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# The visual system of the minnow (*Phoxinus Phoxinus*), with ~~special~~ specific reference to its relationship to colour change and behaviour

by

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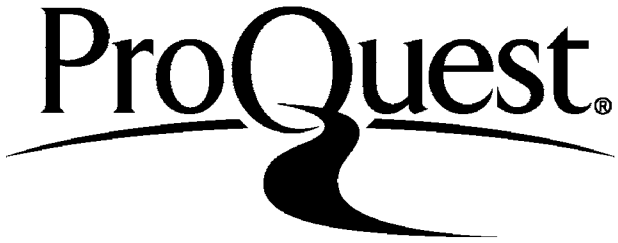

## ABSTRACT

The anatomy of the retina was studied and counts were made of the retinal elements. It was found that the rods single and double cones decrease in number centrally and the triple and quadruple cones increase centrally. The visual acuity calculated from the counts do not agree with the acuity figures given by other workers. The dorsal or ventral parts of the retina were removed surgically or destroyed by high intensity light. The chromatic behaviour of these fish led to the conclusion that the whole of the retina is important for normal chromatic adaptation to white or black backgrounds.

The anatomy of the optic tract, chiasma, geniculate complex, and optic tectum are described. The fibres from the optic tract were traced into the brain. The ability of the fish to adapt chromatically after cutting the optic tract and/or the ablation of the optic tectum indicated that the fibres which are important in background adaptation enter the geniculate complex from the retina and from there run to the tectum. In the tectum the final interpretation of the background occurs.

The region where the fibres involved in chromatic adaptation pass out of the tectum was identified and the fibres were traced to the medullary centre. This is described.

Encephalograms were recorded from bipolar electrodes in the optic tectum. The surface EEG amplitude appears to be correlated with the retinal input. Recordings from electrodes implanted at different depths showed frequency changes associated with the tint of the background.

A possible hypothesis for the mechanism of the central nervous control of colour change is proposed.

The pattern of the locomotory behaviour of normal, blind, and tectal damaged fish in conditions of limited confinement are described and the rôle of the optic tectum in the control of general and motor behaviour are discussed.

It is experimentally demonstrated that the optic tectum plays an important rôle in the control of the Mauthner cells of the medulla, and the relationship between the tectum and the Mauthner cells is discussed.

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# S E C T I O N 1 : INTRODUCTION

## 1. THE EYE

### (a) Anatomy

A considerable volume of work has been done on the teleostean eye, especially the anatomy of the retina, the more notable contributions being by Franz (1913), Verrier (1928), Wunder (1925, 1930), McEwan (1938), Walls (1942), Lyall (1956, 1957 a,b.), Brett & Ali (1958), Engström (1960, 1961, 1963 a,b.) Engström & Ahlbert (1963), O'Connell (1963), and Blaxter & Jones (1967).

The eye of the minnow has been worked on by von Frisch (1925), Wunder (1925, 1930), Brunner (1935), Lyall (1956, 1957 b) and Engström (1960). They all reported the presence of rods and of single and double cones, but only the work of Lyall and of Engström reported the presence of triple and quadruple cones.

Brunner (1935) made behavioural studies of the visual acuity of the minnow eye and found that the minimum separable visual angle was about 11'. Unfortunately, this work was not extended and this figure only applies to one region of the retina.

(b) The eye and colour change

The eye has been known from the time of Lister (1858) to be the primary organ concerned with colour change in chromatic animals. Pouchet (1876, 1872) found that if the eyes were removed the trout, Salmo trutta, could not change colour or adapt to the background. The first experiments to be performed on the eye, and colour change, were by von Frisch (1911), who covered the eyes of trout, Salmo trutta, ~~eyes~~ with a mixture of lampblack and vaseline. This usually fell off after a period of 15 minutes or less. Covering the whole eye resulted in non-maximal darkening of the whole body; covering the lower half of the eye resulted in greater darkening than that following complete covering of the eye or their removal (p. 18 ); covering the upper part of the eye had no effect. From this he concluded that the dark colouring depends on the contrast observed in a darkened background. The main criticism of this work is that the covers were not impervious to light and that the observation period was too small.

Sumner (1933), working on Fundulus parvipinnis, performed similar experiments to von Frisch but used false corneas made of celloidin which could be fixed over the eye and could be made black or transparent. The colour of the fish was always compared to normal fish subjected to the same conditions without the blinders. By varying the amount of black and transparent celloidin the dorsal and ventral retina region could be separately darkened. Darkening the lower half of the visual field

resulted in pronounced darkening; covering the upper half resulted in no change on a white background, but the fish were paler than controls when placed on a dark grey background. These results confirmed those of von Frisch (1911) and Sumner concluded that they supported the view that the shade the fish assume on a given background is determined by the relative luminosity of the upper and lower portions of the visual fields, the latter acting in a positive sense and the former in a negative sense. The results were far from conclusive, for he reported that many fish had very badly damaged eyes, and that the fish with transparent corneas gave different results from normal fish, and seldom became maximally pale on a white background.

Butcher & Adelmann (1937) also attempted to cover various parts of the eye by using thick paper blinders which were fixed by slipping them under a series of stiches made in a rectangular area around the eye. They concluded that in Fundulus heteroclitus when neither half of the eye is stimulated the skin assumes an intermediate colour; when the light is on the upper part of the retina the skin lightens; when the lower part of the retina is stimulated, in the absence of stimuli to the upper, darkening results.

In a further series of experiments Butcher (1937 a,b, 1938 a, b, 1939) investigated the different portions of the retina in relation to colour responses. By a series of similar covering experiments on Fundulus coupled with superior and inferior illumination, he concluded

that stimulation of the upper retina resulted in lightening and of the lower in darkening and that these form two distinct regions with regard to colour change control. He rotated the eye by 180° and also placed the fish in tubes so that illimination could be reversed and, finally, made cuts to destroy various regions of the eye. When the dorsal region was destroyed the fish was dark on a white or black background and intermediate in tint in total darkness; and when the cut was made in the lower region of the eye the animal was pale on a white and intermediate on a black background. Butcher (1937 a, b, 1938 b) studied the retina of Fundulus, and found that there were single and double cones in the dorsal retina but only double cones in the ventral retina, and that the rods were fairly well distributed. The double cones showed a fairly uniform distribution of about 12 per 100 $\mu$ . He therefore demonstrated that not only are the different regions of the retina physiologically different, but that they also differ anatomically. Finally Butcher (1939) tested the effect of direct and reflected light by confining the fish in glass tubes in crystallizing dishes, the latter being lined with black paper. The dishes were illuminated from above and below and it was possible to limit the area of dorsal and ventral illumination. Some fish paled when the ratio of direct to reflected light was 50:10. He concluded that the paling depends upon the white area seen by the fish, and that paling is more easily elicited when the lower region of the eye is not illuminated at the same time as the upper. According to Butcher, the shade of Fundulus

depends upon the ratio of direct and reflected light entering the eye. As the proportion of reflected light to the upper region of the eye is increased, there is induced a proportional increase in an inhibitory reaction which causes a reduction in the degree of darkening of the fish.

Butcher's work is open to numerous criticisms, the first, and perhaps the most important, being that no accurate record of the colour was taken. The sequence of colours recorded by Butcher was light-lightish-intermediate-darkish-dark. Such a series has no quantitative meaning. Secondly, the results he did obtain were far from conclusive and at best gave only general indications. In nearly all recorded cases the fish showed two tendencies; for example, 77 fish were tested on the black when the light was from below and the upper half of the eye covered. Of these 29 paled and 48 were intermediate, hence only 29 paled, and we have no way of estimating how dark intermediate is. Thirdly, in considering the eye in relationship to colour change he did not take into account the optical properties of the eye. Fourthly, he made no mention of the sides of the containers. It was shown by Sumner (1911) using Rhomboidichthys and Lonhopsetta, that the sides of the containers are important in normal chromatic adaptation.

Butcher was not the only one to propose the ratio hypothesis, for as early as 1904 Keeble & Gamble stated "that on the white and black grounds the animal appeals for pigment-guidance to the amount of light scattered or absorbed from the ground; or, as we put it previously,

it is the reaction to the ratio direct/reflected light".

Sumner & Keys (1929) using Hypsopsetta guttulata, by varing the illumination, obtained results consistent with the ratio hypothesis, but unlike Butcher stated that the ratio must not be construed in any strictly quantitative sense. They went on to say that generally speaking the shade assumed by the fish depends directly upon the light reflected from the substratum and inversely upon the intensity from above.

Hogben & Landgrebe (1940) also considered that the ratio must not be construed in any quantitative sense. They went further than Butcher in the localisation of the retina involved in chromatic responses. By measuring the refractive index of the lens and examining the general optics of the eye of Gasterosteus aculeatus, as well as by using superior and inferior illumination on various backgrounds, they concluded that the photoreceptors concerned with the black background responses are located in the floor of the retina below the optic nerve. The photoreceptors concerned with the white background responses are located in a restricted region in the central retina above and below the optic nerve. With respect to colour change the dorsal region is neutral. These results present many problems, for all the concepts are based on theoretically calculated regions of the retina without taking into account light scattered or reflected from the walls. In fact, the colours of the fish were recorded in an apparatus with walls which

both reflected and scattered the light. The second difficulty in this work is that the conclusions regarding these regions apply only in the experimental conditions; they ignore the fact that the fish will normally be swimming at a variety of depths so that the image falling on the eye from the background will vary.

Not all workers support the ratio hypothesis. Mast (1916) using Paralichthys and Ancylopsetta performed a series of experiments in an apparatus where the illumination could be varied from above and from below the fish. Using this apparatus he found that the fish did not become spotted on a spotted background, that they pale maximally even on the grey paper, and that in other conditions where one would expect darkening according to the ratio hypothysis if the fish is already pale they remained so. He concluded that the reaction of colour change is not as simple as the ratio hypothesis would indicate. Similarly, Danielson (1939, 1941) using Nocomis and Semotilus concluded that although all parts of the eye are not equivalent, the state of the melanophores appear to be determined not by the stimulation of any particular region of the retina but by the contrast in the visual field as a whole.

(c) Blinding and colour change

After complete blinding the majority of fish that have been studied go dark, (Pouchet, 1872, 1876; Butendijk, 1911; von Frisch, 1911; Polimanti, 1912; Bray, 1918; Murisier, 1920; Abolin, 1925; Sumner, 1911,

1933; Parker, Brown & Odiorne, 1935; Parker, 1939; Osborn, 1939).

Von Frisch found in the minnow that the fish were very dark when first blinded but showed a very variable colour within a few days; in darkness they became paler. Abolin however in 1925 reported that after the loss of both eyes Phoxinus exhibited moderately expanded chromatophores.

While the loss of one eye does not effect the ability of many fish to change colour, von Frisch (1911) found that in the trout blinding one eye resulted in darkening one side of the body. Sumner (1933) reported that in the Fundulus if one eye was removed the fish could not adapt fully, to a white background, this effect being general over the whole of the body.

## II. THE VISUAL SYSTEM IN THE BRAIN

### (a) Anatomy

The general anatomy of the brain, especially the visual system, has been studied in a very wide variety of fish by Belloncí (1888), Herrick (1891, 1892), Goldstein (1905), Kappers (1906), Cajal (1909), Franz (1912), Holmgren (1920), Burr (1928), Brickner (1929), Jansen (1929), Jeener (1930), Meader (1934), Leghissa (1955), Ohta (1959), Tandon & Sharma (1963), Schnitzlein (1959, 1964). Experimental work on the anatomy of the degenerating retino-tectal pathway has been performed by Lubson (1921) on Leuciscus rutilus, Ströer (1940), Akert

(1949 b) on Salmo gairdneri, Schwassman & Kruger (1965) on Carassius auratus. Regeneration studies on the goldfish (Carassius auratus) were made by Attardi & Sperry (1960, 1963) and Cronly-Dillon, Sutherland & Wolfe (1966). Electrophysiological studies were made on the retinal tectal projection by Buser & Dussardier (1953) on Ameiurus, Cyprinus carpio, and Tinca tinca, and Schade & Weiler (1959) and Jacobson & Gaze (1964) on Carassius auratus.

The general conclusion from the work on the retinal-tectal projection is that the projection is very regular and, at least in fish with good vision, very precise, so that every point on the retina is represented by an area in the tectum. In general the periphery of the retina is represented by the extreme dorsal or ventral tectum, and the central retina by the central tectum. The main difficulty is that the results of Buser & Dussardier, on the carp and tench, Attardi and Sperry, Legissa and Cronly-Dillon, Sutherland and Wolfe on the Goldfish, Lubsen on Leuciscus and Akert using the Salmo, show that the ventral retina gives fibres, which run in the medial brachium, to the dorsal tectum, and that the dorsal retinal fibres run to give the lateral brachium and end in the ventral tectum. This means that the projection is essentially reversed so that the ventral retina runs to the dorsal tectum. The work of Jacobson & Gaze, and Schwassman & Kruger, however, demonstrated that the dorsal retina is relayed to the dorsal tectum in the goldfish. This conflicting account in the goldfish seems very difficult to explain.

(b) Relationship to Colour change

Very little work has been done on the relation of the central nervous system to chromatic adaptation and this has been almost wholly confined to the minnow.

Von Frisch (1911) performed a series of cutting experiments on the brain of the minnow and found that if the brain was cut at a level just anterior to the medulla the fish paled. This paling could be induced if the cuts were made at some levels anterior to this but if the medulla was separated from the spinal cord the fish darkened. He also stimulated the brain of the minnow with an induction coil and found that if the medulla was stimulated the fish rapidly paled. Stimulation of the tectum or the cerebellum had no effect but stimulation of the diencephalon produced darkening. He concluded that there was a centre, "Aufhellungszentrum", situated in the medulla which caused the fish to pale and that there was possibly another centre in the diencephalon which inhibted the paling centre and so caused darkening.

Dijkgraaf (1949) found that the darkening which appears in the normal minnow after blinding occurs only slightly or not at all if, instead, the blinding is accomplished by the removal of the optic tectum. If in an eyeless darkened minnow the tectum is removed on the one side only, there is no change in the tint of the skin, but after removal of the remaining half of the tectum there is a pronouced paling which partially persists. According to Wichers (cited in Healey,

1957), after complete removal of the optic tectum in the normal minnow, or unilateral removal of the tectum and of the eye on the same side, the fish cannot see, but still exhibits colour changes mediated by the other eye. It would seem from this that the paths of the fibres from the retina to the autonomic chain avoid the tectum. Why, then, does the blinded fish pale when the tectum is removed?

## III. ELECTRICAL ACTIVITY IN THE TECTUM

A large amount of information has been gathered by unit recording from single electrodes as a result of a flash of light by the work of Buser (1949 a, b, 1950), Schade & Weiler (1959), Konishi (1960 a, b), Schade (1962), Jacobson & Gaze (1964), Gaze, Jacobson & Sharma (1967). In relation to light intensity the tectum has been investigated by means of recording encephalograms. Two species of fish have been investigated. Enger (1957) recorded encephalograms from free-swimming codfish Gadus morhua, and Schade & Weiler (1959) and Schade (1962) on the goldfish Carassius auratus. Unfortunately the work of Karamian, Vesselkin, Belekhova & Zagorulka (1967) did not include a teleost fish.

## IV. BLINDING AND LOCOMOTORY BEHAVIOUR

Very little work has been done on the locmotory behaviour of blinded fish. Von Frisch (1911) and Dijkgraaf (1949) reported that blinded minnows feed but that instead of taking the food as it falls from the surface, find it by searching on the floor of the aquarium.

Dijkgraaf (1949) also reported that blinded fish at first collide with the walls of the aquarium but soon learn, and these collisions become very infrequent.

Keenleyside (1955) reported the failure of blinded Phoxinus to school.

Harden Jones (1956) has described the activity of blinded minnows in tanks and found that there was no inherent daily rhythm of activity. The fish were in general active during the day and quiet at night but the behaviour was reversed if they were given air-bricks in which they could ~~can~~ take cover. Woodhead (1956) studied minnows in a light gradient and found that they were restricted in their movements by light above an intensity which lay between 0.2 and 0.002m.c.

No observations have been made on either blinded or normal fish in confined spaces which only allow limited movement.

## V. THE OPTIC TECTUM AS AN INTERGRATING CENTRE

The importance of the tectum as a correlating centre of other functions than vision was proposed as long ago as 1875 by Sir Richard Owen (cited in Dexter, 1966), who wrote concerning the optic lobes of the blind fish Amblyopsis spelaeus, that since they are both present they cannot be exclusively the central ganglion of the optic nerve, nor can their sole function be that of receiving the impressions of the

sense of sight and making them perceptible, as ideas, by the animal. More recently Charlton (1933), working on the anatomy of the blind fish Troglichthys rosae and Typhlichthys eigenmanni, found that in these forms it is only the optic tract and the layer into which the optic tract runs, which are lost, and that the layers 4, 5 and 6 are very well developed (p. 132).

In considering the general fibre connections of the optic tectum ~~recently~~ Schnitzlein (1964) concluded that ~~the optic tectum~~ this region has a very important correlating function.

Sperry (1950), working on the goldfish, found that forced circling produced by visual inversion could be abolished by the ablation of the tectum of the rotated eye and stated that these findings point to the optic lobe as the primary intergrative centre.

## VI. THE OPTIC TECTUM AND MOTOR ACTIVITY

In 1864 Baudelot found that unilateral lesions in the base of the midbrain of fishes are followed by rolling movements to the un-operated side. Two years later Vulpian reported rolling and circus movements following removal of the midbrain roof. Since then a number of workers have reported disturbances in balance following tectal lesion or removal (Traube-Mengarini 1884, Loeb & Bethe 1899, Loeb 1891, 1901, Polimanti 1911, 1912 a, b, Reisinger 1915, Rizzo 1929, 1932) and others have reported disturbances in breathing movements (Springer 1929).

Muskens (1930) made lesions in the midbrain of the goldfish and noted the forced movements which followed. He distinguished such movements as being predominantly in the horizontal and vertical planes and correlated these types of movement with specific regions of the midbrain.

More recently Kirsche & Kirsche (1961) performed an extensive series of experiments on Carassius carassius, in which they removed progressively larger areas of the optic tectum by means of a rose-tipped drill. They reported extensive bending of the body to the unoperated side, circus movements, and turning on their axes of motion, the more extensive the lesion the greater the disturbance found. In some cases the fish recovered and appeared normal after 30 - 60 days but on raising the temperature they showed abnormalities again; hence the fish were only normal under constant conditions. Botsch (1960) reported similar disturbances in motor patterns following tectal damage.

Steiner (1888), Rizzolo (1929) and Dijkgraaf (1949) did not report any disturbance following the removal of the tectum. Dijkgraaf removed the optic tectum, including the torus longitudinalis, on one side and on both sides in the minnow (Phoxinus). As long as the operation is carried out without damaging the torus semicirculares, which lie below the tectum, the animals swim normally, maintain balance and feed without hesitation. After the removal of the tectum on one side the fish is blind on the other side, and when swimming slowly there is a slight

tilting of the fish to the seeing eye. Dijkgraaf stressed the importance of the tori semicirculares whereas Kirsche & Kirsche (1961) and Botsch (1960) make no mention of them, and from the photomicrographs presented by these last three workers considerable injury could be seen in both the tori and also in the valvula cerebelli. Lesions in the valvulae may also give rise to locomotory disturbances as well as impaired vision and hearing (Karamian 1949).

Chauchard & Chauchard (1927 a) investigated the motor significance of the tectum by electrical stimulation. They found that if Mugil auratus was stimulated in different parts of the tectum movements of various parts of the body were elicited, e.g. stimulation of the anterior region of the median surface of one lobe produced movements of the tail towards the opposite side and the spreading out of the caudal fin. Using Trigla gurnardus they found that by stimulating various points in the tectum they were able to set in action the pectoral fin rays which are used for crawling on the bottom. They concluded that they were definite localised motor areas in the optic tectum. In another paper (1927 b) they reported chronaxie values of the order of 0.0002 sec. These results have been confirmed in general by ten Cate (1931), and ten Cate & ten Cate (1931) on elasmobranchs and more recently by Kirsche & Kirsche (1961) on the Carassius carassius.

Akert (1947, 1949 a, b) did not agree with the conclusions of Chauchard & Chauchard (1927 a) and in a series of experiments on Salmo

gairdneri, by stimulation of the tectum, he obtained first eye movements, then movements of the paired and unpaired fins, and finally beats of the tail. The whole succession gave the impression of active movements toward some goal. Akert also studied the retino-tectal projection and correlated this with the results of the stimulation, to find that the projection of the temporal visual field agreed well with the area associated with contra-and ipsiversive turning. He concluded that physiologically this area, excited by a stimulus arising in the corner of the temporal field, brings about a position of the eyes and of the body axis so that there is either a turning toward or away from the object perceived.

As there appear to be differences between the results of Dijkgraaf, Akert, and the other workers, Healey (1957) has suggested that it is conceivable that some lesions in the tectum may be followed by positional and motor irregularities rather because they remove certain elements from the total complex modifying system, and throw the output of the remainder into a state of unbalance, than because they stimulate specific regions. On the other hand, removal of the entire tectum or of one complete lobe may not introduce any unbalance modification by way of the tecto-spinal tracts.

## VII. HABITUATION IN TELEOST FISH

Mobius (1873) was the first to record this type of learning in teleost fish. He found that when a pike, Esox lucius, was kept

separated from a number of minnows by a glass partition, it ceased snapping at them through the glass after a while and, when the glass was finally removed, never attacked them.

Fear responses have been recorded by a number of workers (Hoar 1958; Morris 1958; Russell 1931; Braun 1961; Barlow 1962) but waning of response to a repeated stimulus, i.e. habituation, has only been reported a few times (Breder & Halpern 1946; Keenleyside 1955; Barlow 1962; Rodgers, Melzack & Segal 1963; Russell 1967 a, b), and of these the only detailed studies of response decrement are by Rodgers et al (1963), and Russell (1967 a).

Russell (1967 a) studied the fear response of Lebistes to a repeated shadow stimulus and found that the first and most characteristic feature was the "jerk response", which was a multiple tail beat. Frequent repetition of the shadow (2 minute intervals) resulted in the intense tail-beat of the early responses giving way to a less intense response, and finally the only orientation responses were elicited. A significant decrease in the number and intensity of the jerks occurred after 40 stimuli.

Rodger et al (1963) found that goldfish, Carassius auratus, responded to a pressure wave and to visual stimuli to give the tail-flip, but that the most effective stimulus was the pressure wave, and the visual stimuli alone only gave the response when the fish was swimming

at the surface. They gave the fish ten taps a day at the rate of one a minute, and demonstrated significant response decrement over a period of 15 consecutive testing days.

It seems from the work of Retzlaff (1957), Graham & O'Leary (1941), Retzlaff & Fontaine (1960), Berkowitz (1956), and Wilson (1959) that this tail-flip is due to the activity of the Mauthner cells in the medulla. Each Mauthner cell receives afferent fibres from the eighth cranial nerve (Rëtzlaff 1957) and also from the optic tectum (Bartelmez, 1915; Kappers et al, 1936). It seems therefore that the tectum can elicit the tail-flip response but the exact relationship between the tectum and the Mauthnerian apparatus is not known.

## VIII. THE OPTIC TECTUM AND LEARNING

Sears (1934) studied the effect of lesions of the optic tectum upon purely visual conditioned reflexes in the goldfish. A jet of water was used as the unconditioned stimulus, and light as the conditioned. He found that partial ablation of the optic cortex had no effect on either the eye-movement reflex or the start reflex to bright light. Preoperatively learned responses were retained without apparent change after the operation, and there was some evidence that the process of experimental extinction took place more rapidly following the removal of the optic cortex. He did not, however, remove the whole of the tectum and left the anterior part untouched together with large amounts of the lateral parts.

Sanders (1940) performed a series of optic lobe ablations on the goldfish, training them in a situation with olfactory, and then optical stimuli. He found that the removal of large areas of the tectum or cuts made at its anterior border causes disturbances in the second order learning. He concluded that there exists in the teleostean optic tectum a mechanism capable of second-order olfactory-optic learning.

Dijkgraaf (1949) trained minnows to respond to an auditory stimulus and demonstrated that their response did not depend upon the optic tectum, the complete removal of the tectum on both sides causing no deterioration in the training to a frequency of 1650c.p.s.

More recently Botsch (1960) demonstrated the importance of the tectum in learning by a series of experiments on the carp, Carassius carassius, using point and line models. He trained them to go to a particular pattern and them tested their retention and transposition before and after tectal removal. The results showed that after extirpation of 20 - 75% of the tectum the fish could still learn visual discrimination but needed on average 36% more training to bring them up to the statistical steady learning-success of normal controls. He also found that the post-operative loss in performance agrees with the extent of the lesion, concluding that the findings confirm Lashley's (1931) "rule of mass" against localisation.

Prosser (1965) was unable to show any conclusive results when he tried to analyse the tectum electrophysiologically following the conditioning of the fish to various temperatures.

## IX. CONCLUSION

This consideration of previous work on the relationships in teleost fish between the visual system, colour change and certain aspects of behaviour leads to the conclusion that very little information is available and that with regard to all of them what is available is highly inconclusive. The work to be described is an attempt to analyse the visual system of the minnow by a variety of methods in order to thow further light on some aspects of these relationships.

A number of experiments were carried out to try to explain the following problems.

1. No measurements have been made of the number of receptor cells in the eye of Phoxinus. The eye of the minnow has not been studied in relation to colour change. (Section 1 a, b. page 12 ).

2. No accurate record of the colour of blinded minnow has been taken. (Section 1 c. page 18 ).

3. Except for a few observations by Wichers (cited in Healey, 1957), and by Bhargava (1967) the visual system of Phoxinus has not

been studied. (Section II a. page 19 ).

4. Neither the fibre pathways to the medullary centre, nor the brain centres anterior to the medulla which are involved in chromatic adaptation are known. (Section II b. page 21 ).

5. No recordings have been made of the electrical activity of the brain of the minnow. (Section III. page 22 ).

6. No observations have been made on either blinded or normal fish in confined spaces which only allow limited movement. (Section IV. page 22 ).

7. It would appear from the discussion in Section VII. page 27 that the tectum can elicit the tail-flip response but the exact relationship between the tectum and the Mauthnerian apparatus is not known.

8. The relationship between the tectum, learning and motor activity is inconclusive (Section VIII. page 29 , Section VI. page 24) and many of the results contradictory. Further experiments are therefore necessary to confirm or deny the previous findings.

## S E C T I O N 2 : GENERAL METHODS AND HISTOLOGY

### I. SOURCE AND GENERAL TREATMENT OF THE FISH

Fish were collected during the summer months from the river Lea in Hertfordshire. They were used for most of the experiments, but as they had a very high infestation of the brain parasite Diplostomium, they were not used for the recordings in part 7 page 214 , or for the retinal work. Fish for the encephalogram recordings and eye work were collected from the river Chess outside Rickmansworth. These had only v very few, if any, parasites in their brains or eyes.

The fish were kept in large sinks in the laboratory and supplied with running water and air. These sinks were an intermediate grey colour on the inside in order to ensure that the fish maintained the ability to show a good range of colour change. The fish were fed about three times a week on minced ox heart and about once a week on heart mixed with stabilised wheat germ (Bemax) to provide them with the necessary vitamins.

All the fish used were at least 6cm long and of both sexes. Comparing them with the tables given by Frost (1943) indicated that they were adults of about three years of age. Before any of the fish were used in experiments they were tested to make sure that they performed normal colour change and that they formed a homogeneous group in their extent of chromatic adaptation.

## II. ESTIMATION OF THE COLOUR OF THE FISH

The minnow shows a marked degree of excitement-pallor when handled. The method where the colour of the fish is compared by the naked eye with a series of nine standard grey tints derived from the Ostwald White-Grey-Black series (Healey 1967) was used therefore. These grey tints are given numbers ranging from 0 (very light grey), to 8 (very dark grey); the six intermediate greys form a series where the difference between each grey appears to be equal to the experimenter. For the sake of convenience these numbers are referred to as the Derived Ostwald Index (D.O.I.) and they have the following values in terms of white content:

| D.O.I. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| % White | 71 | 45 | 28 | 18 | 11 | 7.1 | 4.5 | 2.8 | 1.8 |

In general, the fish kept on a grey background do not show the

whole range within a period of 30 minutes, and for these fish the effective scale used in testing was more like D.O.I. 1 to 7.5, and in practice it proved very difficult to estimate below 1 or above 7.5.

These standard greys were made with Ilford photographic paper and mounted on both black and white card ~~so as~~ to enable comparison ~~them~~ with the fish on either background. This series is shown in fig. 1 p. 37.

To test the colour of the fish they were placed in litre beakers containing 8cm of water and these were then placed in larger containers made out of metal and painted white or black. These large containers contained water and were 8cm deep. The illumination was by means of a 40w Osram frosted bulb, suspended about 18 ins. above the surface of the water. The background could then be changed by transferring the beakers with the minimum disturbance to the fish. During the experiments where the fish were kept for long periods on a black or white background, they were placed in experimental aquaria. These were made of glass, and measured 35 x 20 x 22 inches, and were painted black or white on the outside, and ~~were~~ partitioned internally into 4 equal compartments by means of black or white perspex.

Unless specially mentioned, the temperature was not controlled and ranged from 15 to 20°C.

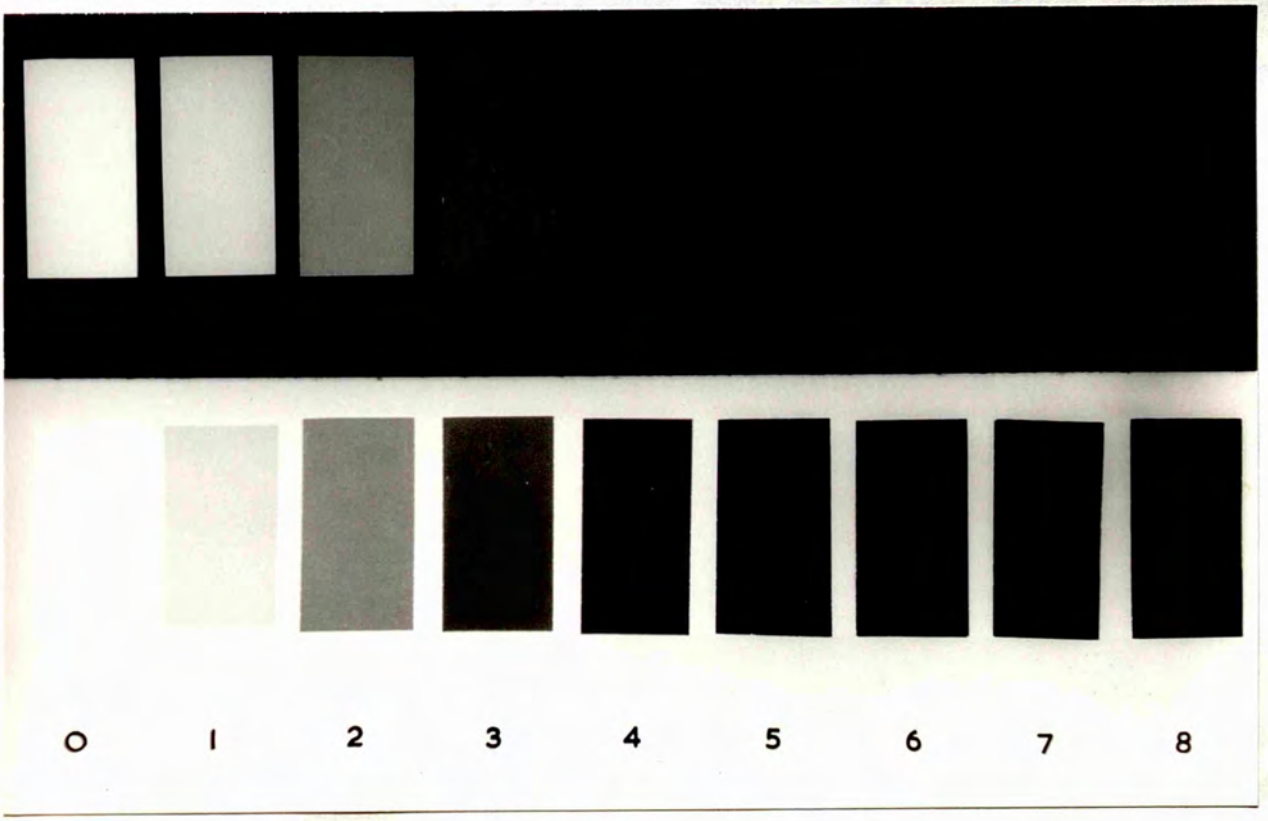
Fig. 1

The Derived Ostwald series of greys mounted on white and black card.

Fig. I

The Derived Ostwald series of greys mounted on white and black card.

Fig. 1



## III. OPERATIVE TECHNIQUE

During the course of the operations the fish were anaesthetized using MS.222 (Tricaine methane-sulphonate. Sandoz). A wide range of concentrations was tested on the fish and it was found that an 0.008% solution in tap water was sufficient to anaesthetise them fully so that they did not respond to a cut being made in the body wall. Using this concentration the fish take a somewhat variable time to become deeply anaesthetised, but usually this is about 2 minutes. They showed a very rapid recovery, and would commence to swim after being returned to tap water in about 4 minutes. The fish will survive for more than an hour in a 0.008% MS.222 solution, becoming fully dark and showing a slight decrease in the respiratory rate.

It was reported by the manufacturers (Sandoz), that a solution of MS.222 was fairly stable at room temperature, the activity only decreasing slightly. In practice, these very dilute solutions were found to be so unstable that within a week the solution was no longer effective. It was therefore always made up freshly before a series of experiments.

After a fish was anaesthetised in a beaker it was placed on the operating board. This consisted of a zinc tray measuring 4 x 5 inches filled with paraffin wax. In the wax were embedded two rows of pins and

in the centre of the wax a groove was cut so that the fish would fit into it. Respiratory water was fed in direct through a tube in the mouth, and this served to hold the fish still while, in addition, an elastic band was attached to the pins and over the fish to hold it more securely. The fish was prevented from drying by placing moistened filter paper all round it. The respiratory water was contained in Winchester Quart bottles on a shelf above the table and syphoned down into the mouthpiece. An additional syphon was placed on this shelf containing ringer (Young 1933) whose composition was:-

NaCl.....5.5 grams

KCl .....0.14 grams

$CaCl_2$.....0.11 grams

made up to a litre in tap water. The ringer ran into a fine glass tube with a fine point and was used to wash away the blood during operations.

The surgical removals were made using very fine knives manufactured by J. Weiss and Co. In teleost fish, wound closure becomes a problem and in the case of most of the tectal removals the two frontal bones, and in many cases part of the parietal bones, were removed. These are the main roofing bones to the skull. These bones were removed using fine forceps after attempts to use the dental drill or trephine proved to be unsatisfactory. Malyukina (1962) used a vaseline/paraffin wax mixture and poured it over the skull, whereas Horsch (1936) used a gelatin/paraffin wax mixture to close the skull. Both of these methods

were tried on the minnow but neither worked, and the mixture soon fell off to leave the brain exposed. Finally an acrylic cement was used similar to that used by Enger (1957), and this gave good adhesion provided that certain precautions were taken. The skin was first scraped off the dorsal surface of the skull from a point just anterior to the orbit to a point at the back of the parietals, as well as being removed laterally to the point of attachment of the operculum. The muscles between the anterior part of the operculum and the orbit were removed and cleaned down to the bone. Although these muscles are connected with the operculum, when they are removed the opercular beat is maintained by the remaining muscles in an apparently normal fashion. The perosteum was then scraped from the bone, and the bones were dried with 50% alcohol, re-scraped, and dried with filter paper. This procedure was performed before the skull bones were removed and at the completion of the operation the remaining bones were dried with filter paper and the cement applied. The cement (Simplex acrylic denture repair material. Dental Fillings Ltd.) would only adhere to the bones provided that the preoperative drying was performed. The alcohol hardened the mucus and periosteum and facilitated their complete removal. When dry the cement (15 minutes) was waterproof, and sterile and gave good adhesion to the minnow skull for as long as required, which was 21 days.

All operations were performed under a binocular microscope.

## VI. HISTOLOGICAL PROCEDURES

(a) Fixation and Embedding

The fish were killed by decapitation at the end of the experiment, the cement being removed, and the head dropped into the fixative. A number of fixatives were used, depending on the staining technique. The fixative used in the Nauta technique (Guillery, Shirra & Webster, 1961). was 10% formol-saline, which was neutralised with an excess of lithium carbonate, together with a few drops of Bromo-thymol-blue. The Bromo-thymol-blue is blue when alkaline but orange when acid and is used as an indicator because of the formol-saline should not be used when acid. The tissues were fixed for at least four, but usually six, weeks. The same fixative was used in the Klüver & Barrera method but here the fixation period was only two weeks.

For the Holmes technique Formalin-alcohol-acetic was used on the brain.

For the work on the eyes, the latter were removed by cutting the optic nerve, and attendant muscles and were dropped into the fixative. In the case of the retinal counts, aqueous Bouin for 48 hours was used, and for Klüver & Barrera 10% formal-saline for two weeks was used. The lens was not removed before fixation as this increased the risk of detaching the retina.

In all the fixatives except Bouin no washing was necessary. Bouin-fixed eyes were washed for a period of 12 hours in distilled water before dehydration.

Alcohol was used for the dehydration and consisted of two changes of 30%, 50%, 70%, 80%, 95%, and 100% for two hours in each.

All tissues were cleared in cedarwood oil.

The brains for the Nauta and Holmes techniques were embedded by transfering them to a mixture of:

1 part cedarwood oil

1 part benzene

1 part 45° paraffin wax.

for one hour. The tissues were then transferred to a bath of 54° paraffin wax for one hour and this was followed by two more baths before being blocked out.

In the Klüver & Barrera technique on the eyes and brain the embedding was essentially the same as for the Nauta but Paraplast (Brunswick Laboratories) was used instead of the paraffin wax. Paraplast is a mixture of paraffin wax, and plastic polymers with a melting point of 56-57°C. It reduces tissue shrinkage and allows easier section cutting.

Sections of brains were cut in general at 8μ and of eyes at 4μ.

The sections were mounted on slides using glycerine albumin. With the Nauta method great care had to be taken in the mounting of the section because of the prolonged treatment with ammoniated alcohol. The original method recommends a 20% solution of glycerine albumin but the most consistent results were obtained using a 10% solution, provided that the sections are blotted firmly with a moist piece of filter paper and dried well.

(b) Staining Techniques

1. Klüver & Barrera (1953)

This gave good results with both eyes and brain, and as far as I know, it has not been used on teleost material before. Slight modifications were made on the basic 1953 method, and, instead of using Luxol Fast Blue MBS, (GURR) Luxol Fast Blue G (Matheson, Coleman and Bell) as recommended by Salthouse (1964) was used.

The schedule was as follows:

1. Remove wax and hydrate to 95% alcohol.
2. Stain in 0.1% Luxol Fast Blue made up in 95% iso-propyl alcohol for 18-24 hours at 58-60°C.
3. Wash in 95% alcohol for 30 secs.
4. Transfer to 70% alcohol for 60 secs.
5. Differentiate until single fibres sharply defined.
   a. Place in lithium carbonate (0.01%) for 1 min.
   b. Transfer to 70% alcohol ( this stage is critical and the fine differentiation takes place in the alcohol)

c. Return to distilled water.

Repeat a,b,c until the desired result is obtained.

6. Counterstain in 0.2% Cresyl Fast Violet (Chroma) for 10 mins.

7. Different in 95% alcohol.

8. Dehydrate, clear and mount in D.P.X.

A 0.01% lit ium carbonate solution was used instead of the 0.05% recommended in the original.

The neurons and glial cells stain a reddish violet shade and the fibres a deep blue, and with careful differentiation very clear results could be obtained.

In the retinal work Luxol Fast blue MBS (Gurr) was used, but the staining was limited to a period of about 1 hour at about 40°C. The outer segment stains a dark blue, whereas the lentiform body, myoid and the nucleus of the cones stain a reddish violet.

The technique is based on the findings of Klüver (1944) that there are naturally occurring porphyrins in the myelin sheath. Klüver & Barrera (1953) found that it was possible to stain myelin sheaths with porphyrin derivatives, and Luxol Fast Blue MBS is an alcohol-soluble amine salt of a sulphonated copper phthalocyanine ( a tetrabenzotetraazoporphyrin). Salthouse (1964) recommended the use of Luxol Fast Blue G, which gives blue-black fibres and stated that a 1.1% solution is iso-propanol binds

the dye to the phospholipids more strongly than in a ethanol. It is interesting to note that the 10% formal-saline which is recommended for the stain fixes proteins and proteolipids of the myelin sheaths but not the phospholipids (Pelckmans, 1964).

Although the technique gave excellent results in some cases it proved difficult to perform, the critical point being the differentiation of the Luxol Fast Blue. The minnow fibres had very little affinity for the stain and although they were over-stained, they would lose stain uniformly in the 70% alcohol. It was possible to return the slides to the Luxol Blue after the counterstaining to give a better contrast between the fibres and cells. This staining difficulty may well indicate a difference in chemical composition of the teleost fibres compared with other vertebrates and differences may be present with the porphyrins or phospholipids.

2. Cresyl fast violet (C.F.V.)

This is made up as a 0.2% solution and used as by Klüver & Barrera (1953).

3. Paraffin Nauta (Guillery, Shira & Webster 1961)

The method used was essentially that described by the authors and was as follows:

1. Remove wax and hydrate slides
2. Place for 6 hours in:-

50% ethyl alcohol.....100 parts

Ammonium hydroxide (Sg.- 0.880)..... 1 part

3. Wash throroughly in 3 changes of distilled water and transfer to:

1.5% Silver nitrate ..... 90 parts

Collidine..... 10 parts

This mixture goes cloudy and should be shaken well. The slides should be left in it for 18-24 hours in the dark at room temperature.

4. Transfer to the following solution:

4.5% Silver nitrate ..... 20mls.

Ethanol..... 10mls

Ammonium hydroxide.....1.8mls

2.5% Sodium hydroxide.....1.5mls

Place this solution in a dish and warm on a hotplate so that at the end of three minutes it reaches a temperature of 40-45°C. (The solution can only be used once.)

5. Transfer to reducing solution:-

10% Ethanol ..... 450mls

equal parts 1% citric acid)
10% formalin ) ... 30mls

6. Wash in distilled water

7. Treat with 1% Sodium thiosulphate, wash, dehydrate, clear and cover.

The degenerating fibres are shown as black granules and the normal fibres are light brown in colour. It has been stressed by Glees & Nauta (1955) that the droplike disintegration is the sole dependable criterion

of axonal degeneration.

The precautions stressed by Wallington (1965) were all taken, namely the preparation of the solution in stage 4 was only done in clean glassware, and immediately before use.

The technique stains axons and is superior to the Marchi technique which only stains degenerating myelin. However, the mechanism of the Nauta is not fully understood. The work of Ciolli (1965) suggests that the chemical groups involved are unsaturated lipids, probably cholesterol esters.

If the Collidine is left out of stage 3 the technique can be used to stain normal fibres (K.E. Webster personal communication). In stage 4 the ammonia concentration can be varied and increased impregnation of degenerating fibres occurs with lower concentrations, with a 2ml concentration very good staining of normal fibres was found.

Both with the normal fibres and degenerating methods counterstaining can be done with Cresyl Fast Violet.

4. Ehrlich's Haematoxylin and Eosin.

This was used in the retinal count work.

5. Holmes Silver technique (1943)

The schedule followed was:-

1. Dewax and Hydrate
2. Place in 20% silver nitrate in the dark for 1-2 hours.
3. Remove sections and wash in three changes of distilled water: total time 10 mins.
4. Impregnate in buffered silver nitrate at 37°C for 18 hrs.
   The solution was:

   Boric acid m/5 ,,..... 65mls

   Borax. 10 $H_2O$. M/20..... 35mls

   Distilled water.....385mls

   1% aq. Silver nitrate..... 5mls

   Pure Pyridine..... 10mls

   The ph of the buffered silver nitrate was approximately 8.2.
5. Reduce for 2-3 mins. in a solution of:

   Hydroquinone.....1.0 grams

   Sodium sulphite cryst..... 10 grams

   Distilled water.....100 ml.

   which is heated to 50°C.
6. Wash in running tap water for 3 mins. Then rinse in two changes of distilled water.
7. Tone in 0.2% gold chloride (yellow) until sections go colourless - approximately 15-30 secs.
8. Rinse in distilled water
9. Reduce in 2% oxalic acid until nerve fibres blackish
10. Wash well and if the results are not satisfactory, repeat stages 8, 9 and 10.
11. Fix in 5% Hypo. 1 min.

12. Wash, dehydrate, clear and mount.

This gave good staining of the fibres and cells.

## S E C T I O N 3 : THE EYE

### I GENERAL ANATOMY

The general anatomy of the eye of the minnow is very typical of teleost fish in general. Serial sections were cut in two planes, one dorsoventrally and the other rostro-temporally. They were cut at 4 μ and stained with Klüver & Barrera or haematoxylin/eosin, the lens being left in the specimen. The general anatomy is shown in fig. 2 page 52 .

No retractor lentis muscle could be found and no definite Area could be found. Brünner (1934) strongly suspected the presence of a specialised region of the retina, an Area, but did not have any good evidence for its existance. In the minnows used in this work there was a region in the temporal part of the retina with a slight increase in the number of retinal elements. However, a distinct Area with very thin cones of high density could not be identified.

Fig. 2

Drawing of a dorso-ventrally cut section through the eye of the minnow in the midline.

Fig. 2

Drawing of a dorso-ventrally cut section through the eye of the minnow in the midline.

Fig. 2

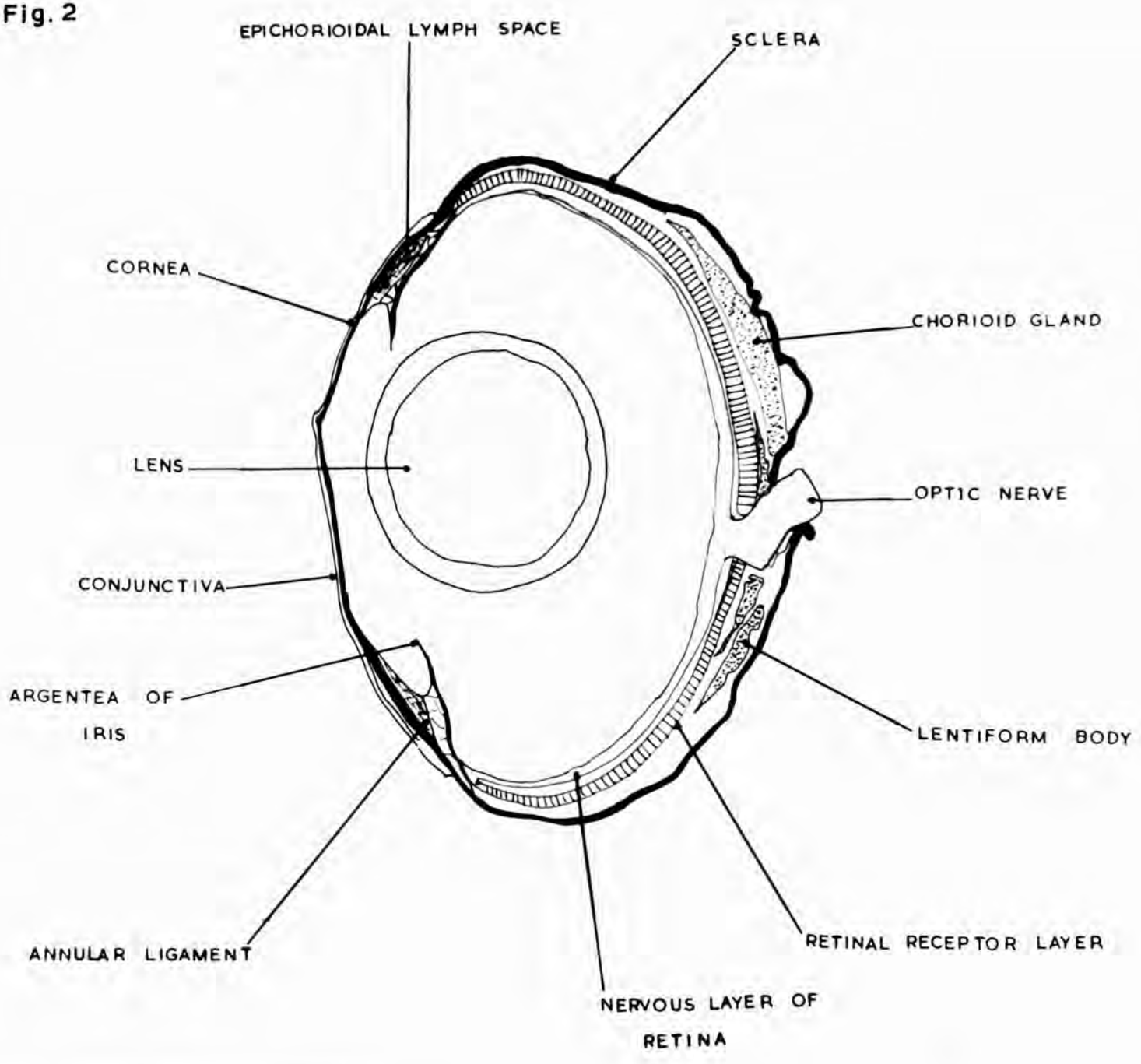

EPICHORIOIDAL LYMPH SPACE
SCLERA
CORNEA
CHORIOID GLAND
LENS
OPTIC NERVE
CONJUNCTIVA
ARGENTEA OF IRIS
LENTIFORM BODY
ANNULAR LIGAMENT
RETINAL RECEPTOR LAYER
NERVOUS LAYER OF RETINA

Fig. 3

Section of the retina stained with Klüver & Barrera and section at 4μ.

Abbreviations:

1. Pigmented epithelium layer
2. Layer of rods and cones
3. External limiting membrane
4. Outer nuclear or granular layer
5. Outer plexiform layer
6. Inner nuclear layer
7. Inner plexiform layer
8. Ganglion layer
9. Nerve fibre layer
10. Internal limiting membrane

GAN. Ganglion cell

AM. Amacrine cell

Bi. Bipolar neurones

HOR. Horizontal cell

L.B. Lentiform body of the cone

O.S. Cone outer segment

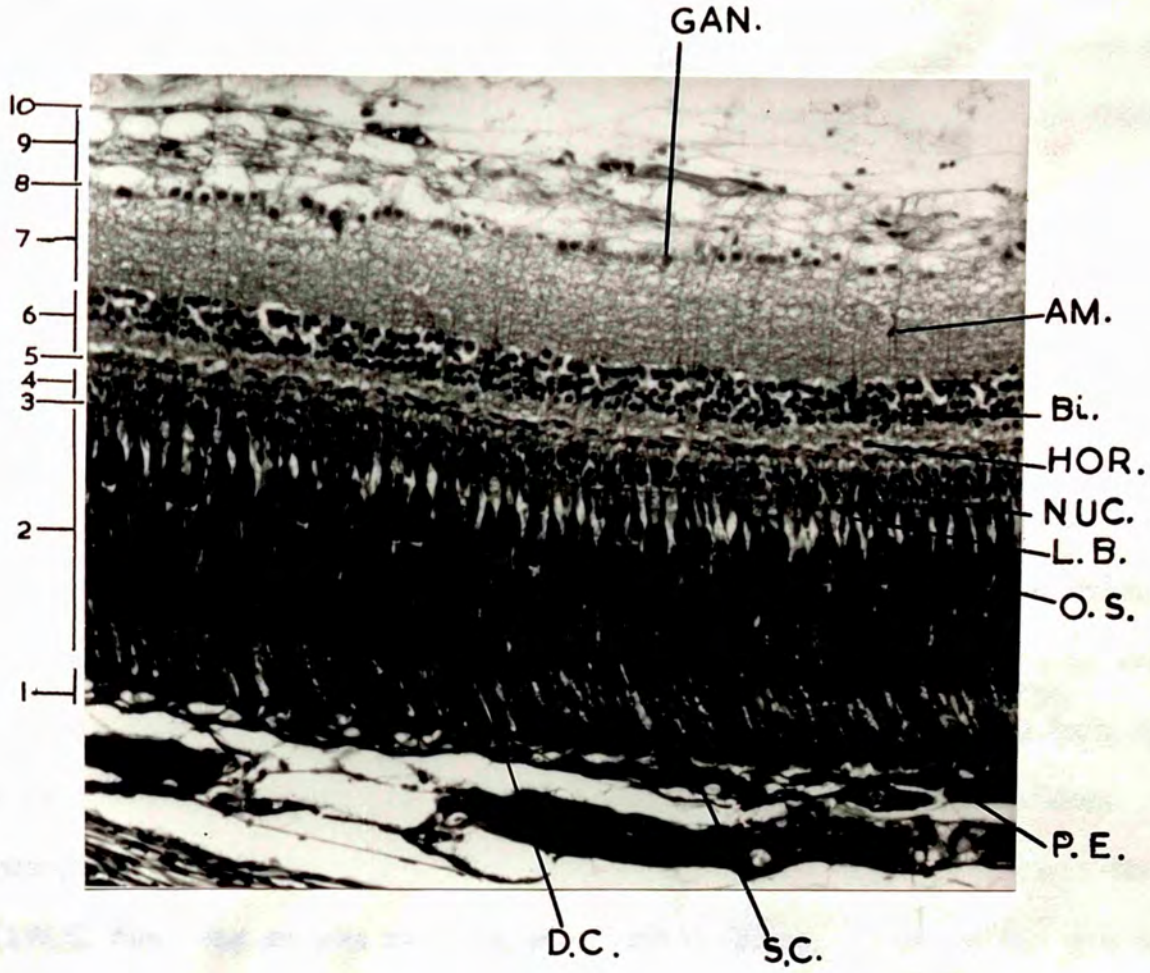
NUC. Nucleus of the rods and cones

P.E. Projections of the pigmento-epithelial cells to surround the retinal receptors.

S.C. Single cone

D.C. Double cone

Fig . 3



The retina itself showed the 10 layers labelled by McEwan (1938) (Fig. 3 p. 54 ). The horizontal cells of the inner nuclear layer were very well developed and constituted a definite layer of cells. There were also a large number of amacrine cells in the inner plexiform layer. This large number of amacrine cells would allow for a greater degree of variety in the interactions of the bipolar and ganglion cells and would enable conduction to take place in all directions at this level of the retina.

## II. DISTRIBUTION OF RETINAL RECEPTORS

The retina was studied more fully by means of counting the retinal receptors. The eyes from a number of fish were removed and a nylon tie was placed in the extreme dorsal part of each one so that it could be orientated correctly when it was embedded. The right and left eyes were fixed separately. To get an accurate picture of the retinal receptors it has been stressed by Lyall (1956, 1967 b) and Engström (1963) that the retina must be cut tangentially. Cutting the eye tangentially results in the rods and cones being cut transversly to their main axes. Fifteen different regions of the retina were investigated, some dorsal and some ventral. Both Lyall (1956, 1957 b) and Engström (1963) removed pieces of the retina but found that in order to get good sections it is necessary to take fairly large pieces. This however introduces the problem of the orientation of the pieces and their position in the retina. In the present work, eyes were cut tangentially, using the whole eye by

Fig. 4

Drawing of the apparatus used to produce the retinal lesions.

Fig. 5

Diagram of the eye mounted in wax with the cutting angle $\alpha$ marked on the outside of the wax.

Fig. 4

Drawing of the apparatus used to produce the retinal lesions.

Fig. 5

Diagram of the eye mounted in wax with the cutting angle $\alpha$ marked on the outside of the wax.

Fig. 4

PHOTOCOAGULATOR

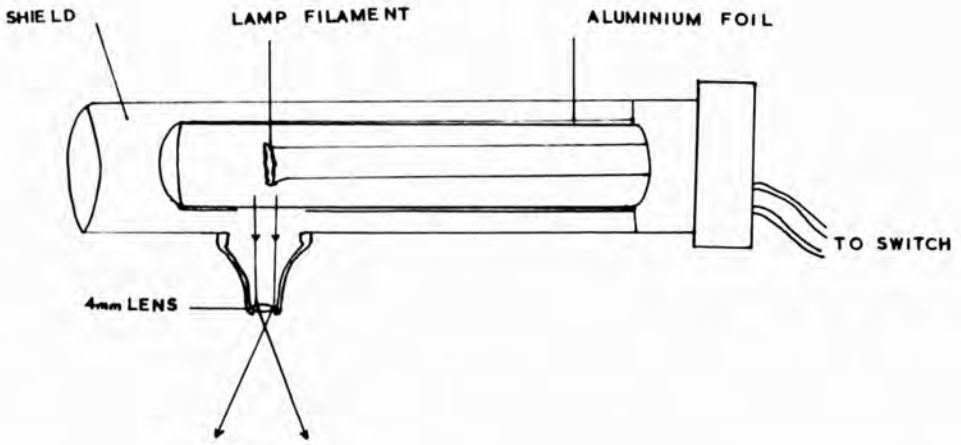
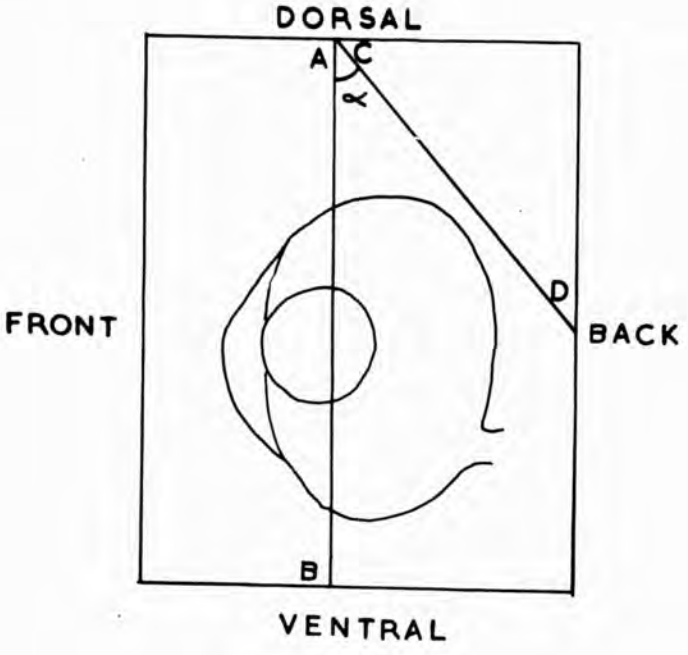



Fig. 5



utilising the dorso-ventral axis and the front of the eye. The eye was mounted in the wax block with just sufficient wax surrounding it to be able to section easily, and arranged so that it lay exactly in the centre of the block as shown in fig.5 page 57 . On the outside of the block the dorsal-ventral axis (AB) could be marked, and providing the rostral-temporal axis is parallel to the front of the block, the cutting angle can be measured and scored directly on the block (DC). The block is mounted on the microtome chuck by the front surface and orientated so that the knife cut is parallel to the cutting angle. By varying the cutting angle ($\prec$) different parts of the retina could be cut tangentially. This procedure was used when the midline regions were investigated, and by mounting the block so that the eye was at $45^{\circ}$ to the front of the block it was possible to cut tangentially in both the rostral and temporal fields of the retina. The cutting angles were determined from scale drawings of the eyes as shown in fig. 6 page 60-62.

The regions of the retina are described with reference to their cutting angle; for example, the region 20D is the region with a cutting angle of $20^{\circ}$ dorsal from the dorso-ventral axis in the midline. The regions used were as follows:-

20D - $20^{\circ}$ from the dorso-ventral axis dorsally

40D - $40^{\circ}$ from the dorso-ventral axis dorsally

DV - Cut parallel to the dorso-ventral axis medially

90D - $90^{\circ}$ to the dorso-ventral axis dorsally.

Fig. 6

A series of drawings $A_3B_3C$ to illustrate the different angles used to investigate the eye.

A. eye cut dorso-ventrally in the midline

B. eye cut in the plane AB of drawing A.

C. eye at either of the $45^{\circ}$ ventral or temporal position.

Fig. 6

A series of drawings A,B,C to illustrate the different angles used to investigate the eye.

A. eye cut dorso-ventrally in the midline

B. eye cut in the plane AB of drawing A.

C. eye at either of the $45^{\circ}$ rostral or temporal position.

Fig. 6A

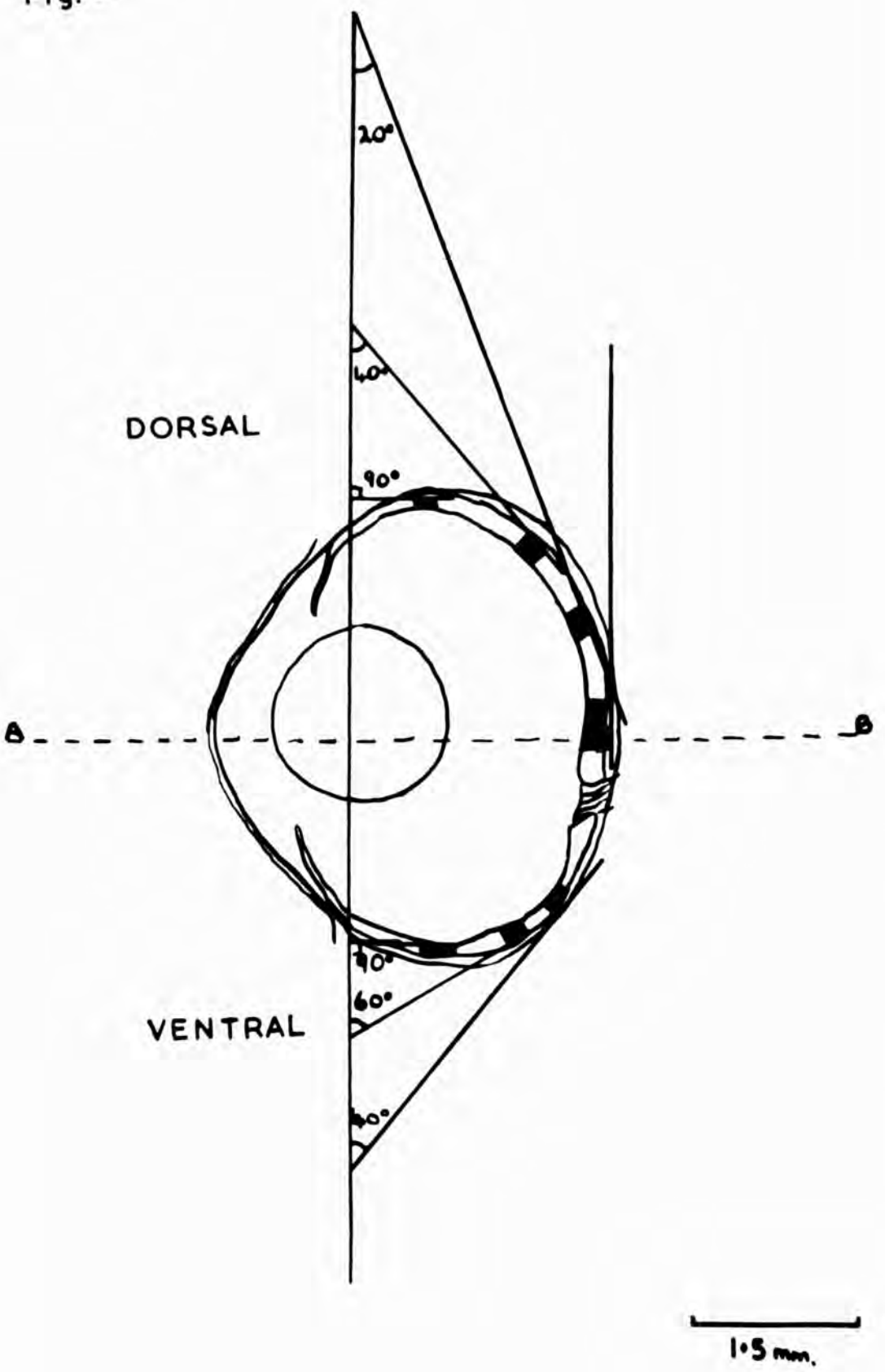


Fig. 6B

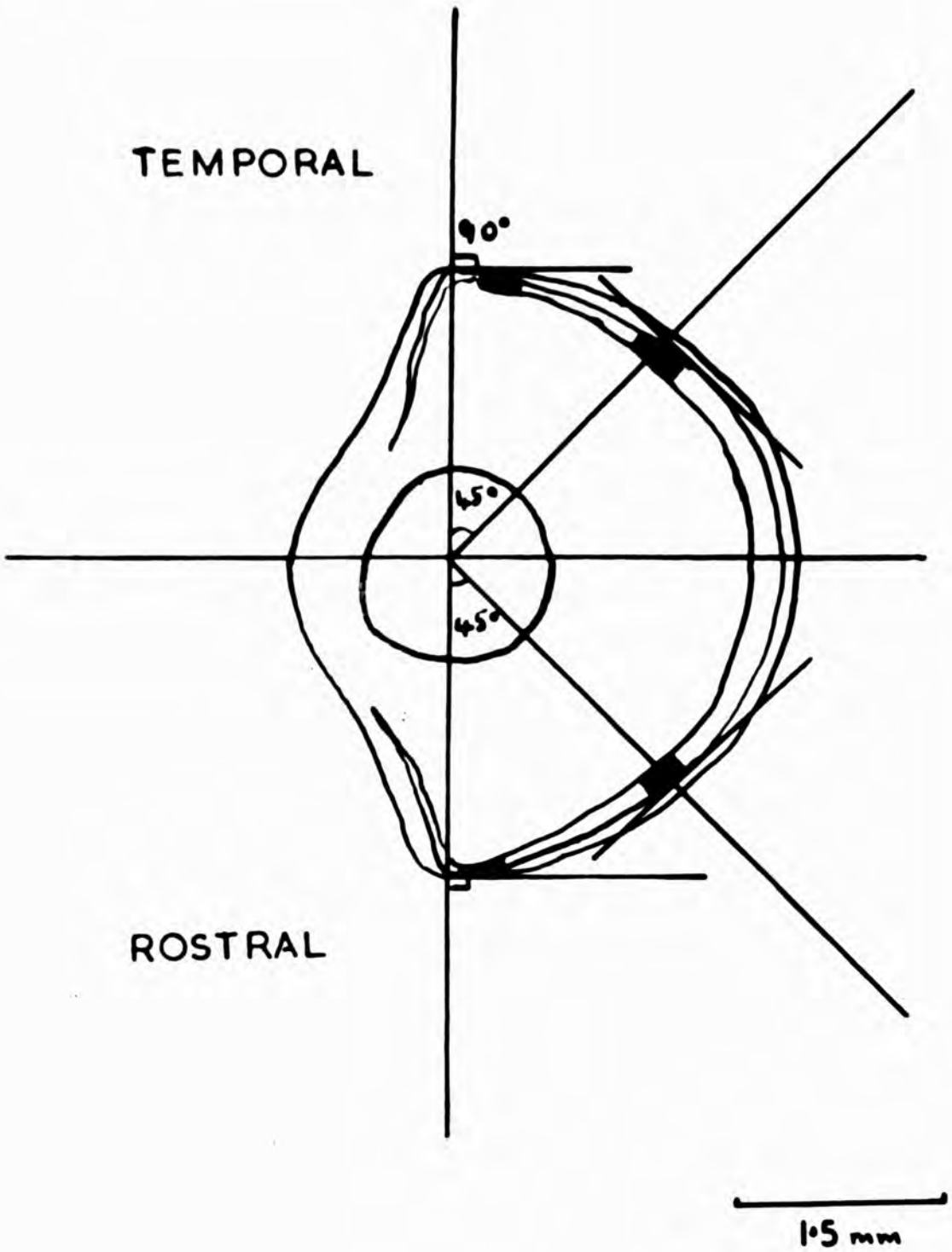
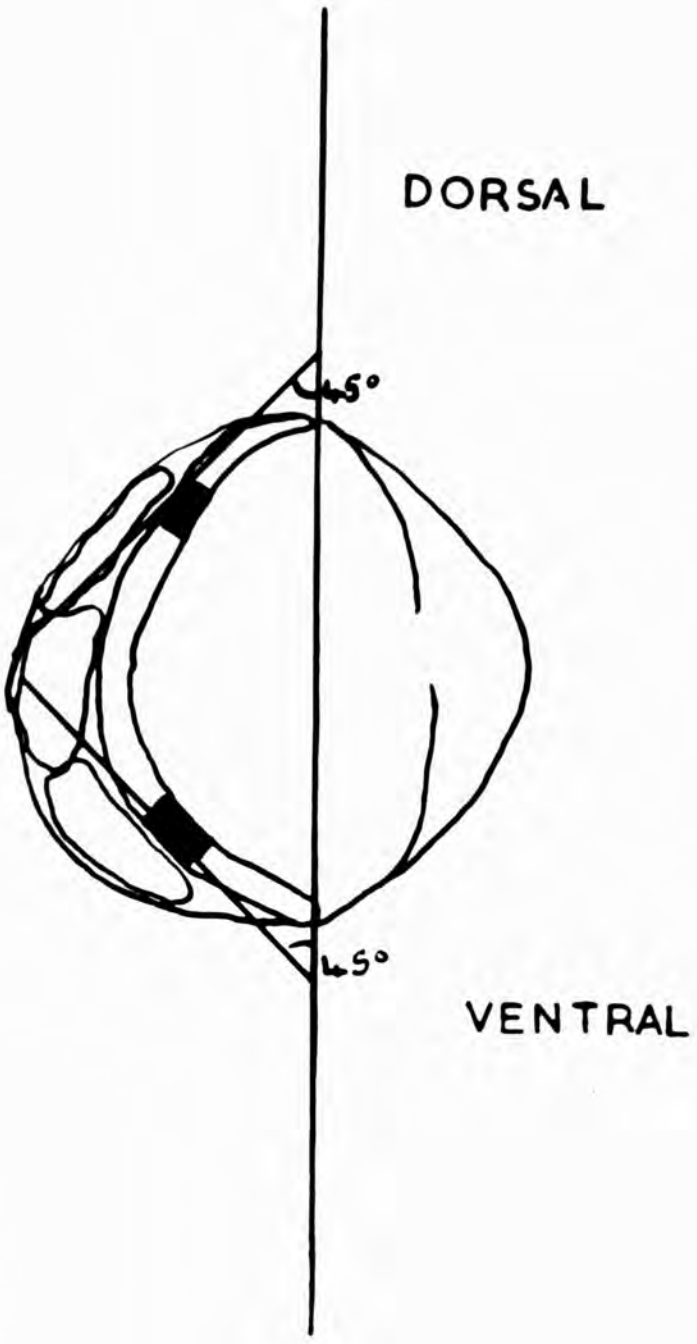


Fig. 6C



40V - 40° to the dorso-ventral axis ventrally

60V - 60° to the dorso-ventral axis ventrally

90V - 90° to the dorso-ventral axis ventrally

//DVR - Parallel to the dorso-ventral axis rostrally

//DVT - Parallel to the dorso-ventral axis temporally

45R45D - 45° rostral and 45° dorsal

45RDV - 45° rostral and parallel to dorso-ventral axis

45R45V - 45° rostral and 45° ventral

45TDV - 45° temporal and parallel to dorso-ventral axis

45T45D - 45° temporal and 45° dorsal

45T45V - 45° temporal and 45° ventral

Counts were made from all the retinal regions cut. The counts were made from an area of $0.01mm^2$ and from each region of the tangental section there were taken at least 3-4 different counts. These counts did not differ from each other by more than about 3 or 4, in a count of 50 for each retinal receptor and, because of this conformity, only the means are given in the table on page 66 .

With these counts it was possible to calculate the visual acuity for the different regions of the retina. The visual acutiy was calculated from the equation:

$$\sin \beta = \frac{1}{f} \left[ \frac{0.1\ (1 + 0.25) \times 2}{\sqrt{n}} \right]$$

taken from Tamura & Wisby (1963)

f = focal length in mm of the lens

n = density of cones per $0.01mm^2$

0.25 = degree of shrinkage using Bouin fixative and staining with haematoxylin/eosin.

$\beta$ = acuity and is the minimum separable angle in minutes.

The focal length can be calculated from Matthiessen's ratio which is $f = 2.5r$, where r is the radius of the lens. Ten minnows approximately 6cm. long showed a lens radius of from 0.68 to 0.8mm. and a mean of 0.72mm.

From the equation it can be seen that so far as the structure of the eye is concerned on its own without reference to the central nervous visual acuity depends upon two factors, the resolving power of the lens and that of the retina. The resolving power of the lens is related to the reciprocal of its focal length and is large when the lens is large. The resolution of the retina depends upon the density of the visual cells (n) and the cross connections between them. If the cross connections are very numberous this may make a difference, for they cannot be quantified and do not occur in the equation. Furthermore, details can only be resolved if their image on the retina is separated by an unstimulated cone. In calculating the acuity of the fish used in the work of Tamura & Wisby (1963) and Blaxter & Jones (1967) the double cones were counted as single cones and the two were equated. The minnow on the other hand, has both triple and quadruple cones and the question arises as to how these should be counted. On the basis of Lyall's (1957 b) theory that these multiple cone types are formed from the fusion of single

and double cones, all the multiple cone types may be counted as single cones for the purposes of calculating acuity. This may well introduce a serious error but so far nothing is known about the function of these multiple cone types.

## III. RESULTS AND DISCUSSION OF OBSERVATIONS ON RETINAL RECEPTORS

The figures in table I (p. 66) are marked on a series of diagrams of the retina looked at from the front (fig. 7 p. 68 ). Only their approximate positions are marked.

In the retina of the Chess minnows, in general agreement with the results of Lyall (1956, 1957 a, b), and Engström (1960, 1963), are present rods and single, double, triple and quadruple cones.

The rods are very difficult to see and the figures are only very approximate. They have the typical teleost structure with an extremely long and thin outer segment, lentiform body and myoid. They show a general distribution characteristic of many other vertebrates in that they occur in large numbers at the periphery of the retina and the numbers decrease centrally. Nowhere in the retina are they the dominant receptor cells and the retina is predominantly a cone retina.

/sq. mms

TABLE I . The number of rods and cones in different regions of the retina and the visual acuity for each region measured from the cone counts. Also shown is the presence (+) or absence (-) of a cone mosaic (p. 72 ).

| Region | Rods | Cones | | | | Acuity | Mosaic |
|---|---|---|---|---|---|---|---|
| | | Single | Double | Triple | Quadruple | | |
| 90D | 21 | 26 | 40 | 0 | 0 | 55' | + |
| 40D | 22 | 12 | 12.15 | 11 | 3 | 50' | - |
| 20D | 14.7 | 10.3 | 11 | 10.3 | 4 | 53' | - |
| DV | 14.2 | 9 | 12.5 | 13.5 | 6 | 46' | - |
| 40V | 12.3 | 5.7 | 27.7 | 9.3 | 4 | 44' | - |
| 60V | 27 | 31 | 45.7 | 4 | 1 | 49' | + |
| 90V | 34 | 45 | 61.7 | 0 | 0 | 43' | + |
| 45R45D | 22 | 41 | 43 | 2.3 | 1.3 | 48' | + |
| 45RDV | 14.3 | 2.7 | 2.6 | 35.3 | 7 | 41' | - |
| 45R45V | 18 | 28 | 58 | 2 | 0 | 38' | + |
| 45T45D | 17 | 43 | 21.5 | 10.5 | 3 | 42' | - |
| 45TDV | 11 | 23.7 | 5.3 | 35 | 3.3 | 38' | - |
| 45T45V | 17 | 33 | 52 | 2 | 0 | 40' | + |
| //DVT | 32 | 37 | 52.5 | 0 | 0 | 40' | + |

Fig.7

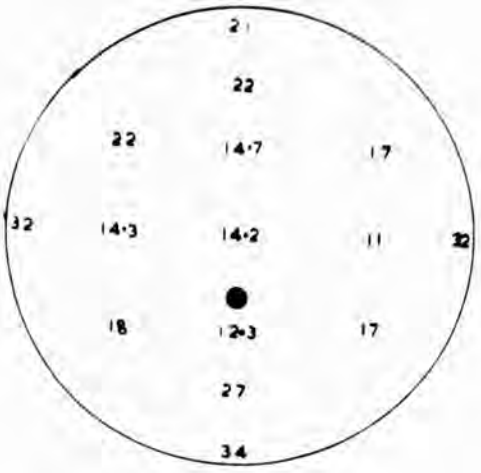
A series of drawings of the retina of the mimmow looked at from the front of the eye. The numbers and approximate positions of the rods, single, double, triple and quadruple cones are marked. The acuity and the presence of a cone mosaic are also shown.

Fig.7

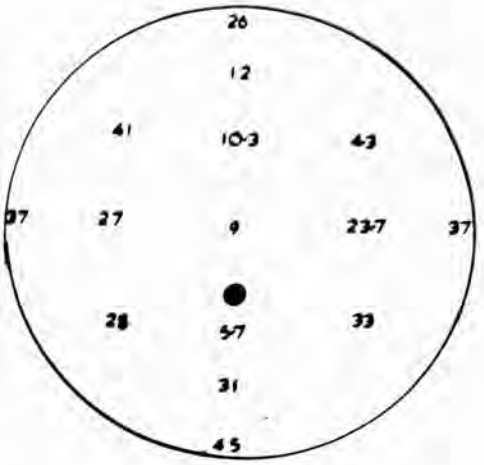
A series of drawings of the retina of the minnow looked at from the front of the eye. The numbers and approximate positions of the rods, single, double, triple and quadruple cones are marked. The acuity and the presence of a cone mosaic are also shown.

Fig. 7

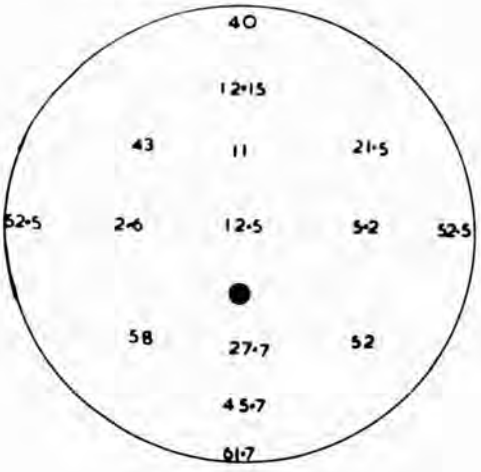
RODS



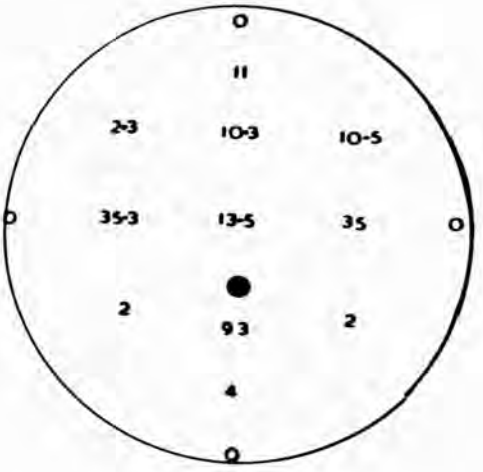

SINGLE CONES



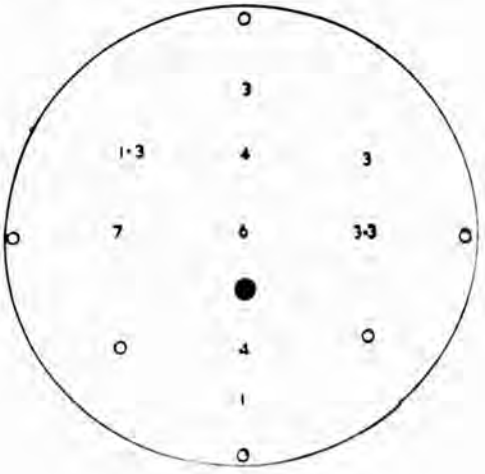

DOUBLE CONES



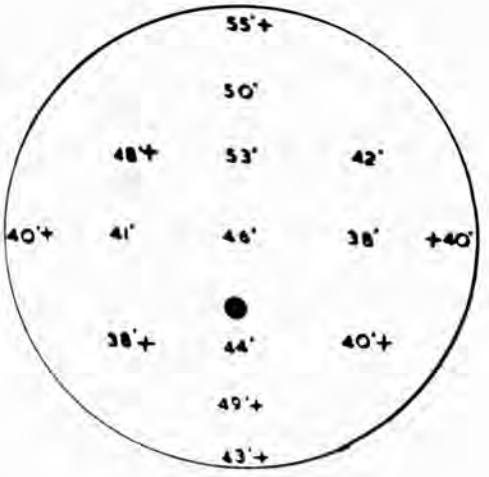

TRIPLE CONES




QUADRUPLE CONES




ACUITY AND MOSAIC [+]



The double cones are unequal, one usually being larger and, together with the single cones, show a tendency to become reduced in the central regions of the retina. This reduction centrally in the retina is associated with the increase in the number of triple and quadruple cones, which are not found in the peripheral parts but form the dominent cone type in the central regions. Taking double, triple and quadruple cones as 2, 3 and 4 single cones respectively, the total number of cones in a given area did not vary greatly throughout the retina. This supports the hypothesis that these multiple cone types are formed by the fusion of double and single cones (Lyall 1957 b), which is in turn based on the assumption that the retina grows from its edge as in other forms (Lyall, 1957 a; Blaxter & Jones, 1967).

Triple cones are essentially composed of three normal cones fused together, (for the general structure of the cones see fig. 3 p. 54 ), and for their appearance in tangential section, fig 8 p 71 ). Engström (1960), and Lyall (1956, 1947 b), both agreed that they were of the linear type and arranged in a straight line with a large central cone and two small ones on either side. The linear type of triple cone was also present in the Chess fish but there was also present the triangular type, generally seen in Gadids.

The quadruple cones are never very dominant in the retina and are composed of a large central cone surrounded by three smaller cones.

Fig. 8

A. Photomicrograph of the central region of the retina of a Ghose mirrow cut tangentially and stained with haematoxylin and eosin. It shows the absence of any mosaic and the presence of linear triple cones (L.T.C.) triangular triple cones (T.T.C.) and quadruple cones (Q.C.)

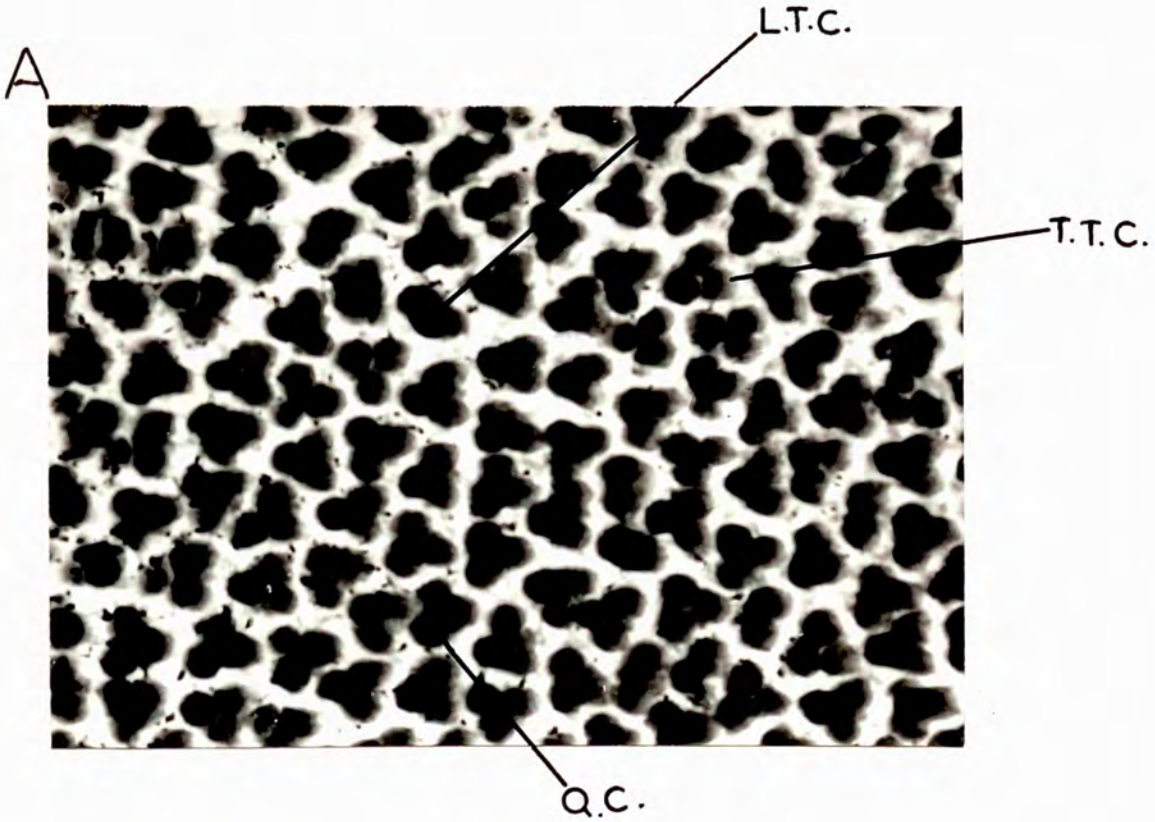
B. Photomicrograph of a tangential section of retina of a Ghose mirrow at the periphery, showing the mosaic of alternating rows of double (D.C.) and single (S.C.) cones.

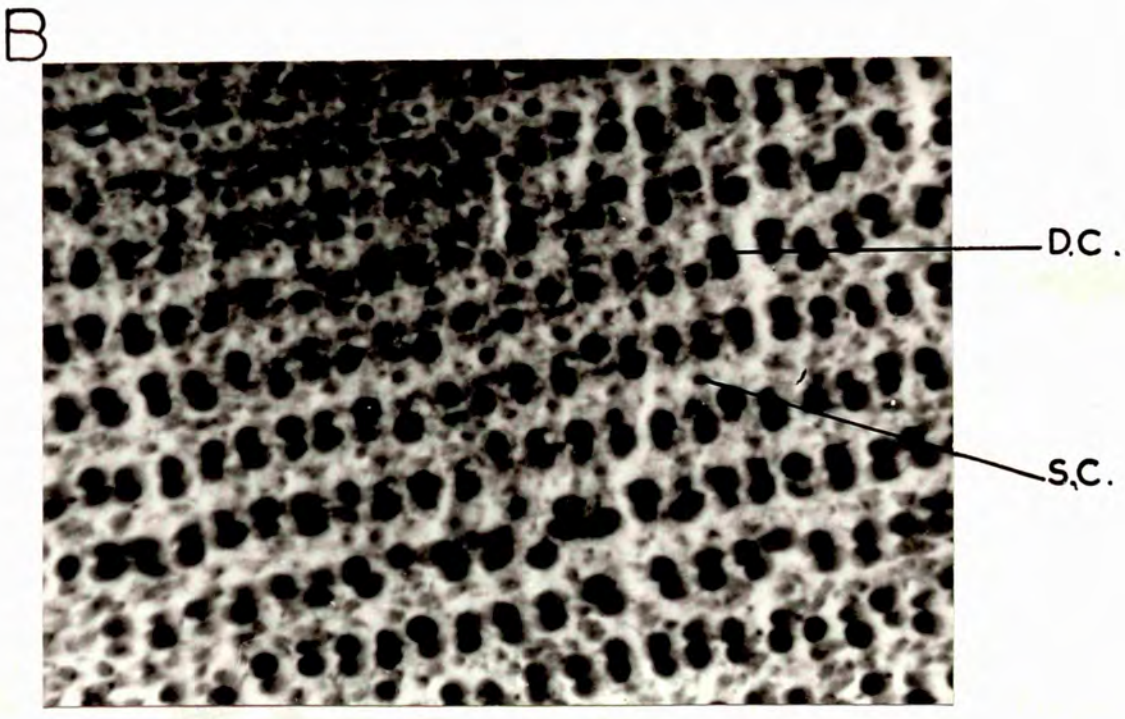
Fig. 8

A. Photomicrograph of the central region of the retina of a Chess minnow cut tangentially and stained with haematoxylin and eosin. It shows the absence of any mosaic and the presence of linear triple cones (L.T.C.) triangular triple cones (T.T.C.) and quadruple cones (Q.C.)

B. Photomicrograph of a tangential section of retina of a Chess minnow at the periphery, showing the mosaic of alternating rows of double (D.C.) and single (S.C.) cones.

Fig. 8




0·05 mm

The minnow retina shows the general teleostean retinal character of a larger number of retinal elements in the temporal field than in the rostral field.

The ventral region of the retina, like that of Fundulus (Butcher 1938), differs from the dorsal region in having very few triple and quadruple cones but a large number of double and single cones.

The visual acuity varies slightly throughout the retina and the area of lowest acuity is in the extreme dorsal part of the retina. This decrease in acuity is to be expected from the results of Hogben & Landgrebe (1940) on Gasterosteus from which they concluded that this region is not used for form-vision and probably can only receive light intensity. These figures obtained for the acuity of the minnow using the formula of Tamura & Wisby (1963) do not agree very well with those given by Brünner (1934), obtained by behavioural experiments. Using a black strip,8cms. from the fish, she found the minimum separable angle to be 0.25mm, giving a visual angle of around 11'. It would seem that the eye works more efficiently than would appear from the calculated figures using the formula and this may well be due to the vast number of cross connections found in the teleost retina.

The mosaic found in the minnow only occurs in the peripheral regions of the retina and consists of parallel rows of alternate double

and single cones. The function of these cone mosaics is obscure. Lyall (1957 b) states that he could not find any functional significance in the cone pattern in teleost retinae, but suggested that it might improve the perception of movement, and that the pattern provides a uniform distribution of cone types which might be important if the various component cones had different functions. Engström (1963) found that cone mosaics are very regular in eyes or regions of eyes which are adapted for acute vision and that the most regular mosaics are found in species which are feeding on fast moving objects. According to him, retinae or regions of retinae which are not adapted to the same extent for sharp vision have a more loosely organised mosaic or no regular mosaic at all. Engström (1963 b) and Walls (1942) have further suggested that the parallel rows of single and double cones which constitute the mosaic pattern seen in the Cyprinids is a primative feature. Engström concluded that the presence of triple and quadruple cones associated with the breakdown of the mosaic in the central regions of the minnow retina to be aberrant. The minnow does not fit all Engström's conditions as it occurs in a wide variety of habitats: lakes (Frost 1943), deep rivers, and shallow, fast-moving streams such as the Chess, where very acute vision would be necessary. All the evidence shows that the minnow has fairly good vision and that the mosaic is present at the periphery and not at the central regions. It seems from consideration of the minnow that the breakdown of the mosaic does not reduce the visual ability of the fish. It seems possible that the same effect as a complex cone mosaic could be achieved by the fusing of the cones and that the benefits of a mosaic

would then be amplified because of the close proximity of the individual cones. This would then imply that instead of the triple and quadruple being aberrant types they are highly evolved alternatives for a cone mosaic. This would also explain the evolutionary significance of their occurrence in such numbers in the visually most important parts of the retina.

## IV. RETINAL REMOVAL AND COLOUR CHANGE

In view of the conflicting results from the behavioural experiments by previous workers (p. 13 ), it was decided to approach the problem of the rôle played by the retina in colour change from another aspect, namely, by surgically removing parts of the retina or by destroying parts of the retina by other means.

The cutting of the optic tract is a simple operation which consisted of making a small cut in the conjunctiva and rotating the eye. The optic tract can clearly be seen and cut with a pair of fine, sharp scissors. The operation does not appear to weaken the fish very much and, unlike fishes operated by Hogben & Landgrebe (1940), they survived for as long as necessary to complete the experiment. In the present experiment, some survived for more than a year. Regeneration was not observed in these fish because the blood vessels running to the eye were also cut.

Fig. 9

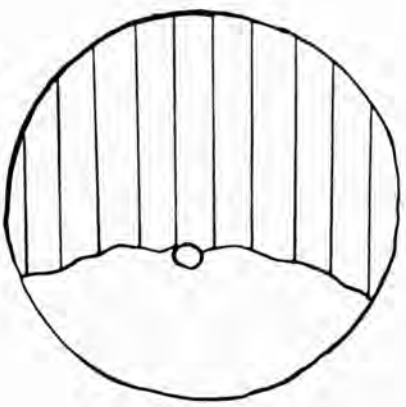
A series of drawings to show the extent of the dorsal retina removed in fish R11, R12, R27, R24, R25, R26, and R19. The retina is viewed from the front of the eye.

Fig. 9

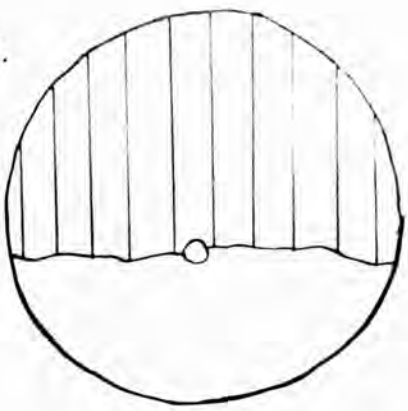
A series of drawings to show the extent of the dorsal retina removed in fish R11, R12, R27, R24, R25, R26, and R19. The retina is viewed from the front of the eye.

Fig.9

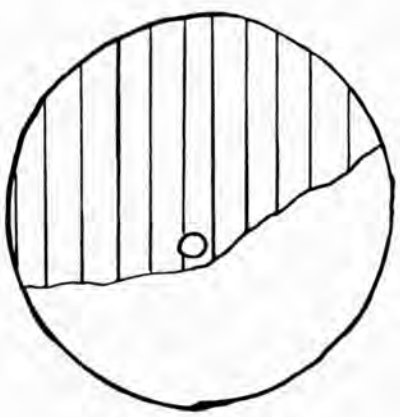
R.11.



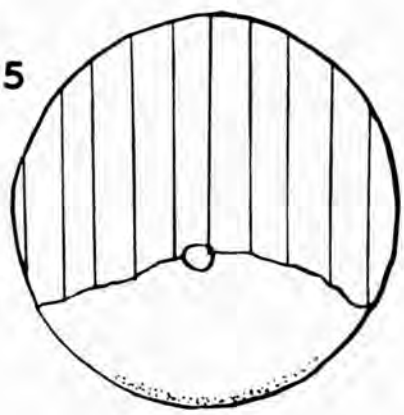
R24.



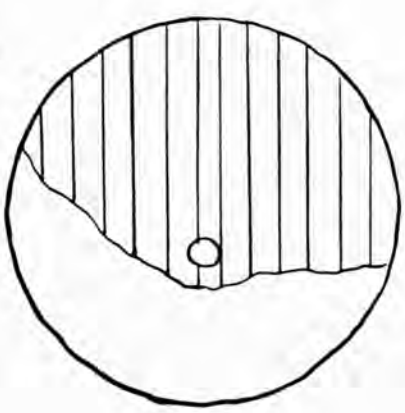
R.12.



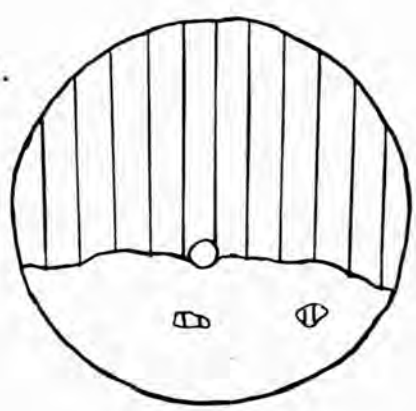
R.25



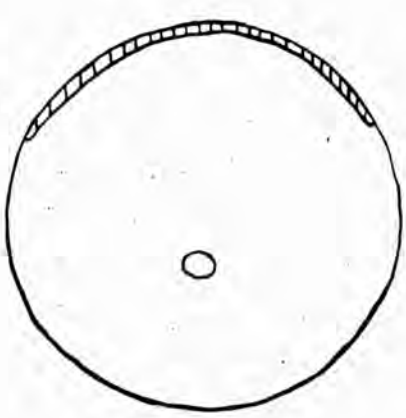
R.27



R.26.



R.19.

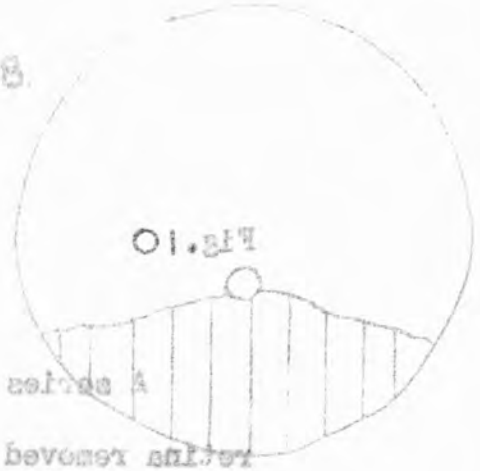


1mm

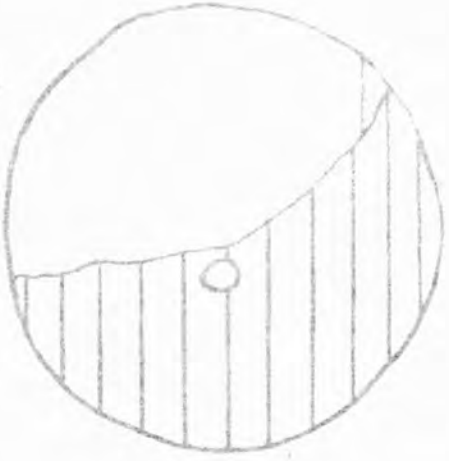
Fig 10

R.7.

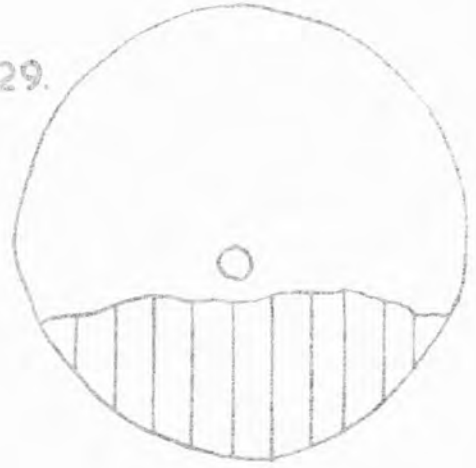
R.28.



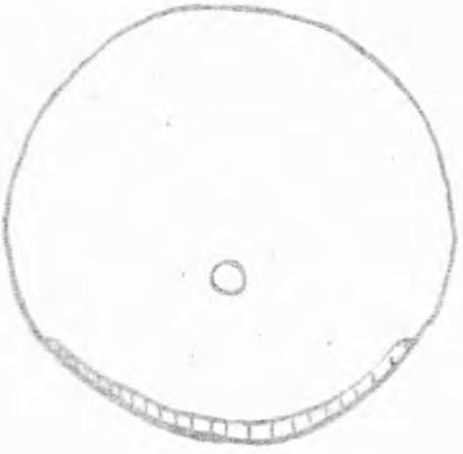
R.31.



R.29.



R.18.



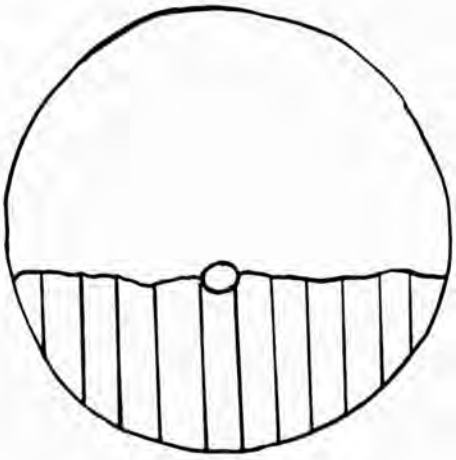
0·8 mm.

Fig.10

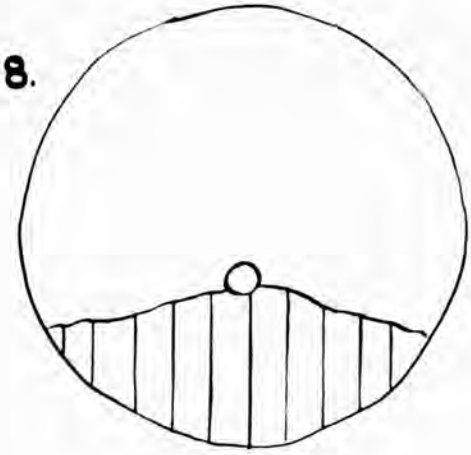
A series of drawings to show the extent of the ventral retina removed in fish R7, R31, R28, R29, R18. The retina is viewed from the front of the eye.

Fig. 10

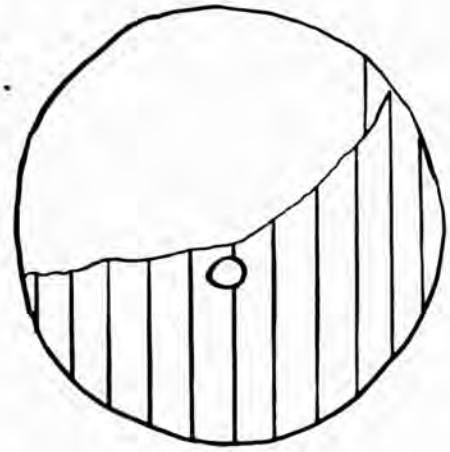
R.7.



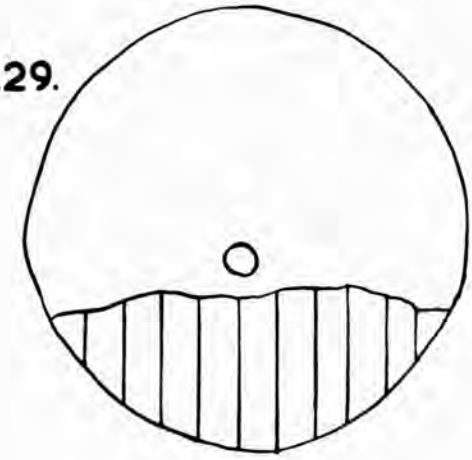
R.28.



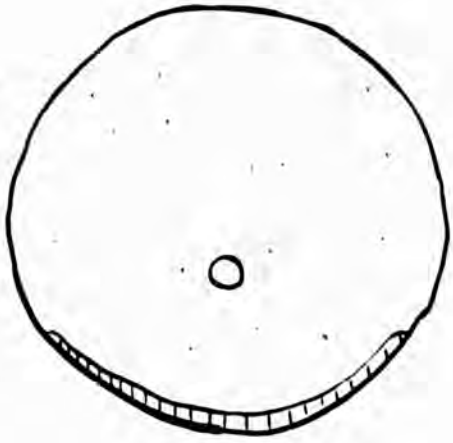
R.31.



R.29.



R.18.



0·8 mm.

16 minnows were taken, and the left optic tract was sectioned and they were allowed to recover for at least 24 hours. These fish were then tested for normal colour change on both white and black in order to confirm that they all could change from D.O.I. 7.5 to 1 well within the normal time (fig. 28 p. 151 ). The fish were then re-anaesthetised and the conjunctiva was cut over the dorsal part of the right eye. A cut was then made in the dorsal part of the right eye at the base of the iris into the vitreous humor. In three of these fish no further damage was done and the conjunctiva was reattached to the surrounding skin of the skull by two stitches of fine nylon. In 7 other fish the dorsal part of the retina was removed by means of careful cutting and scraping, using very fine knives. During the operation the eye was kept bathed in ringer to wash away blood and prevent it from filling the eye, a situation which would have caused total blindness. The ventral part of the retina was treated in the same way in 5 fish. The extent of the removals was determined from serial sections of the eyes and is shown in fig. 9, 10 page 76, 78.

The fish with retinal removals were then tested after 7 and 14 days on both a black and a white background and their colour recorded. At the end of the 14 days the fish were killed and the eye was examined for any degenerative changes. In all except one of the dorsal removals the back of the eye, (fundus) could be clearly seen through the pupil with a binocular microscope and the lens appeared normal. After 14 days,

the wound had completely closed and scar tissue had formed. A section of a 16-day eye is shown in fig. 11 page 82.

8 control fish were treated in exactly the same way as the experimental fish but only a small cut was made in the right eye. The cut measured 0.75mm. long and was made through all the layers in the eye, in 4 dorsally and in 4 ventrally.

The results for all fish are shown in table 2,3,4 .

TABLE 2. The colour of the ventral retinal removed fish at 7 and 14 days post-operation, after having been placed for 30 mins. on a black or a white background.

Experimental Fish.

a. Ventral retinal removals.

| Fish | D.O.I. 7-day | | D.O.I. 14-day | |
|---|---|---|---|---|
| | White | Black | White | Black |
| R7 | 3 | 4 | 2 | 4 |
| R28 | 3 | 5 | 4 | 5 |
| R29 | 5 | 6 | 3 | 5.5 |
| R31 | 5 | 6 | 4 | 6 |
| R18 | 1 | 6.5 | 1 | 6.5 |

b. Dorsal retinal removals.

Fig. 11

Section of fish 87 showing the complete removal of the ventral retina while leaving the dorsal retina intact. Stained with Kultver & Barrera and section at 4μ.

Fig. 11

Section of fish R7 showing the complete removal of the ventral retina while leaving the dorsal retina intact. Stained with Klüver & Barrera and section at 4μ.

Fig. 11

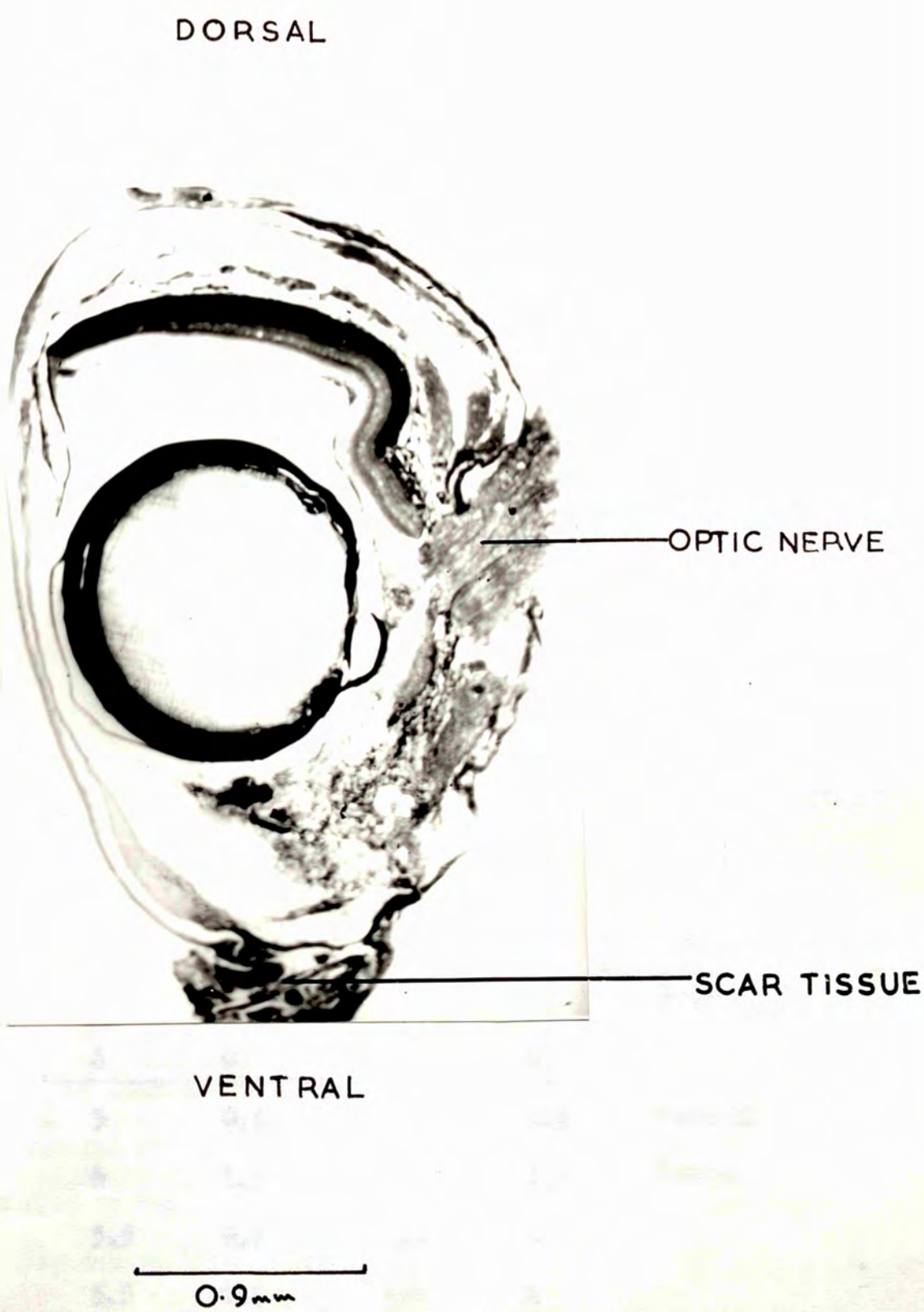


TABLE 3 . The colour of the dorsal retinal removed fish at 7 and 14 days post-operation, after having been placed for 30 mins. on a black or a white background.

| Fish | D.O.I. 7-day | | D.O.I. 14-day | |
|---|---|---|---|---|
| | White | Black | White | Black |
| R11 | 7.5 | 7.5 | 7.5 | 7.5 |
| R12 | 6 | 7 | 6.6 | 7 |
| R24 | 7 | 7 | 6 | 7 |
| R25 | 7 | 7 | 7 | 7 |
| R26 | 7 | 7 | 7 | 7 |
| R27 | 7 | 7 | 6.5 | 7 |
| R19, 32, 33 | 7 | 7 | 7 | 7 |

TABLE 4 . The colour of control fish with a small cut placed in the dorsal or ventral retina. The post-operative D.O.I. was recorded after 7 days, and the period on the black and white backgrounds was 30 mins.

Control fish

| Fish | Pre-operative D.O.I. | | Post-operative D.O.I. | | Treatment |
|---|---|---|---|---|---|
| | Black | White | Black | White | |
| 1 | 6 | 0.5 | 6 | 1 | |
| 2 | 6 | 0 | 6 | 0 | dorsal |
| 3 | 6 | 0.5 | 6 | 0.5 | lesion |
| 4 | 6 | 0 | 6 | 0 | |
| 5 | 5 | 0.5 | 4 | 0.5 | ventral |
| 6 | 6 | 1.5 | 6 | 1.5 | lesion |
| 7 | 5.5 | 0.5 | 5.5 | 1 | |
| 8 | 5.5 | 0.5 | 5.5 | 1 | |

The D.O.I. values were recorded after 14 days because this time corresponds to stage 2 degeneration of the optic tract (fig.14 p. 102). The degenerating fibres from the regions of retinal removal could then be easily traced.

The fish were allowed to remain on a black or a white background for 30 mins. because after this time for normal fish would have become almost completely adapted (fig.28 p. 151 ).

Serial sections of the whole eye showed that in every case there was no evidence of haemorrhage and that the retina appeared normal, was not detached and had normal nerve connections.

The control fish show that the operation has no, or only very slight (1,5, 7, 8) effect on the normal colour change. It would therefore appear that the effect on colour change of the experimental operations were due to the actual depletion of retina.

The results from the experimental fish show that fairly large cuts in the ventral retina have very little effect on colour change, as seen in R18 which had lost its ability to darken by 1.5 on the D.O.I. scale. The larger ventral removals assume an intermediate colour, when they can only darken to values of from 4-6. Not only can they not darken fully but they cannot lighten fully, and in no case did the colour exceed a value of 2 on the D.O.I. scale. The overall ability of the fish to adapt to its background ranged from 1 to 2 degrees.

None of the dorsal removed fish showed any ability to colour over 1 degree seen in R24. In all except R11 the colour was not maximal black.

In only 2 fish was there any partial recovery in the 14-day test; these were R7 and R31 both showing a 1-degree improvement in their ability to lighten from the 7 to 14-day test.

## V. RETINAL LESIONS USING HIGH INTENSITY LIGHT

Direct surgical removal of parts of the retina may well damage other properties of the eye which are not easily seen. For example, it may disrupt the ability of the lens to accomodate. Also, the ringer may well dilute the vitreous humor altering its optical properties. Other methods were therefore tried to confirm the surgical removals. Thermocautery and electrocoagulation were tried but they both proved to be unreliable and involved extensive surgery. Further, in most cases, it was not possible to ascertain what damage had been done until after the eye had been sectioned. The method of photocoagulation, however, did prove to be highly successful. Recently, Geeraets & Ridgeway (1963) reviewed the factors involved in the damage done by a pulsed light source on the retina and concluded that the damage is dose-rate dependant. They constructed a model where the radiation is absorbed by the retina so that the temperature of the pigment epithelium is raised, and damage then results. This would mean that a short exposure to high intensity light results in a rapid heat wave and less damage and a long exposure

to a lower intensity, a slower heat wave and more damage, because thermal damage increases with time.

The methods generally used for producing photocoagulation are the Xenon arc and the Laser beam, but neither of these was available. The apparatus used (fig. 4 p. 57) consisted of a 500w projector lamp covered with aluminum foil except for a small opening which allowed the light to pass out. The projector lamp was housed in a tin-plated iron shield with a hole bored in it and in this hole was attached a 4mm microscope lens. Although this apparatus does not develop a fraction of the energy of a Xenon arc or a Laser, if it is left on for long enough sufficient heat is generated to damage the retina.

The fish were anaesthetised and placed on the operating board and the lens of the photocoagulator was placed very close to the cornea and arranged so that the light was defocused onto the lens of the fish and the lens of the fish then focused the beam onto the retina. The eyes were exposed for a variable period of time to the light. It was found that a 4-second exposure gave a very clear lesion (fig. 12 p. 83).

The photograph shown on page 83 is of a lesion 4 hours after being placed. This shows nicely the disruption of the cones and rods, especially the outer segment and lentiform body which is surrounded by the pigment epithelial cell layer whose processes run in between the rods and cones. Massive damage is also seen in the myoid and the nuclei of the rods and cones. The nuclei of the bipolar cells also appear disrupted and, like

Fig. 12

A. Photomicrograph of the eye to show the retinal lesion, stained with Klüver & Barrera

B. High power photomicrograph of the retina to show the lesion.

Fig. 12

A. Photomicrograph of the eye to show the retinal lesion, stained with Klüver & Barrera

B. High power photomicrograph of the retina to show the lesion.

Fig. 12

A


B

the nuclei of the rods and cones, are small and pyknotic. Up to the level of the end of the bipolar cells there is complete disruption but in the inner plexiform layer of the retina the damage is not so noticeable. This is consistent with the thermal effect theory proposed by Geeraets & Ridgeway (1963), for in the case of the minnow a large pigment epithelium would absorb the light and heat but because of the low intensity of the light insufficient heat is produced to damage more than the layers immediately adjacent to the pigment epithelium.

The lesion was circular and approximately 0.94mm in diameter. Using this technique it was possible to destroy large areas of the retina and to utilise the optical properties of the eye in doing so.

A series of six lesions was placed in the dorsal and ventral retina, in two arcs of three in the right eye, the left optic tract having been cut previously. The arcs were in the approximate direction of light coming from the immediate background and of light coming from overhead. The positions of these lesions are shown in fig. 13 page 91 .

The results of only two fish were obtained.

a. R20 - The six lesions were placed in the dorsal retina of the right eye and showed D.O.I. values, after 7 days, of 7 on black and 5 on white.

B. R21 - The six lesions were placed in the ventral retina of the right eye and showed D.O.I. values, after 7 days, of 5.5

Fig. 13

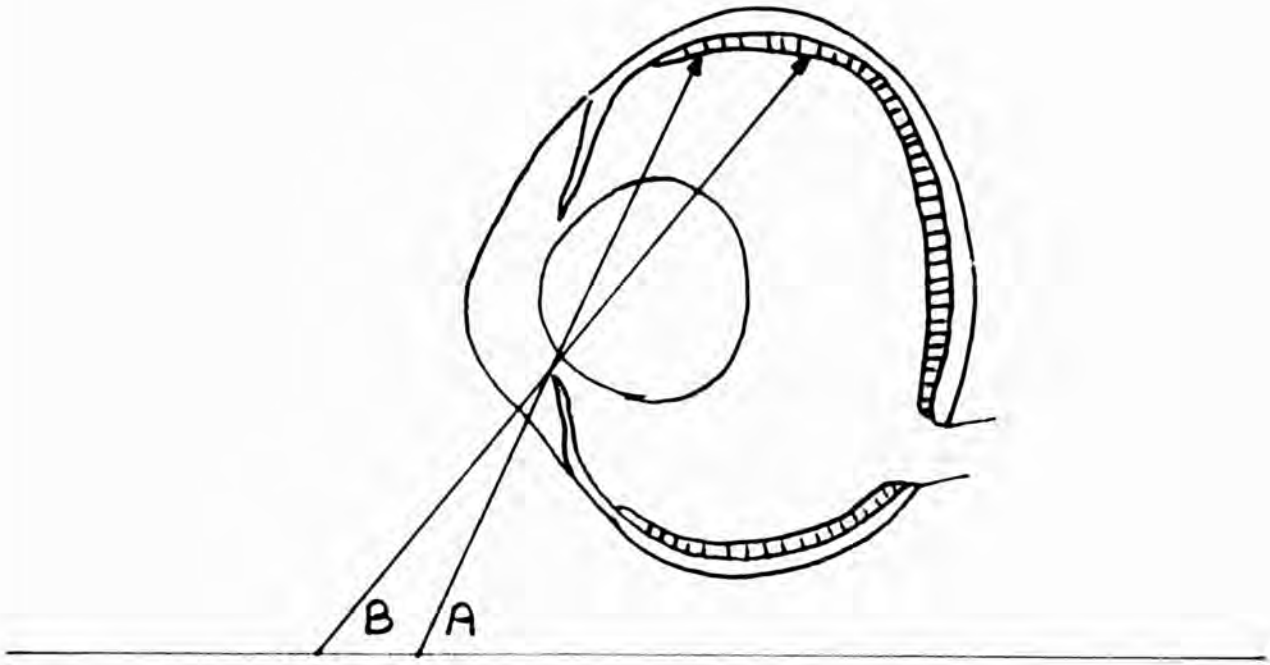
The position of the lesions placed in the eye of fish P20 and #21 to destroy either the dorsal or ventral retina.

Fig. 13

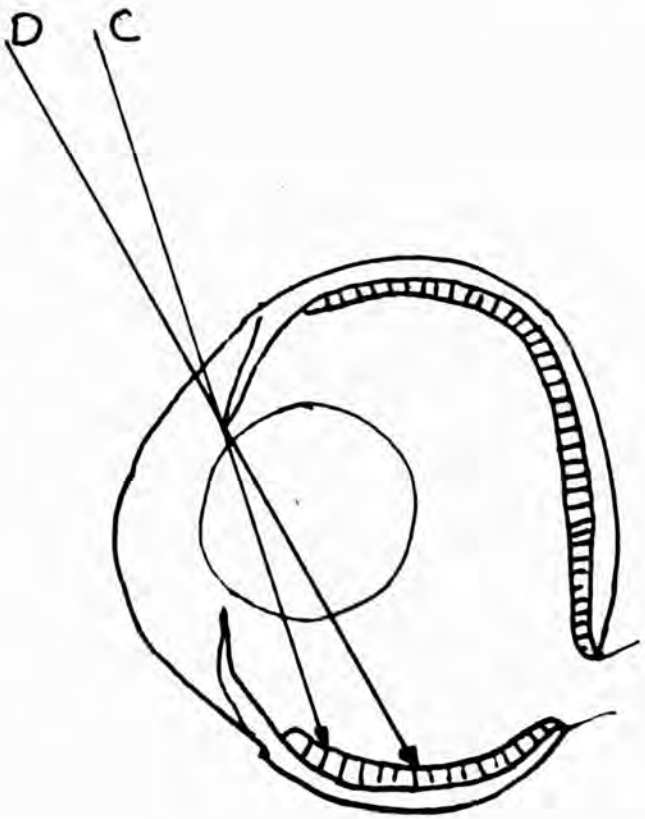
The position of the lesions placed in the eye of fish R20 and R21 to destroy either the dorsal or ventral retina.

Fig. 13

R. 20




R. 21



on black and 3.5 on white.

Control fish were tested with a single lesion, and they showed normal colour change in both rate and direction.

The area destroyed by a single lesion is about 0.73266 sq.mm. which means that the total area destroyed by the six lesions would be about 4.396 sq.mm. The total retinal area was found to be approximately 12.76 sq.mm. which gives the area destroyed by the six retinal lesions at very slightly over one third of the total retinal area.

The reasons why more results were not produced are twofold. Firstly, and most important, was the technical difficulty involved in placing the lesion without a clear picture of the fundus. It was not possible to arrange the apparatus so that the beam could be accurately focused on the retina by defocusing on the lens. Secondly, the apparatus was limited by the absence of a cooling device and hence there was no means of reducing the heat transmitted with the light beam. The two results largely confirm those of the surgical removal. To obtain them a large number of fish were used but all excepting these two were discarded because of the failure to produce lesions or because the heating of the cornea caused it to become cloudy.

The results of the experiments do show that destruction of the dorsal retina prevents the fish from becoming pale on a white background and that

the destruction of the ventral retina prevents the animal from darkening but also from fully paling.

## VI. DISCUSSION OF RESULTS OF RETINAL REMOVAL AND LESIONS.

One must consider whether these results are due to the background response or whether they can be explained in other ways. In all the dorsal retinal removals the fish became dark and in most cases did not show any adaptation to background. However, there is a possibility that these fish were totally blind in which case they would have gone dark for this reason alone (p.135). This, however, seems unlikely from the histological observations, and from the fact that they all went dark, for if they were totally blind then approximately half would have begun the second lightening stage (p.139). In the case of the ventral retinal removals, the fish showed a limited ability to change colour, apparently to an attempt by the fish to adapt to the background, for this could not be produced by varying the light intensity.

The extent of the lesion appears to be an important consideration, for the small lesions in the retina, whether in the periphery or in the central regions, had no effect on colour change. The larger lesions had a marked effect and range from the small ventral cut R18 after the fish could change colour from 1-6.5 to the large removal of the dorsal

retina where the fish could not show any change in colour.

When the dorsal retina region was removed by surgery or by photocoagulation, leaving the ventral retina, the fish assumed a dark colour on the black background, and it was only R24 of the surgical removals which showed any lightening on the white background (table 3 p. 83 ). R20 did show a certain ability to change colour and could pale down to 5 giving the fish the chromatic adaptation of 2 degrees. The photocoagulation fish, R20, had only the extreme dorsal part of the retina removed, unlike the surgical removals where the whole of the dorsal part of the retina was removed. It would therefore appear that active paling in response to an illuminated white background can only occur when the dorsal retina above the optic tract is present, and that the more retina present the greater the ability to pale. In considering the action of the dorsal retina the results of R19, 32 and 33 do not fit with the other results. This apparent anomaly may be due to blood in the vitreous humor screening the retina. Although this was not clear from the sections it proved very difficult to prevent bleeding in these fish with cut retinae and they continue to seep blood into the vitreous humor. When the retina is completely removed the residual blood is negligible.

The removal of the ventral retina leaving the dorsal intact results in the fish assuming an intermediate colour on the black ranging from 4 to 6-5. One the white none of the fish became paler than a value of 2; the fish not only have a reduced ability to darken on a black

background, they also do not have the ability to lighten fully on the white. It must also be noted that the fish which darkened most was R31, and it was this fish which showed the largest ventral retinal removal, and also had the least ability to pale.

The general conclusions from these results seem to be that illumination of the dorsal retina is followed by paling and of the ventral retina by darkening. This situation is further complicated by the fact that for complete paling the ventral retina must be present. With the progressive removal of the ventral retina dorsalwards, an increased inability to pale is found, together with an increased ability to darken. If these facts are taken into consideration with the fact that in the absence of retina input (removal of both eyes) the fish darkens, it would appear that in the case of the ventral retinal removals the fish is responding to the background with all the retina available, the part removed always inducing darkening. This leads to the conclusion that the whole of the retina is important in colour change, and that it is the whole of the visual field which is necessary, and that no area of the retina is any more important than any other.

Such conclusions do not agree with those of previous workers in this field. Retinal differentiation was proposed by Sumner (1933) for Fundulus parvipinnis, Butcher (1937 a, b, 1938 a, b 1939) Fundulus heteroclitus, and Hogben & Landgrebe (1940) Gasterosteus aculeatus. Sumner and Butcher considered that the dorsal retina controls paling

and the ventral retina darkening. From the work on the minnow it is seen that the situation is more complex than this because the presence of the ventral retina is necessary for paling. Hogben & Landgrebe (1940) further divided the retina of Gasterosteus in relation to colour change. They considered the region concerned with black adaptation is confined to the floor of the retina below the optic tract and the white background response is associated with a restricted region of the central retina. They considered the extreme dorsal retina to be essentially neutral with regard to colour change.

On the basis of Hogben & Landgrebe's (1940) work one would expect the fish R21 with the floor of the retina destroyed to have been completely white. In fact, it showed an intermediate colour. The destruction of the more extreme dorsal retina (Table 3 p. 83) which Hogben & Landgrebe considered is neutral to colour change, resulted in the inability of the fish to change colour. Fish R20 with a dorsal retinal lesion could not pale further than D.O.I.5. It may be that the eyes of Gasterosteus and Phoxinus differ in relation to chromatic adaptation, but they do show a similarity in visual acuity (p. 72), and it seems possible that their eyes may have a similar function in colour change. Hogben & Landgrebe (1940) using measurements of the refractive index of the lens calculated that in a container with the top and sides painted black, and the light inferior, the fish would show the darkness response if the dorsal peripheral part of the retina was neutral to colour change. They tested fish under these conditions and found that

the fish paled but not to the same extent as the normal background pallor. The main criticism of this work is the retinal regions are based on theoretical considerations in the absence of light scattering, whereas the testing was performed under conditions which would allow considerable light scattering. If, however, the light in this experiment is restricted to a certain retinal area the results obtained could be explained as partial background paling, and not darkness paling. In the minnow, darkness paling only occurs in the complete absence of light.

The 'ratio' hypothesis proposed that the colour the fish assumes depends upon the ratio of the direct and reflected light. In view of the lack of rigid retinal differentiation as shown by surgical operations, this hypothesis becomes untenable for in the minnow the ventral retina is important for paling, yet receives light from overhead. In the minnow, and probably in other fish, the retina acts as a unit, relaying the total visual field to the brain, where it is interpreted in terms of the relative amount of light reflected from the background. The effect of the retinal removals can then be looked upon as modifying the total visual complex, and the results are what would be expected for a limited visual field. The idea that the eye acts as a unit in colour change was first hinted at by Mast (1916) when he concluded that the colour change reaction is not as simple as would appear from the ratio hypothesis. A similar idea was expressed by Danielson (1939, 1941) who considered that the state of the melanophores is determined by the

degree of contrast in the visual field as a whole.

The relationship therefore of the eye to colour change is one of the interpretation of the brightness of the visual field by the brain, and ideas concerning retinal differentiation and the ratio of the direct/reflected light are far too simple to be considered as applicable.

# SECTION 4

## THE VISUAL SYSTEM IN THE BRAIN

### I. THE OPTIC TRACT

In order to determine which fibres of the optic nerve are afferent degeneration studies were made using the Nauta technique. To determine the optimum time for Nauta technique following nerve section 12 fish had the right optic tract cut and were left in the aquarium at 18°C ± 2°C. Two of these fish were then removed at 2, 4, 7, 10, 14 and 21 day intervals and their brains were fixed, and sectioned, and examined for degeneration.

The two-day fish did not show any breakdown of the fibres. The four-day fish showed the characteristic signs of the first stage of Wallerian degeneration (Johnson, Rossiter, & McNabb 1950; Young, 1942; Noback & Reilly, 1956). This first stage is typified by the large fragmentation products found in the form of ovoids and ellipsoids. This fragmentation of the fibres is the period of axon destruction,

and the physical distruction of the myelin. The seventh and tenth days show the second stage of degeneration in which the myelin and axons are being destroyed chemically. The myelin disappears and there is the formation of cholesterol ester, and unlike the first stage it stains as small droplets. On both the 7th day and 10th day fibres in stage 1 are still present but on day 14 all the fibres in the optic tract show stage 2 degeneration. From the 14th to the 21st day the enzymatic digestion which also characterizes the 2nd stage is seen and considerably less droplets are present. Finally the 3rd merges onto the second, and is seen very clearly in the 6-week fish, and is where the myelin has disappeared, the endoneurium and neurilemma becoming thickened to form the schwann tube and the collagen content of the nerve increases. Fig.14 P102-103 shows all 3 stages in the degeneration of the nerve.

In short, the time course for the degeneration of the optic tract of the minnow at 18°C is none at 2 days, stage 1 at 4 to 10 days, stage 2 begins at 7 days and increases to 14-day where it is best seen, and this merges into the 3rd stage in excess of two weeks.

Previous workers have used the Marchi technique which only stains degenerating myelin whereas the Nauta technique will stain degenerating axons so that one can be reasonabley sure that all the afferents of the optic tract are stained.

The optic tract is circular in a large number of fishes but Kappers,

Fig. 14

Photomicrographs to show the stages in the degeneration of the optic nerve using the Nauta technique.

A. Two day degeneration showing no change in the nerves.

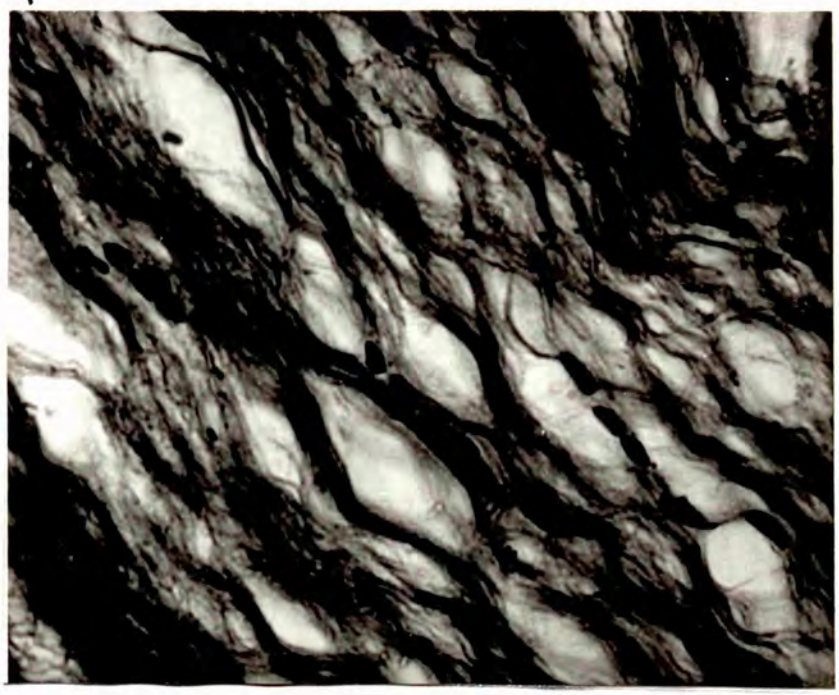
B. Seven day degeneration showing both the large ovoids and ellipsoids of stage one and the droplets of stage two.

C. Fourteen day degeneration showing the characteristic droplets of stage two degeneration.

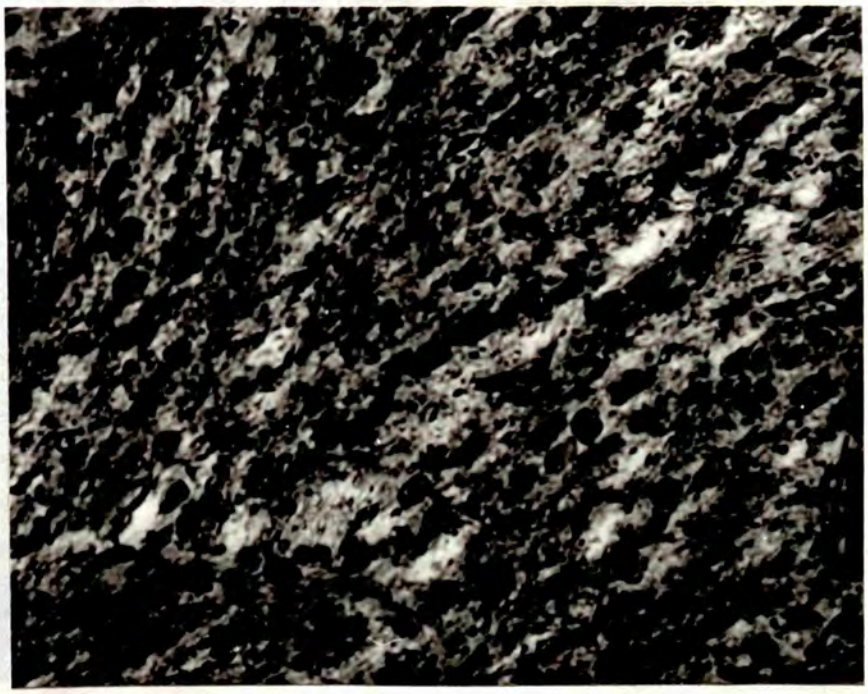
D. Six week degeneration showing only the thickened endoneurium and neurilemma of stage three.

Fig. 14

A



B

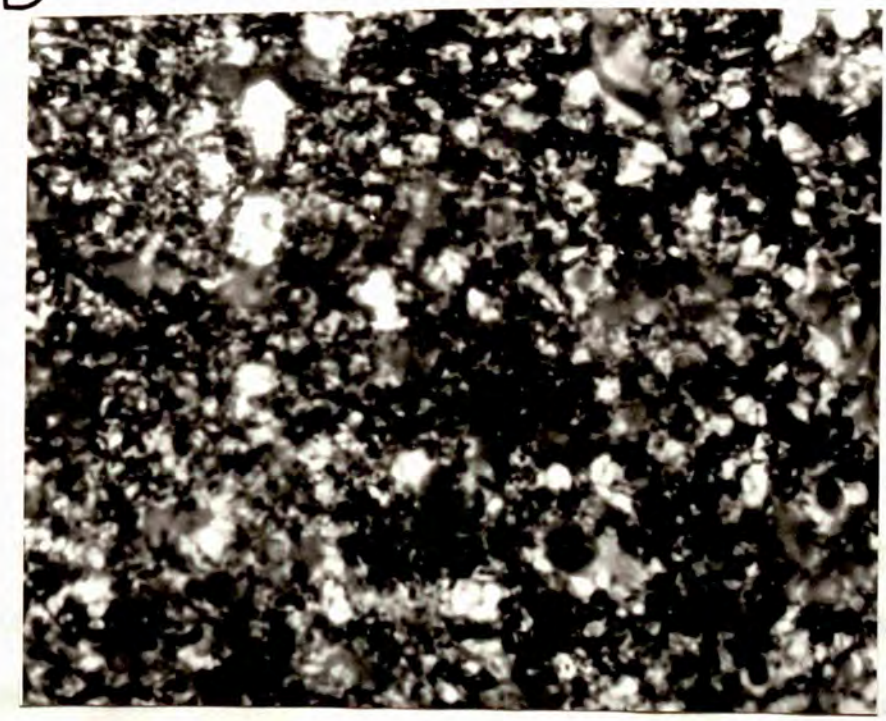


0·01 mm

Fig. 14

C

D



0·01 mm

Huber & Crosby (1936) have reported flattening in the plaice (Pleuronectes platessa), Meader (1934) reported that pleating of the optic tract is common in teleost fishes. The optic tract of the minnow is composed of what appears to be a sheet of nerve fibres which has become folded to give 4 or more lamellae and in general appearance seems to be rectangular in cross section. The lamellae are further divided because the fibres are not distributed evenly but grouped into bundles, shown in fig. 15 p. 106 .

From degeneration studies it appears that the organisation of the retina is essentially preserved in the optic tract. It was difficult to perform accurate work because of this complexity of structure but the dorsal retina is dorsal, and the periphery of the retina is peripheral in the optic tract. These results therefore agree with the findings of Akert (1949 b) on the trout.

## II. THE OPTIC CHIASMA

The optic chiasma shows a wide variety of structures in teleost fishes but it is always complete. The most common condition is where the right tract passes under the left, but in the herring one nerve passes through a hole in the other (Kappers et al, 1936). Lubsen (1921) reported intermingling of the optic fibres at the chiasma in Leuciscus and Meader (1934) found interdigitation at the chiasma in Holocentrotus.

The two groups of minnows used in the experiments showed a

Fig. 15

Transverse section of the optic tract cleared with Cluver & Barrera.

Fig. 16

Diagram of condition 7 and 9 of the optic chiasma.

Fig. 15

Transverse section of the optic tract stained with Kluver & Barrera.

Fig. 16

Diagram of condition 7 and 9 of the optic chiasma.

Fig.15

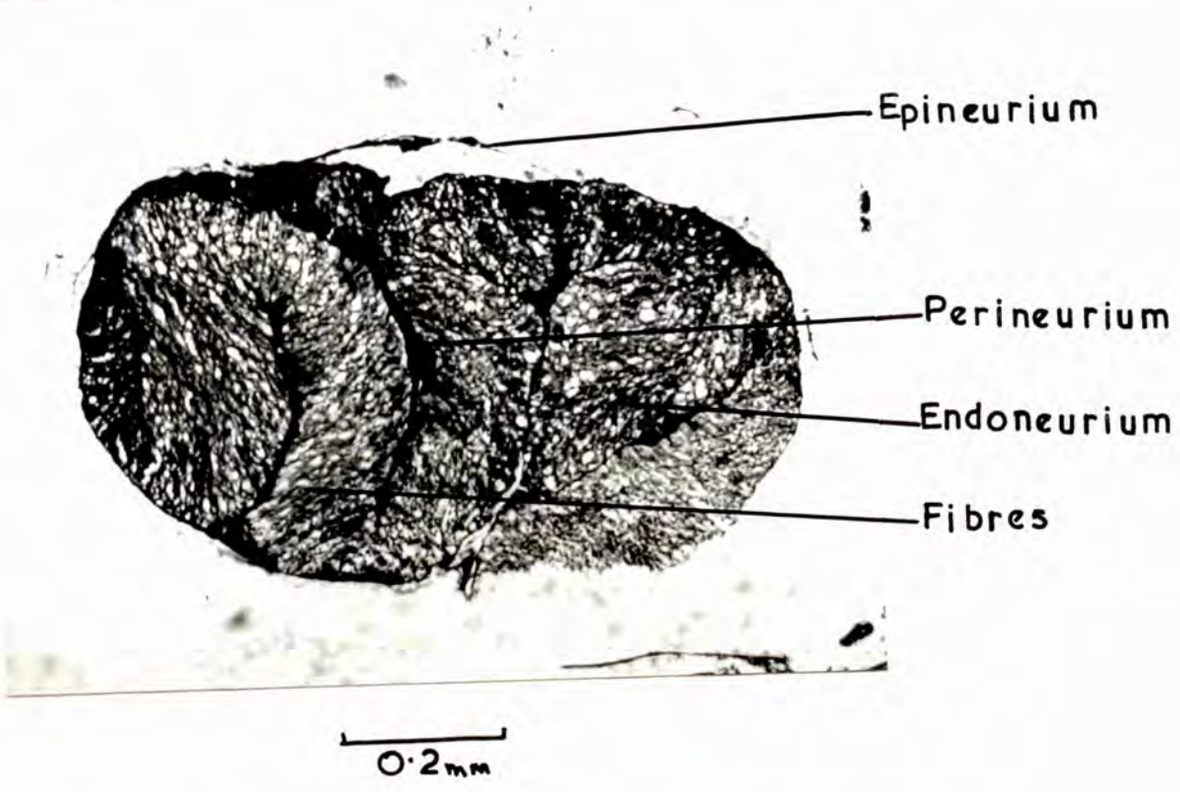
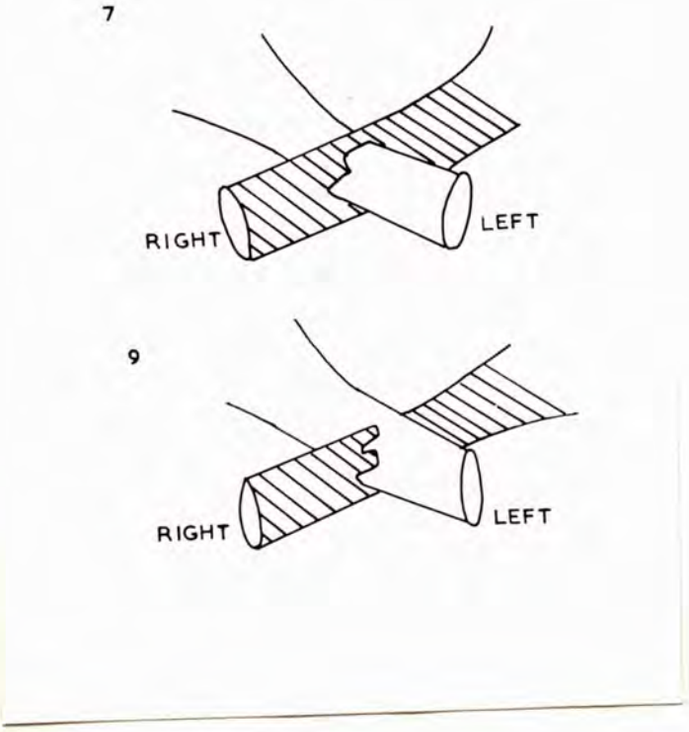

Epineurium
Perineurium
Endoneurium
Fibres
0·2mm

Fig.16


7
RIGHT
LEFT
9
RIGHT
LEFT

number of different types of chiasmata. There appear to be no less than 9 different conditions of the chiasma, and they are as follows:

1. Right passes under left
2. Left passes under right
3. Right passes through a hole in the left
4. Left passes through a hole in the right
5. Both tracts divide into two and then the left runs over and through the right
6. Both tracts divide into two and the right runs over and through the left.
7. Right devides into three and the left into two and then two divisions of the left run through the right.
8. Left divides into three and the right into two and then the two divisions of the right run through the left.
9. Both of the tracts divide into three and they interdigitate with each other.

Types 7 and 9 are illustrated by simple drawings on page 106. fig. 16 .

In the case of conditions 3 and 4, generally in the one which was pierced, the amount below the hole was about 3/4 of the optic tract or 3 lamellae. In the more complex types the proportions of the optic tract in each division varied from fish to fish. In one case of condition 5 the upper division of the left was largest, and the right was equal, but that in a case of 6 the right divides unequally and the lowest

deviation was the largest. In the case where the optic tract divides into 3 as in condition 9, one of these divisions was often very small and only composed of a few fibres.

Types 1 and 2 were the dominant types in the Lea fish, type 1 formed about 60-70% of the population. Type 2 formed 25-30% and types 3 and 4 about 10%. Types 5 and 6 were very rare and only amounted to some 2%.

Fewer Chess were examined but in about 20 fish all the 9 types occurred at approximately the same frequency.

Why there should be so many different types of chiasmata in this species is difficult to explain. Previously no one has reported more than any one type in each species and it seems unlikely that the minnow is unique. These results are from more than 80 dissections of the chiasma. They are not easily seen in sections. Insufficient embryological knowledge is available to explain this condition but interesting possibilities stem from the work of Sperry (1948), and Attardi & Sperry (1960, 1963). These two workers, in an excellent piece of work, showed that regenerating retinal ganglion neurons will grow back into the tectum and have produced strong histological evidence of a chemo-affinity in these regenerating optic fibres. More recently, Sperry (1967) has concluded that the complicated nerve fibre ciruits of the brain grow, assemble, and organise themselves through the use of intricate chemical

codes under genetic control. It would be interesting to perform regeneration experiments similar to those of Attari & Sperry (1960, 1963) on the minnow to see if the chiasma would form in the same way again, and would hence be genetically determined. If it did not form in the same way this peculiar configuration may be the result of environmental feature acting at a very sensitive period of embyonic development. However in that case, why are they all not the same from each stream? If the development of the chiasma is not environmentally determined it must be random. Work on the chiasma of the minnow would certainly throw considerable light on its formation.

## III. THE OPTIC TRACT OF THE BRAIN

At about the level of the chiasma the optic tract rotates so that the dorsal fibres become ventral and the ventral,dorsal. At a level just posterior to the chiasma a small bundle of fibres separates from the main part of the optic tract and runs dorsally to form the medial lamellae of Meader (1934), homologous with the fasciculus medialis tractus optici of Bellonci (1888), Kappers (1906),Franz (1912) and Jansen (1929). The rest of the optic tract runs dorsocaudalward along the external wall of the diencephalon. The optic tract then divides into two approximately equal divisions, a dorsal bundle, the fasciculus dorsalis, and a lateral bundle, the fasciculus lateralis. A further group of fibres leave the optic tract medial to the fasciculus dorsalis but these fibres are highly variable in number and rejoin the fasciculus dorsalis more dorsally.

Figs. 17, 18, 19.

These transverse sections of the trunk of the rhinen
stained with the Weigert method. The insertion show the optic
tract and its relationship to the knot of the cerebellar com-
plex and its position relative to the rest of the brain.
Fig. 17 is the most anterior and 19 the most posterior.

Abbreviations:

| | |
|---|---|
| T. | Optic tectum |
| C.C.L. | Corpus geniculatum mediale |
| C.C.L. | Corpus geniculatum posterius |
| N. RUB. | Nucleus habenularis |
| TEGM. | Tegmentum mediale |
| FASC. RET. | Fasciculus retroflexus |
| FASC. DOR. | Fasciculus dorsalis |
| FASC. LAT. | Fasciculus lateralis |
| N. PRE. | Nucleus praeopticus |
| MED. LAM | Medial lamella |
| COMM. TRANS. | Commissura transversa |
| COMM. MIN. | Commissura minor of Meynert |
| L.T.R. | Lateral forebrain bundle |
| BRA. T. MED. | Brachia tecti medialia |
| BRA. T. LAT. | Brachia tecti lateralia. |

Figs. 17, 18, 19.

Three transverse sections of the brain of the minnow stained with the Holmes method. The sections show the optic tract and its relationship to the tectum and geniculate complex and its position relative to the rest of the brain. Fig. 17 is the most anterior and 19 the most posterior.

Abbreviations:

| | |
|---|---|
| T. | Optic tectum |
| C.G.L. | Corpus geniculatum mediale |
| C.G.P. | Corpus geniculatum posterius |
| N. HAB. | Nucleus habenularis |
| EMIN. | Eminentia medialis |
| FASC. RET. | Fasciculus retroflexus |
| FASC. DOR. | Fasciculus dorsalis |
| FASC. LAT. | Fasciculus lateralis |
| N. PRE. | Nucleus preopticus |
| MED. LAM | Medial lamella |
| COMM. TRANS. | Commissura transversa |
| COMM. MIN. | Commissure minor of Herrick |
| L.F.B. | Lateral forebrain bundle |
| BRA. T. MED. | Brachia tecti medialis |
| BRA. T. LAT. | Brachia tecti lateralis. |

Fig. 17

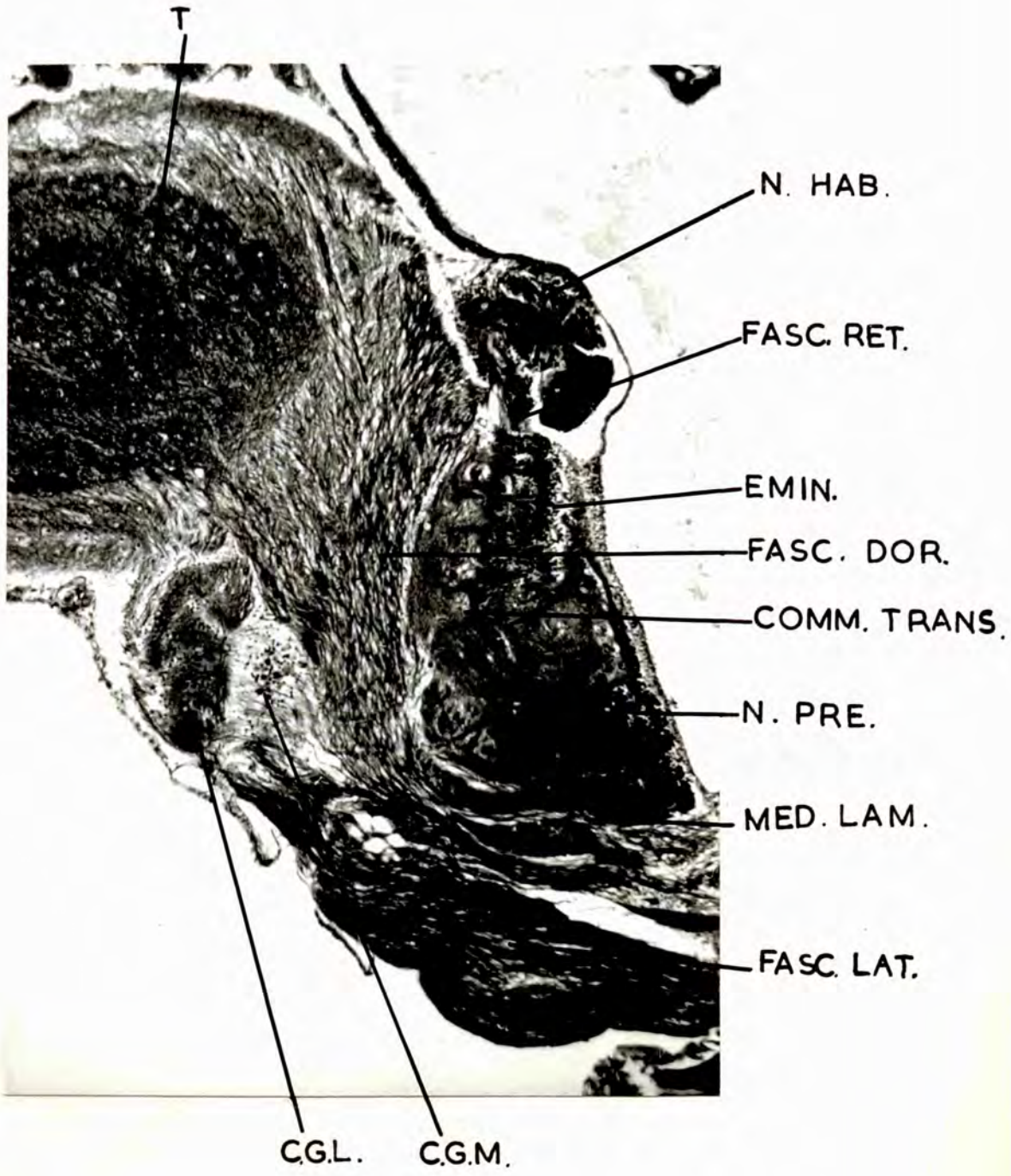

T
N. HAB.
FASC. RET.
EMIN.
FASC. DOR.
COMM. TRANS.
N. PRE.
MED. LAM.
FASC. LAT.
C.G.L.
C.G.M.
0·3 mm

Fig. 18

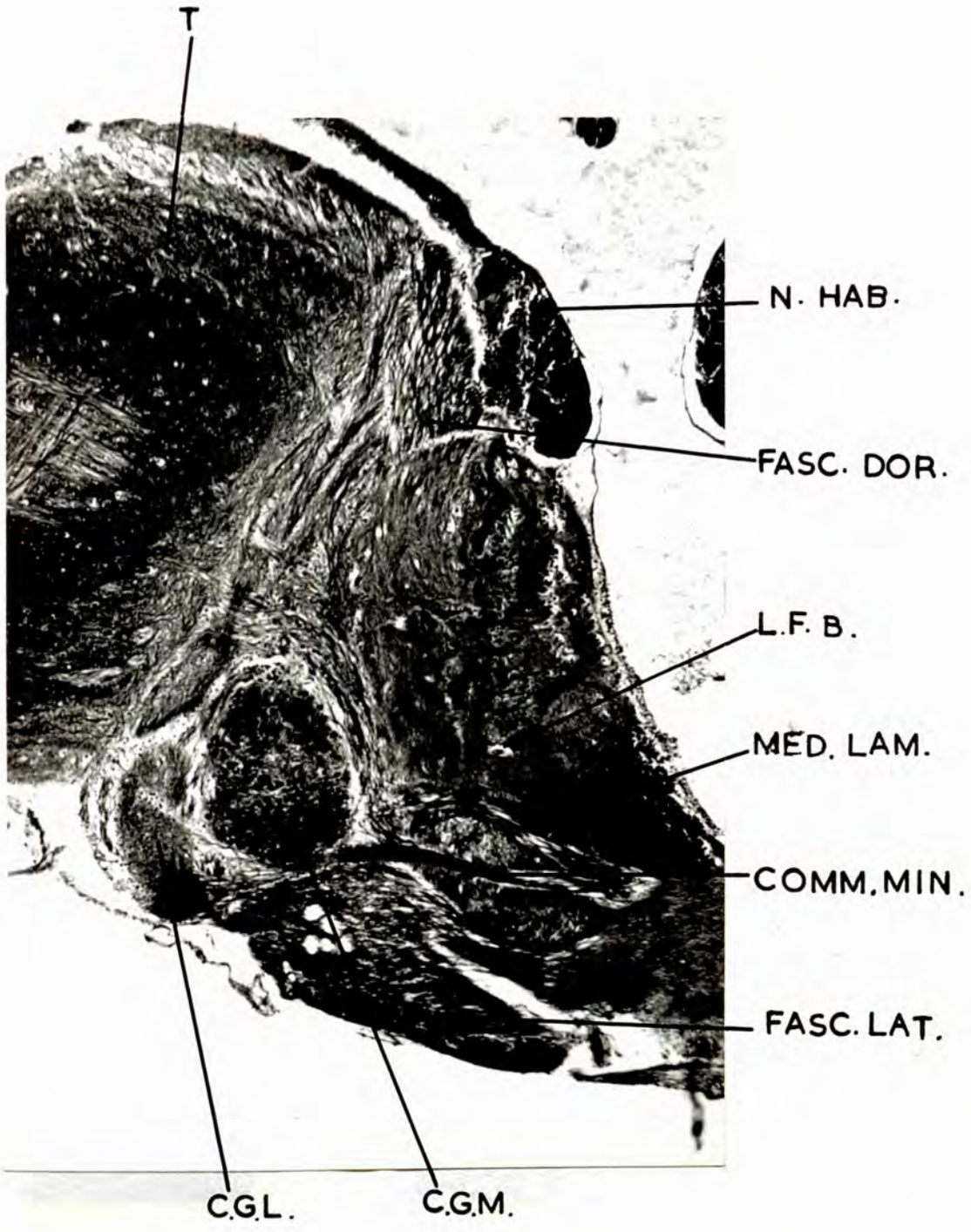
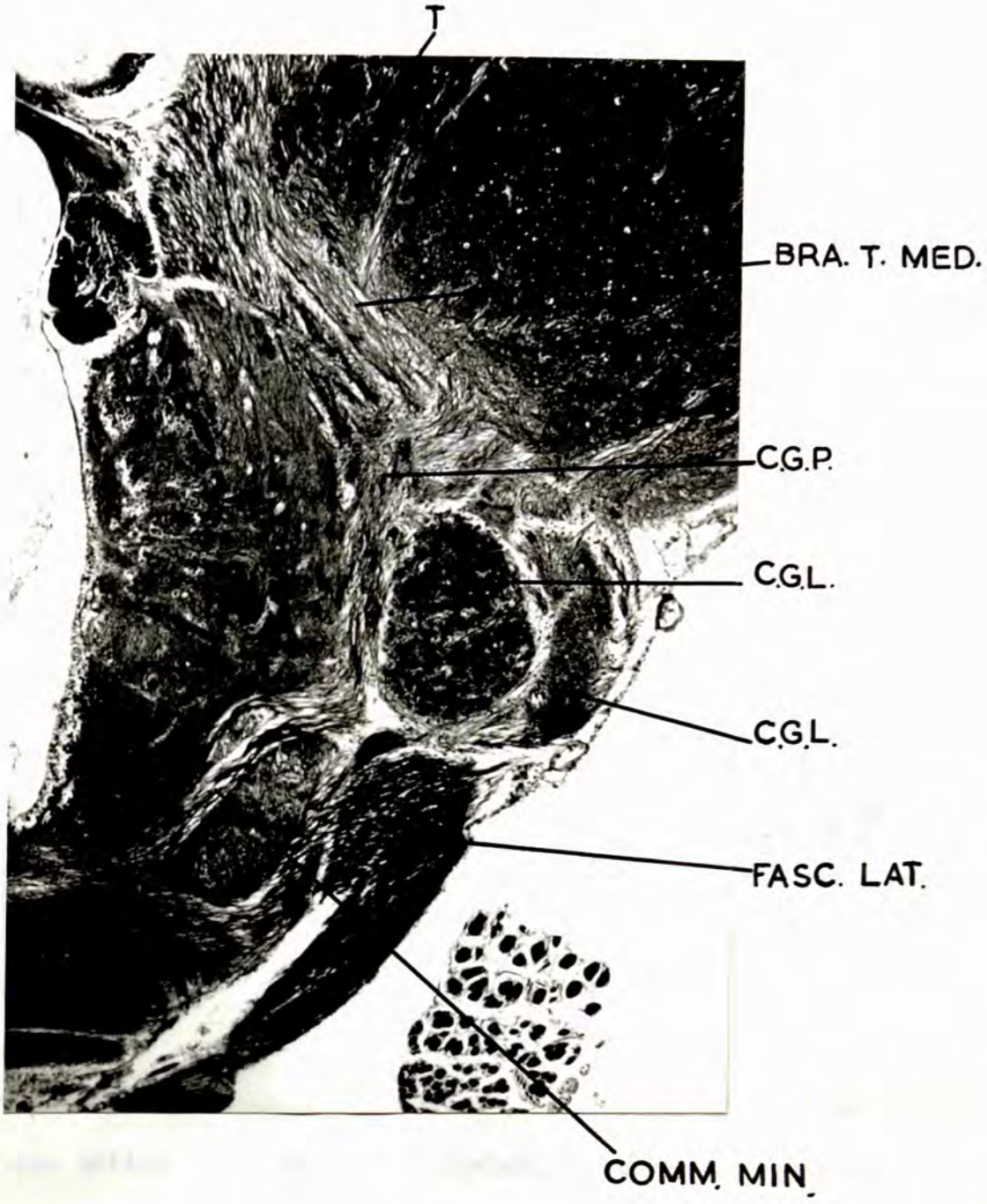


Fig. 19



The medial lamella enters the fasciculus dorsalis without giving off any fibres at about the level of Herrick's commissure. These divisions of the optic tract are clearly seen in fig. 17, 18, 19 ~~a, b, c, d~~ (p.111-3).

Not all the fibres from the optic tract end in the tectum. A number end in the geniculate complex and have been labelled by Meader (1934) the fasciculus geniculatus. The do not form a distinct bundle in the minnow.

The only tracts to be positively identified in the minnow are the medial lamellae, fasciculus dorsalis, fasciculus lateralis, and connections to the geniculate complex. Other fibres which were not found, and are probably not present, were the fasciculus dorsomedialis, which was traced to the tegmentum of the midbrain (Jansen 1929), fibres to the torus semicircularis reported by Van der Horst (Meader 1934), and fibres to the ventral thalamus reported by Lubsen and by Kappers et al. (1936).

## IV. THE GENICULATE COMPLEX

Between the forks of the optic tract lies a group of neurone masses which have been called the geniculate complex, of which a number of conflicting and confusing accounts have been given in the literature.

In the minnow, the geniculate complex appears to be composed of 3 parts, lateral, medial and dorsal.

(a) Corpus geniculatum laterale (C.G.L.)

This is a large nuclear body lying in the lateral part of the dorsal thalamus. It has been called the corpus geniculatum laterale ipsum by Meader (1934) and corresponds to the geniculate of most other authors. It is the most anterior of the 3 parts of the geniculate complex in the minnow and in transverse section appears to be half-moon shaped, but while in longitudinal section it is essentially 'U'-shaped. The neurones in this body are both large and small and are arranged in a complex pattern (fig. 20 p.118 ). The larger neurons occur mainly at the periphery and in a region in the dorsal part of the body. The distribution of these larger neurones matched that of the incoming optic tract fibres. Although synapses were not clearly seen between these large neurons and the optic tract, it seems probable that they do in fact synapse with the optic tract.

The small neurones occur in the greatest numbers in the central and more medial parts of the body. In the medial region of the body there are no direct fibres from the optic tract but there are efferent fibres running to the Commissure of Herrick and to the medial and dorsal bodies. These small neurones may well give rise to the efferent fibres and provide for the interconnections necessary for the working of the geniculate complex.

(b) Corpus geniculatum mediale (C.G.M.)

This is a large spherical body situated directly medial to the CGL

and although it does not extend so far anteriorally it does extend much further posteriorly. Like the CGL, it is composed of 2 types of neurones, large and small, (fig.20 p. 118), but there are fewer small neurones and the large neurones are larger than in the CGL. The distribution shows the same perttern as the CGL with the large neurones lying adjacent to the optic tract.

The question of homology of this group is complex. In general size and structure it appears to correspond to the nucleus anterior thalami of Goldstein (1905), Charlton (1933) and Holmgren (1920) but not to the nucleus anterior thalami of Kappers (1906). It also appears to be homologous with the corpus glomerulosum pars anterior of Brickner (1929) and with the nucleus rotundus of Schnitzlein (1959). Much of the confusion lies in the fact that many of the workers present their results in the form of drawings and not photomicrographs of the specimens. The second reason which may have led to so much confusion stems from the fact that they all use a very wide variety of techniques and the same structure can appear very different when stained by a variety of methods.

In the minnow the fact that there is direct cell contact between the CGL and the CGM throughout the most of their lengths would indicate that they are intimately related (fig.20 p. 118). The fibre connections between the CGM to the CGL are very well developed (fig.21a p.123) and they both receive fibres from the optic tract and tectum and give fibres to the tectum (fig.21,22 p.123,125). In view of their neurone anatomy, fibre

Fig. 20

Series of sections anterior to posterior along the geniculate complex stained with C.F.V. to show the distribution of the cells.

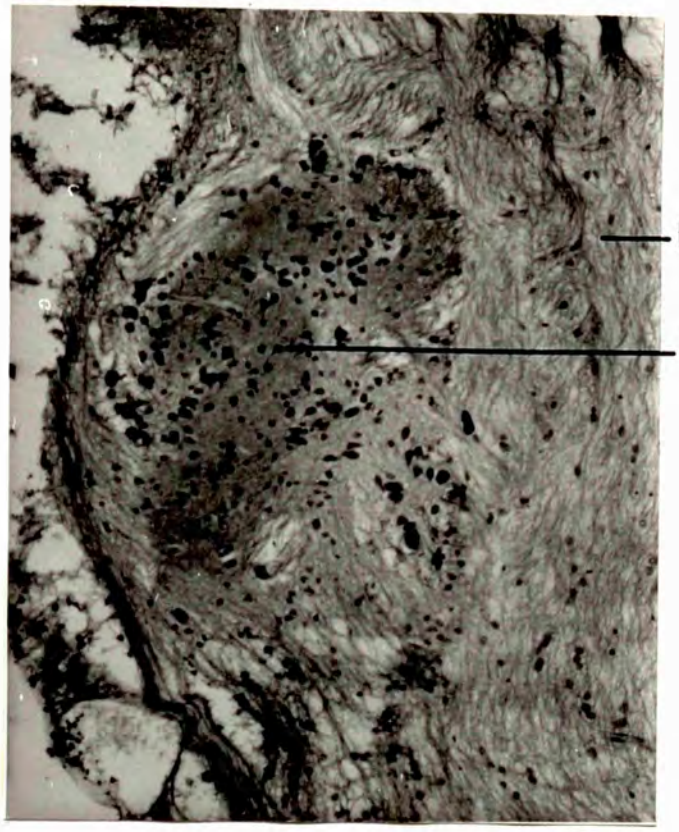
A is the most anterior and is at a level where the C.G.M. is only just present.

Abbreviations:

| | |
|---|---|
| C.G.L. | Corpus geniculatum laterale |
| C.G.P. | Corpus geniculatum posterius |
| C.G.M. | Corpus geniculatum mediale |
| FASC. DOR. | Fasciculus dorsalis |
| FASC. LAT. | Fasciculus lateralis |
| COMM. MIN. | Commissure minor. |

Fig. 20

A




0·1mm.

B

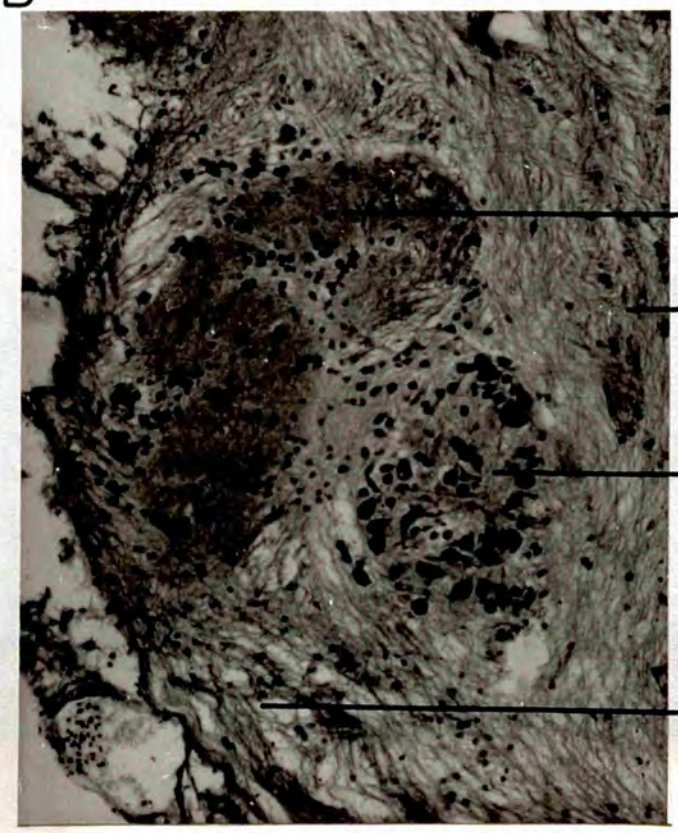
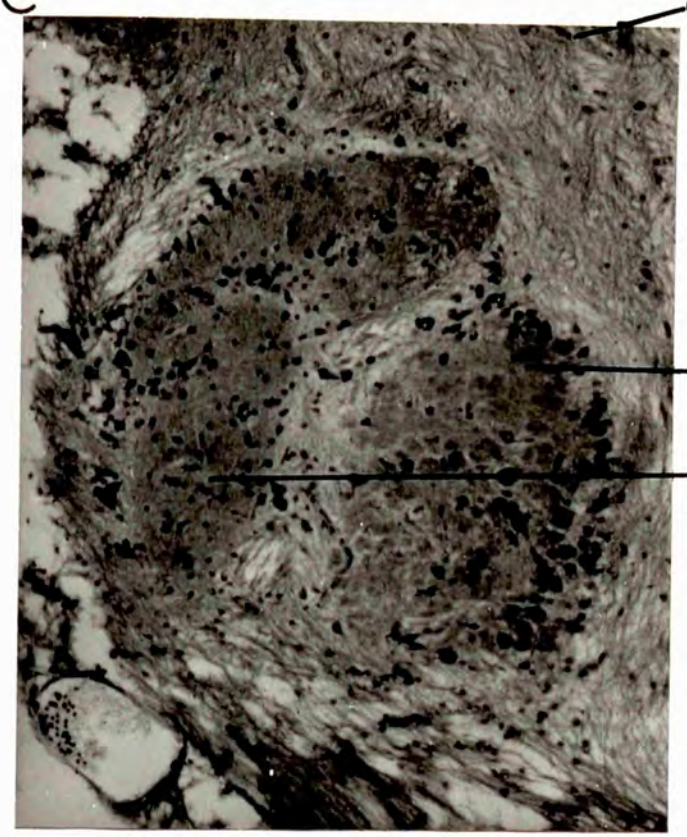


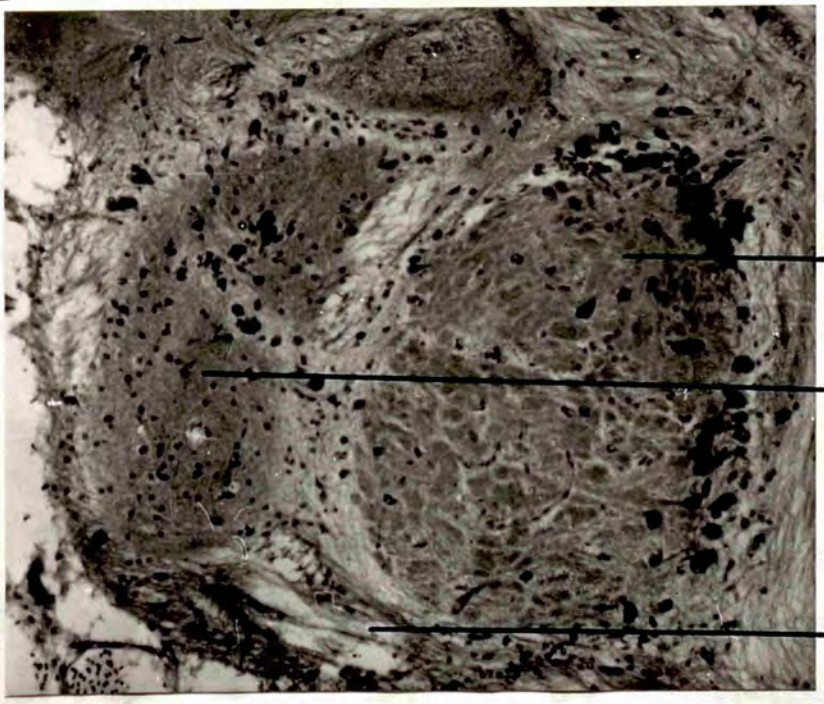
Fig. 20

C




0·1mm

D



connections and general anatomy, it seems justifiable to consider that they are essentially different parts of functionally the same body. In view of these conclusion it seems that the C.G.M. may well be homologous with the nucleus intermedius of Goldstein (1905) and of Holmgren (1920), but very well developed in the minnow, and not homologous with the nucleus anterior thalami of these two authors. It does however appear to be the corpus geniculatum pars ventralis of Meader (1934). The most recent work on teleost brains concerned with this region was performed by Schnitzlein (1959) and from his illustrations the C.G.M. would appear to have the same size, general position and neurone structure as his nucleus rotundus. Schnitzlein failed to find fibre connections that are present in the minnow and concluded that the C.G.M. was not part of the visual system. He did make the observation that it is very variable and not present in the Catfish, very large in the Goldfish, and reduced in the Darter, but that in this last fish, the C.G.L. is very large and folded. From this it could be said that his nucleus rotundus is possibly related to the visual system, for the Catfish has a poorly developed sense of vision, and although the Darter has very good vision the other parts of the geniculate complex are very well developed.

(c) Corpus geniculatum posterius

This is composed of a few large neurons diffusely arranged and lying dorsomedially and caudally to the rest of the complex. It is homologous with the corpus geniculatum posterius of Meader (1934) with the nucleus pretectalis of Schnitzlein (1959), Goldstein (1905) and Kappers (1906).

In view of its fibre connections and general position, it seemed best to include it with the rest of the complex.

(d) Fibre connections of the Geniculate complex

Bellonci (1888) thought that the lateral geniculate nucleus only received collateral of the retinotectal fibres and the work of Schnitzlein (1959) failed either to confirm or to deny this and Kapper et al (1936) seem to agree that they are only collaterals. The optic fibres which supply the lateral geniculate body were believed by Zeeman & Lubsen (Kappers et al 1936) to originate in rostral quadrants of the retina.

In the minnow there is definite evidence to show that they are retinogeniculate fibres and not collaterals of the optic tract. These direct fibres are shown on fig. 22 p. 125, stained by the Holmes method and they were also visible in the degeneration studies. Fibres from all parts of the optic tract run to the geniculate complex and the latter appears to receive a fairly uniform projection from the retina. This has also been demonstrated in a series of experiments involving ablation of various parts of the optic tectum combined with unilateral blinding. (Section 6 p. 186).

The different parts of the geniculate complex give fibres to the other parts of the complex (fig. 21 p. 123). The geniculate complex also gives rise to fibres to the tectum and this is especially well seen in

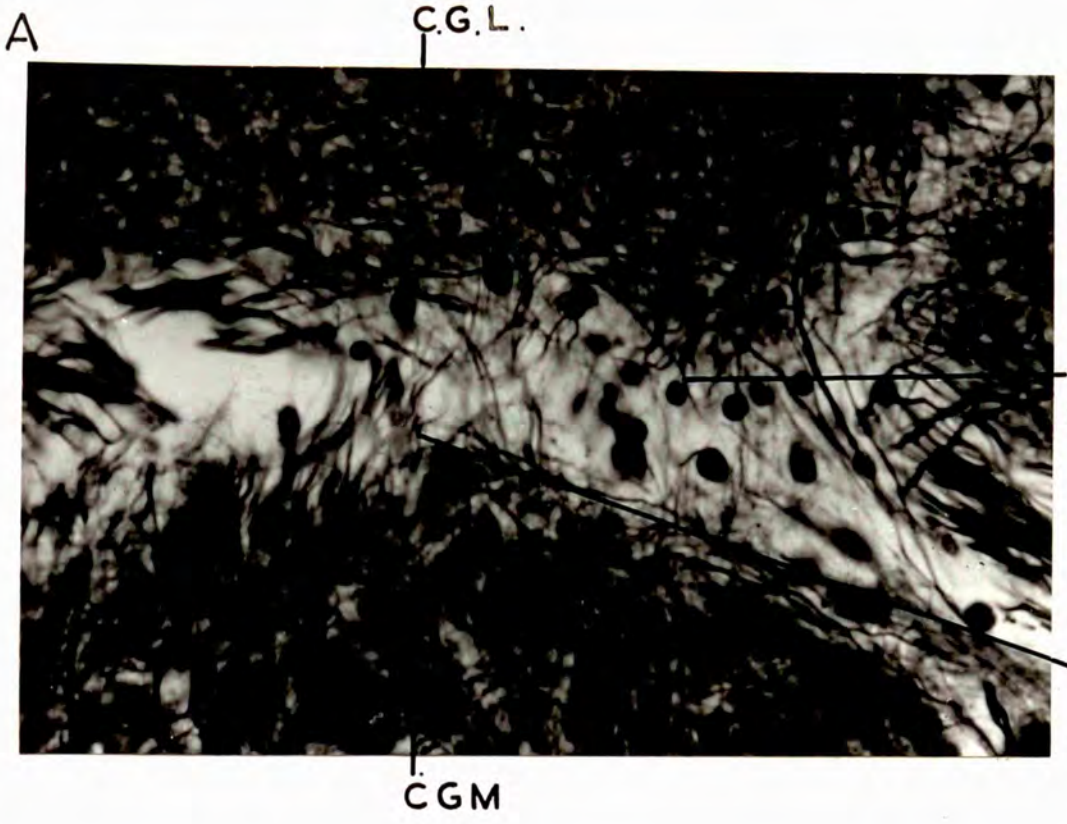
Fig. 21

A. Section of C.G.M. where it makes contact with the C.G.L.
Stained by the Holmes method.



B. Section of the C.G.M. where it is contact with the optic nerve.
Stained by the Holmes method.

Fig. 21


A
C.G.L.
Fibre from C.G.L. running to C.G.M.
Fibre from C.G.M. running to C G L
C G M

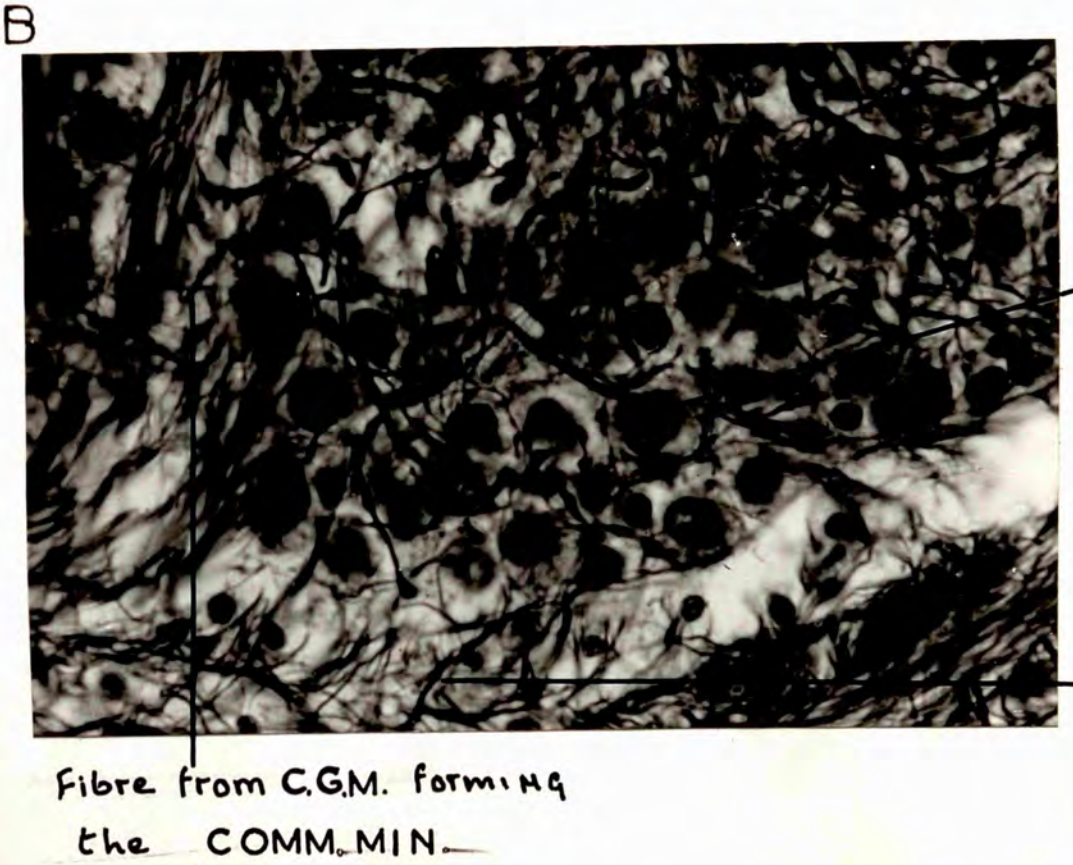

B
Large neurone of C.G.M.
Fibre from optic tract
Fibre from C.G.M. forming the COMM. MIN.
0·02 mm

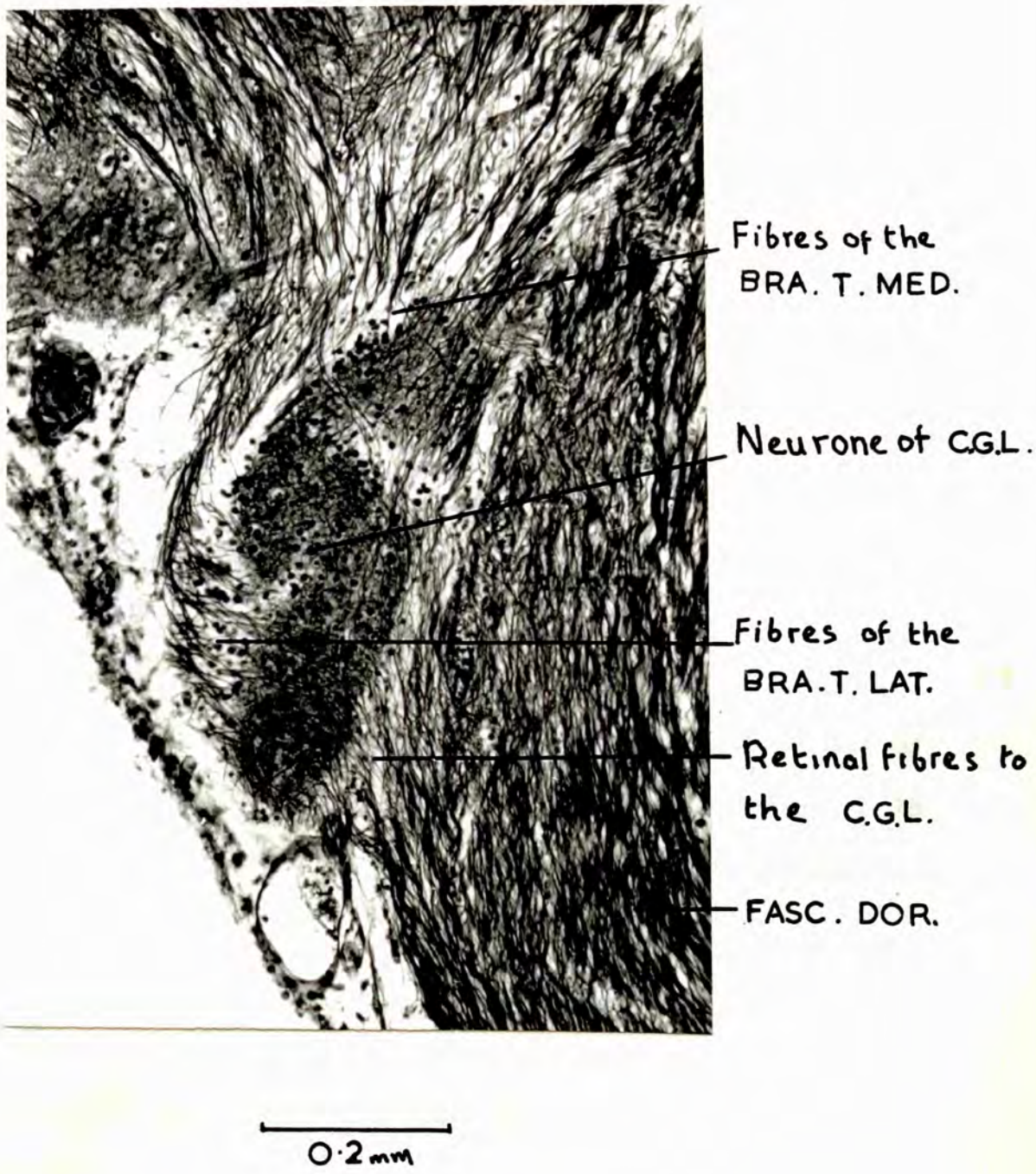
Fig. 22

Section of the C.G.L., stained by the Holmes technique, showing its relationship to the optic tract and tectum.

Abbreviations:

| | |
|---|---|
| BRA. T. LAT. | Brachia testi lateralis |
| BRA. T. MED. | Brachi tecti medialis |
| C.G.L. | Corpus geniculatum lateralis |
| FASC. DOR. | Fasciculus dorsalis. |

Fig. 22


Fibres of the
BRA. T. MED.
Neurone of C.G.L.
Fibres of the
BRA. T. LAT.
Retinal fibres to
the C.G.L.
FASC. DOR.
0·2 mm

C.G.L. In the more anterior sections these fibres form a diffuse fan-like distribution to the tectum, but more posteriorly two definite tracts are present, a medial tract and a lateral tract. These tracts were very well described by Charlton (1933) and named the brachia tecti lateralis and medialis which correspond to the tractus geniculo-lobaris and tectalis of Holmgren (1920). A large number of fibres run from the tectum to the geniculate complex.

The geniculate complex on each side is joined to that on the other side by a large and well developed commissure, the commissure minor of Herrick. Fibres from the whole of the complex with the possible exception of the C.G.P., are found in it (fig. 21 , p. 123). Fibres from the complex also run in the transverse commissure which runs below and more caudal to Herrick's commissure.

Fibres from the geniculate complex also run to a wide variety of other regions in the brain, well developed fibre bundles running to the eminentia medialis, hypothalamus, and ventral thalamic nuclei. The spino-geniculate connections could not be seen, nor could the connections to the ganglion isthmi reported by Kudo (1923).

## V. THE OPTIC TECTUM

The optic tectum is a large bilobed plate of tissue which forms the roof of the optic ventricle and covers the rest of the mesencephalon dorsally and, to a certain extent, laterally. Because of the optic ventricle the optic tectum is only connected to the rest of the brain at certain points and a large amount of its structure is determined by this. The connections are in the extreme anterior region and in the lateral regions extending from a point just anterior to the middle of the tectum to the posterior of the tectum.

The two bundles comprising each optic tract run into the tectum. The fasciculus dorsalis runs along the dorsal midline of the tectum and gives fibres to its dorsal regions. The other bundle, the fasciculus laterlis, runs along the extreme lateral margin of the tectum giving off fibres to its lateral regions.

The optic tectum has been examined by a number of workers and the work by Huber & Crosby (Kappers et al 1936) has divided into into 6 primary layers, using the same terminology as that used for higher vertebrates. Leghissa (1955) has divided it into seven primary layers and considerable agreement with this is shown in the work of Tandon & Sharma (1963) who also divided it into 7 layers.

In the minnow the 7 primary layers labelled by Legissa fit very well

Fig. 23

Transverse sections of the optic tectum to show the arrangement of the cells and fibres.

A stained with C.N.V. to show the cells;

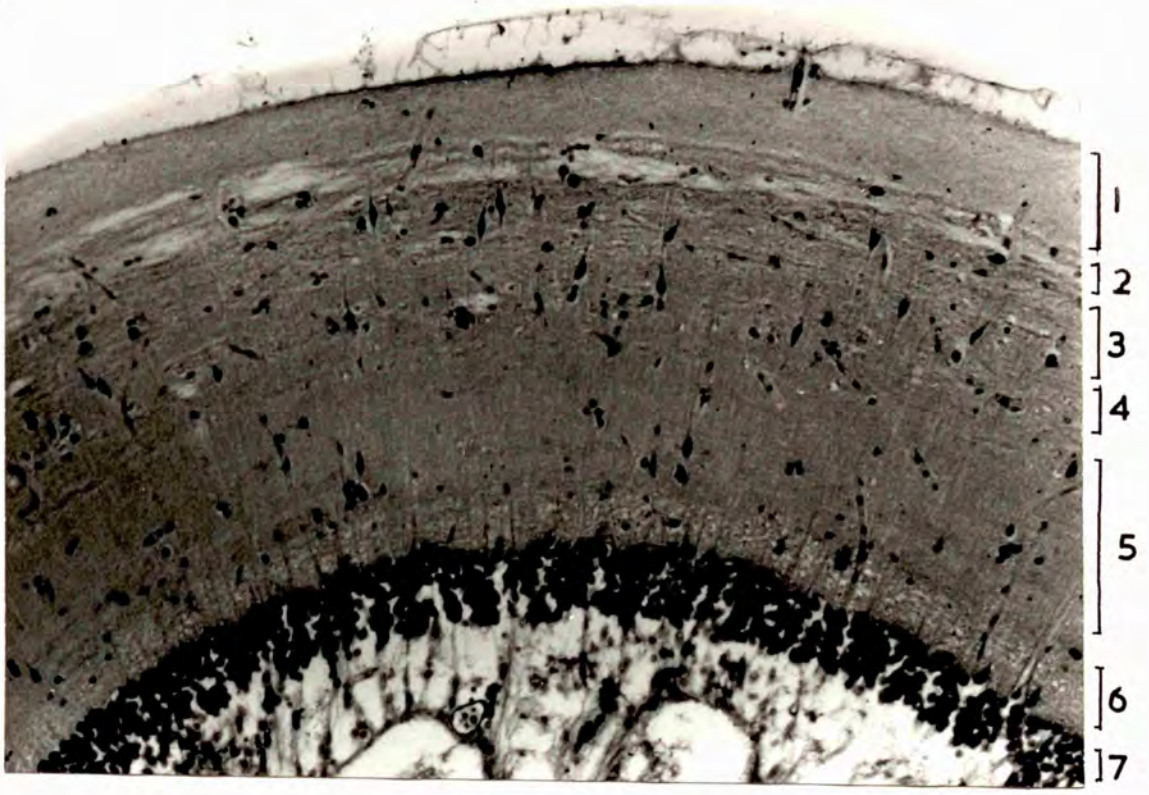
B stained with unsupressed Nauta to show the fibres.

Both were cut at 8μ.

1. Stratum fibrosum marginale
2. Stratum plexiforme et fibrosum externum
3. Stratum griseum externum
4. Stratum plexiforme internum
5. Stratum griseum internum
6. Stratum fibrosum profundum
7. Stratum griseum periventriculare.

Fig. 23

A




B

0·1mm

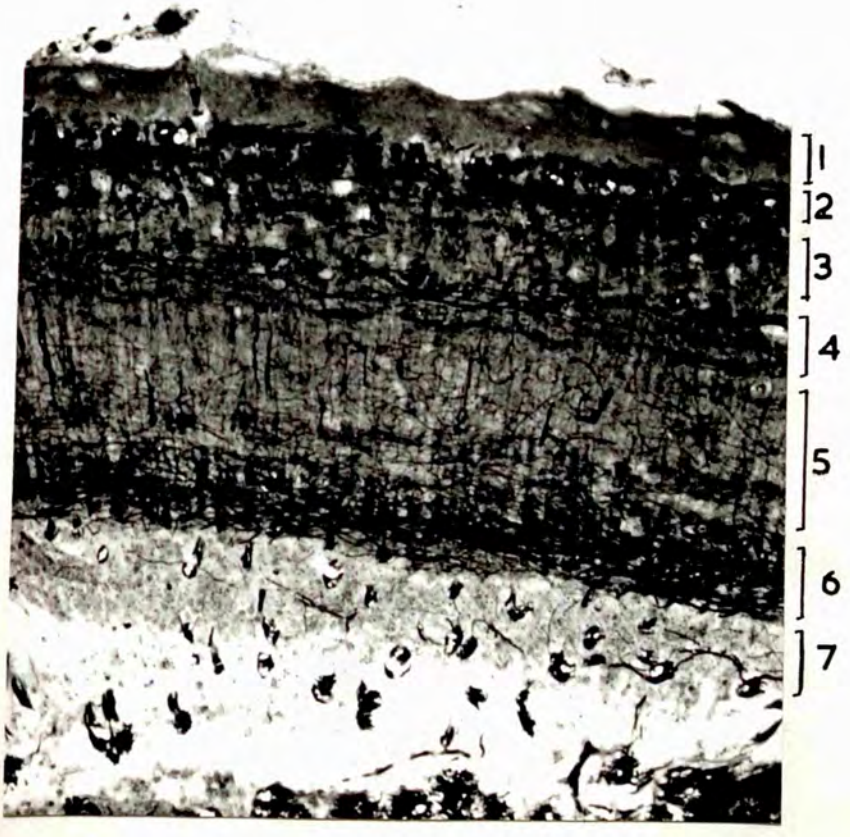
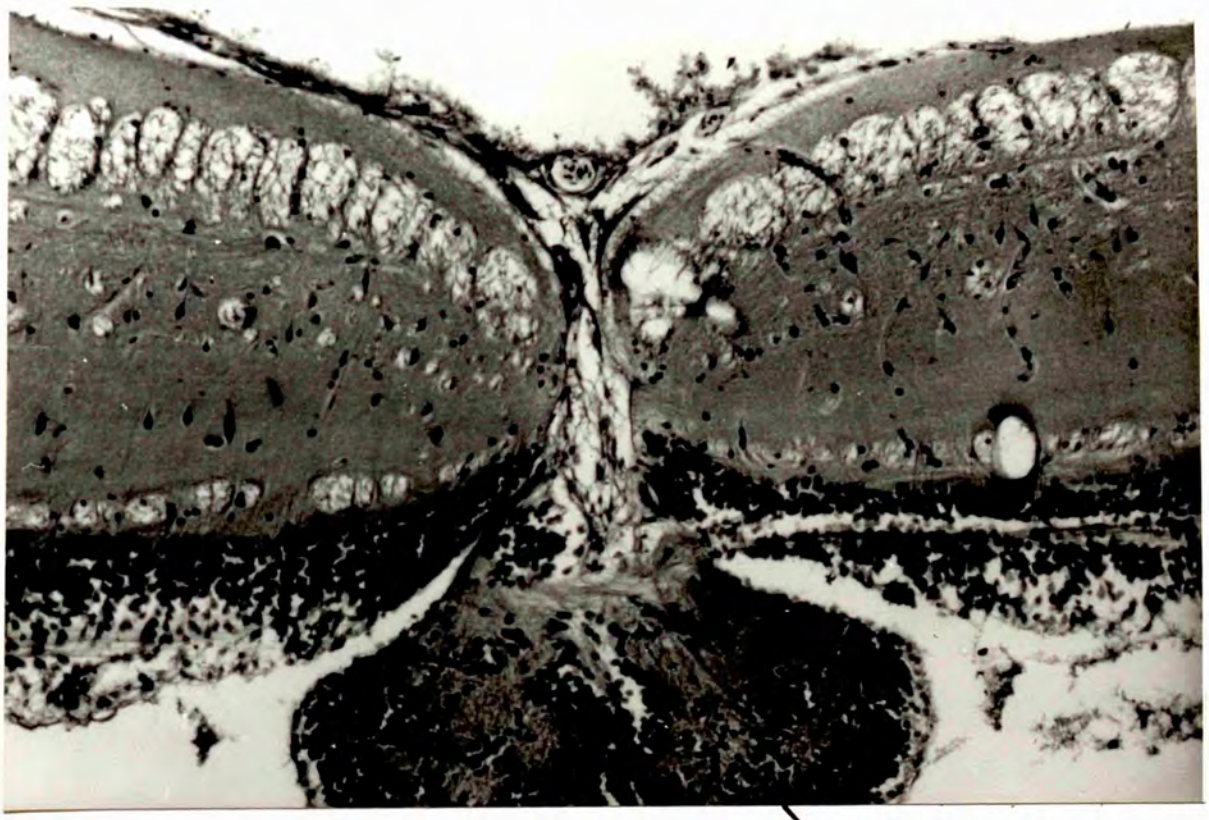


Fig. 24

Section of the tectum in the midline stained with C.F.V.

Fig. 24




0·1 mm.

with the histological picture. Because of the difficulty of staining both cells and fibres with the same stain, separate stains for the same region are shown in fig. 23 (p. 129).

The layers are as follows:

1. Stratum fibrosum marginale

This consists of a fairly thick layer of non-myelinated fibres, and from it a conspicuous bundle of fibres runs across the tectum to the torus longitudinalis. The neurones in this layer are few in number and generally have only two dendrites.

2. Stratum plexiforme et fibrosum externum.

This contains fibres from the optic tract as they make synapse with the dendrites of the next layer down. Evidence that it also contains fibres from the geniculate complex is provided by the appearance of normal fibres in this layer following the complete section of the optic tract. This thickness of this layer varies considerably throughout the tectum. It is thickest at the anterior part of the tectum and in the extreme dorsal and ventral regions where the main bundles of the optic tract run. As the tracts run across the tectum they give off fibres so that the latero-dorsal regions are very thin. There are also a few small to medium sized neurones present.

3. Stratum griseum externum

Here the terminations of the optic tract run and form a very ill-defined layer. There are numerous large bipolar neurones, fusiform in shape, and several small pyramidal neurones. This layer is very reduced in size associated with the large size of layers 2 and 4, at the anterior

and extreme dorsal and ventral extremities (fig. 24 p. 131 ).

4. Stratum plexiforme internum

This layer contains fibres running in most directions and is the region where most of the other afferents run into the tectum. It contains fibres from the geniculate complex, spinal cord and medulla, cerebellum, ganglion isthmi, hypothalamus, and the thalamic nuclei, especially the eminentia thalami.

5. Stratum griseum internum

This is a layer of fibres and cells with most of the fibres running from layer 6 to layers 2, 3 and 4. Fibres run in other directions and it appears to be the main correlating layer of the tectum. The neurons are both pyramidal and fusiform, large and small, and mono-, bi- and multi-polar. This layer is reduced where layers 2 and 4 are thickened, it is however, the thickest layer, constituting one third of the total tectal thickness in the lateral regions.

6. Stratum fibrosum profundum

Being composed of the main tectal efferent fibres, it is thickened at the lateral and anterior regions of the tectum. Fibres from this layer run to the thalamus, hypothalamus, cerebellum, medulla, spinal cord and mesencephalon.

7. Stratum griseum periventriculare

This is a layer of small spherical neurones which give rise to many efferent fibres and send fibres to most of the other layers of the tectum, especially layer 3.

The torus longitudinalis is well developed and appears as a bilobed structure in the midline of the tectum (fig. 24 p. 131 ). Ohta (1959)

has concluded that the development of the torus longitudinalis in teleosts is related to the development of the tectum and vision.

The main fibre connections as observed in the present work on the minnow agree well with the previous description of other species, Burr (1928), Schnitzlein (1964), Kappers et al (1936) and Pearson (1936). In Phoxinus, fibres run to the eminentia medialis, geniculate, preoptic nucleus, cerebellum, mesencephalon, hypothalamus, oculomotor region, habenular nucleus, medulla, spinal cord, ganlion isthmi, and probably others not definitely identified. The tectum of Phoxinus receives fibres from the thalamus, spinal cord, ganglion isthmi, medulla, hypothalamus, and cerebellum (as found by Tuge 1934 in the Goldfish). The secondary trigeminal tract, first identified in fish by Pearson (1936), was found.

The commissural systems in Phoxinus are also very well developed, especially the posterior commissure which has tectal and pretectal fibres, and the commissure horizontalis of Franz (1912) which connects the torus semicurcularis and the posterior tectum from one side to the other, and which crosses very far anterior just behind the chiasma.

The optic tectum resembles that of other teleosts in appearing to be one of the main correlating and co-ordinating centres of the brain.

No precise work was done on the retino-tectal projection so that nothing need be added to the conclusions presented on page 20 .

## S E C T I O N 5 : BLINDING

Throughout these experiments on the minnow a very large number of complete blindings were performed on both Chess and Lea fish. These blindings were all performed by cutting the optic nerve on both sides. On recovery from the operation the fish commenced to darken within 30 mins. after the effects of the anaesthetic had worn off. At 30 mins. they were still quite pale, usually D.O.I. of 2 or 3, but this pale condition did not last long and within the next hour the fish had gone noticeably dark to a D.O.I. of 4 or 5, and this slow darkening continued for the next 24 hours, finally reaching a value of 6.5 - 7.5. Most of these fish were used for other experiments (p.153) but 14 of these fish had their colour recorded for a period of time. The results are given in table 5 p. 136.

These fish were tested on both black and white backgrounds but they remained the same colour. Two fish not shown in this table reached a value of 1.5 within six weeks post operation.

The results of fish 1 to 6 are shown graphically in fig. 25 p.138. They show that there is a wide variation in this paling response not only in extent but also in time. The paling, when it does occur, is stepwise.

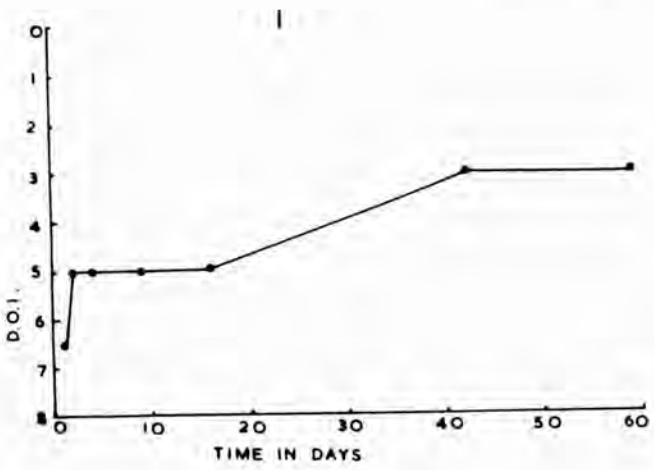
TABLE 5. The colour of 14 minnows following complete blinding expressed as D.O.I values.

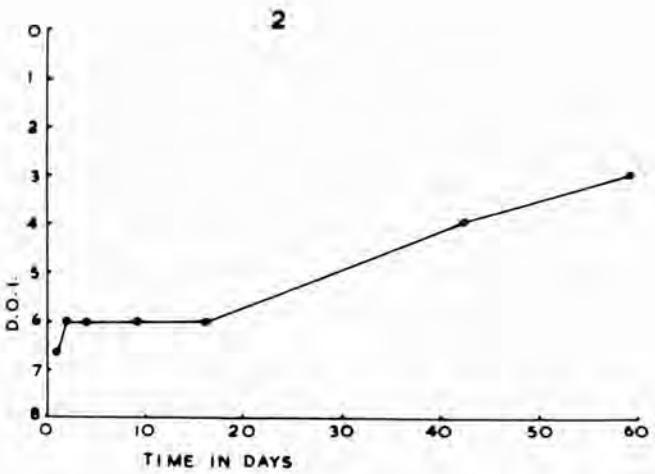
| Fish | Time in Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 9 | 16 | 42 | 59 |
| 1 | 6.5 | 5 | 5 | 5 | 5 | 3 | 3 |
| 2 | 6.7 | 6 | 6 | 6 | 6 | 4 | 3 |
| 3 | 7 | 6 | 5 | 4.5 | 4 | | 2 |
| 4 | 7 | 6 | 6 | 5.5 | 5 | | 3 |
| 5 | 7 | 6 | 6 | 6 | 6 | 4 | 4 |
| 6 | 7 | 7 | 7 | 7 | 7 | 5 | 4 |
| 7 | 7 | 7 | 7 | 7 | 7 | 6 | 5 |
| 8 | 7 | 7 | 7 | 7 | 7 | 6 | 5 |
| 9 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 10 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 11 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 12 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 13 | 7.5 | 7.5 | 7.5 | 7.5 | 7 | 7 | 7 |
| 14 | 7.5 | 7.5 | 7.5 | 7.5 | 7 | 7 | 7 |

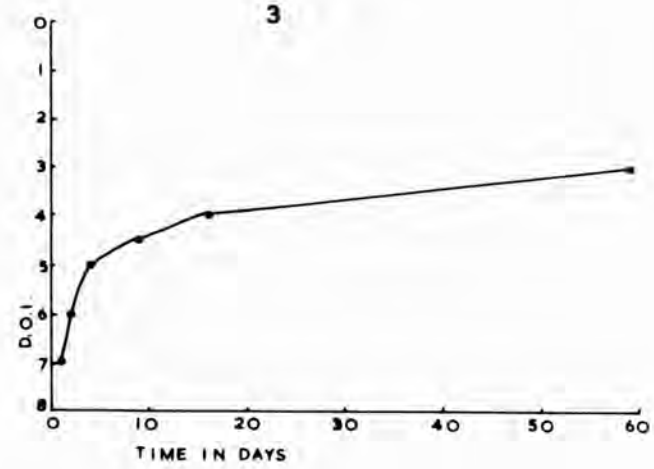
Fig. 25

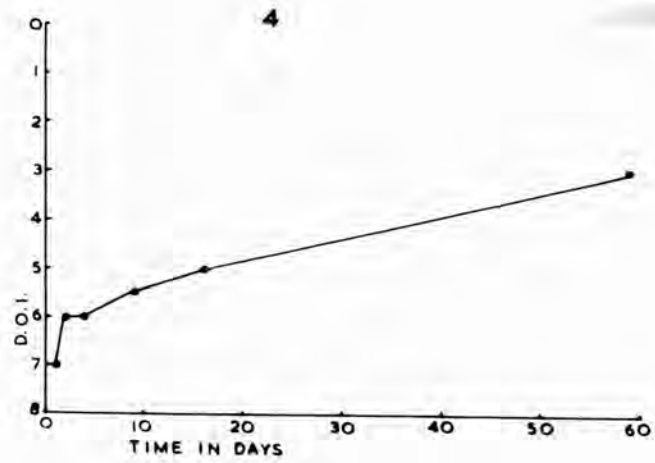
A series of graphs of fish 1 to 6 showing the relationship between colour and time after blinding. The blinding ~~being~~ performed by cutting the optic nerve.

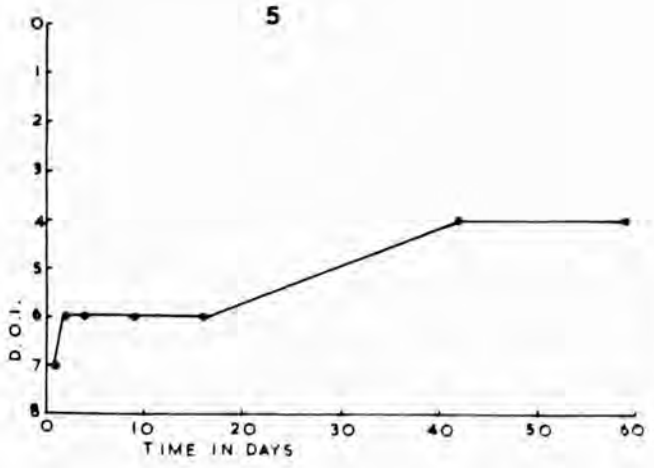
# Fig. 25

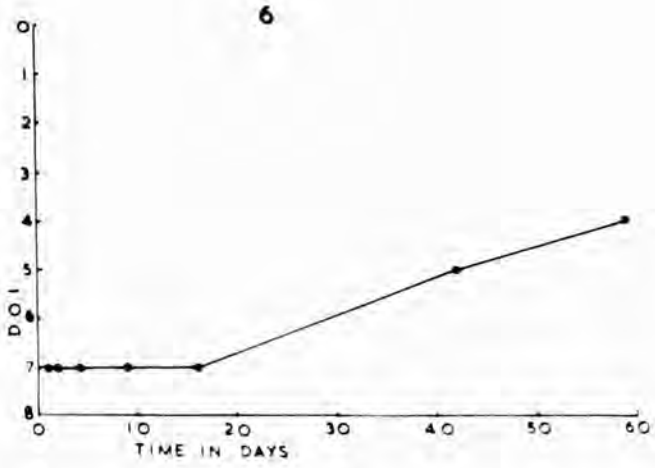

1
D.O.I.
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
TIME IN DAYS


2
D.O.I.
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
TIME IN DAYS


3
D.O.I
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
TIME IN DAYS


4
D.O.I.
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
TIME IN DAYS


5
D.O.I.
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
TIME IN DAYS


6
D.O.I
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
TIME IN DAYS

The temperature was controlled at $18^{\circ} \pm 2^{\circ}$. With the exception of 6, 7 and 8, there is an initial fairly rapid lightening for the second day and this corresponds in time with the absence of any degeneration stainable in the optic tract (p. 99 ). In fish 1, 2, 5, 6, 7 and 8, this is followed by a period of no change, and this corresponds to the period of stage 1 and the early part of stage 2 in the degeneration of the optic nerve. From the 16th day onwards there is a distinct paling and this corresponds to the time of the advanced stage merging into stage 3.

The results do show that there are two groups of minnows; those that remain dark after blinding and those that lighten after a variable period of time. Further it appears that in the population about half pale and half remain dark.

Experiments were carried out to see if the external conditions of illumination intensity had any effect on the colour of the blind fish. Fish were placed in the dark for a variable period of time up to 24 hours and they were subjected to a considerable range of light intensities. The light intensities were produced by arranging for a 20W, or 40W or 100W bulb to be placed at 1 ft and 2 ft. above the surface of the water, together with a 60W placed 10 ft. above the surface of the fish. In no case did the fish show any different colour to that at the beginning of the experiment. From this it would appear that the shade assumed by the fish was not affected by external conditions of illumination.

To try to explain the differences in the colour of blind fish a series of anatomical investigations were carried out. Two fish which had remained dark for six weeks and two fish which had lightened to values of 1.5 on the D.O.L. scale, were killed, fixed and stained and then compared with normal fish that had been killed and stained at the same time and in the same way. The main differences in the brains of the blind fish and the normals were in the absence of the optic tract in the former. In the other structures of the brain the blind and normal fish appeared to be identical and, what is very interesting, no differences could be found between the two groups of blind fish. This is only taking into account the gross anatomy of the neurones and fibres connections and since the stain used would not indicate any differences in degree of activity of the brain structures. This will be further discussed on page 233.

These results have confirmed that blinding of the minnow results in the fish darkening and that after a period of time the colour of the fish is variable. This essentially confirms the results of von Frisch (1911) but although he did not study this response in detail. The paling response of minnows in darkness, reported by von Frish (1911), Scharrer (1928) and Shafer (1964), was not observed in the Chess or Lea minnows following blinding but it has been reported by Healey (1945) that this response is very variable in minnows from different localities and that fish from lakes in Wales did not show it and neither did Munich minnows, although he confirmed that the paling was shown in minnows from Vienna.

In general it can be said that blinding of the minnow results in darkening only for a short period in some fish and that the population appears to be composed of two distinct types, the colour of which is determined by the internal physiological condition of the central nervous system or pituitary complex.

# S E C T I O N 6

## THE OPTIC TECTUM AND COLOUR CHANGE

### I. REMOVAL OF THE OPTIC TECTUM IN NORMAL FISH

15 minnows were taken and the optic tectum was completely removed, the skull sealed, and the fish placed in experimental aquaria. The colour was then recorded at intervals of time on both black and white backgrounds for a period of 11 days, after which the fish were killed and fixed, and the brains were sectioned and stained. 4 Fish were killed after 3 hours to compare them with the fish left for 11 days.

Of the 11 fish kept for 11 days, only 3 showed useful results and the rest showed various degrees of degeneration. This degeneration resulted in large amounts of necrotic tissues in the mesencephalon and diencephalon in which no definite structures were clearly visible. In some fish the "lace-like" necrotic tissue had been walled off and below this normal tissue was present. This degeneration could possibly have been due to the entry of water under the cement, but one would have

Fig. 26

Transverse section through the brain of the minnow at the geniculate complex to show removal of the tectum without damage to other brain structures. Stained by Klüver & Barrera technique and sectioned at 8μ.

Abbreviations:

G.C.L. - Corpus geniculatum lateralis

C.G.M. - Corpus geniculatum medialis

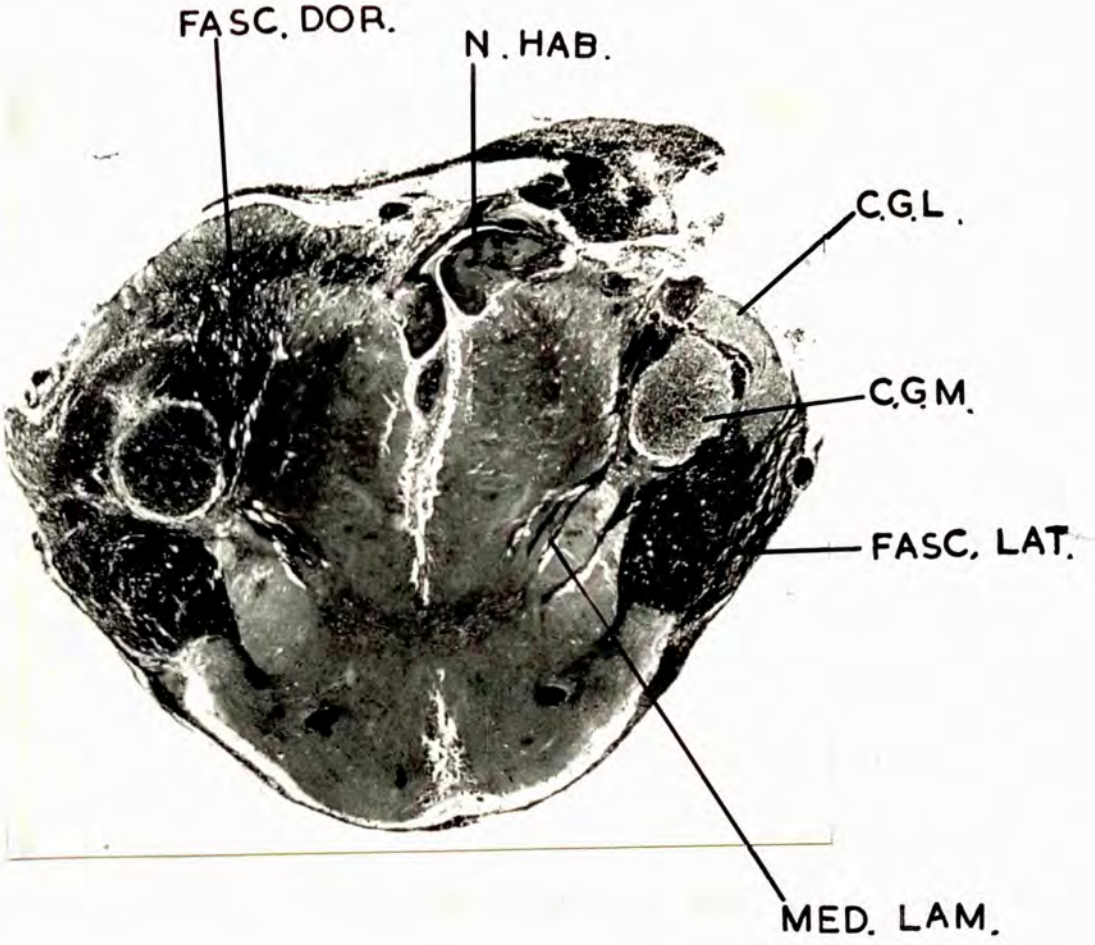
N.HAB. - Nuclius habenularis

FASC.DOR. - Fasciculus dorsalis

FASC. LAT.- Fasciculus lateralis

MED. LAM. - Medial lamella

Fig. 26



expected it to be more extensive, and in which case the fish would not be expected to live long. It seems more likely that the blood supply had been disrupted and that in the 3 fish in which there was no degeneration, the blood supply was adequate.

Because of the degeneration, the results are confined to 4 fish which were killed after 3 hours and 3 fish which survived for 11 days.

In these 7 fish, the tectum was completely removed and, as far as could be seen, the rest of the brain was intact. The geniculate complex was present and intact in all of these 7 cases. (Fig. 26 p. 144). All 7 fish paled within two hours after the operation and the degree of paling varied from 1 to 4.5 on the D.O.I. scale, giving a mean of 2.7. The pallor was maintained for at least 24 hours without change. The fish were tested for normal colour change on black and white backgrounds and in no case could any show any adaptation to the background. A very slight change was noticed in a few fish but this was only due to the light intensity; fish on black could be induced to pale by 1 or 2 degrees by placing the light at a distance of 6 inches from the water as compared to the normal 18 inches.

The results of the 3 fish for 11 days are given in Table 6 p. 146

In these long-term tectal removals there is no recovery of colour

TABLE . The colour of fish 15, 16 and 18 following total tectal removal on black and white backgrounds.

| Fish | White | | | Black | | | White | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24hrs | 48hrs | 96 hrs | 24hrs | 48hrs | 72hrs | 24hrs | 48hrs | 96hrs |
| 15 | 1.5 | 3.5 | 5 | 6 | 6.5 | 7 | 6 | 6 | 5 |
| 16 | 1.5 | 2 | 2 | 4 | 3.5 | 3.5 | 3 | 3.5 | 3.5 |
| 18 | 3 | 3.5 | 4 | 5 | 5 | 6 | 6 | 5 | 5 |

These figures were plotted on a graph (fig. 27 p. 148).

Fig. 27

The colour of three fish after the removal of the tectum, the fish being placed on both black and white background.

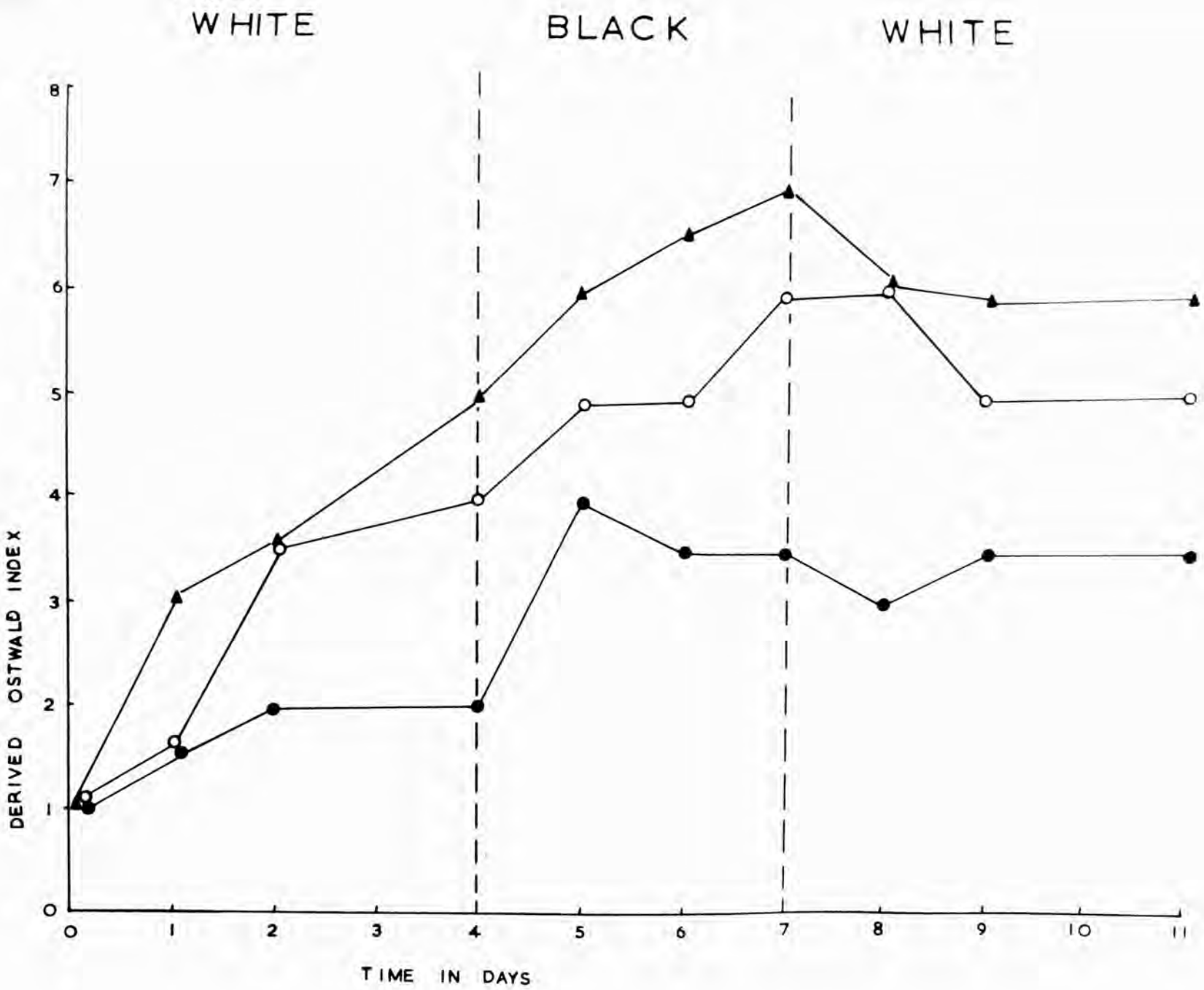
Symbols used:

Fish 15 - ▲

Fish 16 - ●

Fish 18 - ○

Fig. 27


WHITE
BLACK
WHITE
DERIVED OSTWALD INDEX
TIME IN DAYS
0
1
2
3
4
5
6
7
8
9
10
11

change and the initial paling is not maintained. The 11 day tectal removed fish underwent darkening regardless of the background, this occurred while still on white in the case of fish 18 and continued on black. In no case was there more than doubtful lightening on white and the fish retained the general colour that was present on the black. The final colour was highly variable, all three fish showing different colours and none being near the extremes of the colour range possible. However, there are too few results to allow any definite conclusions. Although the results of the other fish have not been presented here, they all show the same spread of tint and no extremes were found.

## II. UNILATERAL TECTAL REMOVAL AND UNILATERAL BLINDING

The optic tectum was completely removed on the right side in 5 fish (later checked histologically). These fish were allowed to recover and then tested after one hour. In every case the fish could perform normal colour change in both extent and direction.

In 10 fish the left optic tract was cut and the animal allowed to recover. On the following day 5 of the fish had the tectum removed on the left side. All of these fish could perform normal colour change in extent and direction (fig. 28 p. 151 ). The other 5 had the right half of the tectum removed and similarly showed normal colour change.

In the case where the optic tract was cut on the left and the optic tectum removed from the same side, the fish was essentially

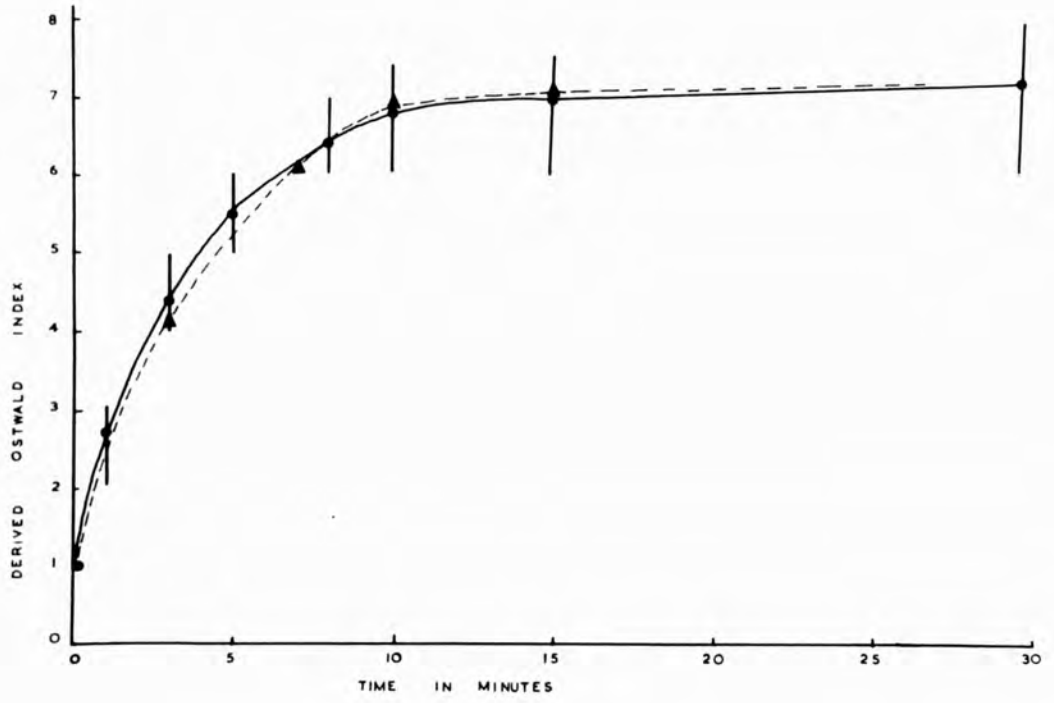
Figs. 28 A, B .

Colour change of normal fish ● , and of fish with the left optic nerve cut and the left lobe of the tectum removed ▲ .

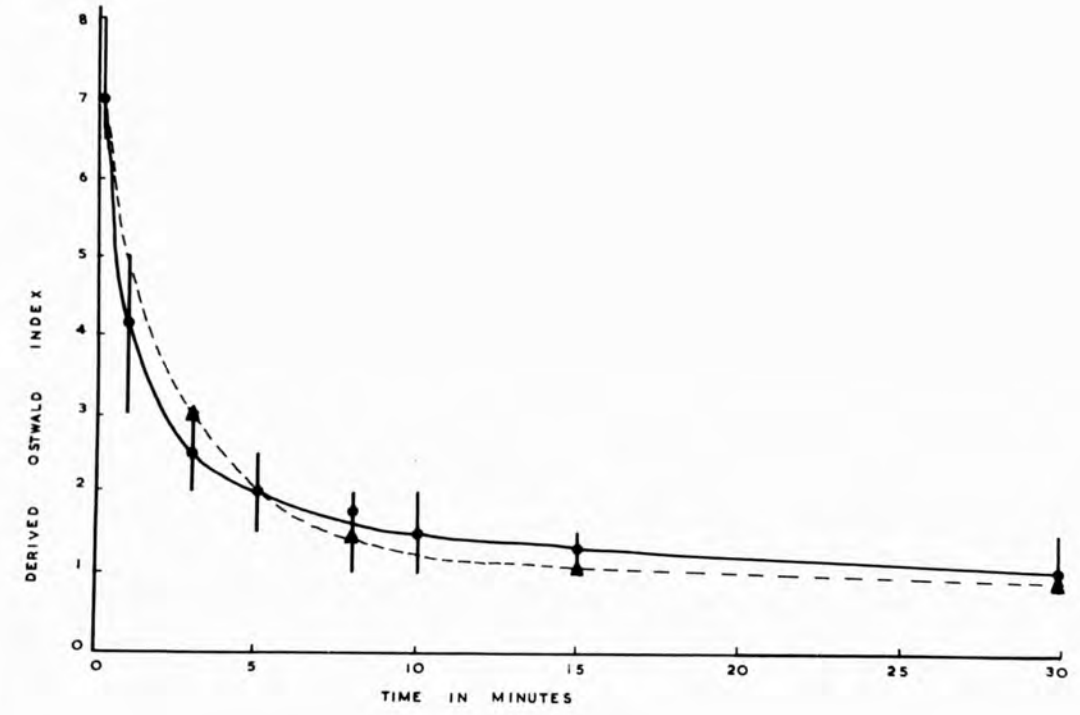
28 A is from white to black and 28 B from black to white.

Fig. 28

A


DERIVED OSTWALD INDEX
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
TIME IN MINUTES

B


DERIVED OSTWALD INDEX
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
TIME IN MINUTES

blind because of the complete chiasma.

Control fish were tested with no tectal removals, but with the skull bone removed and the skull cemented over and in all cases they could perform normal colour change.

## III. TECTAL REMOVAL IN BLIND FISH

Five minnows were blinded by cutting the optic tract and allowed to recover and darken for 24 to 48 hours, by which time they had reached a value of 7 to 7.5. These darkened blind fish then had the tectum completely removed. A few other blind fish acted as controls, having the skull bones removed but the tectum left intact.

In all the fish with the tectum removed and in none of the controls marked paling occurred to a value of 1 to 1.5 D.O.I. within 2 hours.

In a group of 34 fish which had been blinded for 24 to 48 hours the left lobe of the tectum was removed. On recovery a few of the fish showed a slight paling but the majority did not show any difference to the preoperative condition. In the fish which did pale slightly the preoperative colour exactly matched the postoperative colour within one hour.

Four blind fish were taken and more localised removals were

performed. The removals are shown in fig. 31a page. 164 A, B, C, D.

A - this fish had the anterior part of the tectum removed. The fish stayed fully dark throughout the whole of the observation period of 3 hours and maintained a constant D.O.I. of 7.

B - this fish had the posterior part of the tectum removed and 2 hours after operation had reached a D.O.I. value of 1.5. The initial D.O.I. was 7 so that the fish had almost fully paled. Examination of the brain showed that the dorsal part of the tectum had been removed but that there was still present a small part of the lateroventral tectum with intact fibre connections to the torus semicircularis.

C - the dorsal part of the tectum in the posterior region was removed leaving the lateral part intact. The fish had an initial D.O.I. of 7 and a final figure of 2.5.

D - this fish had the dorsolateral part of the tectum removed in the posterior region. The fish had an initial D.O.I. of 7 and a final D.O.I. of 2.5.

The findings from this experiment show that the removal of the dorsal part of the posterior part of the tectum results in complete paling of the blind minnow.

## IV. ISOLATION OF THE TECTAL REGION TO CAUSE PALLOR IN BLIND MINNOWS

A lesion was placed in the dorsal posterior part of the tectum of 23 minnows blinded 24 hours previously.

(a) Method

Normal blinded minnows were anaesthetised and the left lobe of the tectum was removed. The wound was not closed and the fish were allowed to recover in ringer for at least an hour. The initial removal did not affect the D.O.I. of most of the fish but three fish did show slight paling down to 5, these fish also appeared agitated and were discarded.

The fish were then re-anaesthetised and the right posterior part of the tectum exposed. The lesion was then placed in the right tectum, the position being determined by an eye-piece graticule in the upper lens of a binocular microscope. The graticule was a simple grid that enabled the position to be marked on a drawing of the brain on squared paper. The lesion was placed directly, using the hand, and with practice it could be placed to within 0.06mm of the required position. It was not necessary to place them more accurately because the exact position was determined from the sections cut of each lesion and because it was only necessary to cover the area adequately.

In making the lesions the apex of the cerebellum was taken as the reference point. This was very easy to see in the sections and was relatively constant in position with reference to the rest of the tectum. The anterio-posterior axis is called the X axis and the lateral axis the Y axis. The apex of the cerebellum forms the zero point on both axis and the lesions which are more anterior to this are given

positive values while those that are posterior to it are given negative values.

The lesions were made by the technique of micro-chemical cautery devised by Clark & Scott (1962) for the diencephalon of the frog. Because the tectum is a large, fairly thin structure it was decided to make large lesions completely through it. Micro-pipettes were made by drawing out fine glass tubing and attaching a small rubber bulb at the other end. The tips of the pipettes were cut as an acute angle to aid in the moving through the tectum. The outer tip diameter was 0.24mm and the inner tip diameter 0.12mm.

A band marked at one centimeter from the tip enabled a standard amount of acid to be used in each lesion, namely 0.112mm$^3$. The pipette was filled by capillary action with dichloracetic acid ($CHCl_2COOH$), and the pipette was pushed through the tectum slowly, injecting the acid all the time. Using this method very discrete lesions could be produced which were circular in outline and had a diameter of 0.2mm $\pm$ 0.04mm. The larger lesions did not give any greater pallor than the smaller.

The final D.O.I. was recorded at least two hours after recovery from the anaesthetic. Each result is experessed in terms of the number of degrees of paling (D.O.P.) and is the final D.O.I. subtracted from the original D.O.I. The D.O.P. is used because there are slight differences in the initial D.O.I. figures.

Fig. 29

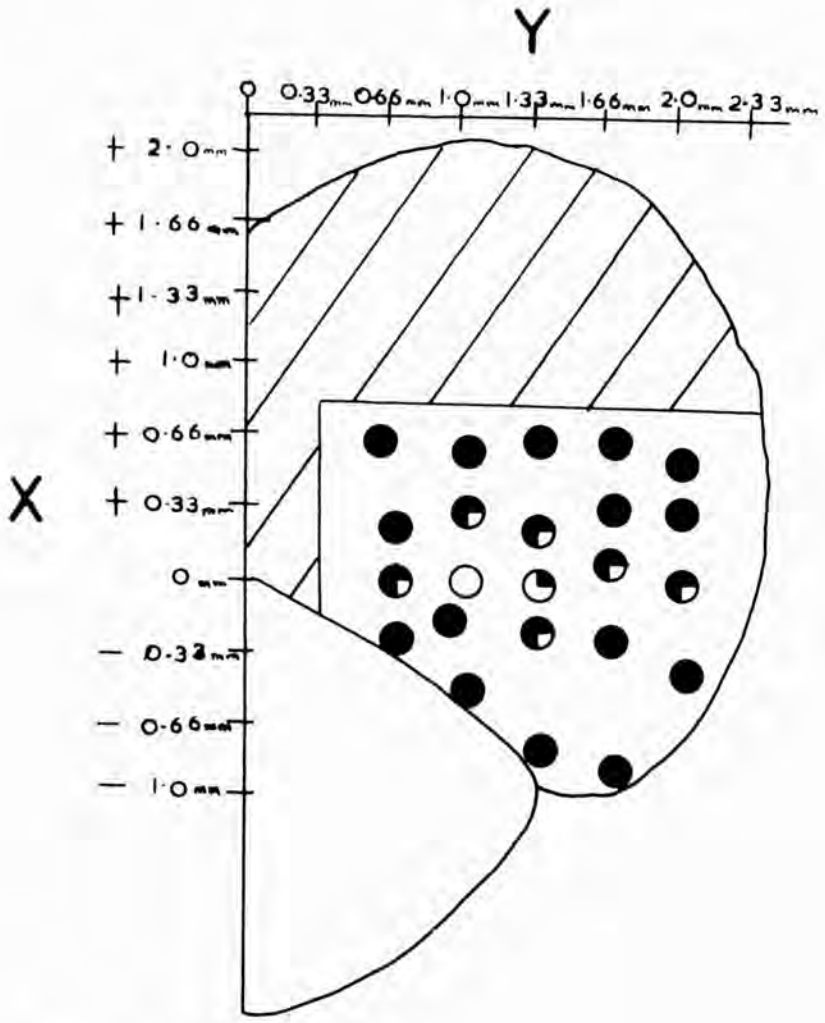
In a summary of the tectal lesion placed in right tectal lobe of blind fish after the complete removal of the left lobe. The degrees of pelting is the difference between the initial and the final D.O.I. values.

Fig. 29

Is a summary of the tectal lesion placed in right tectal lobe of blind fish after the complete removal of the left lobe. The degrees of paling is the difference between the initial and the final D.O.I. values.

Fig. 29

# SUMMARY OF LESIONS




DEGREES OF PALING

0 —
1 —
2 —
3 —
4 —

(b) Results

The positions of the lesions and the D.O.P. are summarised on page 157 fig. 29.

To test the effect of the acid, three lesions were performed without it. They yielded the same results as lesions with the acid but it was difficult to see just what had been removed. The question as to whether the acid destroys more than is immediately visible is one that applies to all methods of lesion making. In the present case, the lesion was constantly washed with ringer to prevent the spread of the acid. In the fish used for the medullary centre experiment (p. 199) the tectal lesion was fully healed and composed entirely of glial cells after 16 days and no damage could be seen in the surrounding tissue. It therefore seems likely also in the lesions described in this section that the dichloroacetic acid did not do any damage to the surrounding nervous tissue and that the results are due to the part removed and not the effect of the acid.

A number of lesions were repeated to verify the original results and in all cases the results were identical. The lesions repeated were XOY1mm, X+ 0.18mmY1.6mm, X 0 Y 2mm, XO Y 0.6mm, X XO Y1.33mm.

In a few cases the lesioned fish paled immediately after recovery from the anaesthetic (X+0.35mm Y1.6mm, X+0.64mm Y1.66mm, X-0.36mm Y2mm, X-0.6mm.Y1.33mm, X-0.33mm.Y0.66mm.), but then began to darken and at

Fig. 30

Photomicrographs of 4 tectal lesions stained with Luxol Fast Blue and Cresyl Fast Violet, and sectioned at 8 μ.

A - X+0.3mm. Y1.3mm.

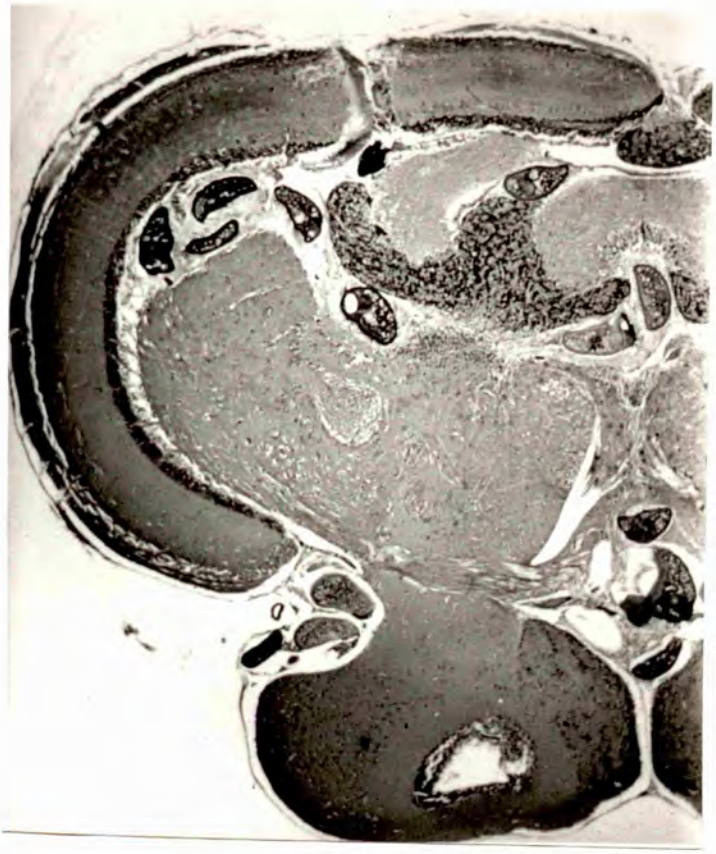
B - X0 Y1mm.

C - X0 Y1.33mm

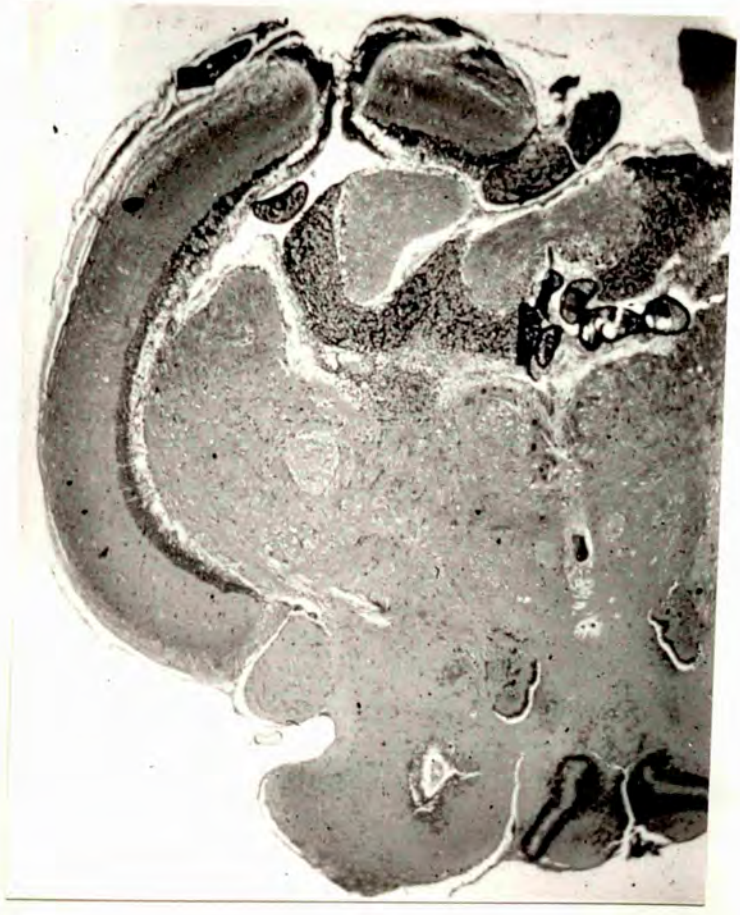
D - X0 Y2mm.

Fig, 30

A



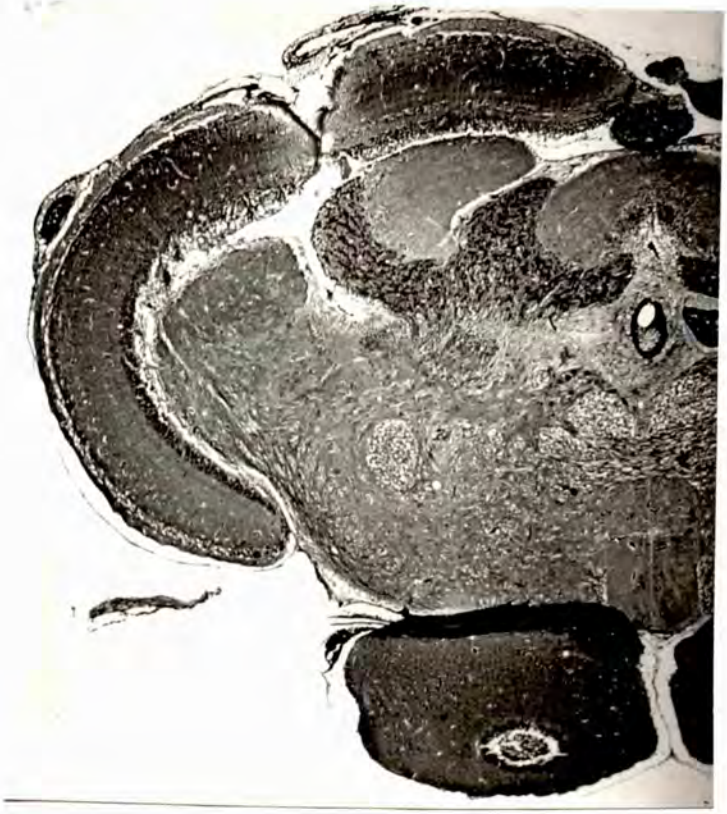
B



0·5 mm

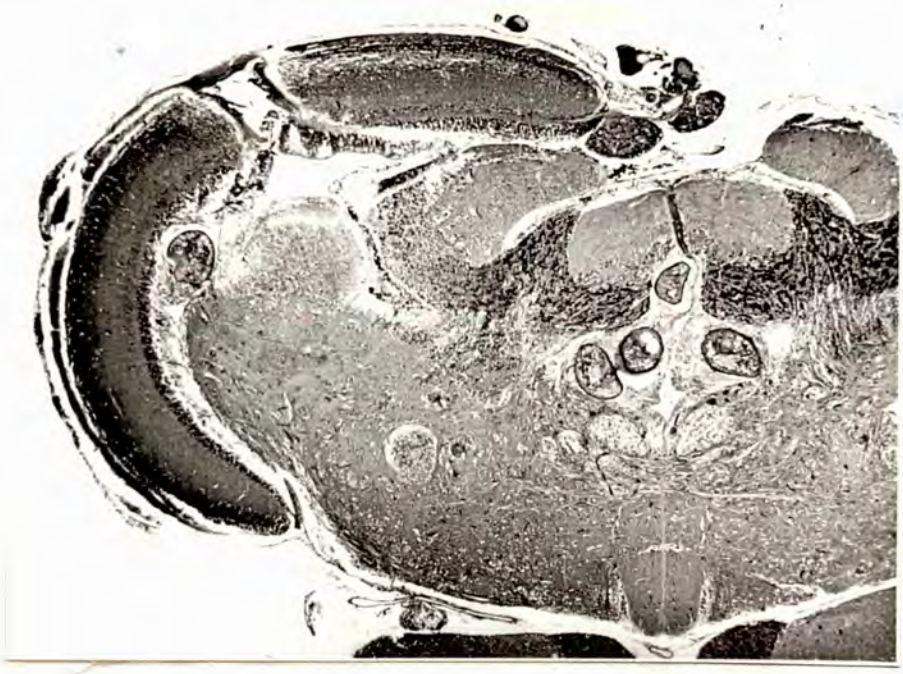
Fig. 30

C



0·5 mm

D



the end of the 2 hours had reached the same colour as the original. Only the persistent change in colour brought about by the lesion is recorded.

Four degrees of pallor represents a change of D.O.I. of approximately from 6 to 2. The fish used in this experiment were kept in stock tanks of an intermediate colour, and the effective range for these fish was from D.O.I. 7 to 1, a D.O.P. of 6. The D.O.P. recorded in fish XO Y1mm was 4 and this is essentially only 2 degrees below the maximum of any given fish in this group.

A group of lesions is illustrated on fig. 30 (p. 160). The results show (fig. 29 P. 157) that there is a small discrete region in the dorsal posterior part of the tectum whose removal results in almost maximal paling of a blind fish. The region is represented at the centre by lesions XO Y1mm and XO Y 1.33mm (Fig. 30 p. 160) giving values of D.O.P. 4 and 3 respectively. These two lesions are bordered by a group of lesions giving values of only 1 D.O.P. these being XO Y0.66mm, X+0.33mm Y1MM, X+0.3mm.Y1.33mm, X-0.25mm.Y1.33mm, X+0.1mm.Y1.66mm, XO Y2mm. Apart from these lesions none of the other lesions gave any degrees of paling.

## V. THE REMOVAL OF PARTS OF THE OPTIC TECTUM IN NORMAL FISH.

The fish in this group were treated differently to those in the groups previously mentioned. Parts of the tectum were removed and the

Fig.31 a, b, c, d, e, f, g, h.

Show the dorsal and the right and left lateral views of the tectum and adjacent brain areas.

Abbreviations:

T. - Optic tectum

C. - Cerebellum

P. - Pineal body

O.L. - Olfactory lobe of the forebrain

O.T. - Optic tract

V. - Vagal lobe of the medulla

In all these figures the shaded areas are the areas removed and the stippled areas are the regions which are present but the operation damaged or separated the fibres running from these areas to the torus semicircularis.

a - Fish A, B, C, D

b - Fish 67, 35, 34, 27

c - Fish 34, 65, 37, 64, 36

d - Fish 33, 40, 47, 56, 60

e - Fish 49, 37, 45, 41

f - Fish 61, 55, 48, 54

g - Fish 73, 74, 75, 76

h - Fish 68, 69, 70, 71, 72

Fig.31 a, b, c, d, e, f, g, h.

Show the dorsal and the right and left lateral views of the tectum and adjacent brain areas.

Abbreviations:

T. - Optic tectum

C. - Cerebellum

P. - Pineal body

O.L. - Olfactory lobe of the forebrain

O.T. - Optic tract

V. - Vagal lobe of the medulla

In all these figures the shaded areas are the areas removed and the stippled areas are the regions which are present but the operation damaged or separated the fibres running from these areas to the torus semicircularis.

a - Fish A, B, C, D

b - Fish 67, 35, 34, 27

c - Fish 31, 65, 37, 64, 36

d - Fish 33, 40, 47, 56, 60

e - Fish 49, 57, 45, 41

f - Fish 61, 55, 48, 54

g - Fish 73, 74, 75, 76

h - Fish 68, 69, 70, 71, 72

Fig. 31a

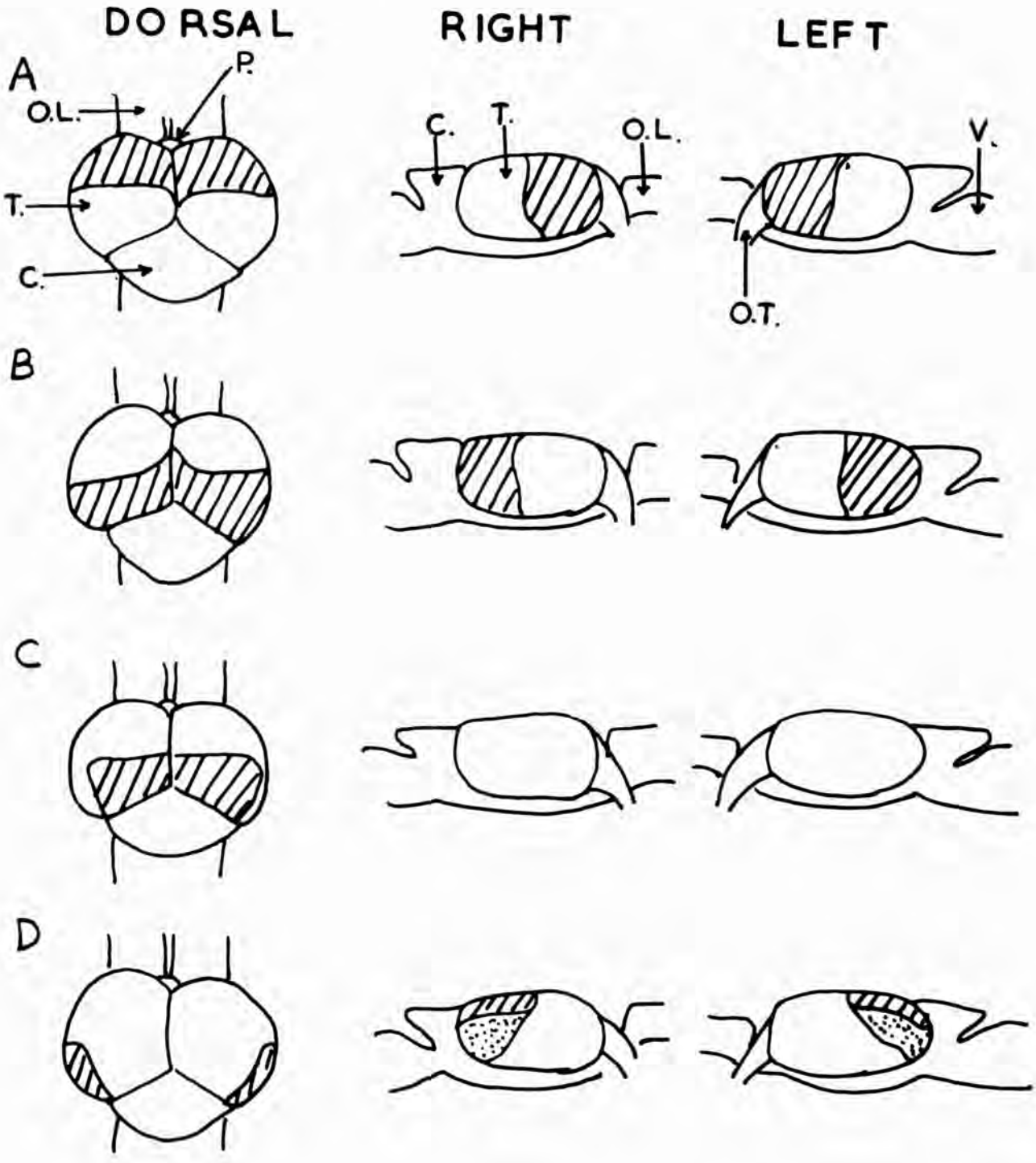


Fig. 31b

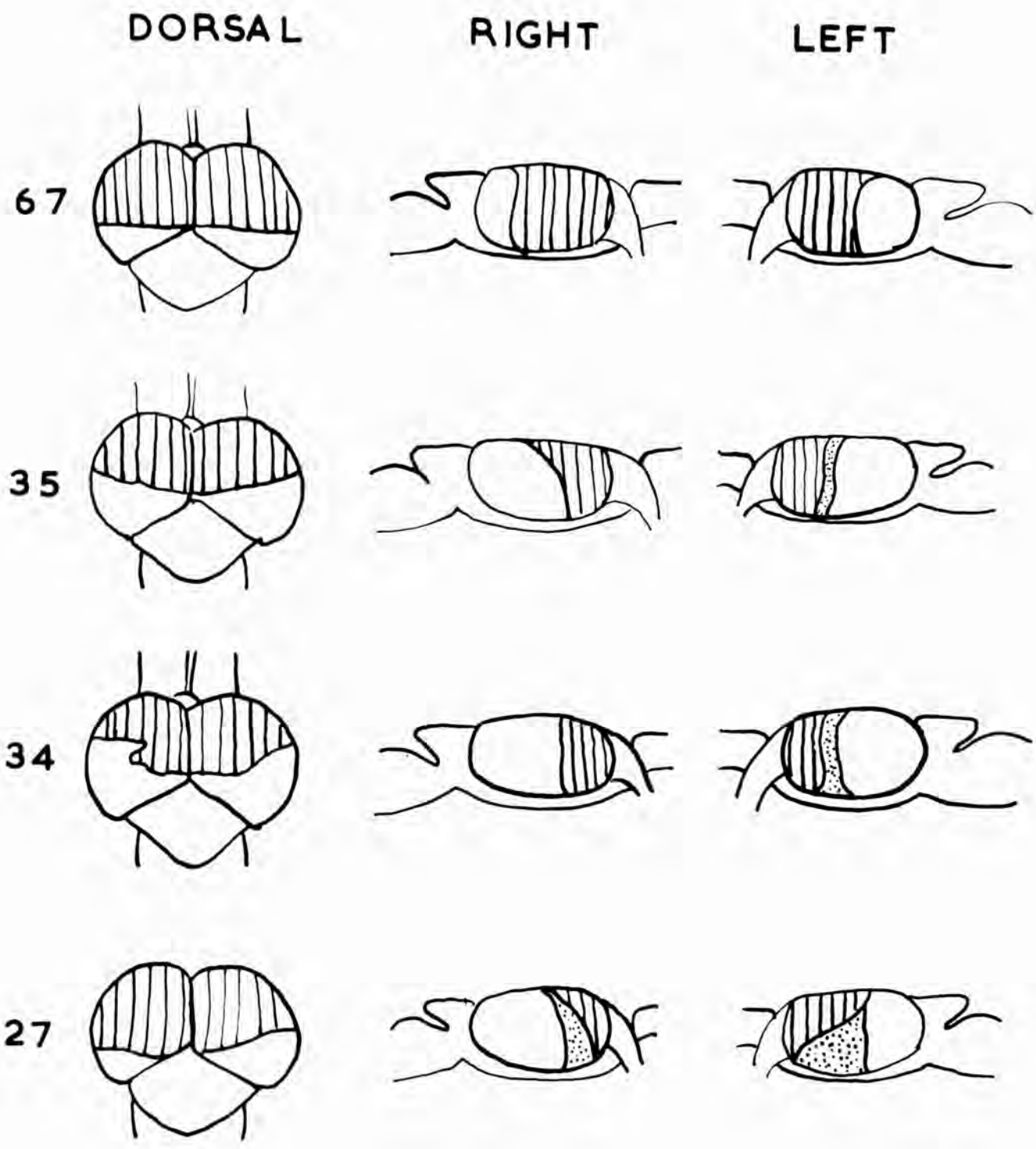


Fig. 31c

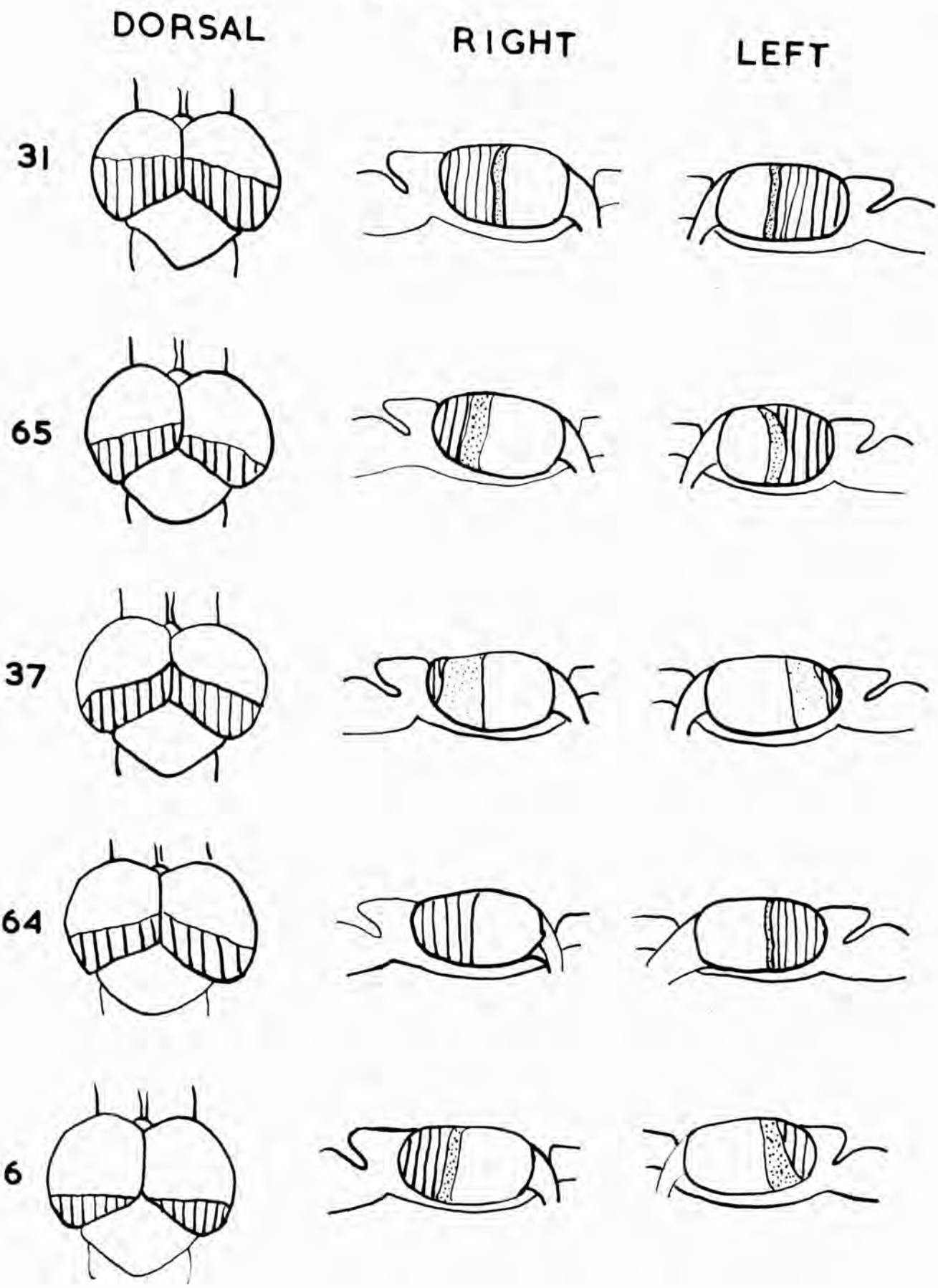


Fig. 31d

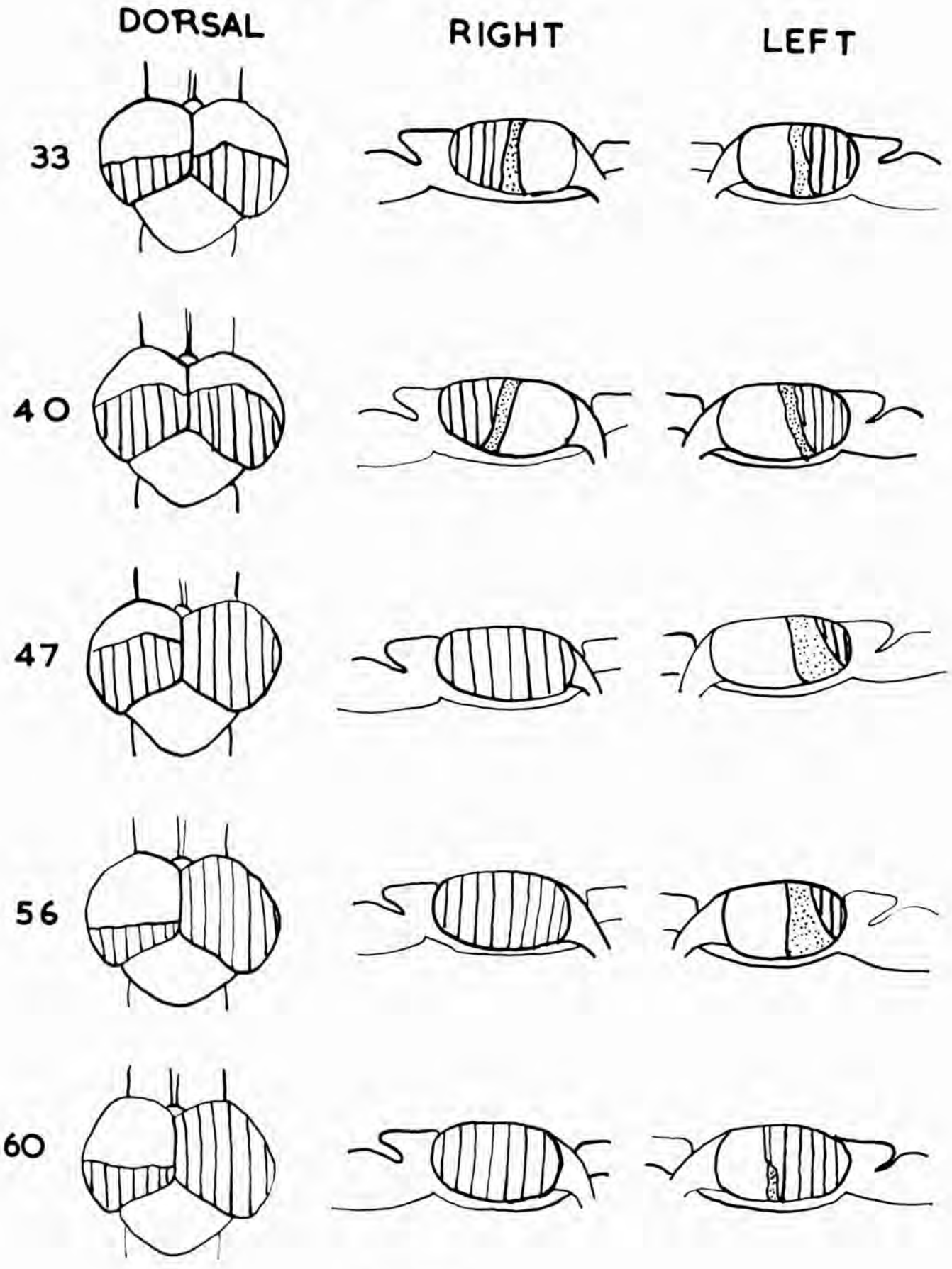


Fig. 31e

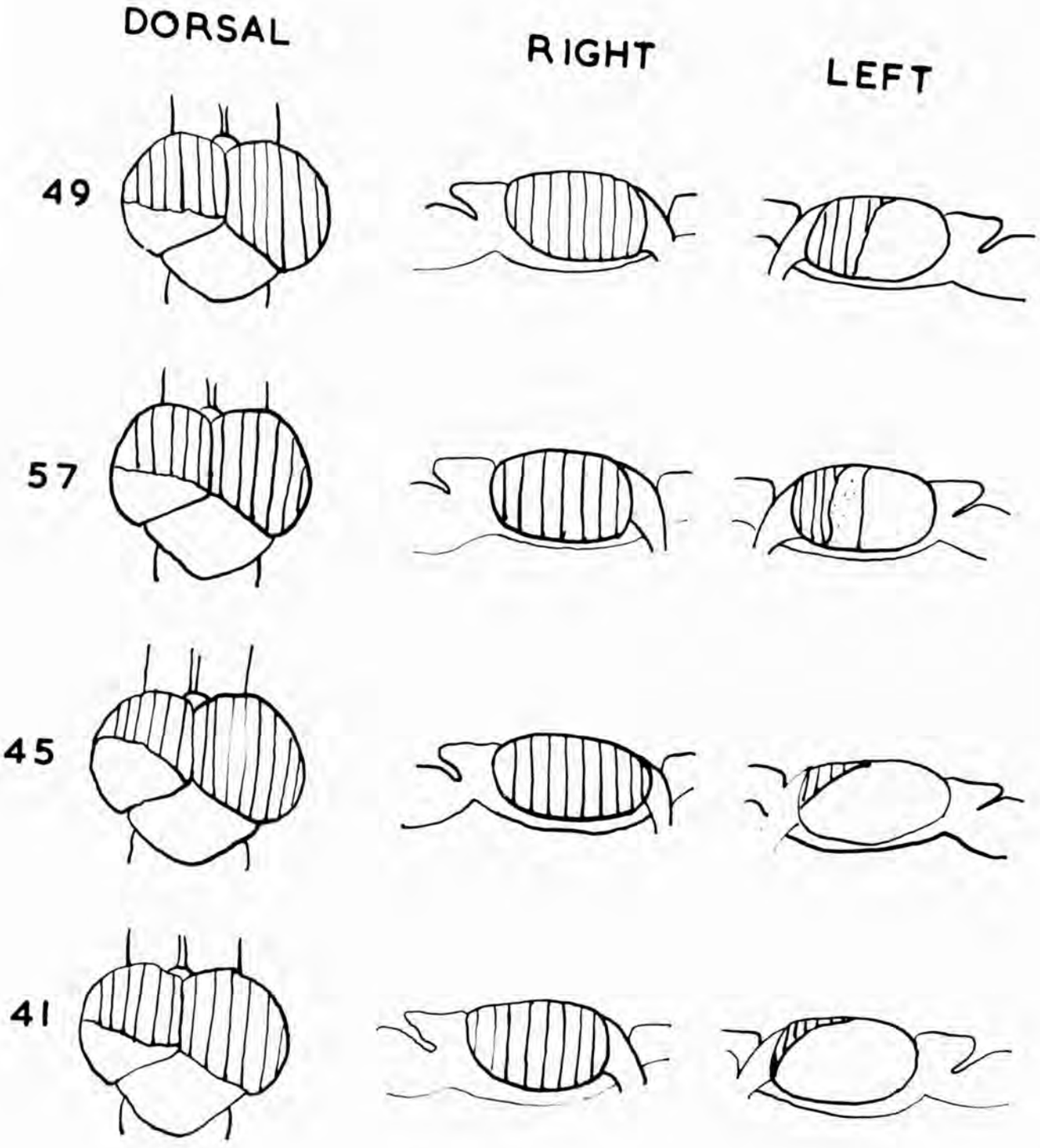


Fig. 31

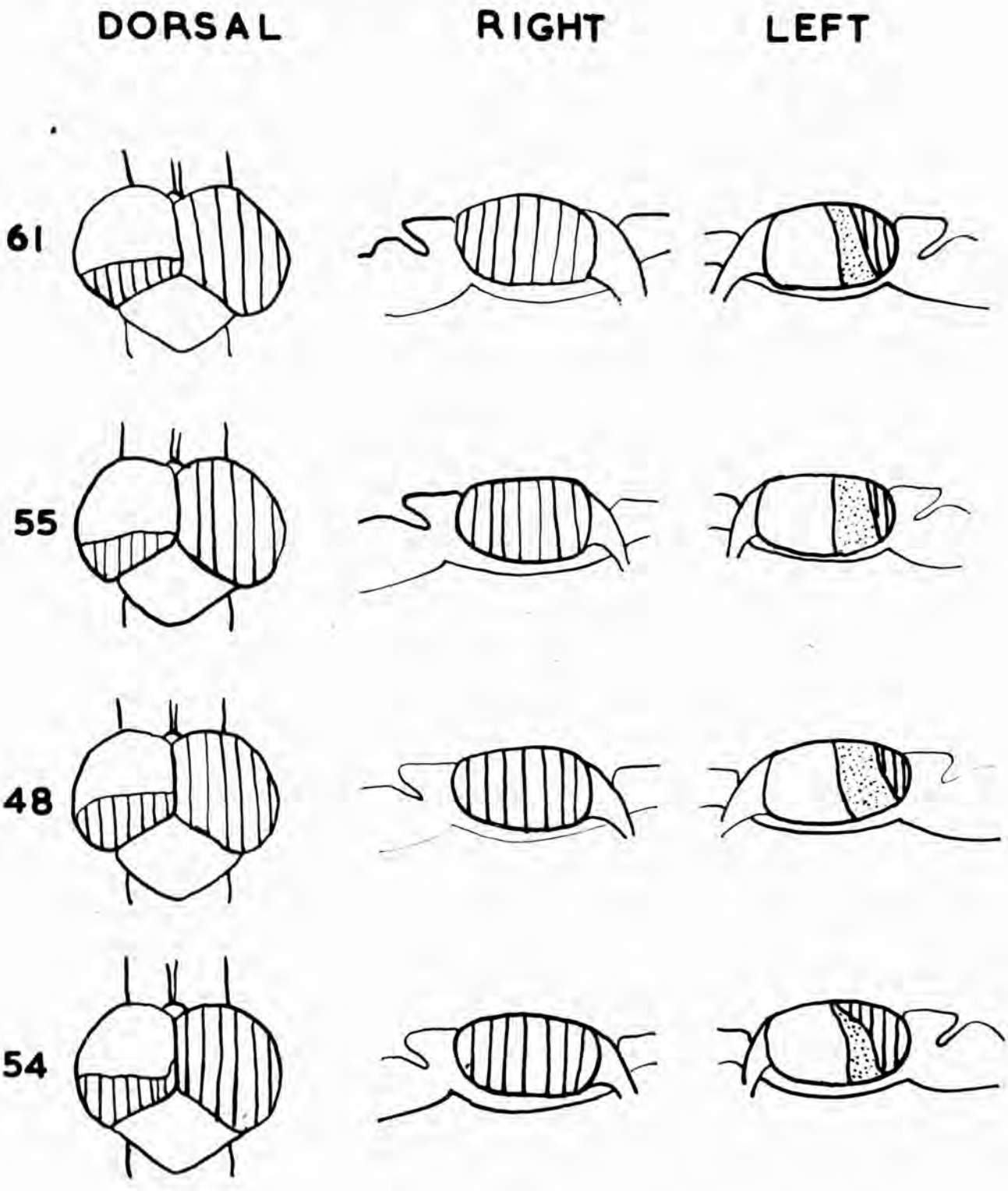


Fig. 31g

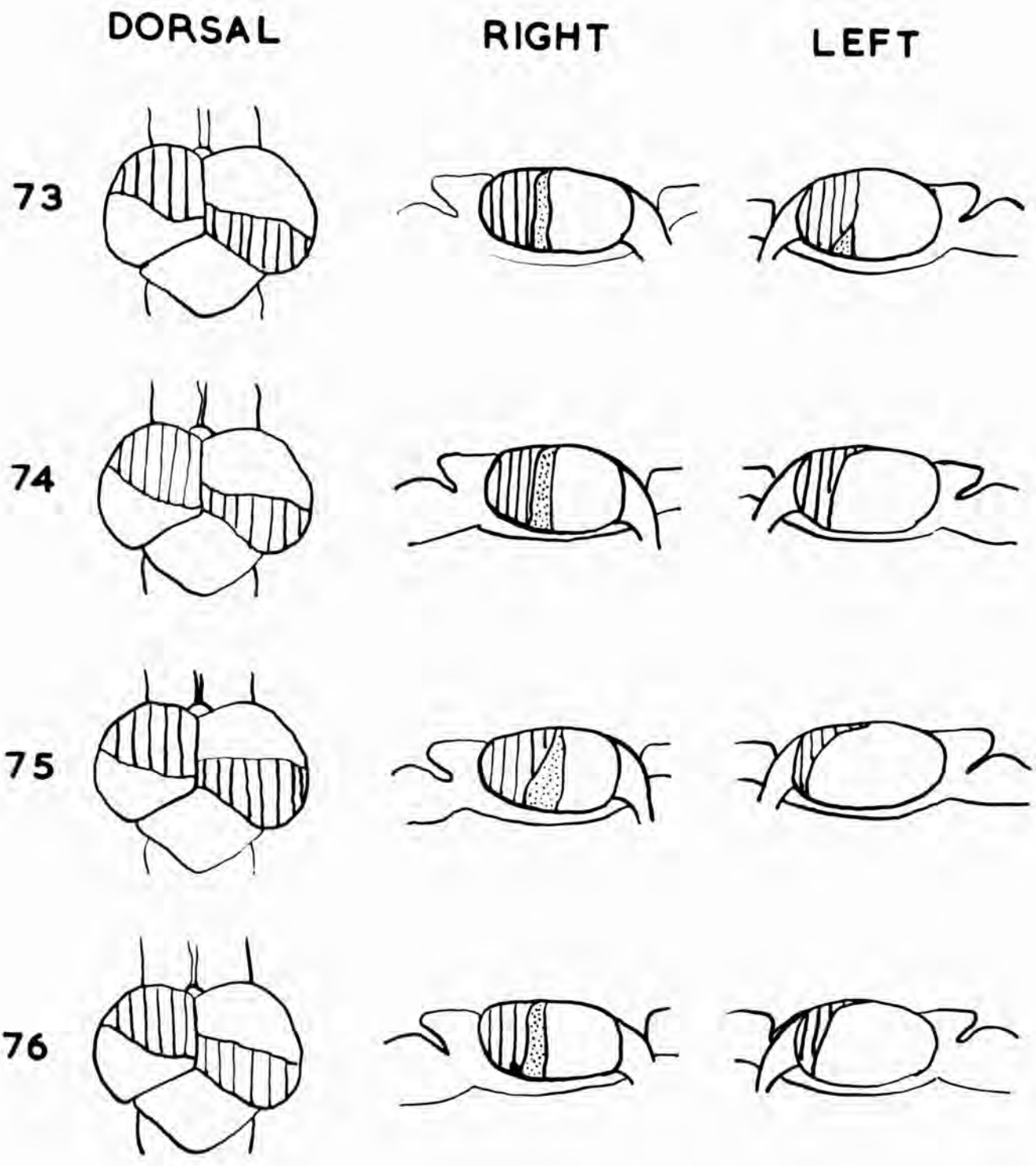
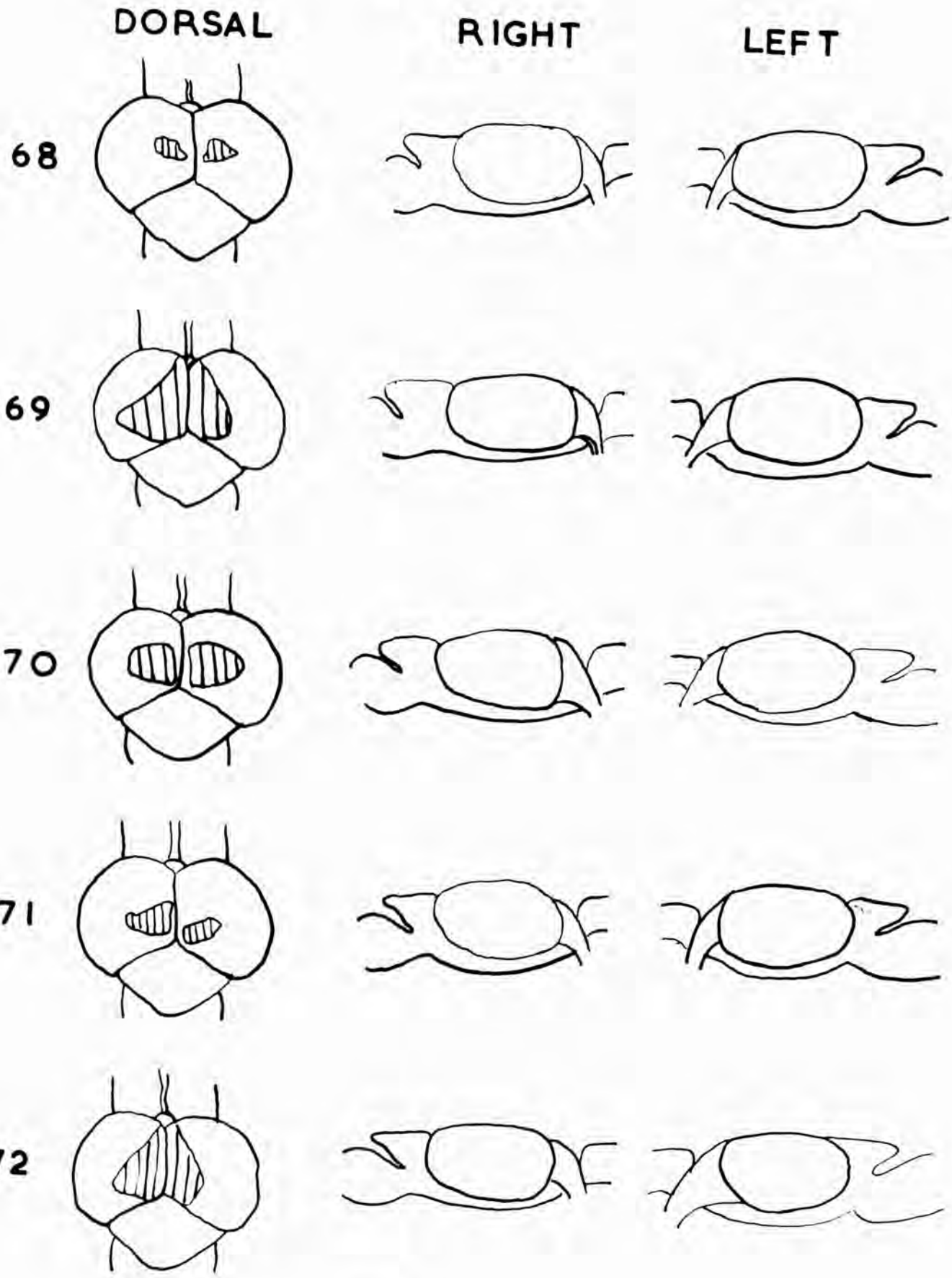


Fig. 31h



skull was sealed with the cement. After 24 hours the fish were tested on black and white for 30 minutes. Of the 51 operated fish only 31 were of use, 20 fish had some damage to other parts of the brain, especially the torus semicircularis and the geniculate complex, and were therefore discarded, and 10 fish died immediately after operation.

(a) Removal of the anterior tectum

The anterior tectum was removed from 4 fish in order to observe the effect of the presence of the posterior tectum along. The removals are shown on fig. 316 (p 165). The results for colour adaptation are given in table 7 .

TABLE 7 . The colour of minnows following the removal of the anterior tectum.

| Fish | Black | White | % tectum present |
|---|---|---|---|
| 27 | 3 | 2.5 | 46 |
| 34 | 4 | 3 | 53 |
| 35 | 1.5 | 1.5 | 57 |
| 67 | 4 | 4 | 25 |

There was a considerable variation in the colour of the fish in this group. All the fish showed some degree of pallor and none of the fish showed any tendency to change colour with the background. The mean D.O.I. value of 2.9 for the group was very nearly the same as the

2.6 mean seen 24 hours after complete tectal removal (p.145 ). There does not appear to be any correlation between the amount of tectum removed and the D.O.I. values. In all cases the posterior tectum was not in contact with the geniculate complex, or the optic tract, and in all cases the geniculate complex was intact. In two fish, 34 and 35, the region identified as the active region in blind fish by means of lesions (p. 162) was present and intact on both sides.

(b) Removal of the posterior tectum

7 fish were used in this experiment. The removals are shown in fig.31c,d (p. 166-7), and the results for background reversal are shown in table 8 .

TABLE. 8 . The colour of minnows following removal of the posterior tectum

| Fish | Black | White | % tectum present |
|---|---|---|---|
| 31 | 2.5 | 1 | 45 |
| 33 | 2 | 2 | 47 |
| 36 | 1.5 | 1 | 74 |
| 37 | 1.5 | 1 | 72 |
| 40 | 2 | 1 | 44 |
| 65 | 3 | 2 | 65 |
| 64 | 3 | 2 | 52 |

The variation in this group is very big but, as with the

anterior removals, the fish had a general tendency to pale. The mean D.O.I. for the group was 1.8, the mean being taken from the black and white record. This is one degree lighter than the colour following total tectal removal, but it is too slight to be important.

Both 36 and 37 are interesting because very little tectum was removed but the fibres passing out of this region to the torus semicircularis were destroyed.

(c) Left posterior tectum only present.

4 fish were used in this group, the removals shown in fig. 31c (P. 164). The results on the different backgrounds are given in table 9.

TABLE 9. The colour of minnows with only the left posterior part of the tectum remaining

| Fish | Black | White | % tectum present |
|---|---|---|---|
| 41 | 6 | 5 | 30 |
| 45 | 4 | 2 | 31 |
| 49 | 4 | 4 | 18 |
| 57 | 3 | 3 | 25 |

The mean D.O.I. for this group was 3.8, which is much higher

than the D.O.I. following either the anterior or the posterior removals. Although the anterior tectum was removed the tectum was usually in contact with the fasciculus lateralis running from the dorsal part of the eye to the tectum.

(d) Left anterior tectum only present

7 fish were used in this experiment. The removals are shown in fig. 31df (p. 167, 169). The results for the background reversal are given in table 10.

TABLE 10. The colour of minnows with only the left anterior part of the tectum remaining.

| Fish | Black | White | % tectum present |
|---|---|---|---|
| 47 | 6 | 3 | 27 |
| 48 | 6 | 3 | 36 |
| 55 | 4 | 3 | 36 |
| 54 | 2 | 2 | 27 |
| 56 | 4 | 2 | 31 |
| 60 | 2 | 1 | 20 |
| 61 | 4 | 2 | 34 |

This gives a mean of 3.1 degrees, similar to that found in the group in which only the left posterior portion of the tectum remained.

In all the fish tested there was a slight change in shade when the animals were transferred from black to white. This was especially marked in 47 and 48. In all the cases this could be brought about on either background by changing the light intensity. In the case of the fish 47 and 48 the change from 6 to 3 occurred in about a minute, which is much too fast for a normal colour change. It would appear then that this slight change from black to white is not due to a change in the background but to a change in the total light intensity and that the mechanism involved does not include the normal visual system.

(e) Removal of the anterior left and the posterior right tectum

~~Teh results~~ The removals from 4 fish after this operation are shown in fig. 31g (p. 170). The results for background reversal are shown in table 11.

TABLE 11. The colour of minnows on black and white backgrounds following the removal of the anterior left and the posterior right parts of the tectum

BLACK TO WHITE

| Fish | 0 mins | 3 mins | 5 mins | 9 mins | 12 mins | 20 mins |
|---|---|---|---|---|---|---|
| 73 | 5 | 2 | 1.5 | 1 | 1 | 1 |
| 74 | 6 | 2.5 | 2.5 | 2 | 2 | 2 |
| 75 | 7 | 4 | 3 | 2.5 | 2 | 1.5 |
| 76 | 3 | 2 | 1.5 | 1 | 1 | 1 |

WHITE TO BLACK

| Fish | 0 mins | 3 mins | 6 mins | 9 mins | 12 mins | 20 mins |
|---|---|---|---|---|---|---|
| 73 | 1 | 3 | 5 | 5 | 5 | 5 |
| 74 | 2 | 4 | 6 | 6 | 6 | 6 |
| 75 | 1.5 | 4 | 6 | 6 | 6.5 | 7 |
| 76 | 1 | 3 | 3 | 3 | 3 | 3 |

The results are shown in the graphs on fig. 32 33 (p. 179 180). These results were not due to any intensity effect for when they were tested over a variable range of intensities none of them showed any change of tint. The second important feature is that the time for this change is within the normal time for colour change.

The results do show that the fish attempt to adapt to the background. In the case of fish 75 there was almost normal colour change in both extent and pattern and 74 was almost normal, only lacking the ability to pale by 0.5 of a degree. Neither 73 nor 76 could change colour over the normal range but the rate of change was the same as that of normal fish.

The amount of tectum present in all these fish was approximately equal to just over 50%.

Figs.32 and 33.

Results of changing the background: fish 73, 74, 75 and 76.

Symbols used:

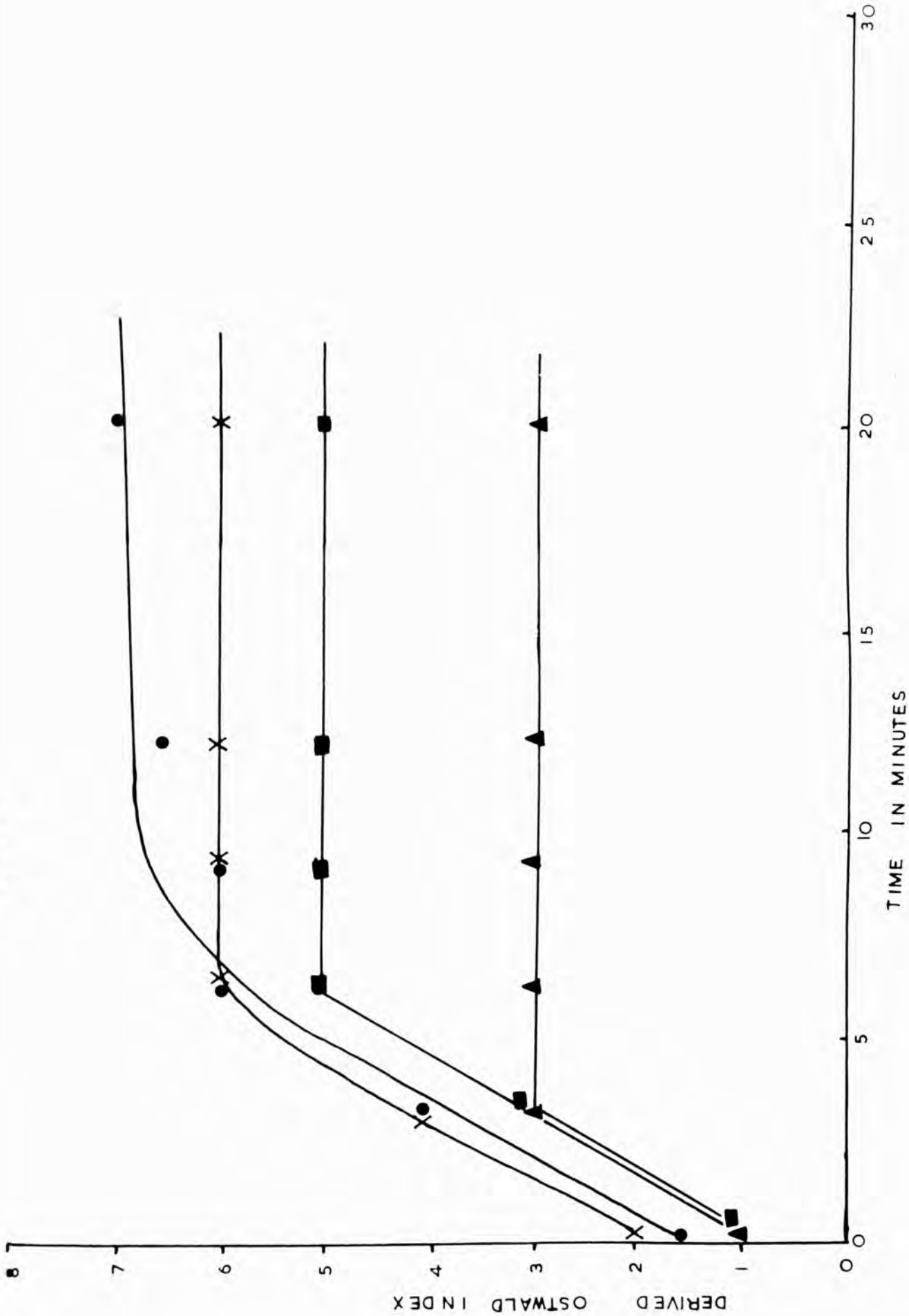
73 - ■

74 - X

75 - ●

76 - ▲

Fig.32 is from white to black and 33 black to white.


DERIVED OSTWALD INDEX
TIME IN MINUTES
0
1
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0
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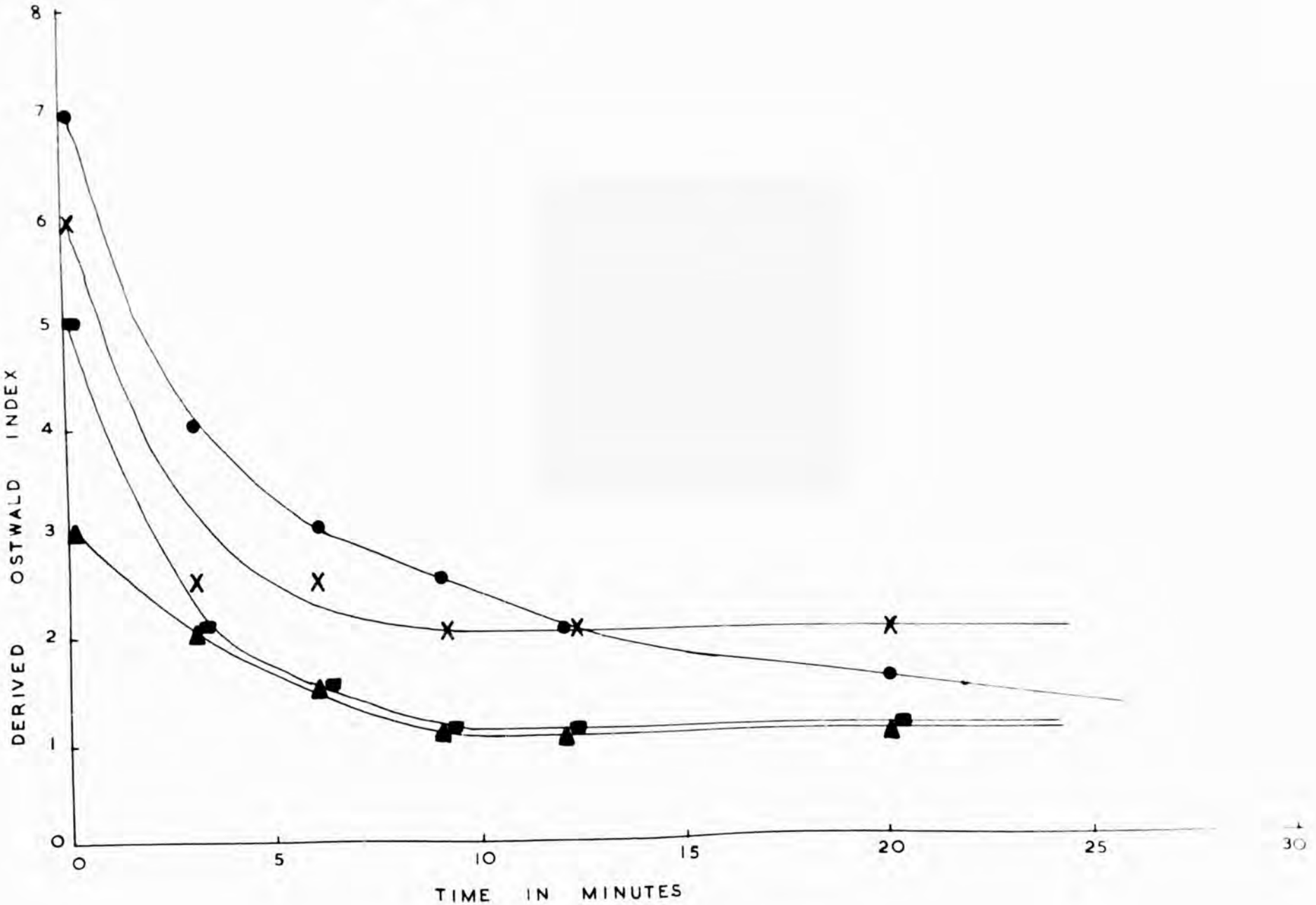
10
15
20
25
30

Fig.32

Fig. 33


DERIVED OSTWALD INDEX
TIME IN MINUTES
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30

(f) Removals from the dorsal tectum

To investigate the activity of the tectum by small removals presents certain problems. Firstly, lesions in the midline will remove or disrupt the incoming optic tract fibres. The effective area put out of action is far in excess of the lesion, and there is no way of estimating the extent of this. Secondly, the removal must not effect the active region in the posterior part of the tectum, for damage to this results in no colour change, and a permanent pale tint regardless of background. The anterior dorsal region between the positions Y0.3mm - 2.33mm is the only region that is easy to operate on and the results bear some relationship to the amount of the tectum.

5 fish were used in this group. The details of the removals are shown in fig. 31h (p. 171).

The results for the background reversal are given in Table 12.

TABLE 12. The colour of minnows on black and white background following the removal of small parts of the dorsal tectum.

| Fish | BLACK TO WHITE | | | | | | % tectum present. |
|---|---|---|---|---|---|---|---|
| | 0 mins | 3 mins | 6 mins | 8 mins | 12 mins | 20 mins | |
| 68 | 6 | 3 | 2 | 2 | 2 | 2 | 96 |
| 71 | 7 | 2.5 | 1.5 | 1.5 | 1.5 | 1.5 | 95 |
| 70 | 6 | 4.5 | 4 | 4 | 4 | 4 | 91 |
| 72 | 5 | 3 | 3 | 3 | 3 | 3 | 86 |
| 69 | 4 | 2 | 2 | 1.5 | 1.5 | 1.5 | 84 |

| Fish | WHITE TO BLACK | | | | | | % tectum present. |
|---|---|---|---|---|---|---|---|
| | 0 mins | 3 mins | 6 mins | 8 mins | 12 mins | 20 mins | |
| 68 | 2 | 4.5 | 5 | 5.5 | 6 | 6 | |
| 71 | 1.5 | 5.5 | 7 | 7 | 7 | 7 | |
| 70 | 4 | 5.5 | 6 | 6.5 | 6.5 | 6.5 | |
| 72 | 3 | 5 | 5 | 5 | 5.5 | 5.5 | |
| 69 | 1.5 | 2 | 3 | 3 | 3.5 | 4 | |

These results are plotted as graphs in figs. 34, 35 (p. 184-5). The colour changes were responses to the background and could not be induced by changes in light intensity.

The removals fall into three groups:

1. Small removals confined to the region between Y0.3mm and Y1.3mm and not involving the active region (p. 162). Both fish (68, 71) show more or less normal colour change in extent and direction.
2. Larger removals which did not seriously affect the active region. Fish 70 shows a removal of only 9% of the tectum but the ability of the fish to change colour is greatly reduced. The fish has an inability to pale below a value of 4 and shows a range of only 2.5.
3. Large removals where the active region is effected on at least one side. Fishes 69 and 72 show an inability to change colour by more than 2.5 as in fish 70 but in the former two fish there is a progressive shift of the curves to the white end of the chromatic range.

Figs.34 and 35 .

Results of changing the background of fish 68, 71, 70, 72, 69.

Symbols:

68 - ●

72 - +

70 - ■

71 - ▲

69 - ○

Fig.34 is from white to black and fig.35 from black to white.

Fig.35

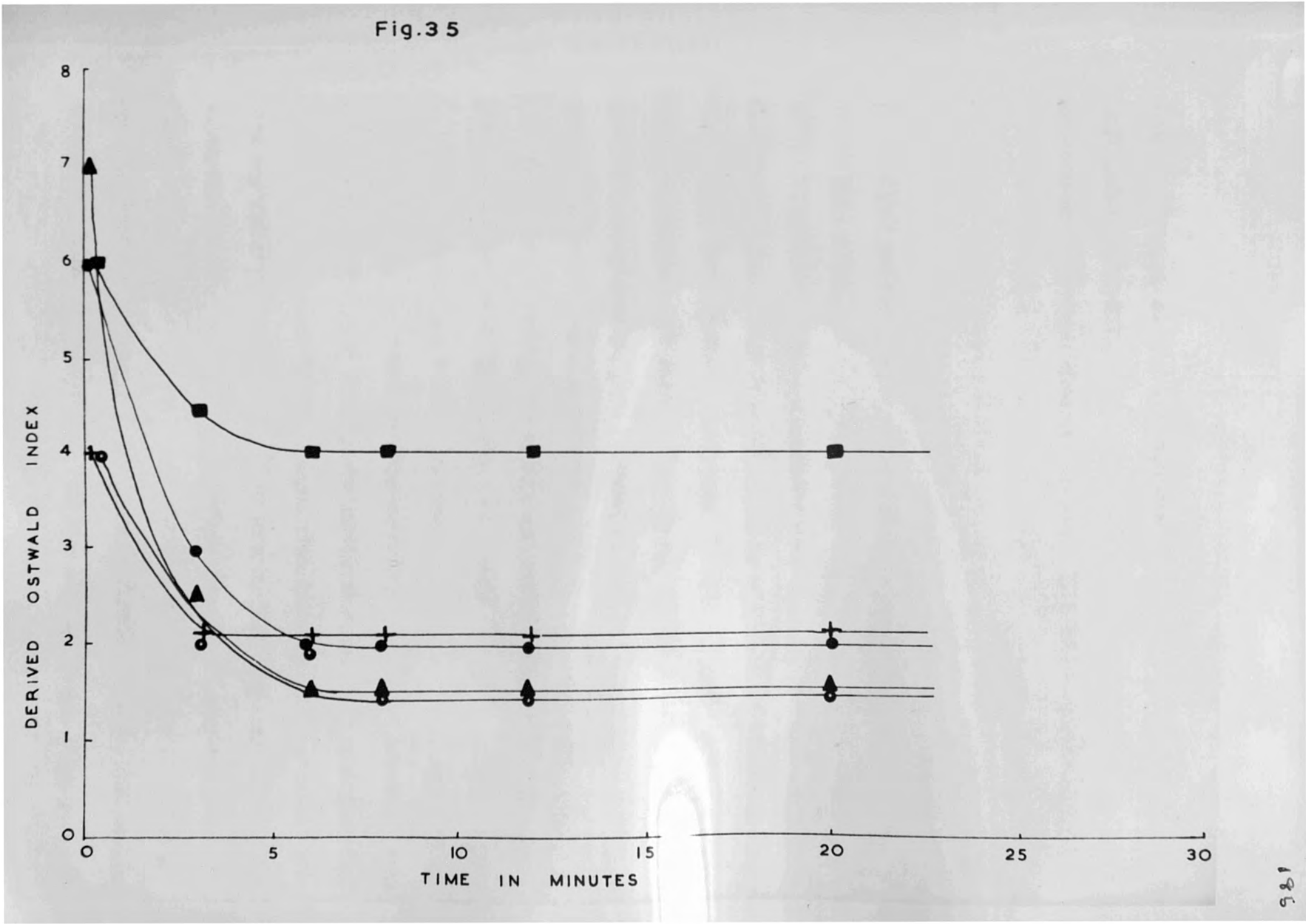
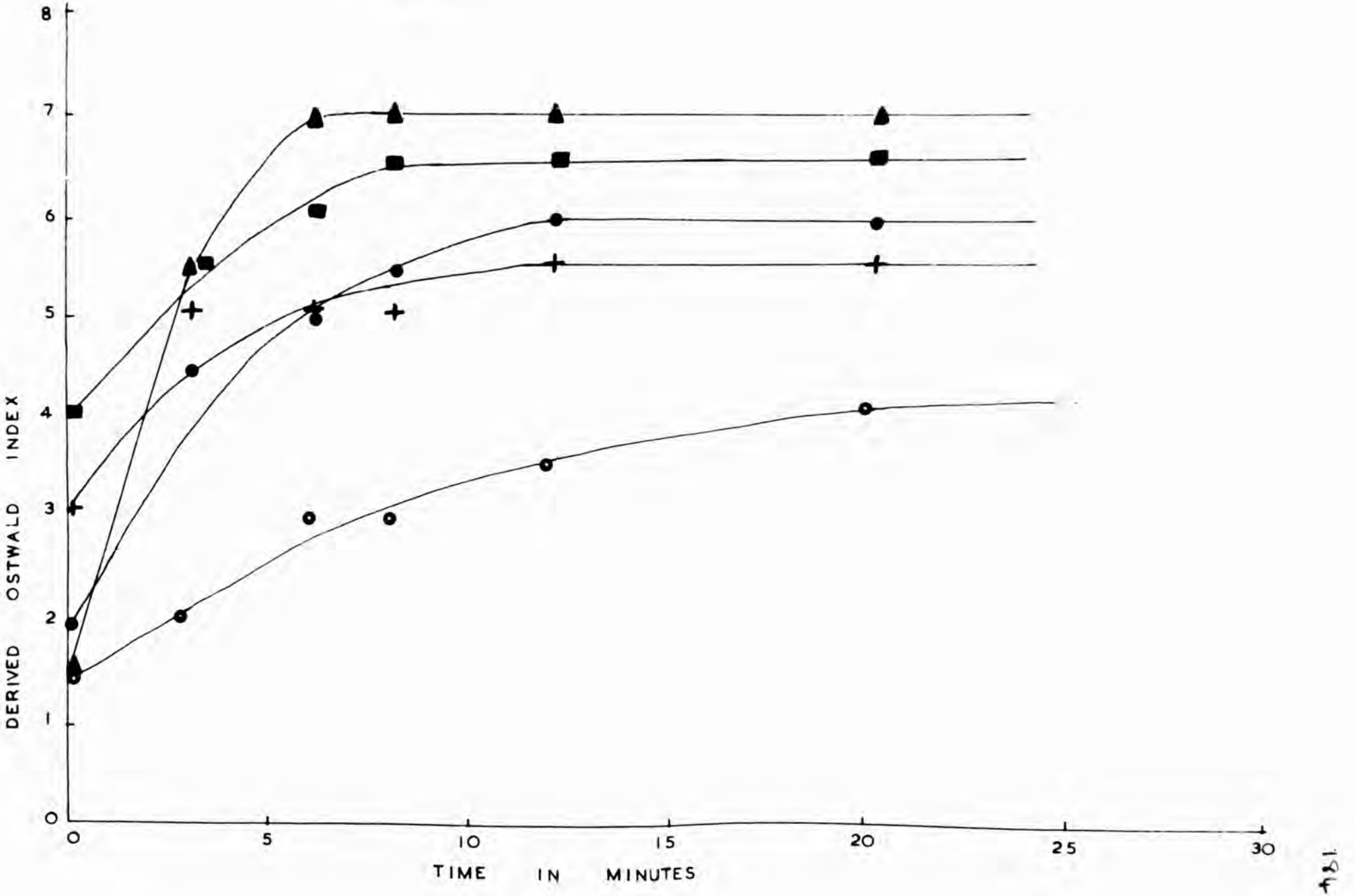

DERIVED OSTWALD INDEX
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
TIME IN MINUTES

Fig. 3 4


DERIVED OSTWALD INDEX
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
TIME IN MINUTES

184

With 70 the range is from 4 to 6.5 (91% tectum), 72 is from 3 to 5.5 (86% tectum), and 69 is from 1.5 to 4 (84%). These last figures approach the very pale state of the total tectal removals.

## VI. DISCUSSION.

(a) Fibre pathway between the retina, geniculate complex and the tectum.

Minnows change colour normally when blind on one side regardless of whether the blinding is accomplished by a removal of the optic tectum or by cutting the optic tract. In this respect the minnow differs from the trout which, according to von Frish (1911), when one eye is removed, goes dark on the opposite side of the body, a condition which he associated with the complete chiasma. Sumner (1911) reported in flatfish that the blinding of one eye resulted in the fish assuming an intermeditate shade on a white background.

Following the removal of the optic tectum, the fish went pale, as reported by Dijgraaf (1949), but unlike the fish used by Dijgraaf they could not perform colour change. The fish without tectum did not show any ability to adapt to its background, from which it must be concluded that the tectum is essential for chromatic adaptation. The observation by Wichers reported by Healey (1957) that the minnow with one eye removed and the optic tectum removed from the same side could

change colour was confirmed. Further observations of the preparation showed that this colour change was normal in rate and extent. The removal of the eye on one side and the tectum on the same side means that the fish is essentially blind in regard to the direct retino-tectal fibres. There is thus the problem that the fish cannot perform colour change without the tectum but that direct retino-tectal fibres are not involved. What is necessary is for some intermediate body to be present which could relay a large amount of information. From the anatomical study of the optic system in the minnow the only body which could perform this function is the geniculate complex. It is ideally suited to its function as it receives numerous connections from all parts of the retina and sends great numbers of considering fibres to the tectum. Thus it should be able to relay a considerable volume of information from the retina to the tectum. The geniculate has the size and complexity which would be necessary to convey all the information necessary.

Each geniculate complex is connected to that on the opposite side by means of a large fibre bundle, the commissure minor of Herrick. The presence of this large fibre bundle could explain how the fish with the optic tract cut on one side and the tectum removed from the same side can change colour normally, the information being relayed through it to the geniculate complex on the opposite side, and hence to the tectum. It is also possible that the fibres from one geniculate are relayed directly to the tectum on the other side by means of the transverse commissure which contains fibres from the geniculate and gives fibres

to the optic tectum.

The proposal that the geniculate complex in fish plays a rôle in the perception of light intensity has never before been made and, in fact, no specific function has been suggested for the teleostean geniculate. The present experiment would appear to establish this and so raise interesting problems of homology. Franz (1912) reported that the geniculate necleus of fish is the homologue of the mamallian corpus geniculatum lateralis, but Schnitzlein (1959) considers that this remains open to question. Functionally the geniculate complex in fish presumably serves the same function in relation to light intensity as the mammallian corpus geniculatum lateralis, and it may well be that the geniculate has similar functions throughout the whole vertebrate series. This would suggest that this arrangement is very basic to the mechanism of vision and that the nucleus geniculatum lateralis is directly homolgous throughout the vertebrate series.

(b) The possible mode of action of the geniculate complex.

Most of the present work concerns the mode of action of the tectum with regard to colour change and, in this connection, one may briefly consider the possible mode of action of the geniculate complex. Granit (1955), mainly from work on the frog, proposed that in view of the lack of a one-to-one correspondence between the discharge characteristics of individual afferents and psychophysical brightness function,

that the perception of brightness is probably based on a statistical average of the frequency responses of a whole assembly of visual fibres. This frequency analysis has been investigated on the cat by a series of experiments by Arduini & Pinneo (1962 a, b, 1963 a, b). These two investigators used very large electrodes and recorded from the optic tract, optic chiasma and geniculate nucleus, and by a mathematical analysis of the results gave figures for the general tonic activity of a large number of units. They proposed that in the retina, in a steady state of dark or light adaptation, all types of inhibitory and excitatory units fire randomly. The level of tonic activity represents a measure of balance between the two antagonistic systems. When the level is high a greater number of random excitatory interactions take place than random inhibitory reactions. The effect of the steady light upon the retina will depend upon the relative balance between the two systems. This complex excitatory-inhibitory system proposed by Arduini & Pinneo may well explain the failure of the ration hypothysis when applied in any rigid manner to colour change (p. 98 ). Arduini & Pinneo (1963 a) further studied the geniculate nucleus in relation to changes in illumination of the retina and found that the level of activity in the lateral geniculate nucleus was inversly related to the level of illumination. They went on to state that it is the number of impulses per unit time which characterizes the activity during maintained states.

Brooks (1966) considers that a working hypothesis is that the

amount of impulse activity in the visual system as a whole is associated with the perception of diffuse brightness and brightness differences.

De Valous, Jacobs & Jones (1962) and Jacobs (1966) have approached the problem from a different angle and have studied the recordings from the activity of the single cells of the lateral geniculate nucleus of the monkey. De Valous et al. (1962) found that the neurones discharged periodically under both conditions of light and dark adaptation. Brief intensity changes in one direction produce either a decrease or increase in the firing rate, depending on the direction, and this increase is proportional to the log. of the intensity. Jacobs (1966) proposes that the steady-state firing of the excitatory cells and the inhibitory cells of the geniculate nucleus provide the information as to the terminal brightness function. This has been taken further by the work of Maffei & Rizzolatti (1967) who have speculated that the operation of integration observed at the geniculate level could be due to the geniculate synapse acting as a decoding device.

It would appear therefore that the geniculate receives information as to the brightness of objects by means of the overall activity of the retinal output, and the geniculate indexes the brightness input in terms of the firing rate of the excitatory and inhibitory neurones, as indicated by their tonic activity. The function of the geniculate nucleus would be to decode this tonic activity from the retina and relay the

integrated function to the other brain centres. What relationship these findings have to teleost fish remains to be determined but the work on recordings from the tectum, Section 8 (p. 214) indicates that the problem of steady state brightness perception is dealt with in a similar manner; i.e. in terms of tonic activity as in mammals. It is also interesting to note that the overall retinal output of the minnow is important for the correct interpretation of the background, and this would further suggest that the mechanism may well be similar to that in mammals.

(c) Colour change and light intensity

In many of the observations of the fish under different light intensities the animals would tend to darken in the lower intensities and lighten in the higher intensities. This was discussed on page 176 , and is not due to the background but due to the overall light intensity. Healey (1945) has reported that minnows show a primary local darkening response to increase in the light intensity. This can be shown in small areas of skin. The results from the minnow are therefore opposite to those associated with a primary response. The answer may lie in the functioning of the pineal organ. Von Frisch (1911) first reported that this could affect the colour of minnows but the work of Healey has shown that this is very variable in minnows from different sources. The fish used in these experiments did not show any pineal activity with regard to darkening on illumination, and blind

fish did not show any change of colour on being placed in darkness. This leads to two conclusions: either the pineal is not active or its activity is reduced and it is completely dominated by the tectum. The second of these possibilities goes against the behaviour of the minnows with tectal removal, for in this case, illumination causes paling.

(d) The possible mode of action of the tectum: relations with the medullary paling centre.

Blinding affects the colour of the minnow in different ways depending on the method of blinding. The fish darkens if the blinding is by cutting the optic tracts but lightens if the blinding is accomplished by the complete removal of the tectum. The colour both after section of the optic tract and after the tectal removal is highly variable, but the fish without tectum never shows the extremes found in the fish with optic nerve section. Von Frisch (1911) found that section of the brain above the level of the medulla always resulted in paling and he concluded that there was a paling centre present in the medulla which worked through the automonic nervous system. The removal of the tectum has the same final effect as the cutting of the brain above the medulla in causing paling. One could therefore think that the tectum can act on the medulla inhibiting it, with resulting darkening. The paling of a blind or normal fish following tectal removal is not at the same rate as the paling to a white background and requires more than 2 hours. This pallor following tectal removal is not maximal

and is not maintained for periods of longer than a few hours, and by 24 hours the fish has darkened noticeably. From these considerations it would appear that the tectum not only inhibits the paling centre but also excites it, and that the chromatic adaptation of the fish is due to the excitatory-inhibitory action of the tectum on the medullary centre. In a group of fish which are blinded by the cutting of the optic tract, there are extremes of colour (p. 139 ) and these would, on the present argument, be due to the action of the tectum and not to that of the medullary centre.

The darkening which occurs in the fish with complete tectal removal may well be due to the medullary centre no longer functioning and no longer maintaining the pale state. This begins on the first day after the removal of the tectum but is highly variable and many fish still maintain an intermediate colour. It would therefore appear that not only does the tectum stimulate the medulla but that this stimulation is necessary for the continued activity of the centre.

Healey (1951, 1954) has shown that in the absence of nervous control of the chromatophores produced by spinal section the fish could still change colour by means of hormones. No hormonal colour change was seen in the fish with the tectum removed, so that the tectum appears to control hormonal colour change as well as the nervous colour change. There are numerous fibre connections between the tectum and the

hypothalamus and preoptic nucleus which could result in the control of the hormones released from the pituitary.

The removal of the tectum in blind minnows leads to paling because of the freeing of the medullary centre from the control by the tectum. This would imply that in the blinded state the tectum completely inhibits the centre. It has been found that this inhibition is not removed by the removal of one lobe of the tectum which means that the tectum on one side can control the medullary centre on both sides. How this could come about remains to be determined and no evidence for the fibre connections ~~can~~ could be found, (Section 7 p. 199). The inhibition cannot be removed by the removal of the anterior region of the tectum in both lobes but it is removed by the removal of the posterior tectum on both lobes. This inhibitory region has been further localised by a series of lesion experiments (fig. 29 p. 157) to a small region in the dorsal posterior tectum. The question arises as to whether this tectal region represents a definite medullary controlling region, or whether the removals and lesions have destroyed the fibre connections as they pass out of the tectum. Most of the evidence points to the conclusion that the pallor is due to an interruption of the fibre connections comming from the tectum to the medulla. Firstly, in view of the results of the tectal removal and the varied degrees of colour obtained, the pallor from lesions X0 Y1mm and X0 Y1.33 could be regarded as almost maximal pallor, because it falls well within the variation found in the normal

fish. In view of the results of the retinal experiments where the whole of the retina was important in chromatic adaptation and lesions had to be over a critical size before they affected colour change in the normal fish, it seems most unlikely that the single tectal lesion could have such a dramatic effect on the paling centre if this region did not in fact control the centre. It seems more likely that these single lesions did destroy the fibre pathway as it leaves the tectum to run to the medulla. Removal of the anterior tectum resulted in pallor in the normal fish similar to that following posterior removals. If the dorsal posterior region is the active inhibitory region then there should be darkening when the anterior tectum is removed for this would result in a similar condition to blinding by optic nerve section in which the posterior tectum is without any sensory input from the eye. This leads to the conclusion that the initiation of the darkening in the blinded fish depends upon the whole of the tectum, but that its continued maintainence only needs the posterior dorsal part of the tectum. Therefore, to inhibit the paling centre the initial inhibition needs to be very large, but once this has occurred the paling centre no longer fires at the same rate and the continued maintenence of the darkening only requires very little tectal reinforcement.

The final piece of evidence to indicate that it is the destruction of fibre connections which is important rather than the destruc-

tion of an active region in the tectum is shown by the two fish with posterior removals, 36 and 37. The active region was present but the fish could not perform colour change. In these fish the fibres to the medulla had been destroyed. In conclusion, it seems that the lesions which produce paling in blind fish do so by destroying the fibres which inhibit the medullary centre, and do not damage a definite darkening centre in the tectum.

Very little can be deduced about the activity within tectum in response to background tint, but would appear that at least one lobe is necessary. The lobe need not be intact, and combinations of the remainder of two damaged lobes was also effective. In the fish with the anterior tectum removed from one lobe and the posterior from the other, at least two of the fish, 75 and 74, could adapt normally.

The removal of the anterior and posterior tectum in both lobes resulted in paling, indicating that the anterior tectum has no separate connections with the chromatophore system and that the posterior tectum does not influence the paling centre in the absence of the total anterior tectum in the normal fish.

These results lead to the general conclusion that the tectum acts as a single unit as regards colour change, and that both the anterior and the posterior parts of the tectum are necessary.

The smaller tectal removal did yield some interesting results. Fish 71 showed normal colour change and fish 68 was almost normal change excepting that it did not pale fully (p. 191 ). Fish 70 could only reach a value of 4 and could not pale further, and 72 with only 84% tectum could only adapt from 5 to 3. In all cases the rate of change was normal and only the extent was defective. The result from 72 corresponds fairly well with the results from the retinal removals R28 and R29. (p. 80 ). Fish 69 shows the tendency to be unable to darken and ranges from 1.5 to 4 which agrees well with the retinal removal fish R7. The results for 72 and 69 may be due to the fact that there was extensive tectal removal analogous to the anterior removals. However, the fish still retained a limited ability to change colour. The other explanation is that these results are due solely to the removal of the particular retinal field from the tectum. The extent of the removal of the tectum does not compare with that of the retina removed to obtain the same result, but this may be due to the destruction of the fibres to other parts of the tectum. In fish 70 the area of tectum present was 91%. The area of retina removed in R31, with a similar pattern of background response as 70, was in excess of 50%. In the case of 70 the tectum was not destroyed in the midline, and hence the area placed out of action was not much in excess of that shown (fig. 31 h p. 171 ). It would appear that, like the retina, the tectum can sustain slight damage without any impairment of the ability of the fish to adapt to its background, but that when damage exceeds the order

of 9% of the tectum the animal can no longer adapt. The more this threshold is exceeded the less able is the fish to adapt until it finally assumes the shade of a total tectal removed fish at 74% tectum present.

Further consideration of this work will be presented in the general conclusion to Section 9 (p. 263).

## S E C T I O N 7 : THE MEDULLARY CENTRE

The results from the lesion experiments on the tectum demonstrated that a single lesion could produce almost complete pallor in a blind fish, and it has been reasoned (p.194) that this lesion destroyed the nerves controlling colour change as they pass out of the tectum. Degeneration studies were then performed to trace the fibres destroyed by this lesion to the other centres in the brain.

### I. METHOD

10 normal minnows were taken and lesions were placed in the tectum in position X0,Y1mm as described on page 154. After cementing over the wound the fish were placed in experimental aquaria. They were kept at 18°C and two fish were removed on the 10th, 16th, 19th and 21st days after operation. The fish were killed by decapitation and fixed in formal-saline for a period of 5 weeks. The brains were dissected out, embedded, and cut transversly, and longitudinally (dorso-ventrally) for each survival time. They were then stained using the paraffin nauta, and in many cases counter stained using C.F.V.

Fig. 36

Photograph of a kidney with a tocal lesion and the wound cemented over.

Fig. 37

Section of a 16-day tocal lesion showing the giant cells filling the lesion. Stained with Kuntz and Orcayl Post Violet and sectioned at 8µ .

Fig. 36

Photograph of a minnow with a tectal lesion and the wound cemented over.

Fig. 37

Section of a 16-day tectal lesion showing the glial cells filling the lesion. Stained with Nauta and Cresyl Fast Violet and sectioned at 8μ .

Fig. 36

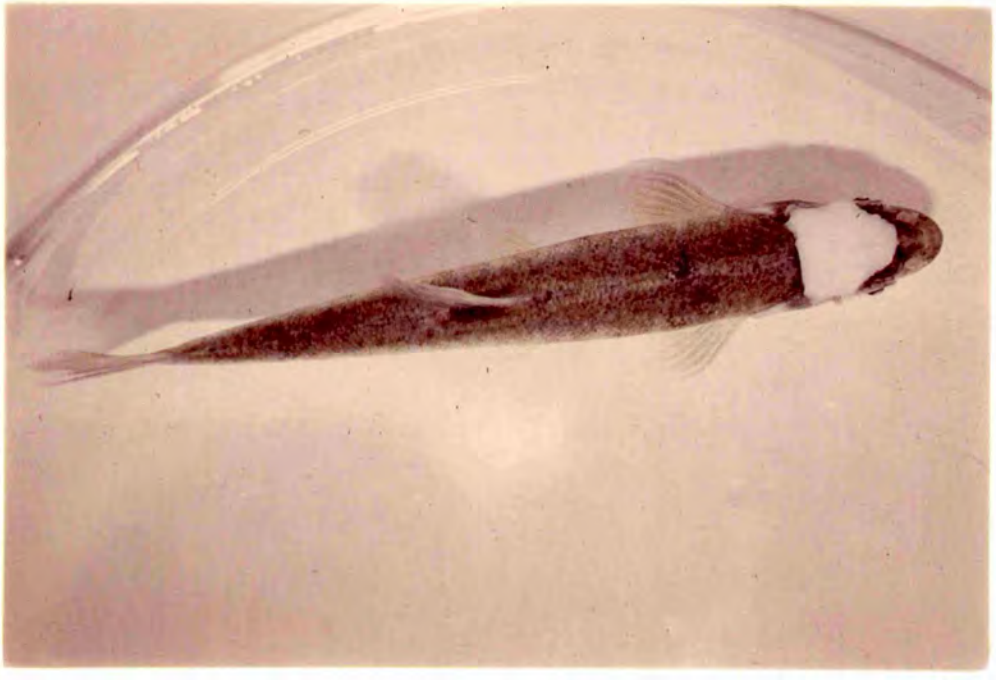
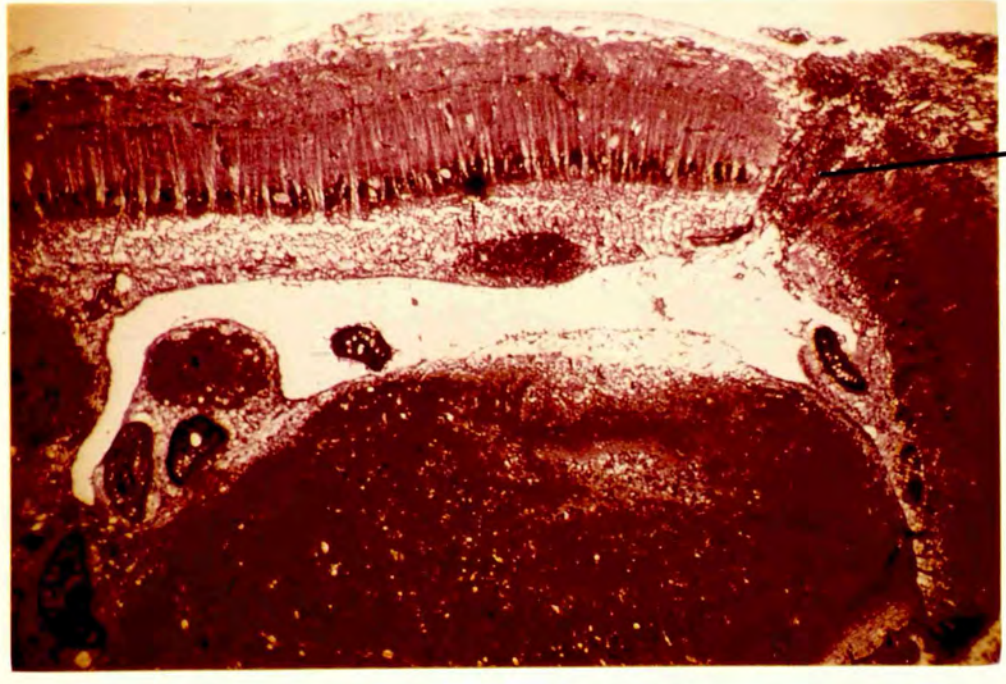


Fig. 37



The optimum survival time for the tectal fibres was 16 days, slightly longer than for the optic tract fibres.

## II. RESULTS

In a few cases the lesion spread and became quite large but in the majority of cases the lesion was discrete and there was complete wound healing. In fig. 37 (p. 201) the lesion can be clearly seen to be full of dividing glial cells. Only the fish with discrete lesions were used in the histological investigation of the degeneration of the fibre tracts.

Considerable degeneration was seen to occur in both the stratum plexiforme et fibrosum externum and the stratum fibrosum profundum throughout the tectum. A distinct bundle of fibres was observed to run out of the tectum laterally and then ventrally through the torus semicircularis to become part of the tractus tectobulbaris ventralis rectus (Kappers et al 1936) (fig. 38 p. 205). Isolated fibres were also found to run anteriorly to the geniculate complex, and to the preoptic nucleus (the tractus tecto-preopticus). A few fibres were also observed running to the hypothalamus. These fibres to the hypothalamus and preoptic nucleus may or may not be important in hormonal colour change. A few fibres were also noticed to run to the ganglion isthmi. These

isolated fibres were however only very few and the vast majority were seen in the bundle of the tractus tect-bulbaris ventralis rectus.

The degenerating fibre bundle is not clear in transverse section but in longitudinal section it appears to course caudally with the rest of the tecto-bulbar tract until it reaches a point at the anterior end of the medulla. A few of the fibres do not end here but continue through the medulla and are seen in the spinal cord constituting part of the tractus tecto-spinalis. Some of the fibres do not reach the spinal cord but end in the medulla, where they could not be clearly seen to enter any definite neuron group. However, the majority of degenerating fibres end in a small group of neurones. This small group of neurones contains both large and small cells, there being approximately 14 large and 100 small. The neurone group is shown in fig.39 (p. 207) together with the degenerating fibres. Both the large and small neurones are arranged along the entire length of the centre and there does not appear to be any definative organisation. The approximate position of this group of neurones is 1.4mm from the apex of the cerebellum caudally, 2.75mm. ventrally from the upper surface of the cerebellum and 1mm from the midline in a 6.5cm fish. Its structure is shown in figs. 40, 41 (p. 209, 211).

Fig. 38

Section of the brain at the level of the apex of the cerebellum stained with Nolsen technique.

Abbreviations:

T.L. - Tenia longitudinalis

V.C. - Velvula cerebelli

T. - Optic tectum

T. S. - Tenia semicircularis

Tr.I.T. - Trectus lutimo-tectalis

Tr.T.S.V.R. - Trectus tecto-bulbaris ventralis rectus

Tr.T.I. - Trectus tecto-lateralis

G.I. - Ganglion latimi

F.L.L. - Fasciculus longitudinalis lateralis

F.L.M. - Fasciculus longitudinalis medialis

L.I. - Lobi inferiores

Tr.T.S. - Trectus tecto-spinalis

Tr.T.B.C. - Trectus tecto-bulbaris cruciatus

Tr.M.C.P. - Trectus mesencephalo-cerebellaris posterior.

Fig. 38

Section of the brain at the level of the apex of the cerebellum stained with Holmes technique.

Abbreviations:

T.L. - Torus longitudinalis

V.C. - Valvula cerebelli

T. - Optic tectum

T. S. - Torus semicircularis

Tr.I.T. - Tractus isthmo-tectalis

Tr.T.B.V.R. - Tractus tecto-bulbaris ventralis rectus

Tr.T.I. - Tractus tecto-isthmalis

G.I. - Ganglion isthmi

F.L.L. - Fasciculus longitudinalis lateralis

F.L.M. - Fascisulus longitudinalis medialis

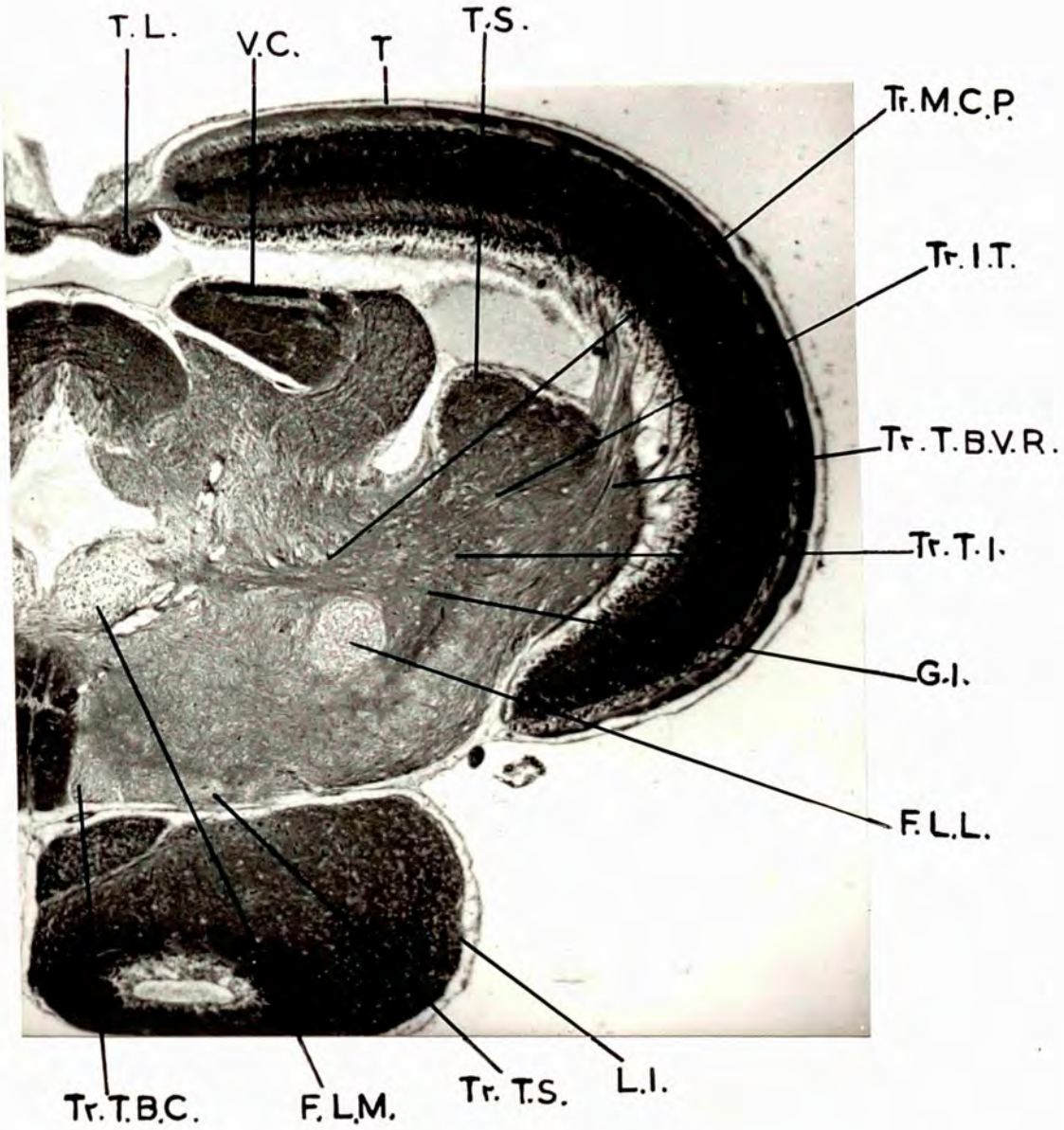
L.I. - Lobi inferiores

Tr.T.S. - Tractus tecto-spinalis

Tr.T.B.C. - Tractus tecto-bulbaris cruciatus

Tr.M.C.P. - Tractus mesencephalo-cerebellaris posterior.

Fig. 38




0·5 mm

Fig. 39

Vertical longitudinal section of the anterior medulla stained with the Nauta and counter stained with C.F.V.

Abbreviations:

C. - Cerebellum

V.L. - Vagal lobe of the medulla

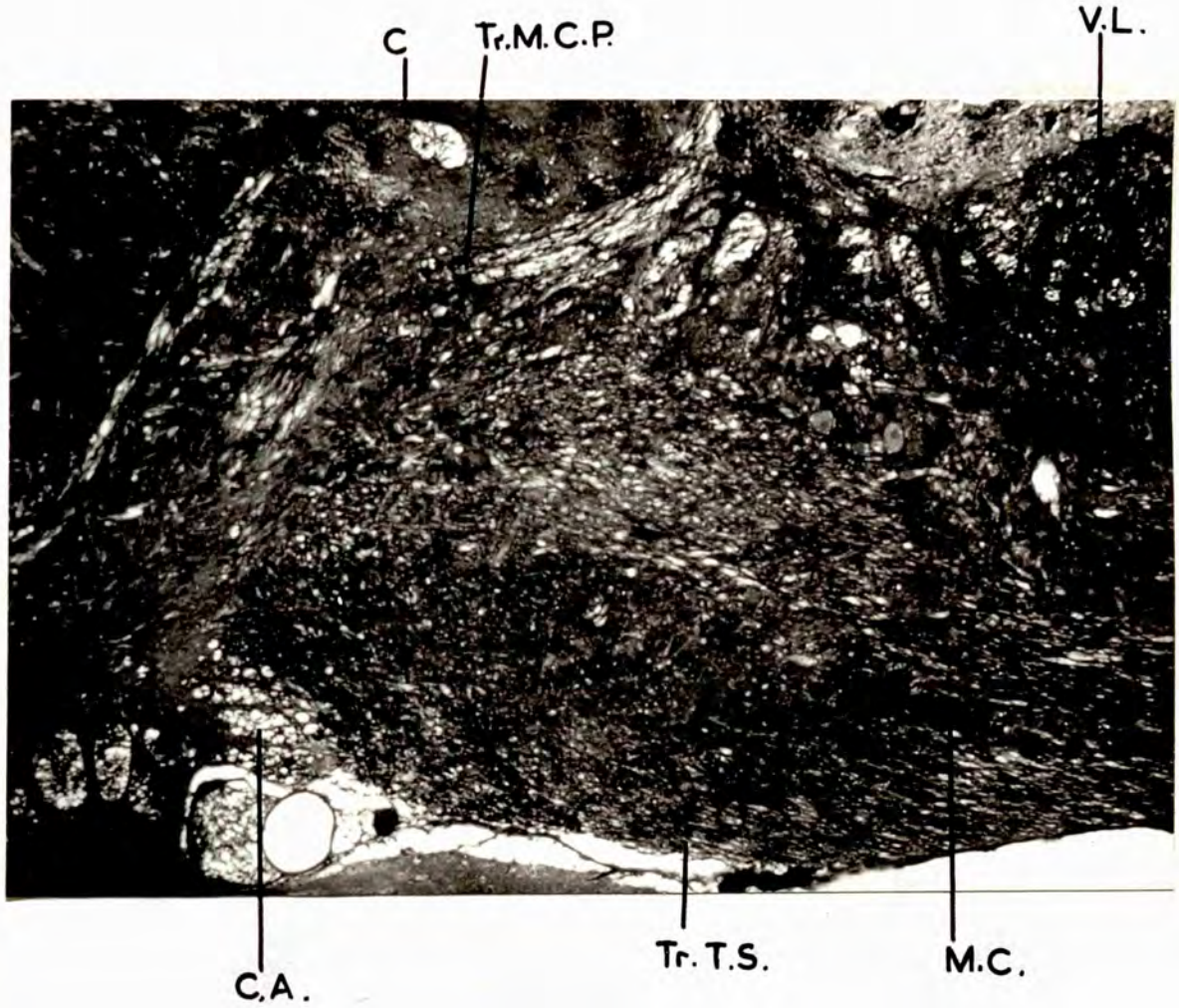
C.A. - Commissure ansulata

Tr.T.S. - Tractus tecto-spinalis

M.C. - Medullary paling centre.

Tr. M.C.P. - Tractus mesencephalo-cerebellaris posterior.

Fig. 39




0·5 mm

Fig. 4 D

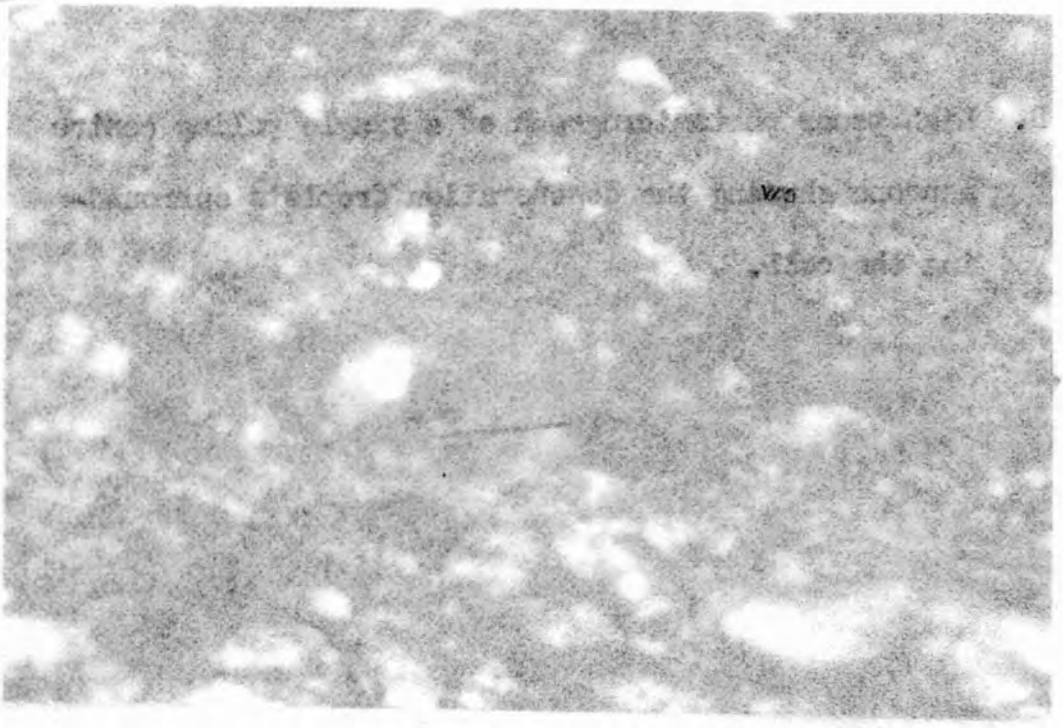
A

Degenerating fibres

Large neurone of paling centre

0 05mm

B



Degeneration droplets

0012mm

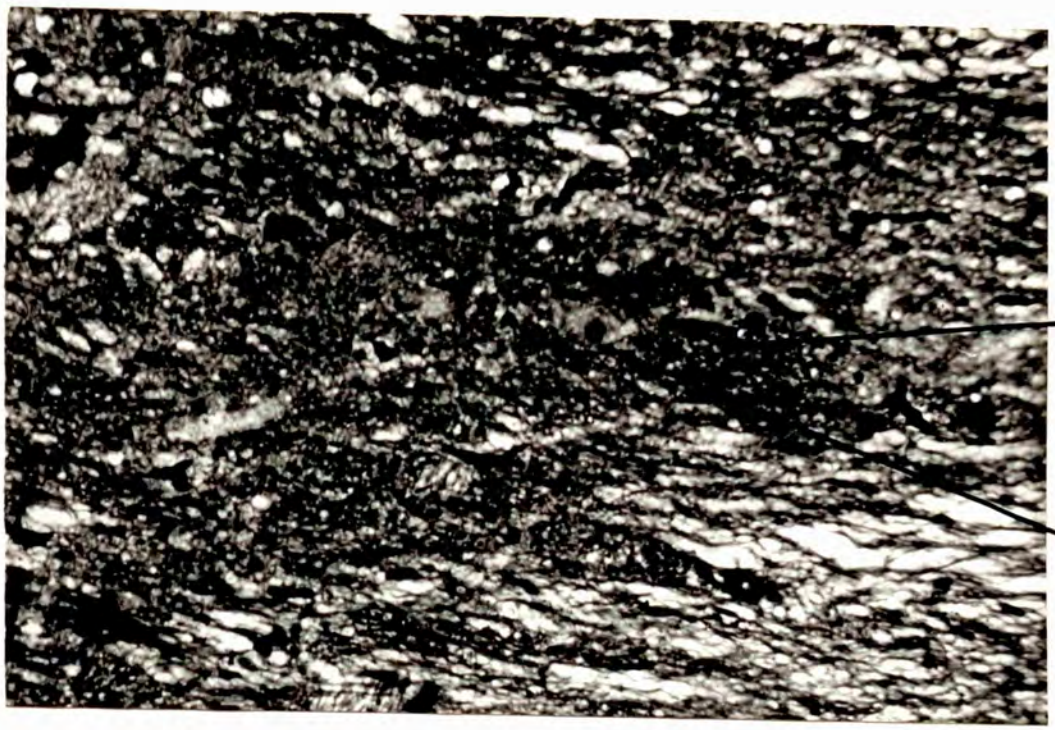
Fig. 40

A. Longitudinal section through the medullary paling centre.
Stained with Nauta and C.F.V.

B. High power photomicrograph of a single paling centre neurone showing the degeneration droplets surrounding the cell.

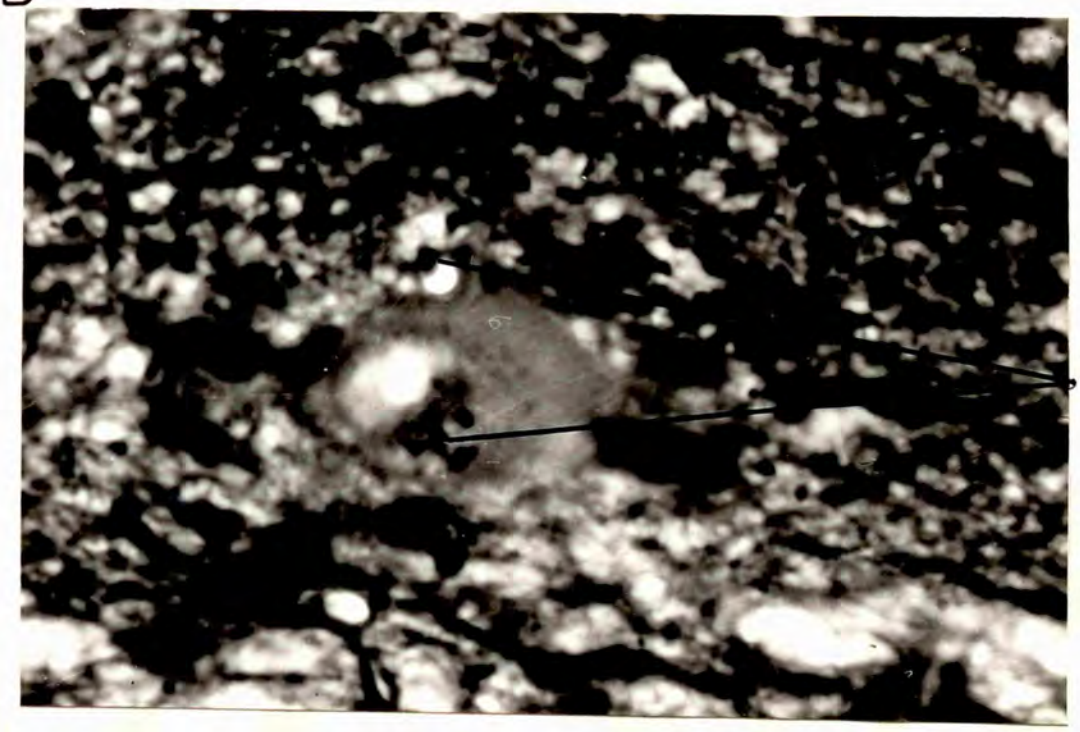
Fig. 4 D

A




0·05mm

B




0·012 mm

Fig. 41

A

B



C



D

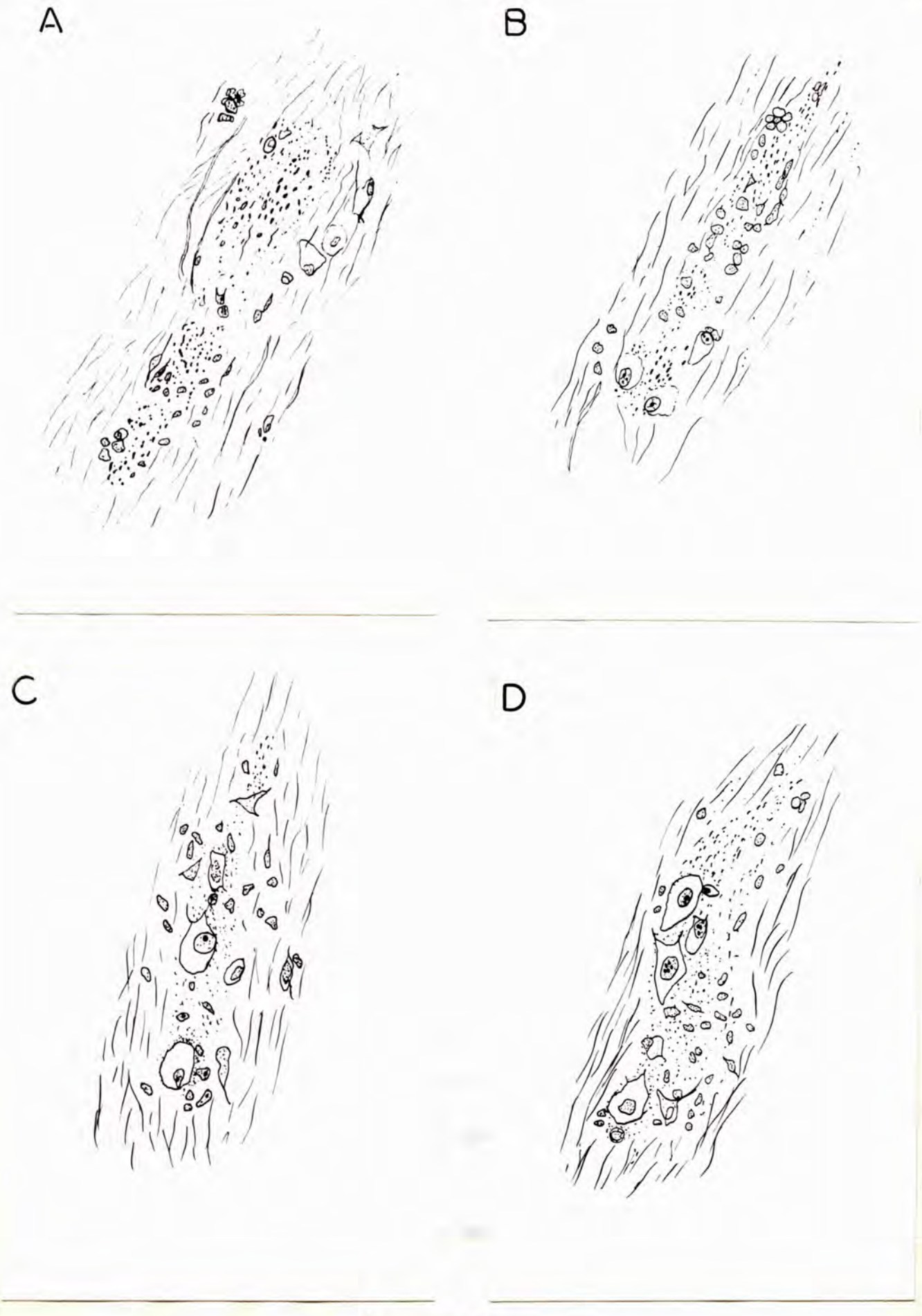
Fig. 41

A series of drawing through the medullary centre to show the cells and the distribution of the degeneration droplets.

'A' is the most lateral of the drawings. 'B' is 32 μ medial to A and 'C' is 32 μ medial to B. 'D' is 16 μ medial to 'C' and no structures were visable 16 μ medial to 'D'.

Fig. 41





## III. DISCUSSION

Although these degenerating fibres and associated group of neurones were found, it is not certain that the latter constitute the medullary paling centre. Further experiments need to be carried out to prove conclusively that it is the colour change control centre of the medulla. The evidence that this can be provisionally accepted as the centre is threefold. Firstly, von Frisch(1911) found that cutting the brain anterior to the medulla resulted in pallor and that stimulation of the medulla electrically resulted in paling. He therefore postulated an "Aufhellungzentrum" that induced the fish to pale. The work presented in this thesis has shown that the removal of the tectum has the same effect as von Frisch's cut anterior to the medulla and that in normal colour change the optic tectum appears to control the medullary centre. It has also been shown that a small lesion in the tectum in position XOY1mm will remove the control of the tectum on the medullary centre. Secondly, the fibre bundle from this region passes to a discrete region of the anterior medulla, and terminates in a small group of neurons. Thirdly, fibres from the centre run into the spinal cord. This neurone group has so far not been identified and not related to any other brain structure; it seems likely that it is the medullary paling centre.

In order to provide conclusive evidence two types of experiment

are necessary.

1. To show that darkening follows the destruction of these specific cells but not following the destruction of neighbouring nervous tissue.

2. To take extracellular recordings from the whole group of cells.

The possible mode of action of the centre will be discussed in Section 9 (p. 264).

## S E C T I O N 8 : ELECTRICAL ACTIVITY IN THE TECTUM

### I. METHODS AND APPARATUS.

Two tungsten electrodes were placed on or in the tectum and the electrical activity was recorded between them. A third reference electrode, which was made of silver, was placed on the dorsal skin about two thirds along the body from the brain. The tungsten electrodes were made from 0.001 in (25 $\mu$) diameter wire. A small piece of wire about 1cm. long was cut from the roll, cleaned using teepol, dried, and attached to the end of an eight inch piece of minature, P.V.C. insulated, copper wire (Radiospares LTD). The P.V.C. was cleaned off the last 4mm of the wire and the attachement was made by forcing the P.V.C. covering apart with a fine pin. When the pin was withdrawn the streched P.V.C. did not return to its original position for a short time and the tungsten wire could then be inserted into the hole made by the pin. The P.V.C. finally closed and this fixed the tungsten. The union was completed by soldering the protruding copper to the tungsten.

The tungsten electrode, and the soldered tungsten/copper

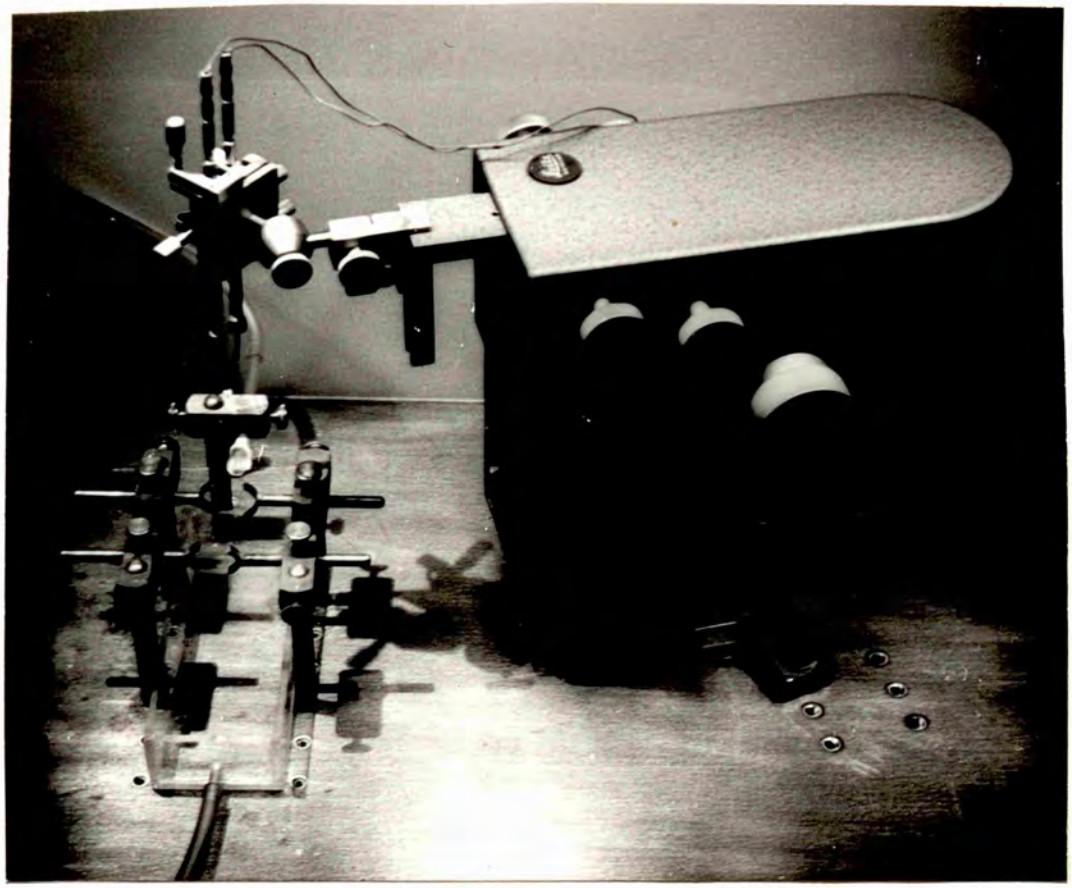
joint were insulated by dipping them in Araldite PZ820 epoxy resin with hardener and cured at 100°C. When the Araldite was dry they were dipped again and re-cured. To test the insulation the electrode was attached to the negative terminal of a 1.5v dry cell battery and immersed in 10% hydrochloric acid; the positive terminal of the battery was connected to a silver electrode and placed in the acid. Bubbling with the release of hydrogen will occur at the uninsulated parts of the electrode. The electrodes were then washed and dried. Using a pair of fine scissors the wire was cut so that the length of tungsten protruding was only 1mm. Using this method, straight electrodes, 1mm long and insulated except for the very tip could be produced in very large numbers without variation in shape or size.

The resistance of these tungsten electrodes was very low and was measured by a very simple technique using a Grass SD5 stimulator, which gave a square wave pulse, and an Oscilloscope. The apparatus was arranged as in the diagram fig. 44 (p. 220).

The resistance of the tungsten electrode is Re, and Ri that of the ringer, which is relatively so low that it can be neglected. R is a calibrated movable resistance made by using a series of known resistances. The height of the square wave on the oscilloscope, Vo, is when the key is open and hence only Re is in the circuit. Vi is the height, and hence the voltage, of the square wave when the key is closed and represents the voltage when both Re and R are in the circuit.

Fig. 42

APPARATUS USED TO POSITION THE ELECTRODES



Now applying Ohm's law to this condition we have:-

$$Re = R \frac{Vo - Vi}{Vi}$$

The resistances ranged from 3 to 4.7 K ohms, with a general mean of 3.64K. With these very low resistances the noise levels of the electrodes were very low and enabled recordings to be made at very high amplification.

The electrodes were positioned using a Leitz micromanipulator and the fish were fixed by two pairs of clamps which were attached to two rods, which were in turn fixed to the same base plate as the manipulator. The apparatus is shown on fig. 42 (p. 216).

The respiratory water was fed into the mouth directly, and was collected in a trough placed under the fish. The mouth piece consisted of a glass tube onto which was stuck acrylic cement modeled on a dead fish in order to firmly hold the head on three sides. These mouth pieces, when made, were polished with a dental drill, in order not to damage the fish. These mouth pieces prevented the head from moving when the fish was mounted in the apparatus. The fish was clamped by two pairs of clamps, one pair fitting at the level of the pectoral fin and the other at the level of the anal fin. When the fish were mounted it could not move and the opercular movements were prevented from affecting the whole head. A piece of filter paper placed on the fish between the clamps served both to collect the water as it

passed out of the opercular cavity, and to keep the fish moist.

Many of the records were made with the fish clamped. The animals did not appear to be distressed when not anaesthetised. In many of the experiments the fish had the electrodes cemented on the skull after being embedded in the brain. The method of drying the skull was that described on page 40 . The general grid using the eyepiece graticule in a binocular microscope, as described on page 154, was used to place the electrodes, and the depth could be measured directly from the manipulator. After the cement had dried the fish was removed from the apparatus and great care had to be taken not to detach the implanted electrodes. The fish were then placed in the apparatus shown in fig.43 page 220, which enabled the background to be changed without disturbing the fish.

The apparatus consisted essentially of a rectangular box made of black perspex, (unshaded in the diagram). The fish was held by means of the tube carrying respiratory water into the mouth and two V-shaped pieces of clear perspex (A). This holding device was fixed onto another piece of clear perspex, C, which was raised from the floor of the box by ledges on three sides. Attached to the free edge of C was an upright plate of black perspex. 'B' was a piece of white perspex which could be slid between C and the bottom of the box E to present the fish with a white background. If B was slid out the fish was

Fig. 43

Diagram of the background reversal apparatus used to record the ERG of minnows with electrodes implanted in the tectum. The clear areas were made out of black paraper.

Fig. 44

Diagram of the circuit used to calculate the resistance of the electrodes.

Fig. 43

Diagram of the background reversal apparatus used to record the EEG of minnows with electrodes implanted in the tectum. The clear areas were made out of black perspex.

Fig. 44

Diagram of the circuit used to calculate the resistance of the electrodes.

Fig. 4 3

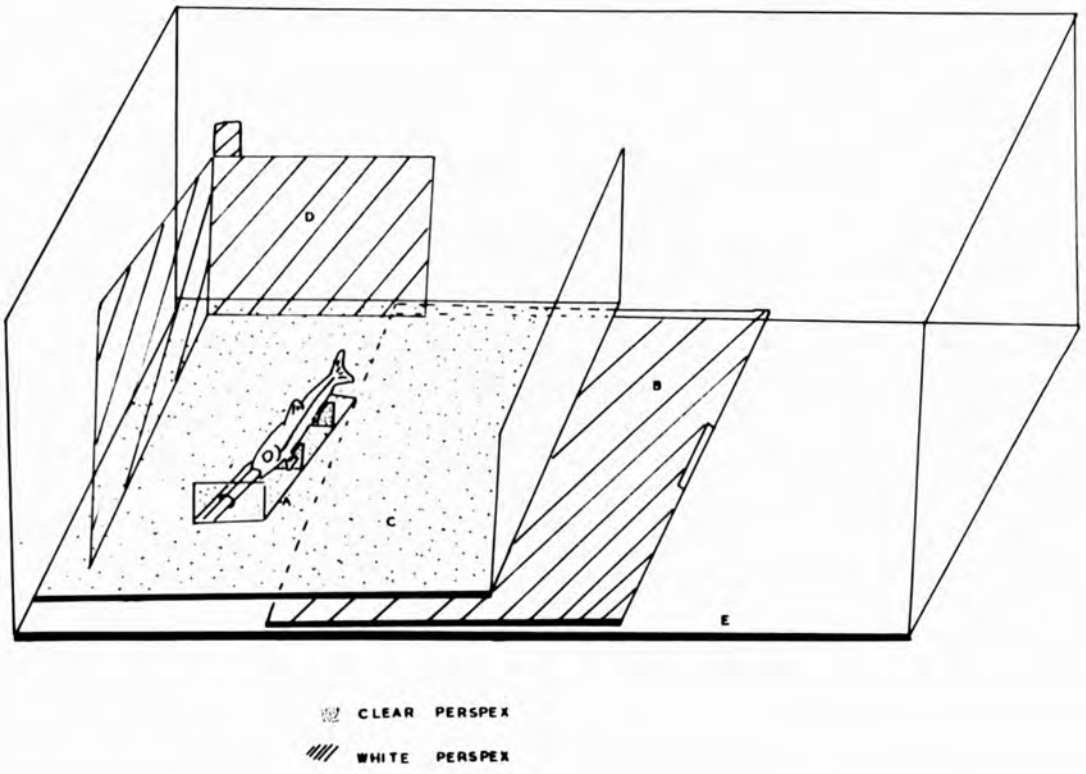
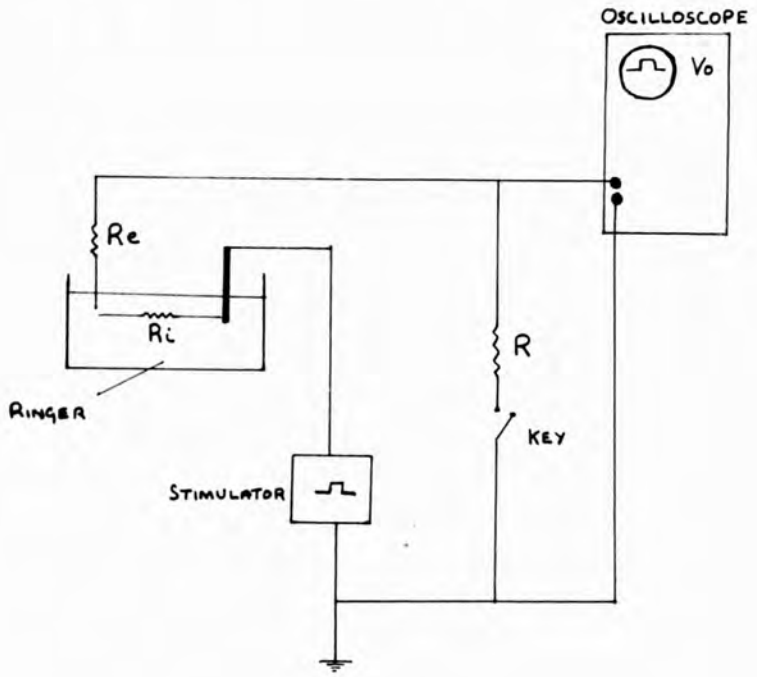

D
B
C
E
CLEAR PERSPEX
WHITE PERSPEX

Fig. 4 4


OSCILLOSCOPE
Vo
Re
Ri
RINGER
R
KEY
STIMULATOR

presented with a black background produced by E. Two plates of white perspex D were made so as to form the two sides of a box and when two of these were put together they formed the walls. When D was removed the sides of the black box formed the black walls.

Before the fish was allowed to recover from the anaesthetic, following the implantation, it was injected with turbocurarine chloride (Burroughs Wellcome). A number of preliminary tests were carried out to determine the most satisfactory concentration. Tested concentrations ranged from 0.33 to 0.083mg/100gm body weight. A concentration of 0.166mg/100gm body weight was found to be most satisfactory and this concentration inhibited all muscular movement except that of the heart and prevented the very strong opercular beat from masking the encephalogram pattern. The action of the curare on the colour change of the fish was complex. Turbocurarine chloride blocks the neuromuscular junction by competing for the receptor sites on the post synaptic membrane. The effect on colour change depends upon the dose and in the region of 0.083mg/100gm body weight the curare blocked the locomotory muscles but not the branchial muscles, there was normal opercular beat. In these fish colour change was normal in both extent and direction. However, if the dose was increased slightly the branchial muscles were affected and normal colour no longer occurred. The fish with the slightly higher dose assumed an intermediate tint, with the macromelanophores in the lateral streak dispersed. The action of the curare in this case is probably not on the chromatophores directly but on the sympathetic ganglia. It is also possible that the inability of the

fish to change colour following the injection of curare is due to a similar mechanism to the learned inhibition proposed by Gray (1956). The second important observation on the action of curare was that if it was injected intramuscularly into the dorsal myotomes in the tail just posterior to the anus the fish took about one minute to become paralysed. If the curare was injected more anteriorly, then the fish was affected more quickly. The mortality rate was higher the more anterior the injection. At the end of the experiment the fin of the fish was examined for circulating blood to confirm that the fish was alive.

The experiments were performed in an earthed cage measuring 2ft x 2ft x 3ft and made of perforated aluminium. The wires from the electrodes were fed into a Tektronix Type A Dual-Beam Oscilloscope. The picture from the oscilloscope screen was photographed using a Cossor camera loaded with Ilford Pan F negative film.

The high frequency of the preamplifier was fixed at an upper limit of 50cy/sec. because no recordable information could be found above this frequency that could be discerned above the electrode noise. The preamplifier was used at its maximum gain of 1000 X.

During the experiments the fish were fed with water directly into the mouth by means of a large 25L. container placed above the cage. In the experiments where the fish were contained in the background

reversal box not only was the water in the main storage container earthed, but also the water surrounding the fish, and the waste water container. To prevent earthing loops from being formed, and hence amplifying the 50cy/sec. mains interference, the most satisfactory condition was when the cage and the water surrounding the fish was earthed to the oscilloscope, and the fresh and waste water earthed to the water pipes of the room.

## II. THE SUPERFICIAL ENCEPHALOGRAM PATTERN

These recording were made with the fish clamped and no curare was used. The fish were not anaesthetised during the recordings. During the recordings the fish were in dimmed light and no attempt was made to control noise. Because of the situation of the apparatus the noise was almost limited to that made by the electronic apparatus used.

The electrodes were mounted in the manipulator and the tips were separated by 0.8mm; they were then lowered onto the tectum so that they just penetrated the surface. 8 fish were used, and the positions of the electrodes are shown in fig. 45 (p. 225). The recordings were made at 2.5cm per second.

The recordings are presented in fig. 46 (p. 227) and show what

Fig. 45

The positions used for recording surface encephalo-
grams.

Fig. 45

The positions used for recording surface encephalograms.

Fig. 45

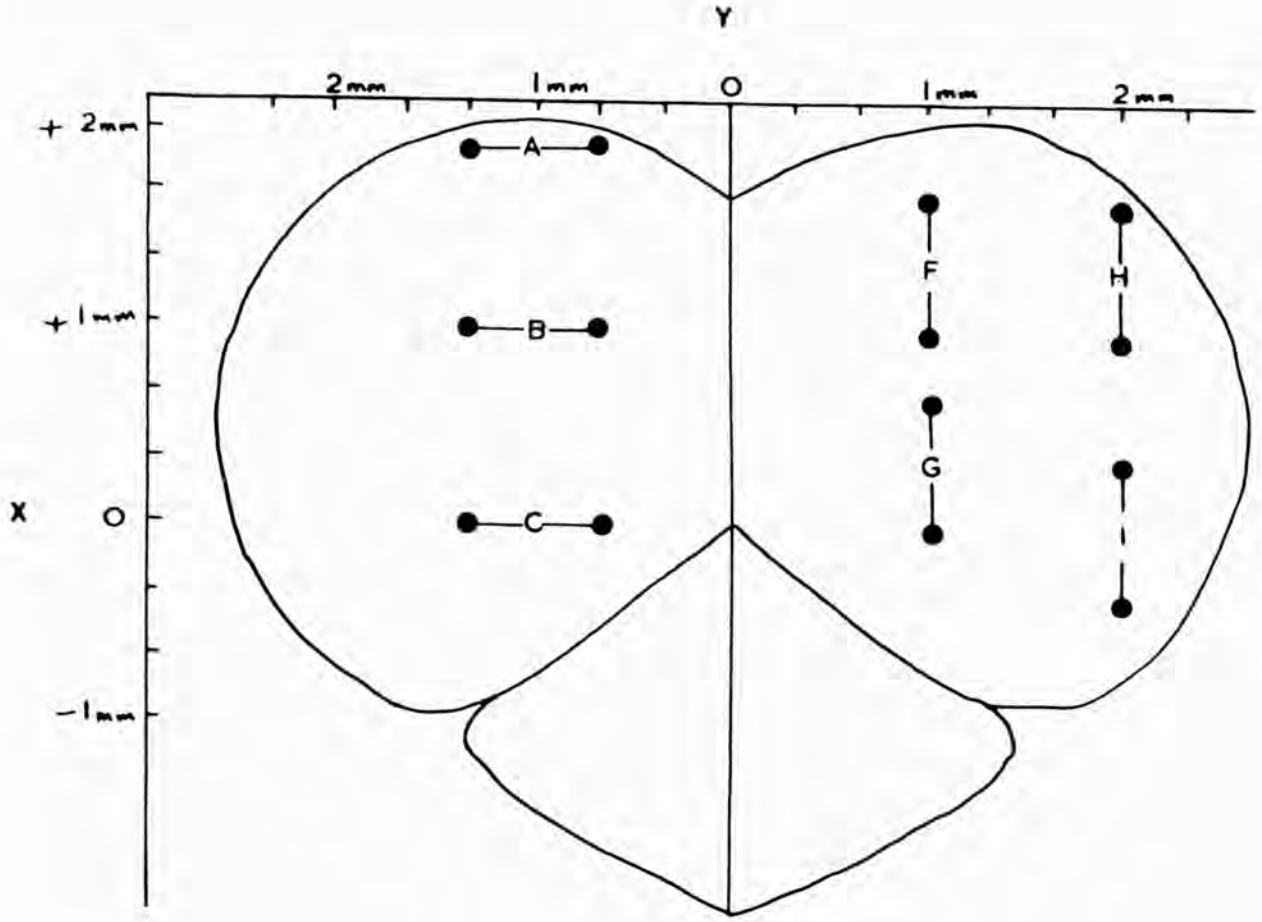
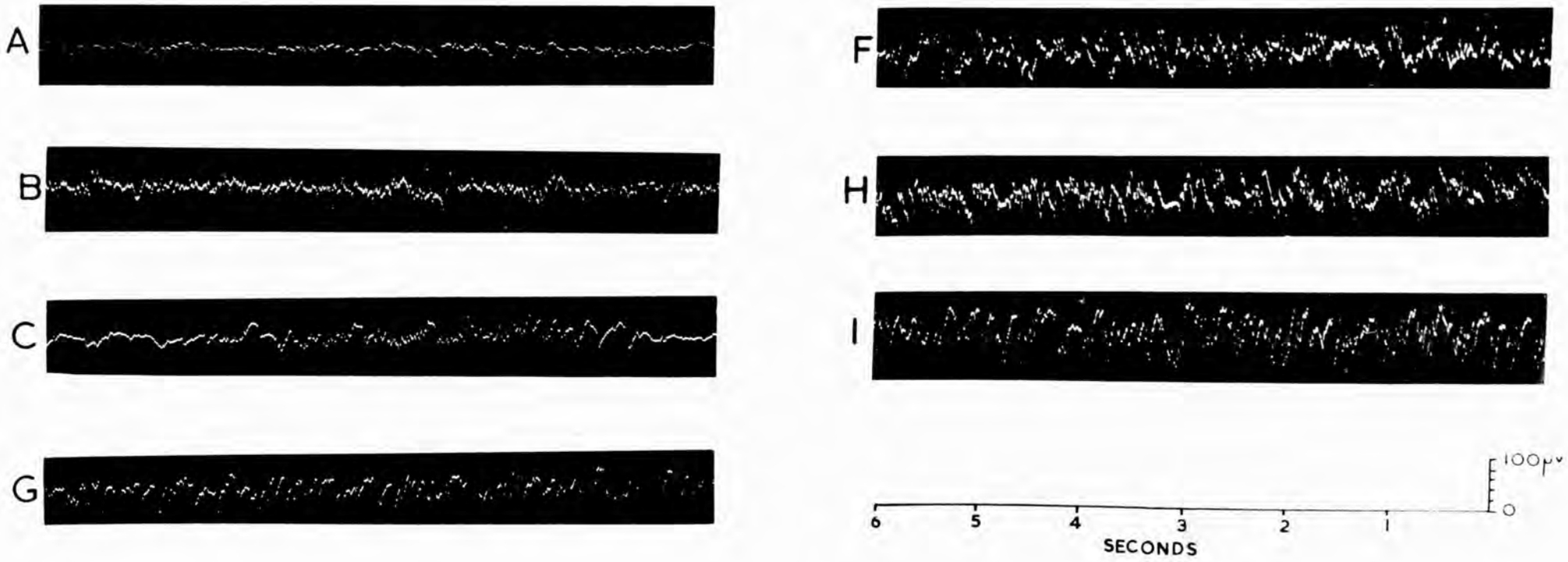


Fig. 46

Surface encephalograms recorded from positions A, B, C, G, E, H, I. (fig. 45 page 225).

Fig. 46

# NORMAL SURFACE ENCEPHALOGRAMS



appear to be two basic rythms, a slow rythm of 6-14cy/sec. and a faster rythm of 18-24cy/sec. The amplitude of the slower rythm was 112-20 v and that of the faster 13-6 v. The general pattern was synchronised over the whole of the tectum.

Not all the regions showed the same recordings, seen clearly in fig. 46 . The amplitude varied considerably over the tectum and, in general, the amplitude of the recordings when the electrodes were aligned antero-posteriorly was greater than when they were aligned laterally. This does not correspond to the position of the optic tract fibres, however, for in recording A the optic tract fibres are running directly anterio-posteriorly when the recordings are lateral. In recordings F and H the electrodes are along the main axes of the incoming nerve fibres, but in G and I the recordings were antero-posterior and the fibres were running laterally. The optic tract layer of the tectum is thickest in A and thinest in both B and C; similar conditions apply to the recordings in G and I.

It would therefore appear that the encephalogram pattern is primarily in the anterio-posterior axis.

A second difference was noticed in that the patterns from different regions of the tectum differ slightly in frequency. Position A shows a variation of 6-12cy/sec. in the LF, with a mean of 7.5, B

with a LF variation of 8-14cy/sec and a mean of 10.5, F with a variation of 6-14cy/sec. and a mean of 9.6, and G, H, and I with a variation of 8-11cy/sec. with a mean of 8.9. The HF rythm recordings show the same variation of 18-24cy/sec. but the means do not differ from each other very much, the values being: A 19.5, B 20.5, F 20.6 and G, H, I, 21 cy/sec.

Finally, in recording C a marked periodicity was found, with definite cycles of high and low activity. The LF and HF activity were both present but there were periods in the record where the HF was apparently absent, and only the LF was seen. These periods were very short.

To test whether the patterns recorded were from the fish and not artifacts, the fish were killed by turning off the water for at least 30 minutes. In all cases no activity could be recorded from these dead fish. On turning on the water supply there was still no recordable activity.

In conclusion, it appears that the activity recorded from the fish is produced by the fish and is in no way an artifact produced by the apparatus.

## III. THE ENCEPHALOGRAM PATTERN FOLLOWING ANAESTHETICS

The MS222 solution used to give deep anaesthesia very greatly reduced the amplitude of the EEG pattern. In all fish tested there was only present a small amount of very low level activity, which could not be discerned above the general noise level of the electrodes. When a recording was taken the fish was always dark because of the MS222.

## IV. THE EEG PATTERN IN DARKNESS

The fish was clamped throughout the experiment. Darkness was simulated by covering the eyes with a lightproof shield which was sufficiently extensive to cover not only the eye but also most of the side of the head. These shields were made out of a mixture of Acrylic cement and finely powdered charcoal. While the cement was still soft it was poured on to a dead fish which acted as a mould and then allowed to harden. When the cement was fully hard it was removed and could be attached to a live fish, providing the fish was of the same size. The shield fitted very well and, as far as could be ascertained, it covered the eye and was completely lightproof.

The results are from three fish and the recording position is

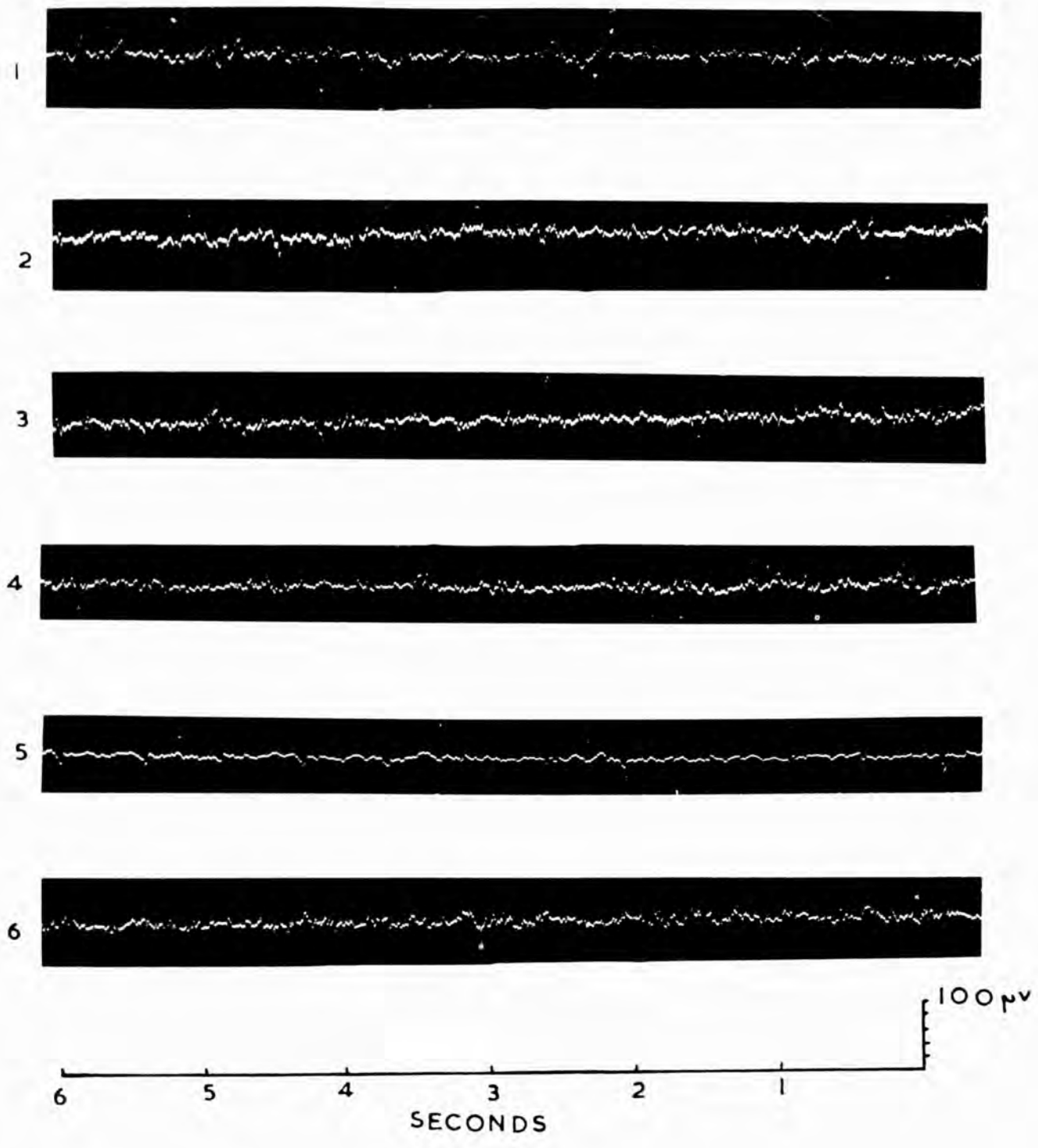
Fig. 47

The EEG pattern in light and darkness. Darkness was produced by covering the eye with a mixture of Acrylite cement and Charcoal.

Fig. 47

The EeG pattern in light and darkness. Darkness was produced by covering the eye with a mixture of Acrylic cement and Charcoal.

Fig. 47




1. IN LIGHT
2. TRANSFERED TO DARKNESS
3. DARKNESS 5 MINUTES
4. DARKNESS 15 MINUTES
5. DARKNESS 30 MINUTES
6. RETURNED TO LIGHT

always G, because it always gave good results. The first record was taken in the light. The eye was then covered and records were taken immediately after covering and 15mins. and 30 mins. after covering. The eye covers were then removed and recordings were taken. The results of one of the fish are shown on fig. 47 (p. 232).

The noticeable change in the patterns is in the amplitude. The initial effect of covering the eyes was that the amplitude increased to a higher level and that this persisted for 15 mins. The amplitude then dropped at the 30 min. record to a very low level and finally, when the covers were removed, the amplitude increased again, but not to the same level as that when the light was turned off.

The fish was fully white at the beginning of the period of the test when the eyes were not covered, but by the end of the 30 mins. covering period the fish assumed an intermediate shade of about a D.O.I. value of 4. All the fish used showed exactly the same result.

## V. THE EEG AND BLINDING

A series of recordings were made from the tectum of 13 blind fish which were clamped in the apparatus used for the previous records.

As in the previous records, no curare was used.

One fish was recorded before and after blinding. Blinding was accomplished by cutting both optic tracts under anaesthetic and the EEG was recorded at 10 mins. and 30 mins. after recovery, (these times were chosen because tectal activity in a normal minnow begins to re-appear about 5 mins. after the anaesthetic has been replaced by normal water.

EEG patterns were recorded from fish which had been blinded for intervals of 5 hours, 24 hours, 48 hours and 5 days, and for each record a separate fish was used because it was not possible to record from the same fish on more than one occasion. A group of eight fish had the EEG recorded after having been blinded for four months. All the records are shown in fig. 48 (p. 236).

The amplitude was greatly affected by the blinding and a comparison of the 10 min. and 30 min. records with those from a normal fish showed that there was almost no activity present. The HF and LF cycles could only just be seen and it was not possible to compare the frequencies. A partial increase in amplitude was seen in the 5 hour fish but this did not increase any further in the 24 hour record. Both the 2 day and the 5 day fish showed increases in amplitude. All the records were taken from the G site. All the fish were fully dark except the 5 day fish which had begun the post-blinding paling and

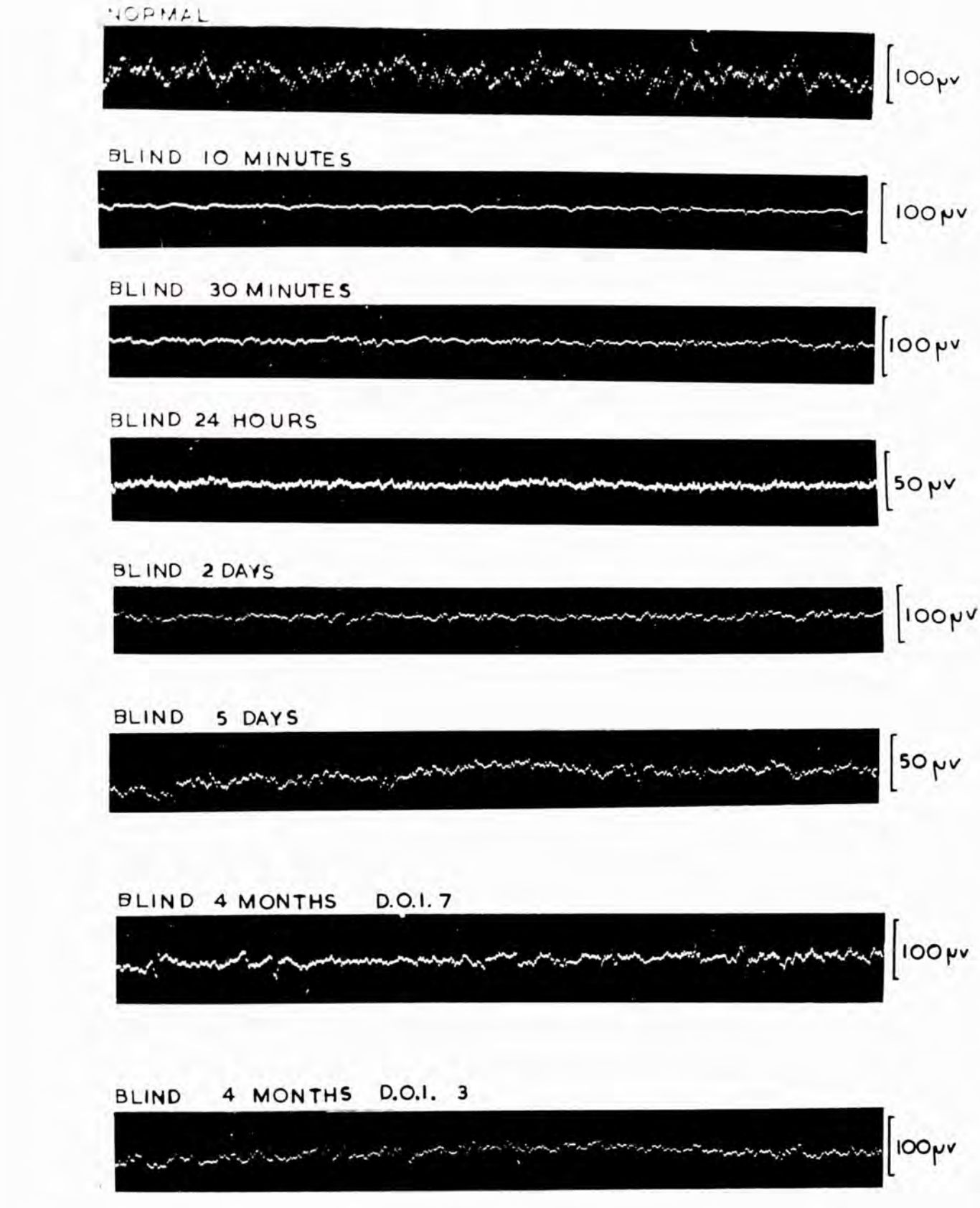
Fig. 48

The EEG of the minnow and blinding. The normal pattern and the 10 and 30 minute blind records are from the same fish and other records are from different fish. Blinding was by cutting the optic tract.

Fig. 48

The EEG of the minnow and blinding. The normal pattern and the 10 and 30 minute blind records are from the same fish and other records are from different fish. Blinding was by cutting the optic tract.

Fig. 48


NORMAL
100μv
BLIND 10 MINUTES
100μv
BLIND 30 MINUTES
100μv
BLIND 24 HOURS
50μv
BLIND 2 DAYS
100μv
BLIND 5 DAYS
50μv
BLIND 4 MONTHS D.O.I. 7
100μv
BLIND 4 MONTHS D.O.I. 3
100μv
0
1
2
3
4
5
6
7
SECONDS

had reached a value of 6.5.

The four month records were taken to see if the EEG pattern had been regained fully and to see if any differences were present between the dark and light fish at this time interval. The amplitude of the EEG pattern is never that of the normal fish but it does rise to a much higher level than it has when first blinded.

The results from the blinded fish were not sufficiently numerous to allow an accurate assessment of the pattern and so enable comparison of the pale and dark fish. They did, however, appear to be a very slight increase in amplitude in the records from the fish which had lightened to values of 3. The fish which remained dark (7.5) tended to have rather smaller EEG records. More work needs to be done to confirm these observations.

The EEG of the minnow therefore is intimately related to the visual input and in the absence of this input the animal does not show the normal EEG pattern.

## VI. THE EEG FOLLOWING BACKGROUND REVERSAL

### (a) Method

To study the effect of changing the background on the EEG

5 regions of the tectum were selected and labelled R, P, Z, T, and V. Region Z is the active region of section 5 (p. 162), and the others serve as controls of this region. No records were taken laterally to those shown because of the difficulty of implantation presented by the operculum. The positions are shown in fig. 49 (p. 239), and the grid references are:-

Z X0 Y0.66mm - 1.33mm

P X+0.66mm Y0.66mm - 1.33mm

R X+ 1.33mm Y0.66mm - 1.33mm

T X+ 1.0mm Y1.66mm - 2.33mm

V X+ 0.33mm Y 1.66mm - 2.33mm

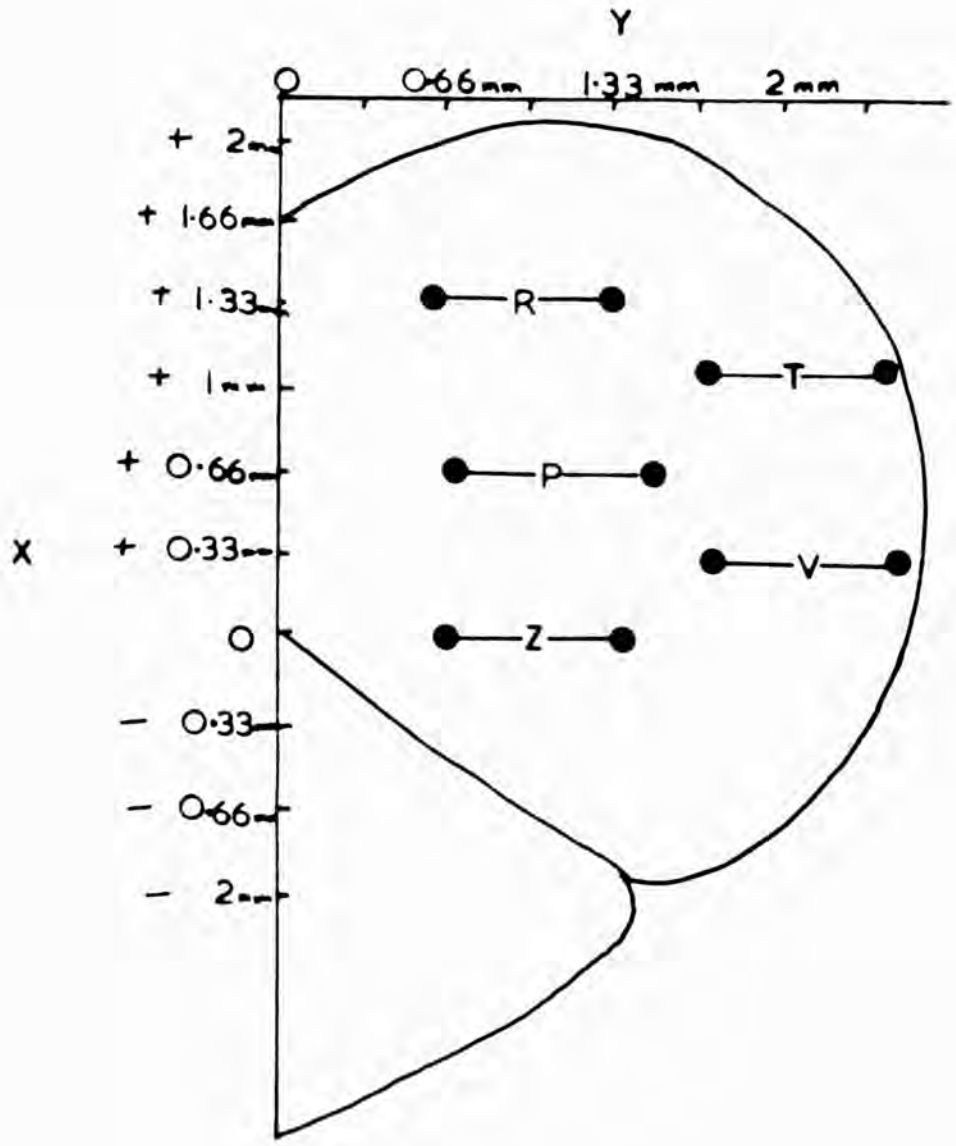
Recordings were made at five depths in each region, as follows:-

1. Stratum plexiforme et fibrosum externum.
2. Stratum plexiforme internum.
3. Stratum griseum internum
4. Stratum fibrosum profundum.
5. Stratum griseum periventriculare.

These layers were chosen because of their distinctness and because of their relationship to the functions of the tectum. The electrodes were lowered the following depths for each layer: 1 = 0.04mm, 2 = 0.13mm, 3 = 0.2mm, 4 = 0.27mm, 5 = 0.33mm from the surface of the

Fig. 49

POSITION OF THE ELECTRODES IN THE BACKGROUND REVERSAL RECORDINGS



tectum. The positions of the electrodes were confirmed by sectioning and staining the brains after the recordings were finished. To clarify the position of the electrodes they were marked by passing a current of 5 micro amps. for 10 seconds through them. The sections were stained with C.F.V.

All the fish used were exactly 6.5 cm long to ensure a conformity in the tectal size. At the end of an experiment the fish was always examined to confirm that it was still alive.

The apparatus used for the background reversal was that described on page 218. The cell used to hold the fish had the advantage that there were no sides and the fish had an unrestricted view of the sides and bottom of the container.

After the injection of curare the fish was allowed to recover from the anaesthetic on a black background. When the fish had fully recovered chromatically (about 20 to 30 mins.) recordings were made (A). The background was then changed to white and another recording was taken (B). Further recordings were made on white at intervals of 5 mins. (C), 10 mins. (D), and 20 mins. (E). The background was then returned to black and recordings were taken immediately (G), after 5 mins (H), 10 mins. (I), and 20mins. (J). The background was then changed to white, and recordings again taken to act as a control to

record B. The recording consisted of about 15 seconds of film at 1 inch per second.

The analysis of the results consisted of the selection of certain amplitude limits and a count of the number of cycles/sec. which fitted these amplitude limits, for 10 seconds, for each record. It was found that both D and I did not differ from C and H and they are therefore not included in the results.

The results are expressed in terms of the variation (Va) and the mean (M) number of cycles in each second for the 10 second count.

(b) Results

Recordings from region Z were repeated on different fish and they agree very well. The results are complicated by the amplitude variation found from fish to fish. In the results very little importance has been given to the amplitude and it may well be that this amplitude variation may be significant. However, in order to test for its significance a much larger number of fish would be needed than were available in the present study. This report on the changes of frequency which occur in the tectum when the background is changed can therefore only be regarded as preliminary.

Depth 1.

TABLE 13. Analysis of the records from region P and Z at depth 1.

| Recording | 40 μv | | 20 μv | | < 20 μv | |
|---|---|---|---|---|---|---|
| | VA | M | Va | M | Va | M |
| B | 1-3 | 2.4 | 6-8 | 6.8 | 18-24 | 20.2 |
| C | 1-3 | 2.2 | 5-8 | 6.4 | 19-24 | 20.8 |
| E | 2-3 | 2.3 | 5-8 | 6.2 | 18-24 | 21 |
| G | 1-3 | 2 | 7-8 | 7.5 | 18-20 | 19 |
| H | 1-3 | 2 | 5-8 | 6.5 | 18-24 | 20 |
| J | 0-3 | 2 | 5-8 | 6.6 | 18-24 | 21.4 |

Z

| | | | | | | |
|---|---|---|---|---|---|---|
| B | 0-2 | 1.1 | 5-7 | 5.4 | 19-23 | 20.7 |
| C | 1-2 | 1.5 | 4-6 | 5.8 | 19-22 | 21.1 |
| D | 0-3 | 1.3 | 3-6 | 5 | 20-24 | 21.2 |
| G | 0-2 | 1.2 | 4-7 | 5.4 | 20-23 | 21 |
| H | 0-3 | 1.4 | 4-6 | 5.2 | 19-24 | 21 |
| J | 0-3 | 1.6 | 4-7 | 5.1 | 20-24 | 21.5 |

The records from the regions R, T, and V are the same as those presented here but the amplitude differs and the recordings from R, T and V show an upper amplitude of only 10 v; it was not possible to

TABLE 14 . Analysis of records from region T and V at depth 2

T

| | 20 μv | | 10 μv | | < 10 μv | |
|---|---|---|---|---|---|---|
| | Va | M | Va | M | Va | M |
| B | | | 6-12 | 8.1 | 26-31 | 28.1 |
| C | | | 6-10 | 7.3 | 25-31 | 25.3 |
| E | | | 7-8 | 7.5 | 24-28 | 26 |
| G | 0-4 | 2.5 | 5-9 | 8 | 21-29 | 25 |
| H | 0-2 | 1.3 | 6-11 | 7.3 | 20-29 | 25.9 |
| J | | | 7-9 | 7.9 | 25-29 | 26.4 |

V

| | | | | | | |
|---|---|---|---|---|---|---|
| B | 0-3 | 1.3 | 6-14 | 8.2 | 21-32 | 25 |
| C | | | 6-11 | 8.3 | 23-29 | 26.7 |
| E | | | 7-11 | 9 | 22-26 | 24.9 |
| G | | | 10-13 | 11.1 | 24-27 | 25-6 |
| H | | | 6-10 | 8.4 | 22-29 | 25 |
| J | | | 5-12 | 8.1 | 25-27 | 25.9 |

divide LF into two different cycles.

Depth 1 records have the same pattern as the superficial recordings, showing the LF of 6-13 cy/sec. and the HF of 18-24 cy/sec. The amplitude of these records was less than that recorded from the superficial work (p.223) It can be seen from these records that the frequencies do not differ from the black to the white and that the same pattern is seen in all the recording sites.

Depth 2

The records from the other regions show the same results as T and V, shown in table 14 (p. 243). The recordings from depth 2 do not differ very much from those of depth 1 but there is an increase in the amount of HF activity so that the variation is now from 20 to 32 cy/sec. and the means are all at least 4 cy/sec. more than in depth 1. The means of the LF also show a slight rise. In both of these records (T and V) there is a slight increase in the LF following the change to the black background.

Depth 3

More variation was found from region to region in this layer than in the others, and this is associated with a very large amount of low frequency activity and amplitude variation, so that the picture becomes confused. In general the characteristic feature in this region is the HF increase with means around the 40 cy/sec. figure. The LF

response shows a slight reduction so that values in the region of 4-5 cy/sec. are the most dominant. None of the records shows any change from black to white and the same general pattern is found in all the records. Region Z shows a marked cyclic activity.

TABLE 15 . Analysis of records from region Z and P at depth 3.

Z

| | 20μv | | 10μv | | <10μv | |
|---|---|---|---|---|---|---|
| | Va | M | Va | M | Va | M |
| B | | | 4-7 | 5.5 | 38-42 | 40.5 |
| C | 0-3 | 1.5 | 4-5 | 4.3 | 41-45 | 42.5 |
| E | 0-4 | 1 | 4-7 | 5 | 39-43 | 40.7 |
| G | 0-4 | 2 | 3-7 | 4.5 | 37-43 | 42.3 |
| H | 1-3 | 2 | 3-6 | 5 | 38-41 | 40 |
| J | 0-4 | 1.5 | 3-6 | 5 | 38-42 | 41 |

P

| | | | | | | |
|---|---|---|---|---|---|---|
| B | | | 2-5 | 4 | 39-41 | 40.2 |
| C | | | 3-6 | 4.5 | 34-43 | 39 |
| E | | | 3-5 | 4.2 | 38-41 | 39 |
| G | | | 2-5 | 4 | 37-43 | 40 |
| H | | | 3-5 | 4 | 39-41 | 40.5 |
| J | | | 3-6 | 4 | 38-41 | 40.7 |

Depth 4

In this there is a reduction in the HF activity to give figures in the region of 16-30 cy/sec. and, as with the other regions, changes in amplitude confuse the picture. All the regions show the same pattern and at this depth there were changes observed when the background was changed. When the fish was placed on the black background from the white, a difference of 10 cy/sec. was observed in the HF rythm and the rythm changed in P from about 17 cy/sec. to 28 cy/sec. This higher frequency on the black occurs directly the background is changed and persists for the whole of the 20 mins. observed. It is found throughout the whole of the tectum. Directly the fish was placed on white the HF rythm was reduced to the 15-25 cy /sec. that it was before the change. The records of LF cycles are not very different from those at the other depths but in region Z when the fish was placed on the white there was an increase in the number of cycles. After 20 mins. on white this frequency decreased (Table. 16 p. 247). These high LF cycles do not appear on the black and when the fish is again on the white they re-appear. This result was not found to occur in the other regions except P and, considering the fact that this is the main efferent layer of the tectum, this result may be significant in colour change.

TABLE 16 . Analysis of records from region Z and P at depth 4

Z

| | 100μv | | 50-100μv | | <50μv | |
|---|---|---|---|---|---|---|
| | Va | M | Va | M | Va | M |
| B | 4-7 | 6 | 3-7 | 4 | 11-15 | 12 |
| C | 5-6 | 5.3 | 4-7 | 4.7 | 12-16 | 12.5 |
| E | 1-6 | 3.2 | 3-7 | 5.6 | 12-16 | 14.9 |
| G | 3-6 | 3.9 | 3-7 | 4.7 | 17-23 | 19.9 |
| H | | 3 | 4-7 | 5.9 | 20-26 | 22.2 |
| J | 1-2 | 1.5 | 5-7 | 6.3 | 19-25 | 22.3 |

P

| | 10μv | | <10μv | |
|---|---|---|---|---|
| | Va | M | Va | M |
| B | 3-7 | 4.3 | 15-25 | 21 |
| C | 4-7 | 6 | 15-25 | 16 |
| E | 5-7 | 6.4 | 13-21 | 17.5 |
| G | 3-4 | 3.8 | 25-31 | 28 |
| H | 3-5 | 3.8 | 20-30 | 25 |
| J | 3-5 | 4 | 23-30 | 27 |

The record from P was of much lower amplitude than that from Z, but it can be seen that if the figures from the 50 v to 100 v are added to the 50 v the results are essentially the same. In P however,

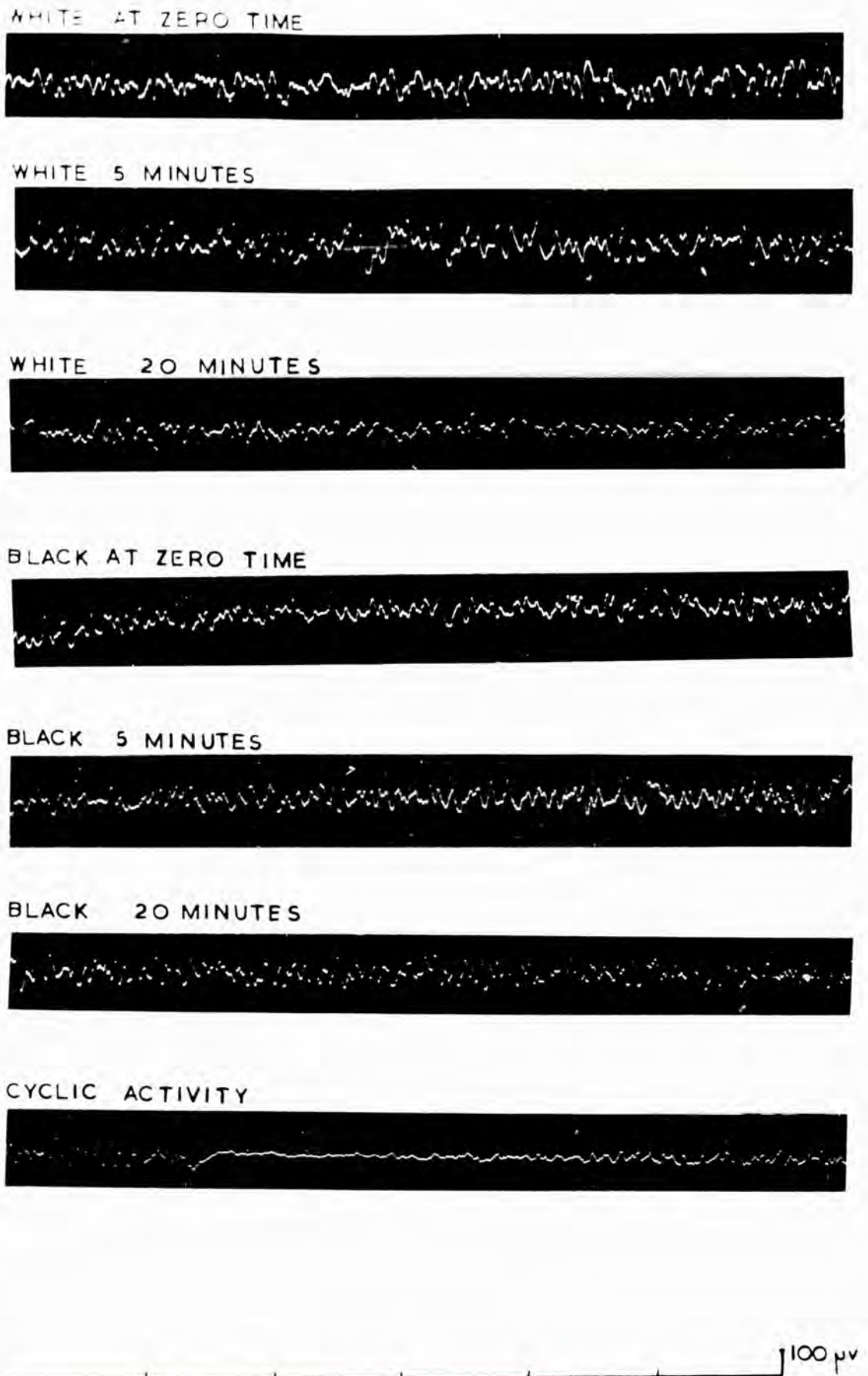
Fig. 50

The REG of depth 4 position 2 during background reversal.

Fig. 50

The EEG of depth 4 position Z during background reversal.

# Fig. 50


WHITE AT ZERO TIME
WHITE 5 MINUTES
WHITE 20 MINUTES
BLACK AT ZERO TIME
BLACK 5 MINUTES
BLACK 20 MINUTES
CYCLIC ACTIVITY
100 μv
0
5
4
3
2
1
0
SECONDS

the increase in the LF on the white is persistent for as long as the fish is on the white background.

Z shows a marked cyclic activity which is seen in the upper three depths but not to such a marked extent. The EGG is suddenly cut off and this is followed by a period of almost no activity, lasting about 1.5 secs. and followed by a gradual return of the normal activity, this return period taking about 3.5 secs. The EGG of Z4 is shown on fig. 50 (p. 249).

Depth 5

The LF does not change with background reversal and shows a 6-10 cy/sec. pattern. The HF activity, however, like depth 4, shows an increase when the fish is on the black and one again the change is in the region of 10 cy/sec. In general, however, the HF activity shows a greater increase in the number of cycles per second that occurs in depth 4.

The records for all the regions are the same and no regional change was seen. Position Z did not in this record show the cyclic activity that was seen in depth 4. Depths 1,2 and 3 are shown in fig. 51 (p. 253), together with the record of depth 5 on both the black and white backgrounds.

TABLE 17 . Analysis of records from region Z and R at depth 5

Z

| | $10\mu v$ | | $< 10\mu v$ | |
|---|---|---|---|---|
| | Va | M | Va | M |
| B | 6-10 | 7.9 | 20-29 | 24.8 |
| C | 7-10 | 8 | 23-26 | 24 |
| E | 9-11 | 10 | 18-22 | 20.2 |
| G | 6-8 | 7.5 | 31-26 | 34.2 |
| H | 6-10 | 8.5 | 30-34 | 32.2 |
| J | 6-10 | 7.4 | 29-39 | 34.8 |

R

| | | | | |
|---|---|---|---|---|
| B | 4-9 | 6.4 | 24-29 | 26.4 |
| C | 7-10 | 8.1 | 19-23 | 20.5 |
| E | 8-9 | 8.4 | 19-25 | 21.1 |
| G | 7-9 | 8 | 30-21 | 30.4 |
| H | 7-9 | 8.2 | 30-36 | 33 |
| J | 7-10 | 8.6 | 30-34 | 32.4 |

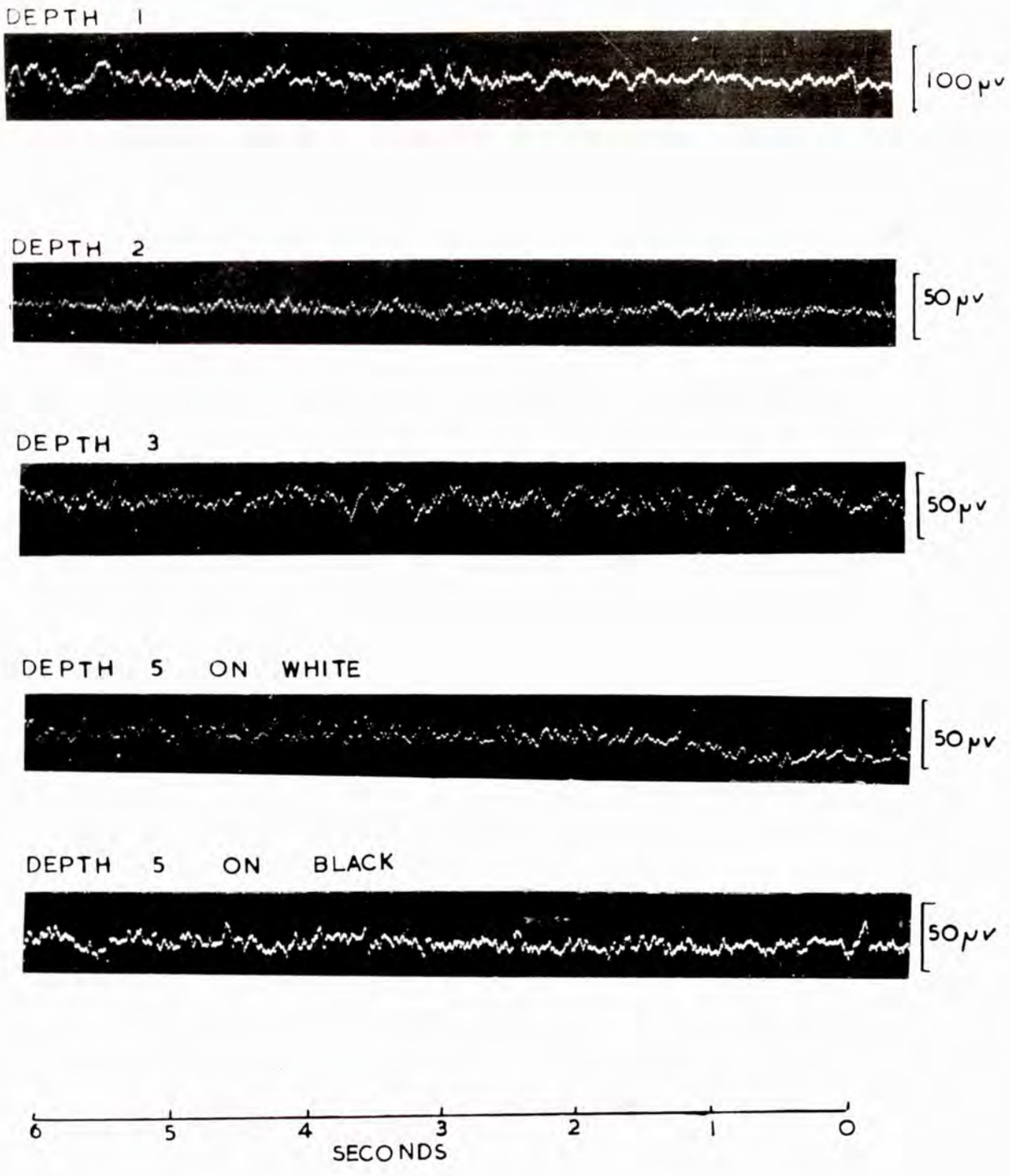
Fig. 51

The NEG of depth 1, 2 and 3 in all positions, and the NEG of depth 5 position 2 on background reversal.

Fig. 51

The EEG of depth 1, 2 and 3 in all positions, and the EEG of depth 5 position Z on background reversal.

Fig. 51


DEPTH 1
100 μv
DEPTH 2
50 μv
DEPTH 3
50 μv
DEPTH 5 ON WHITE
50 μv
DEPTH 5 ON BLACK
50 μv
6
5
4
3
2
1
0
SECONDS

## VIII. DISCUSSION

The superficial EEG pattern in the minnow agrees well with that of the Codfish (Enger 1957), and the Goldfish (Schadé & Weiler 1959; Schadé, 1962; Oshima & Gorbman, 1968). The two rhythms in the minnow are the 6-14 cy/sec. and an 18-24 cy/sec., the codfish being 8-13 cy/sec. and 14-32 cy/sec., and the Goldfish 7-14 cy/sec. and 18-24 cy/sec. The pattern in elasmobranchs is only the 5-11 cy/sec. rhythm demonstrated by Gilbert, Hodgson & Mathewson (1964).

The initial effect of blinding was to reduce the EEG pattern throughout the whole of the tectum. A similar finding was reported in the goldfish (Schadé & Weiler 1959; Schadé 1962), but unfortunately, they did not report the time after blinding when they recorded the EEG. In the minnow the EEG is almost lost after the first 30 mins. following blinding but it re-appears in a reduced form after 5 hours and this improvement continues for several months. In no case did the normal EEG re-appear in blinded fish. Schadé & Weiler (1959) concluded that the reduction of the spontaneous activity following monocular blinding is probably not due to a simple cessation of impulses from the optic tract. It seems possible that the lowered amplitude of the activity observed is due to a spontaneous discharge from the injured ends of the nerve fibres. An unsynchronized bombardment from this source might

cause a decrease in amplitude of the spontaneous activity of the tectum and the suppression of the low-frequency activity. This hypothysis by Schadé & Weiler is essentially that proposed by Parker to explain the action of caudal bands discussed on page 268. In view of the results from the minnow in darkness and the lack of evidence for the presence of persistent injury discharge it seems much more likely that the lowered EEG is due to the reduced retinal input. What is not easy to explain is the resumption of the EEG after a period of time. Claes (1939) worked on the cat and found that after severence of the optic nerves, and cauterization of the papilla of the optic nerve, there were long periods of almost central inactivity lasting for 10-15 secs. in the striate area. These were interrupted by groups of activity of higher voltage but of lower frequency than before. A similar pattern of periods of no activity followed by activity is seen in position Z in the normal minnow. Claes's work on the cat did show that the EEG could not be maintained at the normal level following blinding. Results from the minnow agree with this.

The resumption of activity in the blinded minnow is difficult to explain but it may depend upon the relationship between the geniculate complex and the tectum. It seems from the work of Arduini & Pinneo (1962 a, b, 1963 a, b) and Maffei & Rizzolatti (1967) that the lateral geniculate nucleus of the cat shows similar changes to those shown by

the retina in relation to light intensity. It follows from this that if these conditions apply to the minnow, then, after the cutting of the optic tract, there would be no activity in the geniculate complex. It seems possible that the geniculate complex would after a period of time resume some activity and that this would be fed on to the tectum, which might result in the activity seen in the 5 hour blinding. The geniculate activity may then increase so that the tectal activity increases or the activity of the tectum exerts an influence on the geniculate complex and this increases the geniculate output. A tectal-geniculate system is certainly well developed anatomically and, if it could be demonstrated by recording techniques, it seems possible that such a feed-back system could well be responsible for the increase in spontaneous activity of the blinded minnow.

The results of the EEG on the minnow in darkness lead to some interesting considerations. Schadé & Weiler (1959) reported that the goldfish in darkness showed an increase in amplitude of brain activity over that in the light, but they did not report the time the fish were in the dark before recordings were taken. In the minnow, changing from the light to darkness results in an increase in activity possibly corresponding to the 'OFF' discharge from the retina, which has been reported from the optic tract and the geniculate nucleus of the cat by Arduini & Pinneo (1962 b, 1963 a, b). The activity of the minnow

tectum in the dark still maintains a high degree of activity after 15 mins., which is higher than that seen in the normal fish in the light. This may well be due to the spontaneous discharge of the retina reported by Granit (1955) in the frog and by Kuffler, Fitzhugh & Barlow (1957) in the ganglion cells of the unanaesthetized cat. A high degree of activity in darkness is also seen in the lateral geniculate nucleus of the cat (Maffei & Rizzolatti, 1967) and in the optic tract and lateral geniculate nucleus of the cat as seen by the work of Arduini & Pinneo.

The activity of the minnow tectum decreases after 30 mins., unlike the cat where it is maintained. This result agrees with the work of Adrian & Matthews (1927, 1928 a, b) on the optic tract of the conger eel, Conger vulgaris. They found that the impulses of the optic tract of this fish increased rapidly in frequency when the light was turned off. The rate then declined, at first rapidly, and then more slowly. Finally, in the dark the optic nerve lost all activity. This course of events is exactly what is seen in the EEG of the minnow.

Finally, when the light is turned on the EEG pattern shows a burst of activity corresponding to the 'ON' response of the retina.

In view of the close similarity between the EEG of the tectum and the probable visual input from the optic tract and geniculate complex,

it is interesting to note the observation made by Adrian & Mathews (1928 b). These authors found that when the entire retina of the conger eel was exposed to uniform illumination the action current discharge in the optic tract could lose its usual irregular character and consist of a series of regular waves of a 5-15/sec. frequency. Such waves in the optic nerve are caused by the rythmic waxing and waning in the number of impulses in the nerve fibres. They further suggested that the regular waves are due to rythmic discharges of the ganglion cells, which in their turn are due to the nervous connections between the ganglion cells of the retina. The frequency of these waves corresponds fairly well to the LF of the EEG of the minnow, especially when the latter fish is under uniform conditions of illumination. Enger (1957) has compared the LF rythm with the alpha rythm in man, and has speculated that the 8-13 cy/sec. rythm is associated with the thalamo-reticular system, which he considers to be present in the fish, through reverbrating cortico-thalamic circuites. It may well be that a much simpler situation is present and that the LF is more directly related to the visual output.

The recordings from implanted electrodes showed that the HF and the LF activity varies throughout the tectum and that the region where the highest number of HF cycles occur is in depths 3, and, to a lesser extent, depth 5. At the same time as the HF is increasing the LF is decreasing so that the highest LF values are in depths 1, 2 and 4. If

the frequencies are compared with the structure of the tectum, it is seen that the HF becomes increased where the cells predominate and the LF where the fibres predominate. It seems possible that the large collection of small neurons gives rise to the HF activity and the fibres to the LF activity in the EEG recordings of the minnow.

In depths 1, 2 and 3 no difference was found with back-ground reversal. This can be explained by the fact that this region corresponds to the ventral retinal projection area and it would be expected that background reversal would not affect the illumination of the ventral retina. These layers therefore seem to be both functionally and correspondingly anatomically distinct, namely, they receive the fibres from the optic tract and interpret the visual input. In depths 4 and 5 the number of the HF cycles increases by 10 cy/sec. on the black. Depth 4 is the main efferent fibre layer of the tectum and depth 5 is a thick layer of neurones which give rise to the tectal efferents.

The significance of the HF activity in terms of the control of the paling centre is difficult to envisage. Young (1963, 1965, 1966) has demonstrated that the vertical lobes of the octopus brain have an important inhibitory function and that these lobes have a large number of small cells. He concludes that inhibition may be one function of these small neurones. In the tectum of the minnow the periventricular layer is composed of a large number of small neurones and these may have

a general inhibitory function. The increase of the HF activity in depth 5 on the black would then indicate the inhibition of the paling centre. In connection with this it was also seen in region P and Z there was an increase in the LF activity on the white background.

To conclude on the EEG pattern and its relationship to colour change, several of the findings are very important.

Firstly, in all conditions where the EEG is very reduced the fish darkens, and this is seen when the fish is killed, anaesthetized or blinded. To a certain extent the level of the EEG can be correlated with the colour of the fish. For example, in darkness the EEG is lower and the animal assumes an intermediate shade.

Secondly, it seems that the EEG, especially the superficial LF cycles, is related to the retinal input.

Thirdly, in layer 7 of the tectum there is an increase in the HF activity which is persistent on black, and in the region where the fibres pass out of the tectum to the paling centre there is an increase in the LF activity associated with the white background.

In view of these conclusions a possible hypothesis for the action of the tectum in relation to chromatic adaptation can be put forward.

In the absence of the visual input the cells of the deepest layer, the periventricular layer, exert a general inhibitory influence on the paling centre. This is modified by the retinal input which exerts a general excitatory influence. It would therefore seem that the balance between the excitation and the inhibition would determine the action of the tectum on the medullary centre. One could envisage that the regions of the retina where the light was less intense had less of an excitatory effect than regions where the retina was well illuminated. From this there would be an averaging out process between the retinal inputs and the general tectal inhibition. On the white background the tectum would be fully excited and the HF activity reduced (e.g. in position Z it is only 12 c/sec.). At the same time the LF becomes more prominant. This high level of LF activity is associated with the dominance of the excitation of the retinal input and manifests itself in the excitation of the paling centre.

On the black background the retinal input is insufficient to reduce the inhibitory action of the tectum so that the HF inhibitory activity predominates and the tectum inhibits the paling centre.

This hypothesis is very tentative, for no work was done on the lateral parts of the tectum which receives the dorsal reflected light. It would also be interesting to extend this work not only on recordings from the lateral aspects of the tectum but also to record from the medullary centre and the geniculate complex to confirm the functions

attributed to them in this hypothesis.

# SECTION 9

## CONCLUSIONS ON THE CONTROL OF CHROMATIC ADAPTATION ON THE MINNOW.

### 1. WORKING HYPOTHESIS FOR COLOUR CHANGE

Although most of the results presented in this thesis have been discussed fully, much work would be necessary to verify many of the suggestions put forward. However, by using the results presented here it is possible to suggest a working hypothesis for the mechanism of the control of chromatic adaptation in the minnow.

Having decided in Section 3 (p. 93) that there is no rigid retinal differentiation in the minnow and that the hypothesis regarding the significance of the ratio of dorsal to reflected light is untenable, the relationship between the retina and colour change becomes complex. This does not mean that the relationship between the direct and reflected light is not important but that its importance lies in its relationship to the total retinal output to the brain. It appears that brightness discrimination is analysed in terms of the <u>total</u> tonic

activity of the retinal output. Arduini & Pinneo (1936 a) proposed that in steady light or darkness all types of inhibitory and excitatory units fire randomly in the retina, that they give rise to tonic activity, and that this tonic activity is inversely related to the light intensity.

These levels of tonic activity are relayed to the geniculate. This body may function to integrate the activity from the different regions of the retina and relay it to the tectum. It is in the tectum that the final interpretation of the brightness of the background is carried out. It has been suggested on page 193 that the tectum functions as a complex excitatory-inhibitory system. One could envisage that the incoming fibres from the geniculate complex are of a general excitatory nature and that these act against the inhibitory tendencies of the periventricular neurones. Whichever dominates determines the colour of the fish. When the fish is on a white background the excitatory tendency would be greater than when it is on the black, so that the overall effect would be one of excitation resulting in paling.

The paling centre in the anterior part of the medulla consists of a group of neurones that can spontaneously, without any control from the tectum, cause paling which is never maximal and never at the same rate as normal background paling. However, in general the medullary neurones are under the control of the tectum and the tectum can excite and inhibit them. On a white background the tectum excites the centre

resulting in an increase in its rate of firing and so causing the melanin of the chromatophores to aggregate. On a black background the tectum inhibits the centre and it no longer fires, so the melanin disperses.

Finally, fibres from the neurones of the paling centre run in the spinal cord, pass to the sympathetic chain and reach the melanophores. A diagram of the pathways in the brain is shown in fig. 52 page 267.

Hormonal colour change is controlled by the tectum and fibres from the tectum cause the release of the hormones from the pituitary.

The main difficulty in this hypothesis is the question of the innervation of the chromatophores. The theory that the chromatophores are innervated from the sympathetic system causing aggregation and that dispersion is essentially passive stems from the work of von Frisch (1911). Von Frisch (1911) was aware of the theoretical possibility of dispersing fibres but did not test for their existence. In 1931 Giersberg tested a number of minnows using the sympathetic blocking agent ergotamine followed by electrical stimulation and found darkening to occur. He also tested with acetylocholine and concluded that there were present parasympathetic "dispersing" fibres. Von Gelei (1942) extended this work of Giersberg using the technique of electrical stimulation and the injection of ergotamine and acetylocholine. On stimulation he found

Fig. 52

Diagram of the brain to show the fibre pathways in the brain controlling chromatic adaptation.

Abbreviations:

E. - Eye

C.C. - Geniculate complex

O.T. - Optic tectum

C. - Cerebellum

M.C. - Medullary centre

Fig. 52

Diagram of the brain to show the fibre pathways in the brain controlling chromatic adaptation.

Abbreviations:

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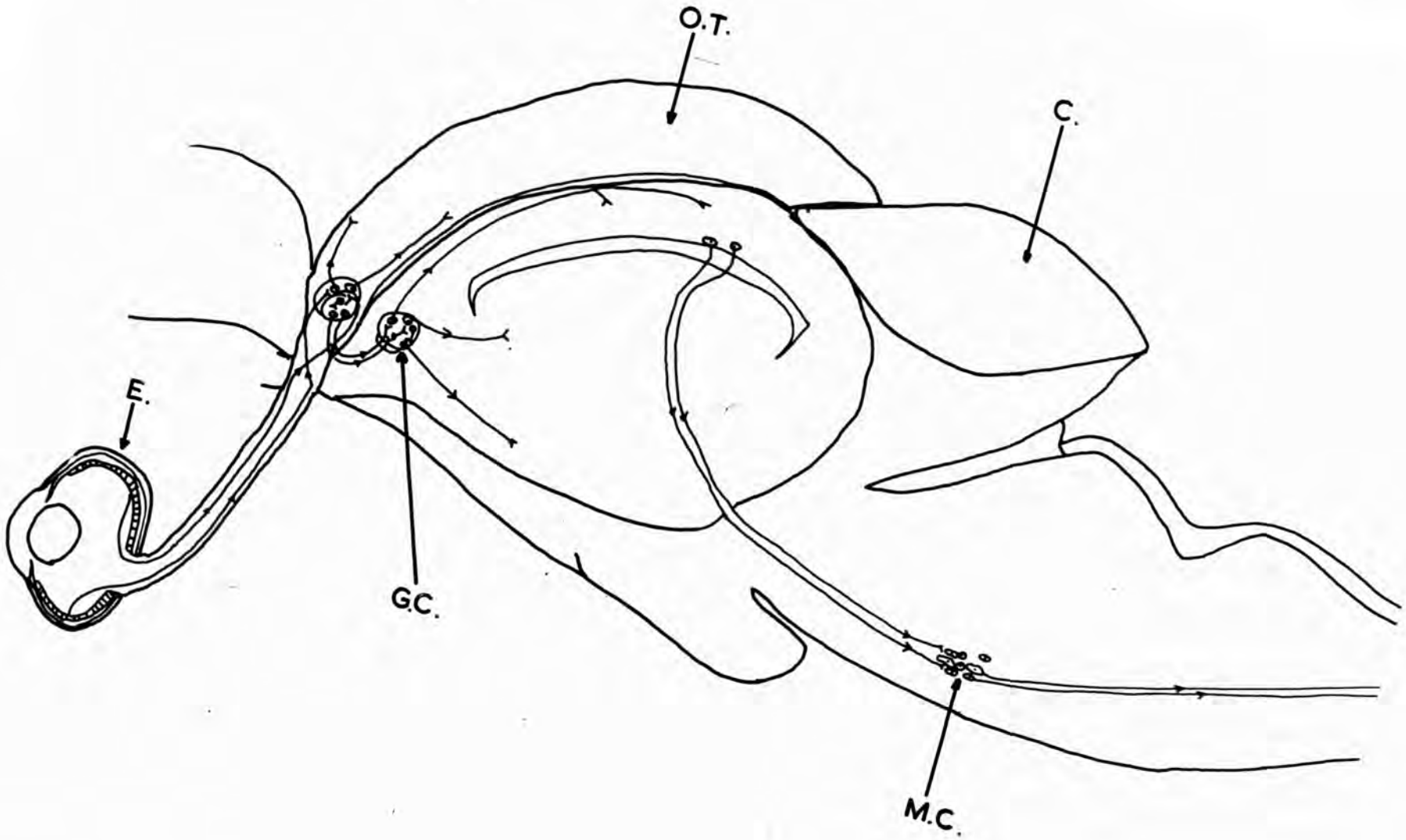
Fig. 52



darkening, and produced a map of dispersing fibres passing out of the spinal cord to the autonimic chain with the first and second spinal nerves; from which point he supposed that they run backwards to pass to the melanophores. However, von Frisch's (1911) earlier observation that the anterior section of the autonomic chain does not appear to result in abnormal colour changes in the posterior region of the minnow would indicate that such dispersing fibres play no significant part in the intact minnow.

Pye (1964 a, b, c) confirmed the observation made by von Gelei that the fish injected with ergotamine and stimulated darken, but he did not find darkening following the injection of ergotamine. Pye found that the arguments put forward by von Gelei for the mapping of the melanophore-dispersing fibres were unsupportable and that his experiments provided no evidence for double innervation. Recently Healey & Ross (1967) and Grove (1967) presented a wide variety of drugs to the minnow, and could not show any real evidence for the presence of cholinergic fibres responsible for darkening.

Parker (1934) claimed to have provided evidence for double innervation. He extended the work of Mills (1932 a, b) on the formation of caudal bands by means of cutting the chromatic fibres in the tail of Fundulus. This cut in the tail produces a dark band of dispersed melano-

phores which finally fade in a few days. Later, Parker (1948) showed that in a previously faded band a further band can be induced by a cut within the original band. Parker also found that the presence of a cold block will prevent the development of the band posterior to the block. He considered that the cutting induces persistent injury discharges to occur only in the dispersing fibres, the cold block preventing their spread and a further cut inducing a further development of the discharges. Sand (1935), Young (1962), and Waring (1942, 1963) have called attention to the difficulties to Parker's hypothesis and stress that this evidence is very inconclusive. Gray (1956) performed a series of tail cutting experiments on the minnow similar to those performed by Parker. He suggested that the development of the caudal bands might result from the removal of central nervous control through nerve section so that some inherent dispersing mechanism of the melanophores could come into play. Later the melanophres might lose their refractoriness to diffusing neurohumours and become hypersensitive. The results are far from being conclusive, for when he performed background reversals after the band had faded, the tail showed a uniform colour.

According to Parker & Rosenblueth (1941) direct stimulation of the chromatophore nerves in Ameiurus resulted in pallor when the stimulation was 8v, at a rate of 15-25/sec, and darkening when stimulating at 6-8v at 1-2/sec. for a period of 15-25 mins. At best these experi-

ments were crude in extreme and Pye (1964 a) failed to get any darkening when he stimulated the superficial ophthalamic nerve of Phoxinus. He has criticized the experiment of Parker & Rosenblueth (1941) on the grounds that they used un-polarized electrodes and that the response times were far too long.

In general there is no conclusive evidence for the presence of dispersing fibres and this suggests a strong possibility that they do not exist. If double inervation is present the work of Healey (1954) Gray (1956) and Pye (1965 a, b) suggests that dispersing fibres may follow the same path as the paling fibres.

If double innervation is present their origin presents a problem. It seems unlikely that they run directly from the tectum because the latter may excite and inhibit the medullary centre. The other possibility is that dispersing fibres arise in the medullary centre and that in the minnow the action of the latter is predominantly excitatory. It would be interesting to see if the medullary centre played the same rôle in other teleost fish.

## II. COMPARISON OF THE WORKING HYPOTHESIS FOR THE MINNOW WITH OTHER THEORIES OF THE CENTRAL NERVOUS CONTROL OF COLOUR CHANGE.

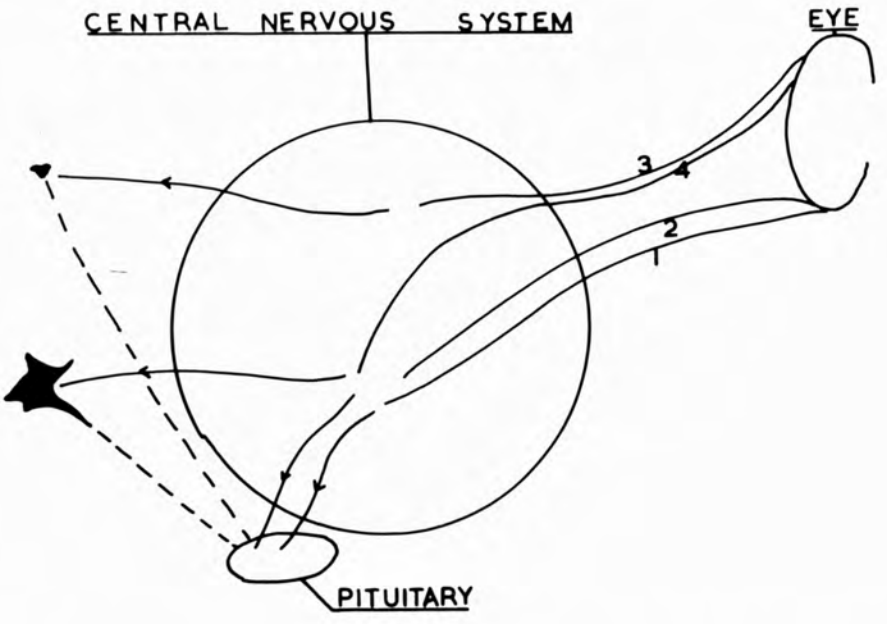
Vilter (1939, 1941) has developed a plan of the chromatic organisation that is based on the antagonism between the dark colour phase and the pale one. These two phases are polarised dorso-ventrally; the dark phase is at its maximum on the dorsal surface of the animal and becomes reduced as we pass ventrally while the pale phase, which is maximum ventrally, decreases as we pass dorsally. He further believes that the retinae of chromatic vertebrates are polarised and that their ventral portions are associated particularly with the ventrally centralised skin changes and their dorsal parts with those dorsally centralized.

From the work on the minnow there is no evidence for any part of this scheme being applicable.

Parker (1948) considered the mechanism for colour change in Anguilla to be composed of four reflex arcs shown in fig.53 p.272, and that for Phoxinus to be essentially similar. These arcs are labelled 1-4 and are as follows:

1. From the ventral retina to the intermediate lobe of the pituitary to release dispersing hormone - retino-pituitary arc.

Fig. 53




THE MECHANISM OF COLOUR CHANGE IN ANGUILLA AFTER PARKER 1948

2. Nervous reflex arc where the terminations release acetylcholine and cause pigment dispersion - retin-cholinergic arc.

3. Dorsal retina to the chromatophores causing the release of adrenalin and is a nervous arc - retino-adrenergic arc.

4. From the dorsal retina to the part of the pituitary where it may excite the production of W- substance described by Hogben (Waring 1940).

Parker supposed that the control is brought about by simple reflex arcs but the system is clearly very much more complex.

The plan proposed by Parker depends upon the existence of rigid retinal differentation and from this comes the false concept of colour change fibres originating from the retina.

The hypothesis presented here differs from that presented by von Frisch (1911) because he suggested the possible presence of a diencephalic darkening centre. This difference may be more apparent than real, but no electrical stimulation was performed on the brain. It may be that stimulation of the tectum would excite it and cause paling and not darkening of the fish. Stimulation of the diencephalon performed by von Frisch could well have effected the geniculate complex and, it, in its turn, could have affected the tectum and so caused darkening.

## S E C T I O N 10 : THE OPTIC TECTUM AND MOTOR BEHAVIOUR

Motor activity and the tectum was reviewed in the Introduction (p. 27 ). Some workers (Rizzolo, 1929; Dijkgraaf, 1949) did not report any disturbances following the removal of the tectum. However, others (Reisinger, 1915; Muskens, 1930; Botsch, 1960; Kirsche & Kirsche, 1961) have reported extensive motor disturbances following tectal damage. In order to resolve these conflicting accounts further study of this problem was undertaken.

### 1. NORMAL FISH.

No study appears to have been made of the behaviour of fish in a limited confined space. The fish were studied in litre beakers under the same conditions used for the colour change tests, with the addition of fine netting over the top of the beaker to limit their vision dorsally. This confinement in beakers essentially provides the fish with a limited environment, whose limits can only be perceived by the fish by touch. As far as possible, all other stimuli were uniform throughout the experiment. The beaker allowed the fish to swim normally

within limits so that it could only move in a straight line for one or two beats of the tail in any direction.

Under the experimental conditions the fish show three types of behavioural patterns.

(a) Resting

The fish rests on the bottom in a characteristic posture, lying parallel to the bottom on its spread-out pectoral fins and its pelvic fins. This period of rest may last for as long as thirty minutes.

(b) Low-level exploratory

This is essentially a slow swimming around the bottom of the beaker. Although the fish remains primarily on the bottom it occasionally makes upward movements, but these do not usually take the fish further than the middle of the beaker. The pattern of behaviour usually consists of one or two beats of the tail which propel the fish forward, followed by a glide period. The next beat of the tail usually propels the fish in another direction and the overall appearance is that of slowly searching the environment.

(c) Escape response

This consists of a series of rapid body movements from side to side at the bottom of the beaker next to the wall. Together with this

side to side movement, the fish swims rapidly up and down the wall of the beaker and gives the impression of attempting to escape; finally the fish may or may not leap out of the water.

To quantify the behaviour of the fish the dominant pattern was recorded every 15 sec. for a period of 15 minutes and this allowed the comparison of individual fish. The type of result obtained by this method is shown in fig. 54 page 279. This shows that the sequence of behaviour was very variable and that no definite pattern was present. The escape response may follow a period of exploratory behaviour but it can also occur directly from rest. The escape response may or may not be followed by a period of rest.

Using these records it is possible to calculate the percentage of the total time the animals spend in each activity. The results for 15 normal fish are shown in table 18 p. 277

These gave the mean values of 48% for the rest period, 24% low-level activity, and 28% for the up-down escape behaviour. The very great variation in these figures limits the conclusions which can be drawn from them. However, it does appear that the resting period in 9 of the fish is dominant, the low-level activity is clearly dominant in none of the fish, and the up-down escape response is dominant in only two. Although it is not shown in these records, all the fish showed all types of behaviour.

TABLE 18 . The general behaviour of 15 confined normal minnows

| Fish | Rest | Low-level | Up-down escape. |
|---|---|---|---|
| 1 | 56.7 | 11.7 | 31.6 |
| 2 | 33.3 | 20 | 46.7 |
| 3 | 23.3 | 43.3 | 33.3 |
| 4 | 41.6 | 30 | 28.3 |
| 5 | 50 | 28.3 | 21.6 |
| 6 | 100 | 0 | 0 |
| 7 | 61.6 | 5 | 33.3 |
| 8 | 40 | 50 | 10 |
| 9 | 3.3 | 11.6 | 85 |
| 10 | 61.6 | 36.6 | 1.6 |
| 11 | 56.7 | 25 | 18.3 |
| 12 | 76.6 | 10 | 13.3 |
| 13 | 11.6 | 48.3 | 40 |
| 14 | 100 | 0 | 0 |
| 15 | 11.6 | 46.6 | 41.6 |

Fig. 54

Diagram of the activity pattern of normal mimosa.

Abbreviations:

BD. - Up-down escape behaviour

LL. - Low-level exploratory behaviour

R. - Rest.

Fig. 54

Diagram of the activity pattern of normal minnows.

Abbreviations:

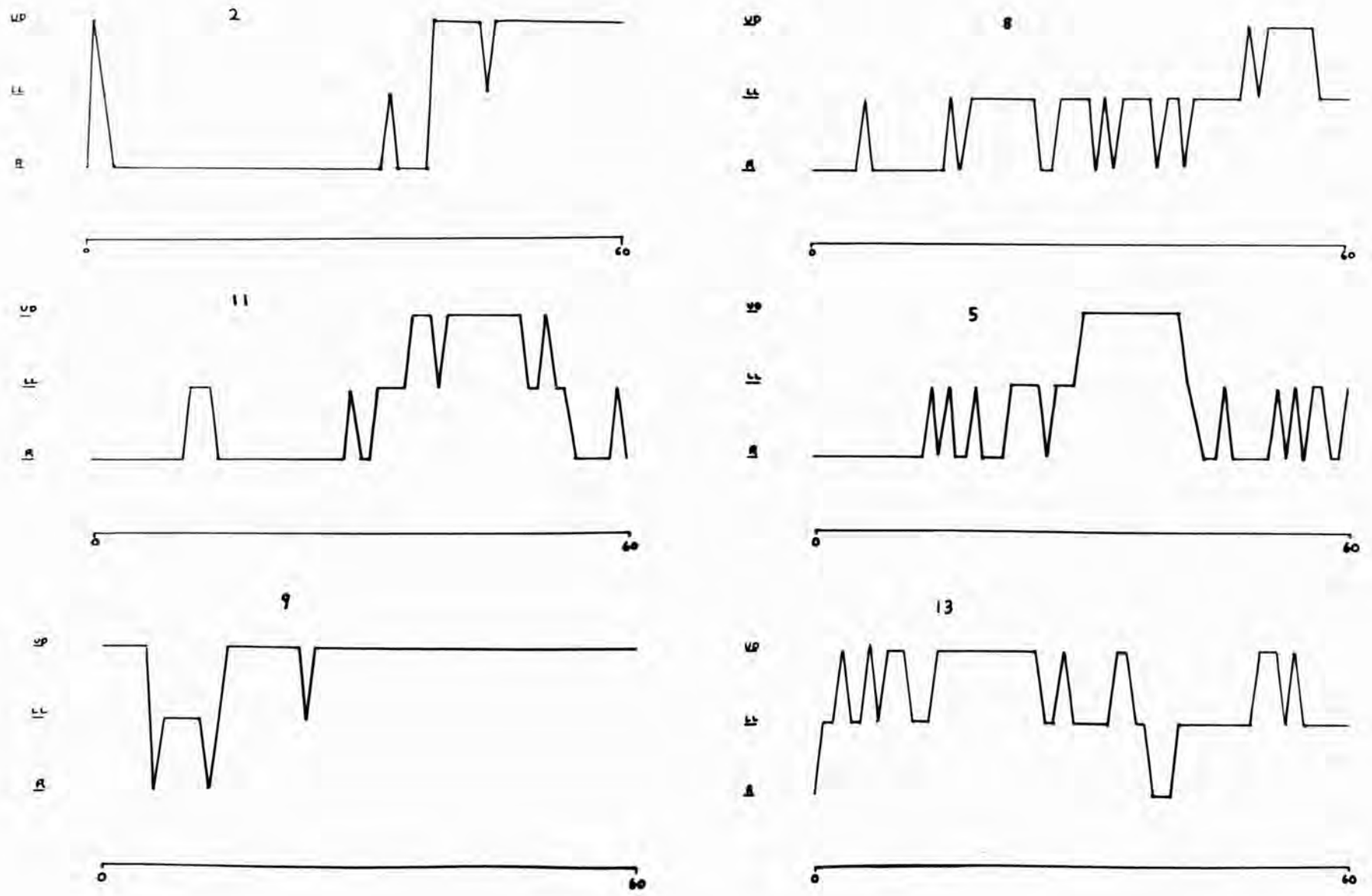
UD. - Up-down escape behaviour

LL. - Low-level exploratory behaviour

R. - Rest.

Fig. 54

# NORMAL MINNOWS



## II. TECTAL REMOVAL IN NORMAL FISH

(a) Removal of the left lobe

5 fish were used in this group. The locomotory behaviour of these fish appeared normal, they did not appear agitated, and they showed all the behavioural patterns seen in normal fish.

(b) Removal of the anterior tectum

4 fish were used in this group. The position and extent of the removals are shown on fig.316 p. 165 . In all 4 fish the locomotion was normal and so was the general balance. The behavioural pattern did show gross abnormalities and in all cases the fish tended to swim close to the surface. This type of behaviour is only seen in normal fish when the oxygen concentration of the water has dropped very low and the fish use the surface water which is richer in oxygen.

None of the fish appeared to be excited and in no case were there observed any rest periods or up-down escape responses.

(c) Removal of the posterior tectum

7 fish were treated in this group, the extent and postion of removals being shown on fig.31ed p. 166 167 .

4 of these fish (33, 36, 37, 64) remained at rest on the bottom for all of the time and only moved when stimulated, and then only for a

very short period of time, usually a few seconds. Fish 31, 40 and 65 swam slowly around the beaker and showed periods of high and low activity. In all 7 fish rest periods were present and there were no up-down escape responses.

(d) The left posterior part only present

4 fish were used. The extent and position of removals are given in fig. 31c p. 168.

No up-down escape response was observed in this group. Fish 45 swam around the beaker and did not show any rest period. 41 swam close to the surface at about a 45° angle and a similar pattern was seen in 49. 57 showed the most extreme surface tendency. None of the fish showed any rest periods.

(e) The left anterior part only present

7 fish were used in this group and the removals are shown on fig. 31d p. 167 169.

One of the fish, 55, rested for long periods and swam only for short periods and the others; 47, 56, 60, 61, 48 and 54, all swam slowly around the bottom or at an intermediate depth.

(f) Posterior right and anterior left removed

4 fish were used in this group and the removals are shown on

fig.31g p.170 . All these fish showed the normal behavioural patterns including the up-down escape response, but they all showed a reluctance to move and when stimulated only moved for a short period of time.

(g) Removal of the complete tectum

10 fish were used in this group. They showed rest and activity periods; the periods of rest are very reduced and only constituted a few seconds in every minute. None of the fish showed the up-down escape response.

(h) Small removal of the anterior tectum

5 fish were used and the removals are shown on fig.31h p.171 . All behavioural patterns are seen in this group. Two fish in this group; 70 and 69, showed a high degree of agitation and made frantic movements to jump out of the beaker at the presentation of any stimulus.

None of these operated fish showed any postural or locomotory defects and it was only in their behaviour that any were noticed.

## III. BLIND FISH

8 fish were tested in the same way as the normal fish 24 hours after blinding and then 10, 20, 30 and 40 days after blinding. The results for the tests are given in Tables 19, 20, 21, 22, 23.

TABLE 19. The values for the general behaviour of minnows 24 hours after blinding.

| Fish | Rest | Up-down escape | Low-level |
|---|---|---|---|
| 1 | 0 | 40 | 60 |
| 2 | 20 | 6.66 | 73.34 |
| 3 | 25 | 0 | 75 |
| 4 | 0 | 70 | 30 |
| 5 | 33.34 | 6.66 | 60 |
| 6 | 0 | 78.34 | 21.66 |
| 7 | 11.67 | 0 | 88.33 |
| 8 | 16.66 | 3.34 | 80 |

TABLE 20. The values for the general behaviour of minnows 10 days after blinding.

| Fish | Rest | Up-down escape | Low-level |
|---|---|---|---|
| 1 | 0 | 75 | 25 |
| 2 | 5 | 45 | 50 |
| 3 | 33.3 | .0 | 66.66 |
| 4 | 0 | 70 | 30 |
| 5 | 63.34 | 0 | 36.66 |
| 6 | 0 | 48.34 | 51.66 |
| 7 | 0 | 13.34 | 86.66 |
| 8 | 0 | 43.34 | 56.66 |

TABLE 21. The values for the general behaviour of minnows 20 days after blinding.

| Fish | Rest | Up-down Escape | Low-level |
|---|---|---|---|
| 1 | 0 | 35 | 65 |
| 2 | 0 | 36.66 | 63.34 |
| 3 | 0 | 30 | 70 |
| 4 | 0 | 68.34 | 31.66 |
| 5 | 3.34 | 1.66 | 95 |
| 7 | 0 | 11.67 | 88.33 |
| 8 | 6.66 | 41.67 | 51.67 |

TABLE 22. The values for the general behaviour of minnows 30 days after blinding.

| Fish | Rest | Up-down Escape | Low-level |
|---|---|---|---|
| 1 | 0 | 15 | 85 |
| 2 | 53.34 | 0 | 46.66 |
| 3 | 11.66 | 18.34 | 70 |
| 4 | 0 | 30 | 70 |
| 5 | 45 | 0 | 55 |
| 7 | 16.66 | 0 | 83.34 |
| 8 | 0 | 70 | 30 |

TABLE 23. The values for the general behaviour of minnows 40 days after blinding.

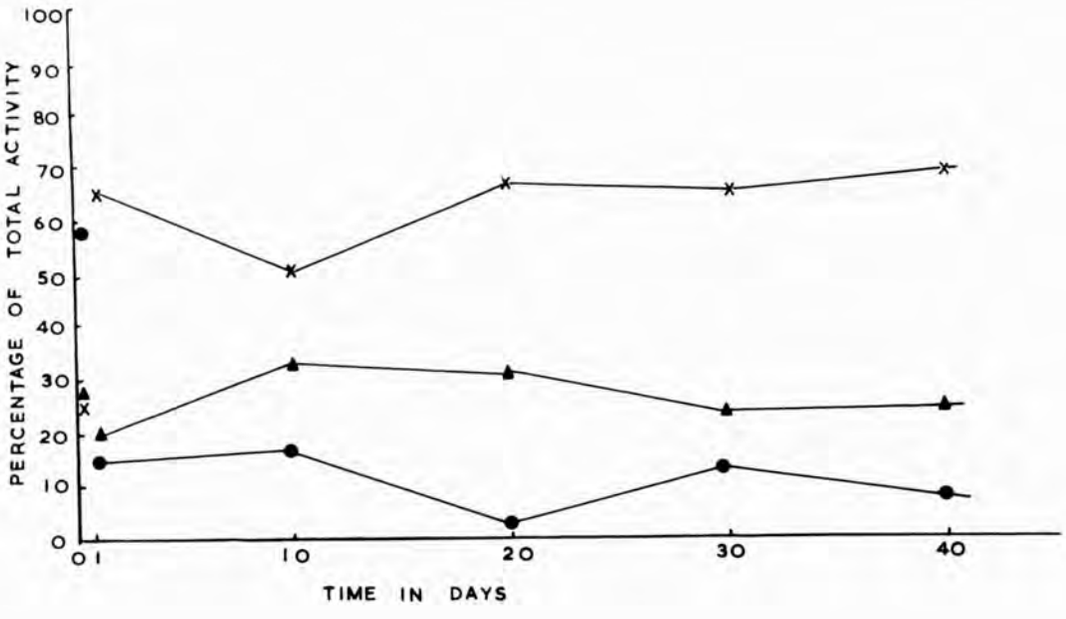
| Fish | Rest | Up-down Escape | Low-level |
|---|---|---|---|
| 1 | 0 | 65 | 35 |
| 3 | 0 | 10 | 90 |
| 4 | 0 | 6.67 | 93.33 |
| 5 | 0 | 16.67 | 83.33 |
| 7 | 40 | 0 | 60 |
| 8 | 0 | 46.67 | 53.33 |

The mean values for the surviving fish are given in table .

TABLE 24. The mean values for the behaviour of 6 fish from 1 to 40 days after blinding.

| Group | Rest | Up-down Escape | Low-level |
|---|---|---|---|
| 24 hour | 14.44 | 20 | 65.56 |
| 10 day | 16.11 | 33.61 | 50.28 |
| 20 day | 1.7 | 31.5 | 66.8 |
| 30 day | 12.22 | 22.22 | 65.56 |
| 40 day | 6.67 | 24.16 | 69.17 |

Fig. 55




The mean activity of a group of blinded fish taken for 40 days after operation.

Rest - ●

Up-down escape - ▲

Low-level exploratory - X

Fig. 56

Diagram of the activity pattern of 24-hour blinded

mimosae.

Abbreviations:

UD. - Up-down escape behaviour

LL. - Low-level exploratory behaviour

R. - Rest.

Fig. 56

Diagram of the activity pattern of 24-hour blinded minnows.

Abbreviations:

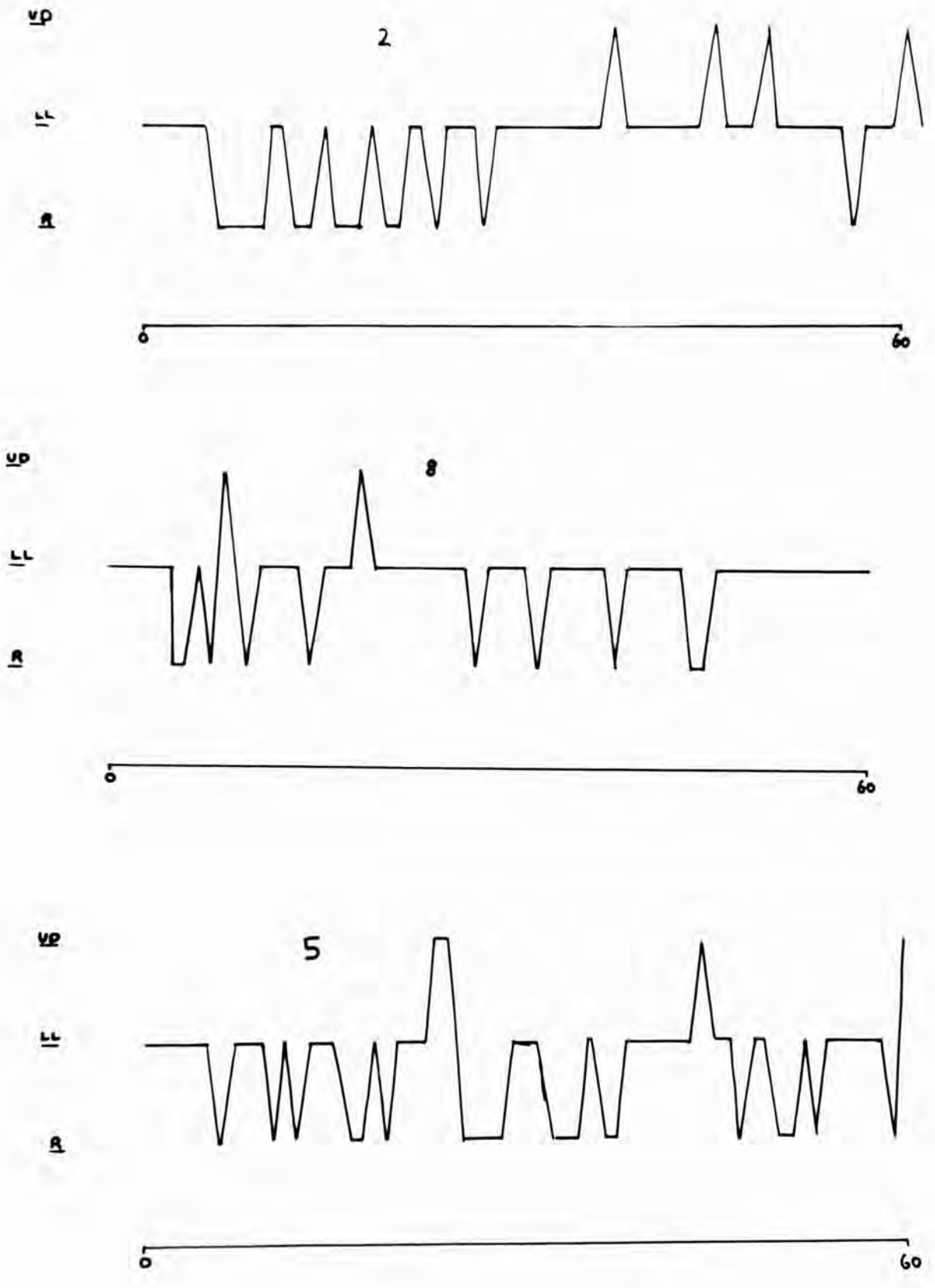
UD. - Up-down escape behaviour

LL. - Low-level exploratory behaviour

R. - Rest.

Fig. 56

24 HOUR BLINDED MINNOWS



These mean figures are plotted on a graph, fig. 55 . page 286. The general activity of the fish after 24 hours is shown in fig. 56 p. 288.

The first feature of these results is the consistency of the mean figures which do not vary very much throughout the whole of the 40 days. In the blind fish the low-level activity becomes the dominant pattern and the rest period is very reduced. The up-down escape response ranges from 20 to 33% of the total activity, a figure that does not differ significantly from that found in the normal fish.

Further comment must be made about the results because by themselves they do not give an adequate picture of the behaviour of the fish. Firstly, the blinded fish swim at a variety of depths, but they always show the exploratory behaviour that is generally seen near the bottom. Secondly, the blinded fish show a steady up and down swimming pattern but this does not constitute the escape response. Thirdly, although the depth varies the direction of each individual movement of the fish is such that the animal appears to be showing a constant exploratory pattern. They thus differ from minnows with tectal removals which, although they show a constant movement, do not show the persistent changes in direction characteristic of the exploratory pattern.

## IV. BLIND FISH WITH TECTAL DAMAGE

A total of 33 fish had small lesions placed in the optic tectum. They are the fish used for the identification of the active region of the tectum in section 6 part IV (p. 153). The fish were blind for 24 hours, after which time the left lobe of the tectum was removed and a lesion was placed in the dorsal part of the right lobe. The results show a wide variability of response and no distinct pattern could be found. Of the 33 fish, sixteen appeared to behave normally, and all the others showed some degree of abnormal behaviour.

### (a) Rolling

4 of the fish showed persistent rolling on recovery from the anaesthetic and in some cases this was coupled with more complex motor defects.

Fish X+0.6mm Y0.65mm on recovery began to swim on its side, and upside down but shortly afterwards it could not maintain this degree equilibrium and underwent pronounced rapid clockwise rolling.

Fish X+0.3mm Y2mm underwent violent rolling only when stimulated by a shock. Otherwise it lay on the bottom, on its right side. The fish rolled clockwise in all parts of the beaker, the period of activity

lasting about 20 minutes. The period of activity following further stimuli became reduced until the fish did not show any activity to the stimulus.

Fish X+0.33mm Y1.3mm showed a further variant. It swam continuously with its snout inclined upwards clockwise and anti-clockwise around the beaker and rolled anti-clockwise.

Fish X-0.12 Y1.6mm showed a clockwise roll and was inclined at a 45° angle to the surface for about two hours after the lesion, after which it ceased to roll but swam with its body inclined at nearly 90° to the surface.

(b) Swimming at the surface.

Three of the fish showed this tendency. Fish X+0.64mm Y1.2mm was normally orientated and X0 Y1.1mm and X+0.18mm Y0.66 were both inclined at a 45° angle to the surface.

(c) Inverted swimming

Fish X+0.33 Y1mm showed periods of rest and activity, the rest periods exceeding the activity. The fish could swim normally but only for short periods, after which it swam in a series of loops and finally inverted. Although it generally followed this sequence of events it could commence to swim inverted immediately following the rest period and show no looping.

## V. DISCUSSION

In comparing normal and blind fish the most noticeable difference is in the resting period, for in normal fish it is dominant but in blind fish it forms the smallest component of the behavioural complex of the animal. This resting period is essentially the habituation of the animal to its environment, so that the blind fish fails to habituate within the test period to the environmental conditions in the beaker. These results largely agree with the observations of Breder & Gresser (1941 a, b) who found that fully blind Characins show very different behavioural patterns to the normal fish. The blind fish do not have any schooling instinct and continually wander in an apparently aimless manner. They concluded that there is apparently no substitute mechanism developed to function for the lost vision. The minnow similarly does not appear to be able to habituate within 40 days to a confined environment devoid of any clues except the sides of the beaker. It would therefore appear that the minnow is essentially a visual animal in in relation to its ability to habituate to such a confined environment. It would be interesting to continue to test more minnows for a longer period. The results from two isolated fish after 4 months gave mean values of 77.5% for the rest period, which is in excess of the normal fish, and may indicate changes in the sense organs, or in the mode of action of the central nervous system.

Before considering the motor and behavioural defects following tectal damage further there is the question of whether the results can be explained by purely supposing that they are due to defects in the visual field. Sperry (1950) found that he could get forced circling and rolling following visual inversion in the Goldfish. In the large number of experiments performed here on the retina various parts of the eye were removed and in no case were there observed any defects in behaviour, or in the motor pattern of the fish. It is therefore concluded that the defects produced by tectal damage are due to the tectal damage, and not to any effect they might have on the visual field.

In all the fish presented here none of them appeared to have any damage to any part of the brain other than the tectum.

In the results one of the most noticeable features was the absence of any body flexure due to the unequal contraction of the myotomes. Such a flexure was reported by Tuge (1934 a) following cerebellar lesions and has been subsequently reported by Kirsche & Kirsche (1961) and by Botsch (1960) following tectal lesions. Although no body flexure was noted in the fish with tectal lesions, it was noticed in the minnow when the lesions had been more extensive, and damage had been present in the valvulae and the torus semicircularis. Close examination of the photomicrographs shown by Kirsche & Kirsche and by Botsch did show definite damage to the valvula and to the tegmentum. It may well be that this damage resulted in the flexure and, in general agreement with

Dijkgraaf (1949), it would seem that this particular motor disturbance only occurs as a result of damage to the valvulae and the torus semicircularis.

The fish appeared to show normal locomotory behaviour following the removal of one lobe of the tectum, in agreement with the findings of Dijkgraaf (1949), Rizzolo (1929) and Steiner (1938). The normal behaviour only seemed to occur if both the anterior and the posterior parts of the tectum were present but they did not necessarily have to be present on the same side. Anterior or posterior removals alone showed defective behaviour but in the 4 fish where the anterior was removed from one side and the posterior from the other, (maintaining the torus longitudinalis intact), the fish showed normal behaviour. Fish with the large tectal removals did not show the gross motor disturbances which occur following small more discrete lesions. The small tectal lesions resulted in most of the disturbances reported by previous workers and included rolling, incorrect horizontal orientation, circus movements, inverted swimming and finally respiratory defects (fish X+0.33mm Y1mm showed a very rapid opercular beat). The disturbances are essentially disturbances in the locomotor co-ordination as a whole and they do not show any tendency toward localisation. These results do not support the view that there is definite localisation of motor areas in the tectum put forward by Chauchard & Chauchard (1929 a). The results from the minnow do support the conclusions put forward by Healey (1957) in that

these positional and motor irregularities occur because they remove certain elements from the total complex modifying system and that they throw the output of the remainder into a state of unbalance. On the other hand, removal of the entire tectum or a complete lobe, may not introduce any unbalance modification by way of the tecto-spinal or the tecto-cerebellar path.

One of the features which was common in all the fish with anterior tectal removals which left the posterior tectum intact was that they all swam with the snout protruding or very close to the surface. This type of behaviour is only seen in fish which are in water where the oxygen tension is very low. Normal fish never swim steadily close to the surface. There are several possible explanations for this. The most likely one is that the fish cannot orientate horizontally so that it constantly swims with its snout protruding. It is also possible that the behaviour is due to an interruption of the blood supply as a result of the operation and the resulting anoxia in various parts of the brain.

One of the most common features of the larger tectal removals was the loss of one or more of the 3 normal behavioural patterns. The fish with one lobe of the tectum or the components of one lobe present (i.e. contributions from the right and left sides) all showed the up-down escape response. This was not seen in any of the group where either the anterior or the posterior tectum had been removed. A number of fish only moved when stimulated, 33, 36, 37 and 64, and then only for a short

period. A large number of fish did not show any rest periods; 47, 56, 60, 61, 48 and 54 and swam continously. The swimming of the fish without rest periods was not normal, and consisted of slow swimming movements continuously around the beaker with no change in direction and no change in depth. They appear to have been more in the nature of a reflex than the normal exploratory movements seen in confined minnows. These results seem to suggest that the tectum not only acts to co-ordinate and intergrate behavioural patterns but also to initiate and maintain the behaviour of the fish, possibly playing a major rôle in motivation and drive. Besides initiating the behviour of the fish it may play an important rôle in the inhibition of behaviour, but this is difficult to demonstrate. Although the resting pattern is dominant in the normal fish it is absent in the tectal removed fish and is reduced in the blind fish. Considerably more work is necessary before it becomes possible to understand the complex and very interesting functions of the optic tectum.

Finally, the last abnormality to be observed was the response of the fish to stimuli. This was observed in fish 70, 69 and the lesions X+0.18mm Y0.66m, X+0.33mm Y1mm, X+0.18mm Y1.6mm, X+0.35mm Y1.6mm. In all these fish instead of hibituating to a series of shock stimuli, discussed in Section II (p. 298), the response to each successive stimulus becomes increased. The first two or three stimuli result in the tail-flip response but the subsequent stimuli result in this response becoming more pronounced so that finally the fish leaps out of the water with

each stimulus. It would therefore seem that the balance between the stimulus and response has been disrupted so that the response is far in excess of the relative strength of the stimulus.

# S E C T I O N 11

## THE OPTIC TECTUM AND HABITUATION.

The term 'habituation' has been given a wide variety of meanings, but is used here in the sense defined by Thorpe (1963), as the permanent waning of a response as a result of repeated stimulation which is not followed by any kind of reinforcement. It is specific to the stimulus and is relatively enduring. In this sense it is applied to the whole animal and its relationship to its environment. Used in this sense it differs from the definition used by Horn (1967): the attenuation of response associated with the repeated presentation of a stimulus and the recovery of the response which may afterwards be induced. Under this definition of Horn's there is no reference to the permanence of habituation, neither does it distinguish it from sensory accommodation. The use of the term 'habituation' is also not the same as the extinction of the orienting reflex of Pavlov (Sokolov 1960), for the orienting reflex is essentially un-specific, and is initiated by increase, decrease or other quantitative changes in the stimulus, and is independant of the modality of the stimulating agent.

## I. STIMULUS AND RESPONSE

The minnow, unlike the goldfish, does not respond to visual stimuli by giving the tail-flip response under normal experimental conditions. The most successful stimulus was found to be a simple shock wave, produced by a single blow of a wooden mallet on a pad of foam rubber, the pad of rubber being placed next to the fish container. The testing conditions were the same as those described for the colour change reversal tests (p.35 ). Care was taken to ensure that the amount of water in the container and the beaker were always the same and that the positions of the beaker and the container were always constant in relation to the stimulus.

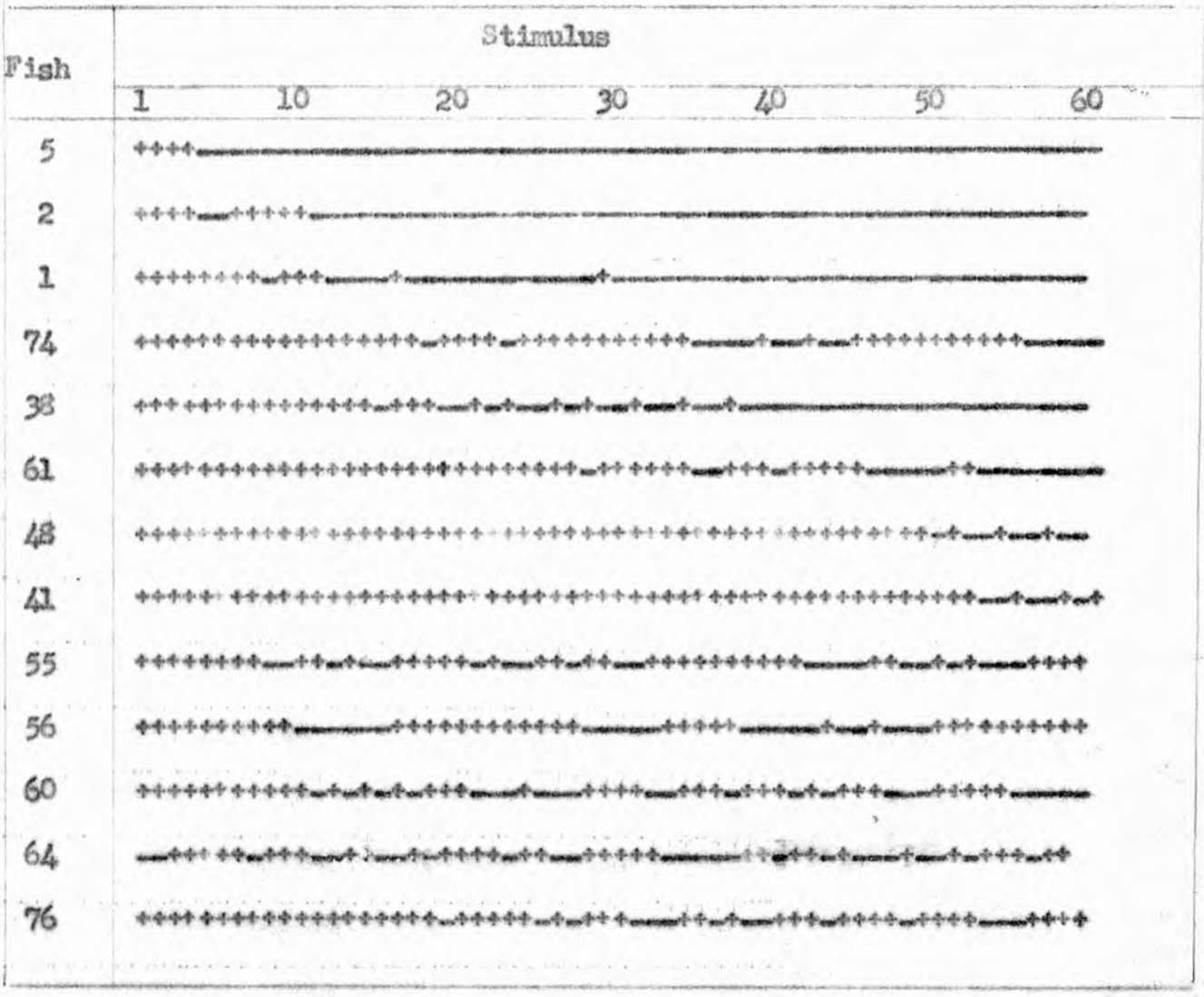
The main difficulty was that the stimulus was not the same intensity throughout the tests. The fish were, however, very sensitive to vibrations of the table and even the slightest tap on the table resulted in the jerk response so that the intensity for the stimulus was always above the threshold for the response. Fish were given very low strength stimuli which were repeated at intervals (one every 3 seconds) until no further response could be obtained. If the stimulus strength was increased further responses were obtained. Finally there was a point were no further responses were obtained regardless of the intensity of stimulus. The stimulus intensity would therefore seem to be important at low intensities, but above a certain threshold it ceases to be im-

portant and the stimulus used in this experiment was above this threshold. In no case did the fish give any further response after it had habituated, even when the stimulus strength was increased.

The stimulus was presented at a frequency of one every 3 seconds for a period of 3 minutes, which means that the fish received a series of 60 stimuli. If the stimulus frequency was increased the fish did not initially respond to each stimulus but only in a very irregular pattern. At a frequency of 2 a second or more, the fish age a single response to the first stimulus and then no further response. Decrease in the stimulus frequency resulted in a longer time to show any waning of response and produced a less clear result. The habituation was not due to the number of stimuli. The number of stimuli within a given time is the important consideration.

Following the stimulus, the fish exhibits a sudden violent tail-flip, the latency period being too short to be measured. The tail-flip was brought about by a single movement of the tail and most of the body to produce a single massive beat which resulted in the fish being propelled through the water for a distance of at least its own length (6cms.). The direction in which the fish moved depended on the position of the fish at the time of the stimulus. After the tail-flip the fish did not move for a short period, the freezing period of Russell (1967), and no record was taken of the time of this freezing period. The freezing period may or may not be followed by a period of after-excitement which, if it occured, could last for 2 to 20 seconds and consisted of rapid swimming

Fig. 60




The response pattern of a number of fish. + is the tail-flip, and - is a negative response to the stimulus. The arrangement of the figure shows the gradual increase in complexity of pattern.

on the bottom or at intermediate depths.

Records of the presence or absence of the tail-flip are shown in fig.60 (p.301) with positive or negative signes. Orientation phenomena recorded by Russell (1967) and by Hodgers et al (1963) were observed.

## II. RESULTS

The waning of response was marked in all those fish which had some tectum present. The results are shown in tables 25 page 304-6 and graphs were plotted to show the relationship between the stimulus to habituate and the percentage of the tectum present (fig.57 p.304). The stimulus to habituate is taken as the last stimulus to give the tail-flip and if the fish did not show this by the 60th stimulus it was given 20 additional stimuli to test whether it could show a complete absence of the tail-flip. A second set of figures is shown plotted on fig. 58 Page 310, to show the number of responses in relation to the percentage of the tectum present.

The stimulus response pattern is shown on Fig. 60 page 301. The most common type of pattern is seen in the fish 2 and 5, where the fish responds to the first stimuli, but after a certain number ceases to respond further. Over no period was the response reduced in intensity nor was there any period giving positive and negative responses. A large number

TABLE 25 a, b, c.

The relationship between the tectum and a shock stimulus. Habituation is scored as positive only when the fish shows a definite stimulus to habituate.

(a)

| Fish | Treatment | Percentage tectum Present | Habituation | Stimulus to habituate | | Response | |
|---|---|---|---|---|---|---|---|
| | | | | No. | Mean | No. | Mean. |
| 1 | | | | | | | |
| 1 | | | + | 5 | | 6 | |
| 2 | | | + | 3 | | 3 | |
| 3 | | | + | 10 | | 10 | |
| 4 | | | + | 1 | | 1 | |
| 5 | Normal | 100% | + | 23 | 8.2 | 20 | 7.7 |
| 6 | fish | | + | 12 | | 14 | |
| 7 | | | + | 11 | | 9 | |
| 8 | | | + | 4 | | 3 | |
| 9 | | | + | 9 | | 7 | |
| 10 | | | + | 4 | | 4 | |
| 11 | | | + | 10 | | 5 | |
| 12 | | | + | 13 | | 10 | |
| 13 | Blinded fish | 100% | + | 4 | 8 | 5 | 6.6 |
| 14 | | | + | 8 | | 4 | |
| 15 | | | + | 5 | | 8 | |
| 68 | | 96 | + | 6 | | 9 | |
| 69 | Small part | 84 | - | - | | 60 | |
| 70 | of dorsal | 91 | - | - | | 60 | |
| 71 | tectum re- | 95 | + | 7 | | 8 | |
| 72 | moved | 86 | + | 12 | | 15 | |

(b)

| Fish | Treatment | Percentage tectum Present | Habituation | Stimulus to habituate No. | Stimulus to habituate Mean | Response No. | Response Mean |
|---|---|---|---|---|---|---|---|
| 20 | | 50 | + | 30 | | 30 | |
| 21 | Left half | 50 | + | 40 | | 40 | |
| 22 | of tectum | 50 | + | 10 | 24 | 10 | 23.4 |
| 23 | removed | 50 | + | 20 | | 15 | |
| 24 | | 50 | + | 20 | | 22 | |
| 29 | | 0 | - | - | | 60 | |
| 34 | Anterior | 53 | + | 30 | 30 | 30 | |
| 35 | tectum | 57 | + | 30 | | 30 | |
| 67 | removed | 25 | - | - | | 60 | |
| 31 | | 45 | - | - | | - | |
| 33 | | 47 | - | - | | - | |
| 36 | Posterior | 74 | + | 12 | | 13 | |
| 37 | tectum | 72 | + | 10 | | 10 | |
| 40 | removed | 44 | - | - | | 45 | |
| 65 | | 65 | + | 27 | | 24 | |
| 64 | | 52 | - | - | | 35 | |
| 58 | | 52 | + | 39 | | 27 | |
| 59 | | 54 | + | 53 | | 44 | |
| 73 | Posterior | 53 | + | 33 | | 28 | |
| 74 | right and | 55 | + | 56 | 43.6 | 46 | 36 |
| 75 | anterior | 53 | + | 38 | | 25 | |
| 76 | left re-moved | 56 | - | - | | 46 | |

(c)

| Fish | Treatment | Percentage tectum Present | Habituation | Stimulus to habituate No. | Stimulus to habituate Mean | Response No. | Response Mean |
|---|---|---|---|---|---|---|---|
| 55 | | 36 | - | - | | 39 | |
| 54 | | 27 | + | 48 | | 47 | |
| 56 | All tectum | 31 | - | - | | 39 | |
| 61 | removed | 34 | + | 49 | | 49 | |
| 60 | except left | 20 | - | - | | 37 | |
| 47 | anterior | 27 | + | 57 | | 56 | |
| 48 | | 36 | - | - | | 53 | |
| 499 | All tectum | 18 | + | 55 | | 43 | |
| 41 | removed | 30 | - | - | | 55 | |
| 57 | except left | 25 | + | 46 | | 22 | |
| 45 | posterior | 31 | + | 55 | | 55 | |
| T1 | | 0 | - | - | | 60 | |
| T2 | | 0 | - | - | | 60 | |
| T3 | Complete | 0 | - | - | | 60 | |
| T4 | tectal | 0 | - | - | | 60 | 60 |
| T15 | removal | 0 | - | - | | 60 | |
| T14 | | 0 | - | - | | 60 | |

Fig. 57

The effect of tectal removal on the ability of the minnow to habituate to a shock stimulus.

Fig. 57

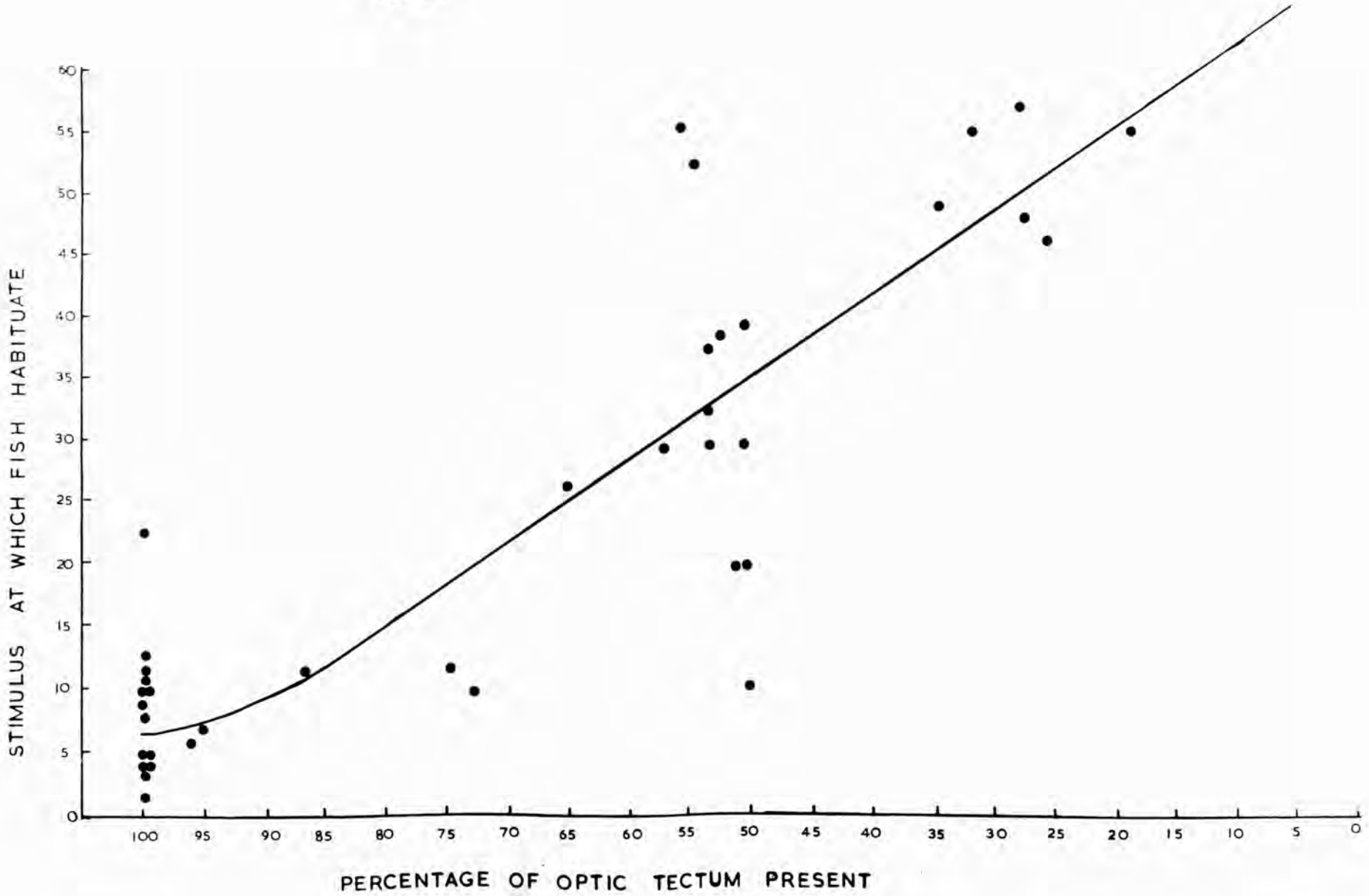
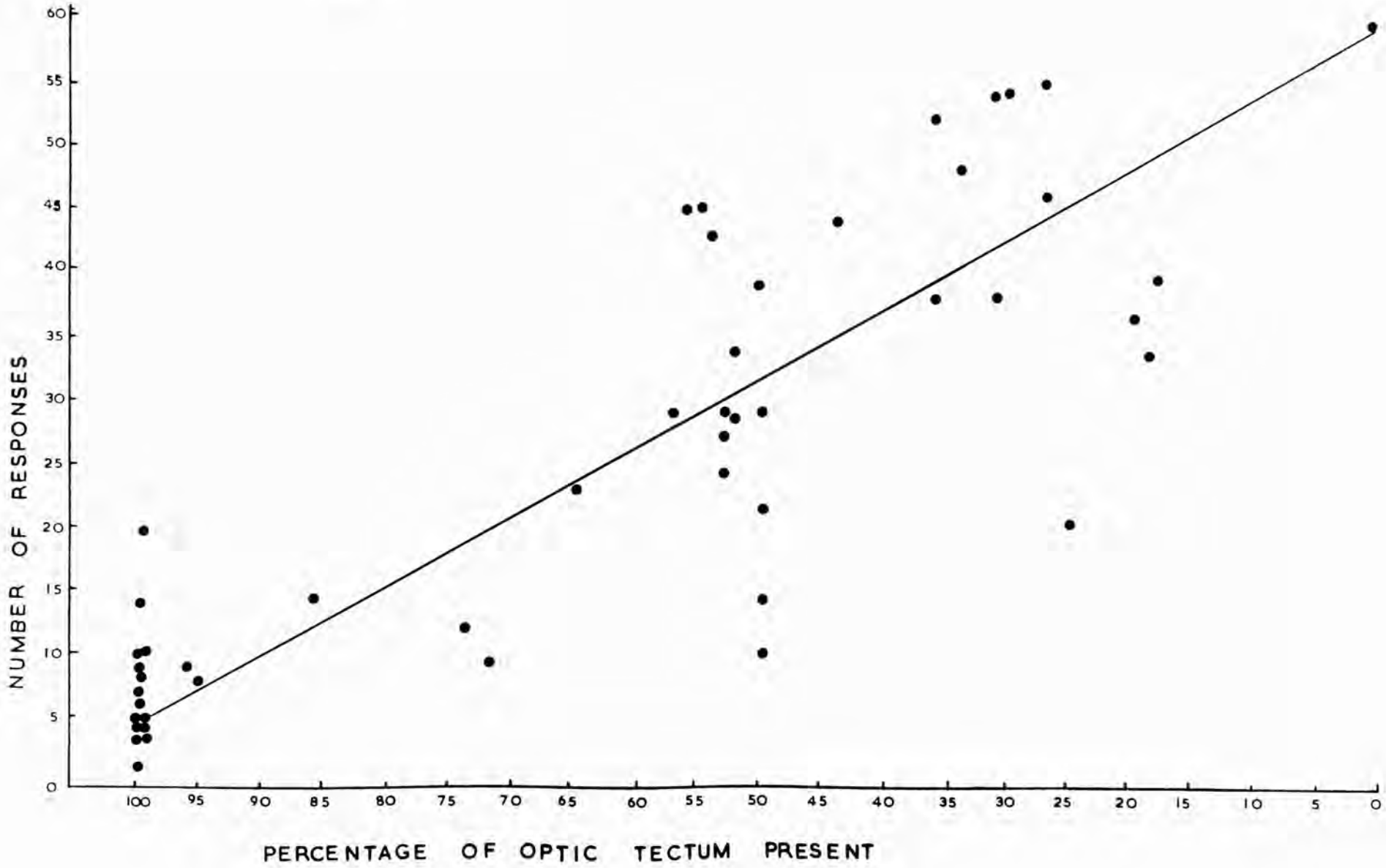

STIMULUS AT WHICH FISH HABITUATE
0
5
10
15
20
25
30
35
40
45
50
55
60
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
PERCENTAGE OF OPTIC TECTUM PRESENT

Fig. 58

The number of positive tail-flip responses given by minnows with varying degrees of tectal damage.

Fig. 58


NUMBER OF RESPONSES
0
5
10
15
20
25
30
35
40
45
50
55
60
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
PERCENTAGE OF OPTIC TECTUM PRESENT

show a more varied waning pattern, 74 shows a similar pattern to 5 but there were several negative responses before the final positive response. In fish 1 and, to a greater extent, 38, the final habituation occurred after a period of positive and negative responses. The difference between the response value and the stimulus to habituate value indicates the type of response waning. Fish which do not show a definite habituation are also included in this table and it can be seen that the response waning pattern is essentially that of the other fish. Many of the fish which do not habituate do, however, show similar values to the fish which do habituate when the number of responses are compared. The only difference in the fish which do or do not habituate is in that in one group the fish are unable to suppress completely the tail-flip response.

The graphs show that there appears to be a definite relationship between the amount of the tectum removed, and the ability of the fish to suppress the tail-flip response to a shock wave. The number of responses shows an increase with the amount of the tectum removed. The stimulus to habituate curve shows clearly that this relationship is far from being linear for the fish with 80% tectum present have the same ability to habituate as do normal fish. What the curves do show is that there appears to be a threshold over which the removal of the tectum affects the response waning of the fish.

A few fish were tested for their retention ability. They were given the same stimuli 1 hour after the first test and they all showed at least a 50% improvement on their previous performance and in some

cases considerably more. Fish 21 showed a complete habituation in the first test after 40 stimuli but in the second test it had almost completely habituated by the 10th stimulus and only gave one more response in the next 50 stimuli.

The relationship between the tectum and habituation is far from being simple. Fish with the anterior tectum present show an incidence of partial habituation of 50% against 20% in those with the posterior tectum present. Also in the group which have 50% removal by means of the removal of the left lobe the mean stimulus to habituate was 24. The fish where the posterior right, and the anterior left were removed, and with 50% present, showed a mean of 43.6 which is almost double the value for the removal of a single lobe.

## III. DISCUSSION

The question arises as to whether these results could be due to fatigue or sensory accommodation. The fish were tested for a further one or two minutes, and the total tectal removed fish did continue to react positively to each stimulus, ruling out the possibility of fatigue. There are two reasons why it is probably not sensory accommodation; the first is that the results did last for a period of one hour and on the second test there was a 50% improvement. The second point is that removal of the tectum prevent habituation under these conditions whereas

the blinding has no effect. It would therefore seem that the response waning in the minnow is a definite example of habituation learning and that it is mediated by the optic tectum. Furthermore, this learning is second-order learning, for the stimulus is by way of the acustico-lateralis system and from this system it is relayed on to the tectum.

Russell (1967) failed to get complete inhibition of the jerk response using visual stimuli, but did report a two-thirds decline in the response to 40 stimuli with a 60% recovery in 2 hours and a complete recovery in 48 hours. Rodgers et al.(1963) tested 10 fish giving them 10 taps a day at the rate of one per minute for 15 days. They found that the fish showed a score of 5 flips on the first stimulus series and on the following days showed a variation of from 0.8 - 4.1 per day. Unfortunately, the results for the minnow are only from a single test so that they could not be compaired with those of Rodgers et al.

Both Russell (1967) and Rodgers et al.(1963) discuss the compensatory fin movements following the presentation of the stimulus, after the fish no longer shows the tail-flip. Rodgers et al.concluded "they continue to maintain a state of vigilance toward the stimuli, in which the disruptive behaviour of the tail-flip response is replaced by more organized orienting responses". This orienting behaviour is capable of alternative explanations, for in the minnow the orientation resembles the righting reflex described by Lowenstein (1932), showing the character-

Fig. 59

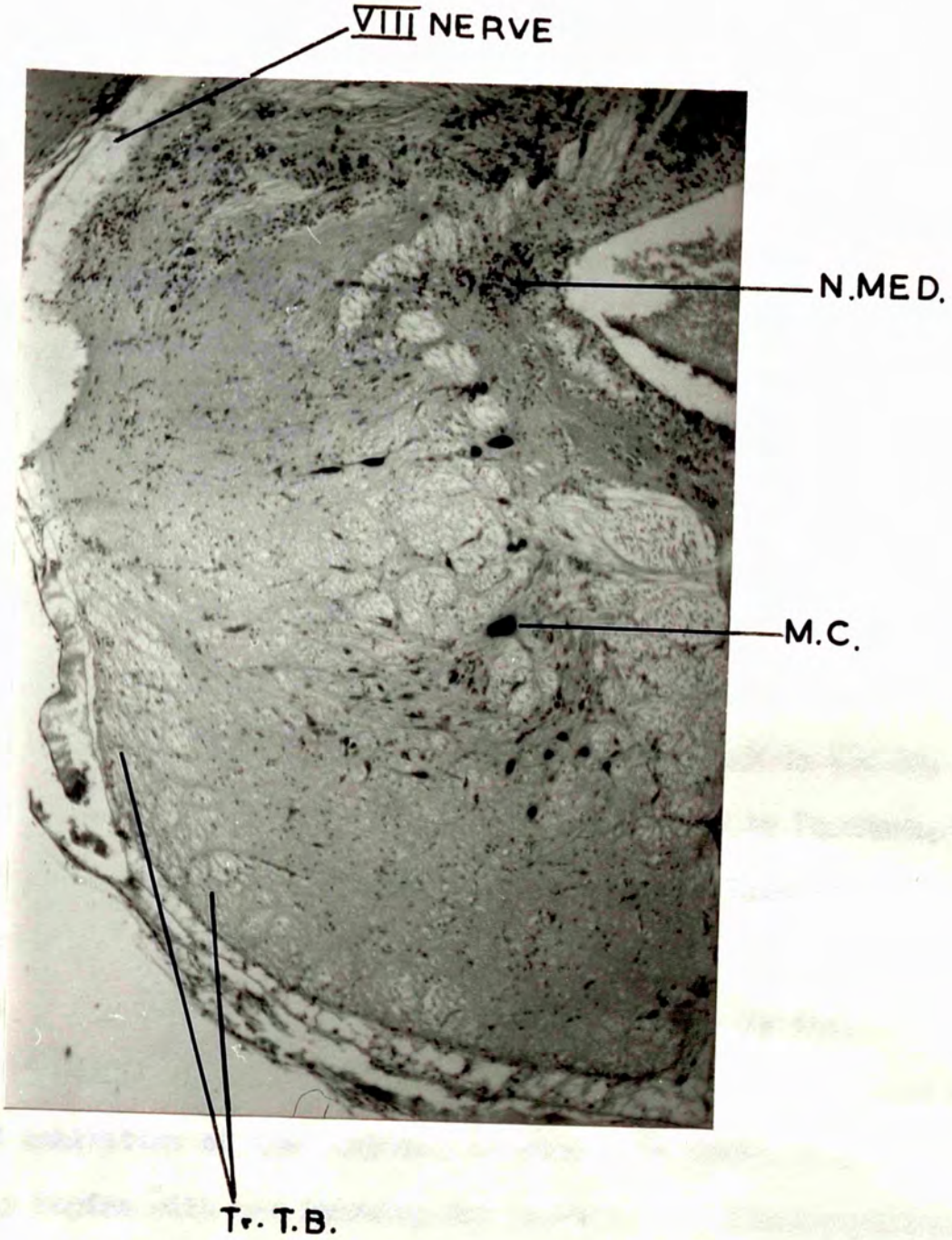
Transverse section of the medulla at the level of the entrance of the VIII nerve, stained with C.F.V.

M.C. - Mauthner cell

N.MED. - Nucleus medialis

Tr.T.B. - Tractus tecto-bulbaris

Fig. 59



istic positions of the fins. It would therefore appear that in the minnow the orienting responses to the stimulus, after the fish no longer shows the tail-flip, are solely intended to maintain the balance of the fish following the water disturbance, as a result of the shock wave, and are not any attempt on the part of the fish to maintain a state of vigilance toward the stimulus.

Anatomically the tectum is connected to the Mauthnerian apparatus by means of fibres in the tecto-bulbar tracts identified by Bartelmez (1915) and Kappers et al (1936) and these have been confirmed in the minnow in the present work. The position of the Mauthner neurone is shown in fig. 59 page 315. Retzlaff (1957) and Retzlaff & Fontaine (1960) found that the mauthner neurons receive afferent fibres from the 8th cranial nerve and that they show reciprocal inhibition so that one of the neurons inhibits the other and an inhibitory feedback is set up. More recent work by Furkawa & Furschpan (1963, 1962) and by Furukawa, Fukami & Asada (1963) have shown that there are at least three types of inhibition present. The position of the tectal control in this complex picture remains to be elucidated. What can be said however is that the experiments on the minnow show that the tectum plays an important part in the learned inhibition of the mauthner neurons. The fibre pathway involved possibly begins with the incoming 8th nerve to the acoustico-lateral regions of the medulla, and from there a bundle of fibres, the secondary trigeminal tract, runs to the tectum. From the tectum inhibitory fibres pass out in the tecto-bulbar tracts which run to the mauthner neurons.

It would seem that the inhibitory action would be required to reach a threshold before it could affect the mauthner neurons, and the response of the mauthner neuron being essentially an all or nothing phenomina, the tail-flip is either present or not. Possibly the successive stimuli build up this inhibition, in which case the removal of the tectum would result in more stimuli being necessary to reach the threshold. Once the inhibition has been built up it is maintained, but in some of the removals the level of inhibition could not be maintained for long periods and positive responses could be found. The presence of these positive and negative responses alternating would suggest that the hypothesis proposed by Horn is only part of the possible mechanism. Horn (1967) proposes that the gradual waning of response of a system of neurones to a stimulus which is slowly and repleatedly applied is a result of a self-generated depression of sensitivity at one or more points in the system. The other part of the mechanism could be explained by the work of Sokolov (1960) who supposes that the response decrement is caused by an active blocking mechanism. On a repeated presentation of a stimulus two processes take place; one the elaboration of the neuronal model of the stimulus situation, and the other the elaboration of the conditioned inhibitory reflex which makes it possible to block these impulses. If there is a change in the stimulus, non coincidence between the reflex and the neuronal model results in the response. Therefore, in the minnow the persistence of the habituation could be explained by the presence of a model of the stimulus situation, and the active blocking of the response, the blocking being carried out by a self-generated depression at one or more points in the system. It would be very interesting to continue these experiments,

to test the fish for long periods of time and to test fish which show the response to visual stimuli so that the stimulus situation could then be varied.

The relationship between cerebral mass and learning in mammals, especially in rats, has been worked on by Lashley (1922, 1924, 1926, 1931, 1934, 1937, 1939). The main work was concerned with the study of maze performance in the rat following cerebral excisions. The findings led him to propose three main conclusions. Firstly, the formation of maze habits is impaired by cerebral lesions, the degree of impairment being closely related to the extent of the lesion, and is independent of its locus. Secondly, the retention of maze habits learned prior to operation is impaired by cerebral lesions, the degree of impairment being again proportional to the extent of the lesion and independent of its locus. Thirdly, the defect in performance produced by any given lesion is a function of the complexity of the task. Lashley considered that localisation in the cerebral cortex must be considered in relation to mass action, for his results show that maze habits based on detailed vision are lost following the excision of a small area of the lateral part of the area striata. Habits based on brightness discrimination are lost only after the destruction of the entire striate area, so that at least brightness discrimination is localised in the striate area. In a recent review of Lashleys work, Zangwill (1961)has shown that the more recent work favours the differential localisation of specific behavioural patterns but that new evidence does not warrant the abandonment of Lashley's

position. Work by Botsch (1960) has shown that a similar relationship occurs (mass action) in the relationship between visual discrimination and the removal of the tectum in the carp, Carrassius. The results shown here on the minnow agree very closely with the mass action concept of Lashley. This close agreement would be expected because of the absence of any definite localisation in the tectum and because of the essential simplicity of the task. In general it can be said that the more tectum removed the longer the fish takes to habituate to a shock stimulus and this is independant of the location of the removal. This is further complicated by the fact that the more the tectum is disrupted the more stimuli are needed before the fish cease to respond. In the group of fish where the posterior right and the anterior left part of the tectum was removed, the mean number of responses were 36 whereas, the mean for the habituation stimulus was 43.6. The fish with the left lobe removed gate the corresponding figures of 24 and 23.4 respectively. Both groups had at least 50% of the tectum present. If this is taken with the evidence of the high incidence of partial habituation in the fish with the anterior tectum only present (50%) it would suggest that the tectum acts as a unit. If necessary, as in the case of removal, the part remaining can assume the rôles of that removed. However, the more it is disrupted the less it can function normally.

# SUMMARY

Only the new findings are listed in this summary.

Section 2

1. It was found that a 0.003% solution of MS222 fully anaesthetised 6cm fish but this dilution was unstable. The fish could survive for more than an hour in this concentration (p. 38).

2. A method for the closure of the skull after surgery was described (p. 39).

3. The first use of the staining technique of Klüver and Barrera on *Phoxinus* showed that the fibres had very little affinity for the Luxol Fast Blue. This may indicate differences in the chemical composition of these fibres (p. 44).

Section 3

4. No specialised 'Area' could be found in the retina (p. 50).

5. A method for cutting the retina tangentially without removing pieces was described (p. 55).

6. Triangular triple cones as well as the linear type appear to be present in the retina of the minnow (p. 69).

7. Counts were made of the retinal receptors and it was found that the rods single and double cones were more numerous peripherally than centrally, and that the triple and quadruple cones were more numerous centrally (ps. 65, 69, 72).

8. The retina is predominantly a cone retina (p. 65).

9. In common with other teleost fish there were a greater number of receptors in the temporal field than in the rostral field of the retina (p. 72).

10. The ventral region of the retina differs from the dorsal in having very few triple and quadruple cones but a large number of double and single cones (p. 72).

11. The visual acuity of various regions of the retina were calculated and the extreme dorsal region was found to be lowest (p. 72).

12. The cone mosaic of the retina was discussed and it was suggested that the quadruple and triple cones possibly function to replace this simple mosaic. The close proximity of the individual cones would serve to amplify the benefits of a complex mosaic (p. 73).

13. A simple apparatus for the production of retinal lesions was described (p. 86).

14. Lesions produced by high intensity light were described (p. 86).

15. The surgical removal of the distruction of the dorsal retina resulted in the fish being fully dark on a black and intermediate on a white background. Similar destruction or removal of the ventral retina resulted in the fish being intermediate on the black and pale on a white background. From these results it was argued (p. 95) that the whole of the retina is important in chromatic adaptation and there was no evidence for any regid retinal differentiation (ps. 93-98).

Section 4

16. The structure of the optic tract of the minnow was described (p. 104).

17. The optimum survival time for the degeneration of the optic tract using the Nauta technique was 14 days at 18°C (p. 100).

18. The structure of the optic chiasma was described and 9 different conditions were found (p. 107).

19. It was found that fibres from the optic tract either end in the optic tectum or in the geniculate complex and no other fibres could be found (ps. 114-126).

20. The structure and fibre connections of the geniculate complex was described and was homologised with other teleosts and other higher vertebrates (ps. 114-126).

21. The anatomy of the optic tectum was described together with its fibre connections (ps. 127-134).

Section 5

22. An accurate account of the colour of blinded minnows was presented showing after the initial darkening half of the fish pale and the other half remain dark (ps. 135-139).

23. The colour of blinded fish could not be affected by external conditions (p. 139).

24. Attempts to find anatomical differences between the dark and pale blinded fish proved unsuccessful (p. 140).

Section 6

25. Following complete removal of the optic tectum the fish at first paled to a mean B.G.I. of 2.7 but after 24 hours they darken to very variable tints (ps. 142-149).

26. Unilateral section of the optic tract coupled with unilateral tectal removal on the same or opposite side did not affect the ability of the fish to change colour (p. 149).

27. The bilateral removal of the anterior tectum from a blinded darkened fish did not affect its colour (p. 153).

28. The bilateral removal of the posterior tectum of a darkened blinded fish caused maximal pallor (p. 153).

29. By a series of lesions an area in the dorsal posterior part of the optic tectum was isolated and found to cause darkening in a blinded minnow because following its removal the fish paled (ps. 153-162).

30. The presence of the anterior or posterior tectum alone on one or both sides caused persistent pallor in normal fish (ps. 172-176).

31. The presence of the anterior tectum on one side and the posterior on the other enabled the fish to adapt chromatically to its background (ps. 176-180).

32. Small removals from the tectum did not effect colour change provided they were below 9% of the total tectum. Larger removals in the dorsal tectum reduced the extent of change until fairly large removals (16% tectum) caused the fish to be pale on all backgrounds (ps. 181-186).

33. The mode of action of the geniculate complex and the tectum in relation to colour change was discussed (ps. 186-193).

Section 7

34. The optimum survival time for tectal efferent fibres to

degenerate for the Naute technique was 16 days at 18°C. (p. 199).

35. A possible medullary paling centre was identified and its structure described (ps. 202-203).

Section 8

36. The apparatus for implantation of electrodes into the optic tectum was described (p. 217).

37. The apparatus for continuous recording during background reversal was described (p. 218).

38. The effect of curare on colour change and on the mortality of the fish was discussed (p. 221).

39. The EEG was recorded from the surface and that it consisted of two rhythms, a 6-14cy/sec (112-20 v) and a 18-24cy/sec (18-6 v), (p. 223).

40. EEG recordings from fish in darkness, under anaesthetic and following blinding showed that the activity of the tectum was directly related to the retinal input and that in all conditions where the amplitude of the EEG was reduced the fish darkened (p. 230-237).

41. EEG patterns were recorded and analysed from various depths and positions in the optic tectum during background reversal. It was found that in depths 1, 2, and 3 no changes were present but in

depths 4 and 5 changes in the order of 10cy/sec were found in the HF rhythm, (ps. 237-253).

42. The ECG patterns were discussed and a possible hypothysis for the action of the tectum in chromatic adaptation proposed (ps. 254-262).

Section 9

43. A working hypothesis was proposed for the control of chromatic adaptation in the minnow (263-270).

44. The hypothesis was discussed in relation to other theories on colour change in teleost fish (271-273).

Section 10

45. The behaviour of normal minnows in a beaker was described and analysed. It was composed of three patterns, a rest period, an up-down escape response and a low-level exploratory response. The rest period was dominant (ps. 274-279).

46. Normal minnows in a beaker after tectal removals show various modifications in behaviour (ps. 280-282).

47. Blind fish in beakers show a different behavioural pattern to normal fish and the low-level activity predominates (ps. 282-289).

48. Blind fish with tectal lesions showed various defects in motor behaviour (ps. 290-291).

49. The results from the analysis of all the fish were (ps. 292-297) discussed in relation to other workers and it was suggested that the optic tectum may be important in motivation and drive as well as being an important correlating centre (p. 296).

Section II

50. The tail-flip response in the minnow was described (ps. 299-300), and the orientation movements following the extinction of the tail-flip, as a result of repeated presentation of the stimulus, are simple reflex balance responses due to the shock wave (p. 313).

51. The tectum was shown to inhibit the Mauthnerian neurones and represents second-order acoustico-optic learning (p. 313).

52. A description of the response decrement of normal, blind and tectal removed fish was presented (ps. 302-312).

53. The relationship between tectal mass and the Mauthnerian neurones was discussed as well as a possible tectal mechanism (p. 316).

# A C K N O W L E D G E M E N T S.

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# AMENDMENTS

1. Publications omitted from the bibliography.

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2. The publication by Hammond (1968) does not appear in the text and is a recent study on the spectral properties of dark-adapted retinal ganglion cells in the plaice, Pleuronectes platessa L. by means of recordings from the optic tectum.