of the adaptation, and thereafter the threshold decreased a little slowly, and finally attained to a plateau state. This type was found in diurnal fishes such as *Kareius*, *Chrysophrys* and *Stephanolepis*. Such differences seemed to be related to the ability which adapted the eye to light.

8) Differential threshold of stimulus light for eliciting ERG was investigated in the light-adaptation with various intensity of adaptation light, in several species, from the results of which a rather great ability of intensity discrimination was found in benthonic fishes, while an inferior ability was found in the fishes of burrowing habit, such as *Anguilla*, *Misgurnus*.

9) During dark-adaptation the recovery of the amplitude of ERG required a long time in general, while that of the threshold intensity was completed more rapidly.

10) The flicker fusion frequency was investigated in several species by using ERG or S-potential. There was a tendency to show a high flicker fusion frequency (*ca.* 33 c/s) in diurnal and actively swimming fishes, but a low one (*ca.* 15 c/s) in benthonic and nocturnal fishes. The flicker fusion frequency increased evidently by light-adaptation, in spite of the decrease in amplitude of the potential. In such benthonic fishes as *Fugu* and *Gymnothorax*, the highest value of flicker fusion frequency was obtained in a relatively low illumination, for example, of about 30 to 60 lux.

11) Some aspects on the retinal structure were given. The shallow water fishes and the diurnal fishes tend to have a thick retina, though in those fishes the threshold intensity is usually high. The fishes inhabiting deeper sea and the nocturnal fishes tend to show the reverse phenomenon.

12) It was found that the external nuclear layer of the retina was much thicker than the internal in nocturnal and deeper sea fishes, and the internal nuclear layer was much thicker than the external in diurnal and shallow water fishes. The ratio of the thickness of the external nuclear layer to that of the internal nuclear layer corresponded to the value of  $L_{10}$  to some extent.

13) In diurnal fishes or shallow water fishes, they have a tendency to show the ganglion cells large in size or a large number of cells in tertiarily neuron layer of their retinae.

14) According to the size of the outer segment of visual receptor, the species were classified into some types. This classification was discussed from the ecological situation of the fishes.

15) Above-mentioned distinctions of the retinal structure between the shallow water fishes or diurnal fishes and the deeper inhabitant or nocturnal fishes, are con-

sidered to be the morphological basis accounting physiologically for the distinctions of the responses to spectral light in the fishes of both types.

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