THE PHOTORECEPTORS OF LAMPREYS

II. THE FUNCTIONS OF THE PINEAL COMPLEX

By J. Z. YOUNG, M.A.

(Department of Zoology and Comparative Anatomy, Oxford.)

(Received 25th January, 1935.)

(With One Plate and Six Text-figures.)

I. INTRODUCTION.

In spite of the considerable amount of work which has been done on the anatomy of the pineal complex of lampreys, and interest which has been attracted by its eye-like structure, no experiments appear to have been made to determine whether the organ is in fact sensitive to light, and if so, what part it plays in the life of the animals. Indeed, very little is known of the functions of the pineal body in any vertebrate. Among reptiles it has a very eye-like structure, and Nowikoff (1907) showed that in lizards following changes of illumination there are changes in the distribution of pigment in its cells. But sudden illumination of the pineal did not produce any movements of the lizard, and therefore, even though the movements of pigment show the organ to be sensitive to light, there is no evidence as to what functions it controls. Tretjakoff (1915) could find no similar movement of pigment in the pineals of lampreys.

V. Frisch (1911) showed that the teleostean fish Phoxinus was still able to change its colour after removal of the paired eyes, and at first he supposed the response to
The Photoreceptors of Lampreys 255

be due to the epiphysis. However, he later showed that the colour changes continued after removal of that organ, so that although light enters through the clear space above the epiphysis, it produces its effect on the cells of the diencephalon itself. Scharrer (1928) discovered that after removal of the paired eyes, Phoxinus not only remains capable of changing colour, but can also be trained to give feeding reactions when illuminated. However, in this case too, the reaction was found to persist after the removal of the epiphysis. There is therefore no single case in which the functions of the pineal eye as a photoreceptor have been discovered.

The present paper describes the movements and internal changes resulting from illumination of various parts of the heads of lampreys, and is an attempt to discover how far the pineal and paired eyes are responsible for these effects.

II. MOVEMENTS OF THE ANIMAL FOLLOWING ILLUMINATION OF THE HEAD.

(a) Effect of illumination of the pineal.

In the previous paper (Young, 1935) it was shown that illumination of the tail of Lampetra fluviatilis or L. planeri is followed by movement of the whole animal. The sensitivity of the head was investigated in the same way as that of the tail, namely by placing the lamprey in a tube, and directing a narrow beam of light on to the region under investigation. In ammocoetes it was found that illumination of the dorsal side of the head, that is to say the region of the pineal, was usually, though not always, followed by movements of the animal. The time of exposure necessary to obtain the reaction, even in the most favourable cases, was always much greater than that needed when the tail was stimulated; the shortest reaction time ever observed being 6 sec. for dorsal illumination as against 1¼ sec. for the tail; in most cases even longer illumination was needed. Moreover, the movements could not be frequently and regularly elicited from the pineal as they could from the tail. The series of reaction times given on p. 256 was quite exceptional: usually, even with intervals of 5 min., the time of exposure needed to produce movements increased very rapidly, the animal ceasing to react at all after very few exposures. In fact the adaptation of the photochemical process, by which it returns to the sensitive condition, proceeds much more slowly in the head receptors than in the tail. In spite of repeated experiments, I did not succeed in so standardising the intervals between exposures as to obtain the reaction from the head region at will. In all animals it is inconstant, and in some cannot be elicited at all. It is possible that the intensity of the illumination used was abnormally strong and damaged the photochemical mechanism, but when weaker intensities were used, no reaction at all could be obtained.

(b) Effect of removal of the pineal.

The matter was further tested by removal of the pineal and parapineal organs. In the earlier experiments an opening was made in the cranium above the pineal, and the latter removed with scissors, but this technique was found to leave too large a hole, through which the brain was apt to bulge. However, if a very small hole
is bored with a needle through the cranium above the pineal complex, then the latter can be caused to protrude and be removed with a fine scissors or scalpel with a minimum of injury to the brain. At the end of the experiments, the heads of the animals were fixed and sectioned for investigation of the extent of the lesion.

It is impossible to remove the pineal complex without any damage to other structures. In all cases the non-nervous roof of the third ventricle (saccus dorsalis) was ruptured, allowing the escape of cerebro-spinal fluid. Removal of the parapineal necessarily involves removal of the small ganglion which is attached to it (gangl. habenulae anterior), but the main habenular ganglion lies posteriorly and was not damaged by the operation. The pineal and parapineal lie very close to the roof of the cerebral hemispheres, which was therefore injured in many of the operations. In most of such cases the damage was restricted to severance of some of the fibres of the commissura olfactoria superior, but in some animals one or both hemispheres had been more deeply incised. Only in one or two cases did these lesions involve any abnormality in the swimming of the animal and in no case did the colour changes (p. 266) differ from those seen in animals from which the pineal and parapineal had been cleanly removed.

It is not considered that any of the results described below were affected by any damage inflicted on structures other than the pineal and parapineal, but at the same time the fact that such damage is almost inevitable must always be borne in mind when interpreting the results of operations in this region.

Altogether the movements of thirteen animals were examined after removal of the pineal complex, two being adult L. fluviatilis, one adult L. planeri, and the rest ammocoetes of L. planeri. In all cases except one, it was found that the head was still sensitive to light, the time of illumination necessary not being noticeably longer than that needed while the pineal was intact. Thus in one ammocoete on the day before the operation, a beam was shone on the pineal with 2 min. intervals, and the animal moved after illumination for 46, 23, 49, 35 and 42 sec. On the day after removal of the pineal, the same animal gave movements after 235 and 42 sec, and 7 days later after 42 and 32 sec.

It therefore seems that although illumination of the pineal may perhaps play a part in producing the motor reactions, yet there are other organs in the head which are also sensitive to light.

(c) Effect of illumination of the paired eyes.

The paired eyes immediately suggest themselves. In adult L. planeri and L. fluviatilis it was found that direct illumination of the eyes was generally followed by uneasy movements of the animal and alteration of the rate of breathing, but not usually by swimming movements such as follow illumination of the tail. Moreover, the time of illumination necessary to produce a movement was far greater for illumination of the eyes than for the tail, the quickest reaction time observed being 11 sec. It is thus clear that, as in the case of the tadpoles investigated by Obreschkove (1921), the activation of the animal in response to illumination is accomplished more rapidly by the photoreceptors of the tail than by the paired eyes. The latter
The Photoreceptors of Lampreys

probably mediate much more complex reactions, either enabling the animal to orientate itself with reference to the direction of the light (topotaxis) or to respond in the appropriate ways to formed stimuli such as prey, animals of the opposite sex, etc.

In ammocoetes, the paired eyes are deeply buried beneath pigment and skin, yet it is possible that light affects them in some way. This is immediately rendered unlikely, however, by the fact that lateral illumination of the head of ammocoetes was only very rarely followed by movements, and then only after very long latencies. However, in order to settle the question, the paired eyes were removed from animals with and without pineals. In neither case was the removal found to abolish the response to dorsal illumination of the head. Thus in an experiment with an ammocoete in which, after removal of the pineal eye, the animals still moved 10 sec. after illumination of the pineal region, the paired eyes were removed by cauterisation. Four days later, illumination of the pineal region still gave movements after 9 sec.

(d) Discussion.

It is thus very unlikely that the paired eyes of ammocoetes are sensitive to light. On the other hand, since animals without either paired or pineal eyes may still move when illuminated, it is clear that some other tissue in the head must be sensitive to light. It is not possible to say exactly where this sensitivity is localised; it might be in the skin or in the tissue of the brain itself. Since the skin over the rest of the body, other than the tail, is not sensitive to light, whereas the spinal cord, if deprived of pigment, is sensitive (Young, 1935, p. 8), it seems likely that the movements following illumination of the head are due to stimulation of some tissue of the brain, by light entering through the pineal foramen. If this is so then the movements following illumination of the head of lampreys are like those described by Scharrer (1928), depending on photochemical processes in the tissue of the brain itself. Scharrer makes the provisional suggestion that the reactions observed by him are due to the liberation of a hormone in the nucleus magnocellularis praeopticus, whereas von Frisch suggested that the colour changes which he observed depended on photosensitive cells in the ependyma of the third ventricle. It is obviously impossible as yet to decide which, if either, of these mechanisms is at work in lampreys.

The fact that the head is very much less sensitive to light than is the tail agrees with the observation that the head may sometimes protrude a little from the mud in which the animal lives, but is withdrawn on sudden illumination. Since the aperture through which the light enters to affect the brain is median, it is hardly likely that the response results in any orientation with reference to the direction of incidence of the light. This would agree with the experiments already described, in which it was found that ammocoetes swimming free in a partly illuminated tank, arrived by random movements in the darkness, but did not swim away from the light.
III. CONTROL OF COLOUR CHANGE BY THE PINEAL AND PITUITARY.

From the experiments already described it is clear that the pineal complex does not initiate movements of the animals, but no consideration has yet been given to the possibility that it may control other responses, for instance changes in coloration.

I have succeeded in finding only one reference to colour change in lampreys, namely by Wild (1903), who reports that *Petromyzon marinus* shows a colour play rivalled only by that of chameleons and Cephalopods.

(a) Melanophores and melanophore index.

No *Petromyzon marinus* being available, the question was investigated with *Lampeatra*. In ammocoetes of *L. planeri* the upper side is a dark brown colour, the belly being yellow; after metamorphosis, the upper part of the body becomes black, and the belly silvery white. The dark colour, both of larvae and adults, is due to melanophores of the usual vertebrate type, lying mostly just below the epidermis. The deeper layers of pigment in the coelomic epithelium and round the spinal chord do not usually contribute to the external appearance of the individuals.

A measure of the depth of pigmentation of the animals can be obtained by recording the degree of expansion of the melanophores, using the arbitrary system of enumeration suggested by Hogben and Slome (1931). On this scale, completely expanded melanophores are counted as stage 5, and varying degrees of expansion are reckoned down to stage 1, in which the pigment is collected into a spherical ball, the animal appearing maximally pale. In the present investigation, the melanophores of the dorsal fins of adult lampreys were examined by transmitted light, whereas in ammocoetes, in which there are no melanophores in the fins, those on the side of the body were examined by reflected light. The animals are so active that it is only possible to make observations after anaesthesia. This was produced by ethylurethane 2–4 per cent., in which the lampreys became immobile in a few minutes, but quickly recovered. Anaesthesia twice daily appeared to leave no ill effects. An error is, however, introduced by the fact that urethane itself has an effect on the melanophores, causing them slowly to expand. Other anaesthetics (magnesium chloride, ether, chloretone) were tried, but as all had the same effect, the error could not be eliminated. The effect is most marked about half an hour after the administration of the anaesthetic, so that the error is very slight when the observations are taken at intervals of 12 hours, but would be much greater at shorter intervals.

The stages were characterised slightly differently from the scale adapted by Hogben, 5 being maximal expansion, 4 melanophores showing a small ball of pigment at the centre, 3 the stellate stage, 2 balls of pigment with a few protruding processes, and 1 complete contraction. Each of the experiments described below was performed on a number of animals, usually ten or fifteen. The melanophore index of each individual was estimated, and the mean and standard deviation of the
mean calculated for the population. In Text-figs. 1, 5 and 6 the vertical lines represent twice the standard error, calculated from

\[ s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}, \quad \text{S.E.} = \frac{s}{\sqrt{n}}. \]

However, in interpreting these figures it must be borne in mind that only small numbers of animals were used.

(b) Daily rhythms of colour change.

The earlier attempts to observe colour changes were made by keeping the animals under constant diffuse illumination in deep zinc tins, enamelled white or black, and provided with a circulation of water. No differences between the individuals on light and dark backgrounds could be detected in this way, larvae and adults of both species all remaining maximally dark when examined on many consecutive days.

The first indication of their capacities for colour change came from the chance observation that the ammocoetes living in their natural habitat are visibly paler at night than during the day. This led to the discovery that both larvae and adults of *L. planeri* show a very striking and regular daily rhythm of colour change, becoming pale during the night and dark during the day. This rhythm is illustrated in Text-figs. 1, 5 and 6. It will be seen that in some cases the change in colour is from almost complete pallor to maximal darkening, though in other cases it is less extreme. It is,
however, a more pronounced effect than the slight daily rhythms of colour change observed by Hogben and Slome (1931) in Xenopus, which were only significant because of their regularity, whereas in the lampreys, although the change varies in extent in different individuals, in many cases the difference between the mean melanophore indices during the day and the night is significant even apart from its regularity over several days. It is, however, noteworthy that the variance between individuals is much greater at night than during the day. This agrees with the conclusion which will be reached below that the process of paling is the active phase of the cycle, and that the phase with expanded melanophores is, so to speak, a resting state to which return is made. In most cases the colour change is readily detectable by the naked eye, the ammocoetes at night having a beautiful yellow colour. In the adults, in which the change is particularly striking, the back, which is dark during the day, assumes at night a shining white colour like that of the belly. In Lampetra fluviatilis, on the other hand, the change is much less marked, and often no change of colour can be detected at night either by the naked eye, or by means of the melanophore index.

The relation of these rhythmical colour changes to the illumination of the animals was shown by keeping them either in total darkness or under continual artificial illumination during the whole 24 hours. The result of the latter experiment was very striking and constant: the melanophores failed to contract as the evening approached, and in fact remained in a state of almost complete expansion for as long as the light was left shining on the bowl. This result was achieved whether the animals were left lying in clean water or buried in the mud, showing that the light can affect them in their normal environment. This experiment also shows the reason for the initial failure to obtain changes of colour by placing the animals on light and dark backgrounds. They are not capable of response to any such subtle differences, but remain maximally dark for as long as they are illuminated. This effect of light is well shown by illumination of the animals during the evening period from 16.00 to 22.00 hours. Instead of becoming pale as they would otherwise do, they remain dark all the evening, and even if left in total darkness, are not found to be pale the next morning (Text-figs. 5 and 6). Thus the diurnal changes may be stopped by illumination for a few hours during the evening.

Transference to total and enduring darkness produced less constant results. In some cases (Text-figs. 5 and 6) the diurnal colour change was completely arrested, the melanophores remaining in the expanded state; whereas in others (Text-fig. 1) the change continued even after many days of total darkness, though usually diminished in extent. This continuance of the rhythms in darkness shows that they must either be due to some internal self-acting factor in the animal, or to some other factor than light, such, for instance, as temperature. Temperature was indeed found to have a slight effect on the colour, lower temperatures favouring marked paling at nights, and higher temperatures tending to inhibit such palings. However, careful observation over long periods showed that there was no regular rhythm in the temperature of the water circulating through the bowls, and the rhythms of colour change persisted when the animals were kept at a constant temperature.
The Photoreceptors of Lampreys

It is, therefore, concluded that the colour change is controlled primarily by the effect of light on the animals, but that this effect is produced by way of an internal mechanism which is capable, to some extent, of continuing the rhythms when the animals are left in total darkness.

(c) Absence of nervous control of melanophores.

This brings us to consideration of the internal factors controlling colour, and in view of what is known of the subject in other vertebrates, search was made (a) for nervous, and (b) for endocrine mechanisms. In order to discover whether there is any nervous control of the melanophores, the spinal nerves of one side were cut close to the spinal cord over a region of several segments. In no case were any significant differences seen between the melanophores of the denervated area and those of the rest of the body, though observations were made up to 6 weeks after the operation. The denervated area was as dark as the rest of the body during the daytime and as pale during the night. It might be expected that even if the melanophores receive no motor nerves yet slight abnormalities would be found in a denervated area on account of changes in the blood supply due to severance of the vasomotor nerves. Such effects, if present, are too slight to be detected by the method of melanophore indices. On the other hand, serious interference with the blood supply, as by a cut across the base of the fin, greatly affects the melanophores (see p. 265).

It is therefore concluded that the melanophores are not directly innervated, and this is confirmed by the fact that faradic stimulation of the spinal cord or spinal nerves at various levels failed to produce any detectable effects on the melanophores. By exposing the spinal cord at the base of the dorsal fin one can obtain a preparation in which single melanophores can be watched under the microscope during the stimulation, and no movements of the pigment could be seen under these conditions.

(d) Control of melanophores by the pituitary gland.

(i) Effect of removal of pituitary. Turning, therefore, to look for endocrine mechanisms, the work of Smith (1916), Hogben and his collaborators, Lundstrom and Bard (1932) and others, at once suggests search for an effect on the melanophores of the posterior lobe of the pituitary gland.

The pituitary of lampreys is an elongated strip of tissue lying along the base of the hypothalamus. In ammocoetes, it is fairly easy to approach for purposes of removal. Under urethane anaesthesia a cut is made in the lower wall of the oral hood, whose two flaps are held apart by hooks. The position of the pituitary on the roof of the mouth can then easily be made out with a low-power microscope, by reference to the large jugular veins. In some cases, the outline of the gland itself is marked by the distribution of pigment round its edges. Total removal can be effected by cutting out a small rectangle of tissue with a very fine blade, but such a piece includes the floor of the cranium, and its removal leaves a hole through which the brain is apt to bulge. To avoid this, the pituitary can be destroyed by lancing with a hot needle. With practice, the area destroyed can be localised, and parts of the pituitary complex destroyed separately.
The operation of removal of the pituitary from adult lampreys of either species is greatly complicated by the difficulty of approach caused by the large mass of muscles connected with the sucker. With care, however, this can be held apart, and if necessary, the tendons can be cut and certain whole muscles removed. The roof of the mouth is thus revealed and the pituitary can be located after cutting open the hypophysial sac.

The effect of total removal of pituitary was the same in all cases. Within an hour the melanophores began to contract, and after a few hours the animals reached a state of great pallor, the melanophores all finally reaching stage 1 (Text-fig. 2 and Pl. I, figs. 1 and 2). All the dermal melanophores are affected in the same way, producing a pallor more extreme than that usually produced during the daily rhythms. Only the melanophores in the coelomic epithelia and round the spinal cord remain expanded, so that the animals assume a beautiful semi-transparent yellow colour, the course of the spinal cord being faintly outlined in black. Usually,

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{Onset of pallor in a single ammocoete larva (135 mm.) after removal of the entire pituitary.}
\end{figure}

as in the case shown in Pl. I, fig. 1, a small area of expanded dermal melanophores remains in the tail, varying in extent in different individuals.

Animals have been kept after the operation for periods up to 11 months without showing any further change of pigmentation. They have also been kept under electric light and in total darkness, and transferred from one to the other, but all without any deviation of the melanophores from their state of maximal contraction.

In thirty-two cases out of the total number of fifty-three operated, the heads of the animals were fixed after death, and cut into serial sections in order to discover exactly what had been removed. The homologies of the parts of the pituitary complex are not very certain (see de Beer, 1926), but it is possible to distinguish a rather small pars anterior containing oxyphil cells, a large "Übergangsteil" formed of columns of basophil cells, and perhaps homologous with the pars tuberalis of Tetrapods, a pars intermedia and a pars nervosa. The anterior, Übergangsteil and pars intermedia lie in a row, but the pars nervosa lies above the pars intermedia, making it impossible to remove without injury to the latter.
The Photoreceptors of Lampreys

Eight of the animals examined histologically had become very pale after the operation, and showed melanophore indices of 1.0 or 1.5 at the time of death. In all of them the pars nervosa was found to be absent, and in only one was there any trace of pars intermedia. In two of these pale animals the pars anterior and Übergangsteil were present, in the rest absent.

Ten of the animals showed no colour abnormalities, the melanophore index being 4.5 during the daytime, and it was found that part, or all, of both intermedia and nervosa were present. The remaining fourteen individuals showed melanophore indices lower than 4 but higher than 1.5, and in all of them the pars intermedia and nervosa had been damaged to some extent.

These results of partial removal of the pituitary complex indicate that a substance responsible for causing expansion of the melanophores is produced either in the intermedia or nervosa. Since removal of these lobes is followed by complete contraction of the melanophores, whether or not the pars anterior and Übergangsteil are present, it seems that these latter tissues do not produce a melanophore contracting principle such as has been shown by Hogben and Slome to be produced in the pars tuberalis of Amphibia. However, this conclusion rests on negative evidence only, and requires confirmation by careful observation of the melanophores of lampreys from which the anterior and/or Übergangsteil have been removed.

A further difference from the state of affairs which follows hypophysectomy in Amphibia is the absence in lampreys of any direct response of the melanophores themselves when placed on varying backgrounds.

(ii) Injection of posterior lobe extracts. The control of the melanophores by the pituitary was also demonstrated by the injection of mammalian pituitary extracts into hypophysectomised lampreys. The preparations used were kindly supplied by Messrs Parke, Davis and Co., Ltd., and included their total extract of the posterior lobe (Pituitrin) and the separate pressor and "oxytocic" principles ("Pitressin" and "Pitocin"). All of these substances were found to cause expansion of the melanophores of hypophysectomised lampreys, but the effect of pitocin was very much less than that of the other two. The procedure adopted was to dilute the commercial preparations with water to the required strength, and to inject the liquid intraperitoneally. As will be seen from Text-fig. 3, there is a relation between the degree of darkening produced and the amount injected. The maximum effect was reached after 2-4 hours, and all trace of it disappeared within 24 hours. Pl. I, fig. 3 shows the effect of injection of 2½ units of pitressin into a hypophysectomised adult Lampetra fluviatilis.

(e) Effects of pituitary and adrenal extracts on isolated melanophores.

Within 2-3 hours after the death of a lamprey all the melanophores begin to contract until eventually the corpse assumes the pale yellow appearance which is characteristic of a hypophysectomised animal. This contraction of the melanophores is presumably due to the absence of the pituitary principle, and this was proved by removing pieces of skin from lampreys and keeping them in solutions containing mammalian posterior pituitary extracts.
In order to obtain suitable saline mixtures, use was made of the measurements of Dekhuyzen (see Young, 1933), who showed that the blood of lampreys in fresh water has a $\Delta$ of 0.487. A saline mixture was therefore used containing:

<table>
<thead>
<tr>
<th></th>
<th>NaCl $M/2$</th>
<th>KCl $M/2$</th>
<th>CaCl$_2$ $M/2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount</td>
<td>190 c.c.</td>
<td>4 c.c.</td>
<td>2 c.c.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>to 1 litre</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Transfer to

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitrin</td>
</tr>
</tbody>
</table>

Text-fig. 3. Effect of injection on successive days of various amounts of pituitrin into an ammocoete larva from which the entire pituitary had been removed 6 days before the first injection.

Text-fig. 4. Three pieces of skin from the fin of $L. \text{fluviatilis}$; $A$ in saline mixture; $B$ in saline with 0.1 units of pituitrin per 3 ml.; $C$ in saline with adrenaline HCl 1/100,000. After 2 hours pieces $B$ and $C$ were interchanged.

From Text-fig. 4 it will be seen that a solution containing 0.1 unit of pituitrin in 3 c.c. of saline mixture prevents the post-mortem contraction of the melanophores, whereas adrenaline 1/100,000 accelerates that contraction. On transferring the pieces from adrenaline to pituitrin it was found that pituitrin is capable of causing re-expansion after adrenaline contraction. It is thus quite clear that pituitrin contains a very active principle causing expansion of the melanophores, and all the evidence goes to show that secretion from the posterior lobe of the pituitary is the

---

1 Galloway (1933) has recently reinvestigated the question of the saline content of lamprey blood. He found concentrations similar to those given by Dekhuyzen, and showed that the heart of $L. \text{fluviatilis}$ will beat well in frog’s Ringer.
normal agent by which the dark condition is maintained. The significance of the contraction caused by adrenaline is more doubtful. It was found that injection of 1 mg. of adrenaline into adult *L. fluviatilis* was followed by marked contraction of the melanophores, producing macroscopically visible pallor. It is therefore possible, though not at all certain, that secretion of this substance plays a significant part in the normal colour change of the animal.¹

Further evidence of the humoral control of the melanophores is provided by experiments in which the blood supply is interfered with. As explained on p. 261 there is no change in the melanophores of an area denervated by section of the spinal roots, but if a cut 1–2 cm. long is made completely through the base of the dorsal lobe of the caudal fin, then within a few hours the melanophores of the area peripheral to the cut become contracted (Pl. I, fig. 4). The line between the normal and affected areas is not sharp, many of the marginal melanophores being in intermediate stages of contraction. Moreover, the pale area is always narrower than the length of the cut. These facts all agree with the view that the contraction is due to the interruption of the blood supply, and hence of the supply of pituitary hormone.

Careful examination of the blood vessels showed that with a cut 1 cm. long there is a very slow venous flow in almost all parts of the affected area, but that a rapid flow persists only at the edges. It is only in such regions with an effective arterial flow that the melanophores remain expanded.

Parker and Porter (1934) observed similar light bands after making cuts in the fins of *Mustelus*, and interpreted these as due to the prolonged stimulation of melanophore-contracting nerve fibres as a result of the act of cutting. They state that it is possible to make such cuts "without serious interference with the circulation", but in *Lampetra* such interference is inevitable, and will adequately account for all the changes observed.

(f) Role of the pineal complex in the colour change.

It still remains to identify the afferent pathway by which the colour change is controlled. Experiments in which the pineal has been removed have shown clearly that it is this organ which is responsible. Text-fig. 5 shows a typical result with ammocoetes. Immediately after removal of the pineal and parapineal by the methods which have already been described (p. 255), the animals became dark, and all daily rhythm of colour change ceased. The melanophore index still showed occasional slight fluctuations, but these were not correlated with changes in the illumination of the animals, but apparently with the temperature, since on each of the rare occasions on which the melanophore index of pinealectomised animals fell to any significant extent, the temperature of the water was exceptionally low. In order to confirm the absence of further colour change after pinealectomy, the animals were placed in electric light and in total darkness, and transferred from one to the other, but in no cases were significant changes of the melanophore index observed.

¹ It may be recalled that Giacomini (1902) has shown that in Cyclostomes cells which represent the adrenal medulla are present, though not aggregated into a compact gland but scattered along the cardinal veins.
This experiment of pinealectomy has been performed with two single ammocoetes and four groups, constituting altogether twenty-eight individuals, all of which showed consistent results, except one in which the daily rhythms still persisted, and in which histological examination later showed that part of the pineal remained. In order to be certain that interference with the colour change was not due to operative shock, or degenerating nerve fibres, the animals were kept and examined at intervals after the operation for as long as 3 months in the case of the two larger groups studied.

The results with the few available metamorphosed individuals of *L. planeri* showed that in these the paired eyes collaborate with the pineal to produce the colour change. After removal of the pineal alone from four individuals, there was a temporary disturbance of the diurnal rhythms, whereas when the paired eyes were also removed, the rhythms ceased altogether (Text-fig. 6).

### (g) Discussion.

It is thus clear that the exteroceptor by which the colour change is regulated is in ammocoetes the pineal (and parapineal?) eye, and that in adult *L. planeri*, the eyes and pineals collaborate to this end. However, it is not certain exactly how the pineal exerts its influence. The two significant facts in this connection are (1) that after removal of the pineals, the melanophores remain permanently expanded, and
(2) that when the animal is left in constant light it remains dark, and when in total darkness, the diurnal cycles either come to a stop in an expanded phase of the melanophores, or at least show greatly reduced rhythms. These facts show that the effect of the pineal on the pituitary cannot be simply a tonic one, such that illumination of the pineal causes reflex accentuation of secretion of the pituitary. On the contrary, the pituitary if left to function alone may produce throughout the entire 24 hours sufficient of the melanophore stimulant to maintain maximal expansion.

It seems that on transference of the pineal from light to darkness, some effect is produced, such that either the secretion of the pituitary is inhibited, or some substance is liberated, which acts antagonistically to the melanophore expanding principle. This is also indicated by the fact that if after a normal day's illumination, followed by pallor at night, the animals are left in total darkness, they nevertheless become dark the next morning. Thus it is the passage from light to darkness which constitutes the active phase of the cycle.

As to the actual mechanism by which the effect is produced there appear to be three possibilities. The passage of the pineal from light to darkness may:

(1) Set up nervous impulses which inhibit for a time the secretion of the melanophore expanding principle of the pituitary; or,

(2) Cause the liberation from the pineal itself of a melanophore contracting or pituitary inhibiting substance; or,
(3) Set up impulses which excite the production of a melanophore contracting principle either in the pituitary or elsewhere.

The first mechanism provides the most direct and satisfactory explanation of the phenomena, in terms of one melanophore controlling substance only. It also allows of an explanation of the persistence of rhythms of colour change in animals left in total darkness.

The second possibility, that a melanophore contracting substance is produced in the pineal itself, is supported by the discovery of McCord and Allen (1917) that the mammalian pineal contains a substance capable of causing contraction of the melanophores of amphibian tadpoles. Further, Tretjakoff (1915) believes that the pineal of lampreys contains secretory as well as nervous tissue. However, no evidence has so far been obtained of the existence of such a substance in lampreys. Two experiments have been made with aqueous extracts of the pineal of individuals killed during the evening, while their melanophores were contracted. In neither case did the injection of such extracts, in amounts equivalent to one pineal per recipient, produce pallor in pinealectomised ammocoetes. If the pineal produces a melanophore contracting principle, then it might be expected that removal of both pineal and pituitary would have effects different from removal of either of them alone. To test this, pituitaries were removed from five lampreys and 14 days later the pineals were also removed. However, no expansion of the melanophores followed the second operation, as it might be expected to do if the pineal produces a melanophore contracting substance.

In fact the observation that the melanophores contract after the death of the animal, or in an isolated piece of skin, suggests that the resting phase of the melanophores is one of contraction, and that it is not necessary to postulate the presence of any melanophore contracting principle. There is no evidence for the occurrence of such a substance in the pituitary (see p. 263), and though adrenaline will cause contraction of the melanophores, this is not proof that it is a significant factor in the normal colour change.

It therefore seems probable that the pineal and paired eyes affect the melanophores by nervous inhibition of the secretion of the melanophore expanding substance by the pituitary.

There remains for consideration the question of the possible biological significance of the diurnal colour change. Since ammocoetes live completely buried in the mud, and emerge only extremely seldom, it seems difficult to understand how a colour change could be an advantage to them, even if it were evident, which it is not, that the pale animals are less easily detectable by night and the dark animals by day. Since the pigment has the function of protecting the tissues from the effects of light, it is clearly advantageous for the melanophores to be expanded when the animals are illuminated, but why should they then not remain permanently expanded as do the internal melanophores round the spinal cord?

In the case of the adult L. planeri, which swim freely about in the water, a protecting colour change would be advantageous, but the pallor at night renders the animals, if anything, more conspicuous as they flash along in the water. It is just
possible that this is indeed the significance of the change which has the purpose of making the animals conspicuous and hence easily recognised by the opposite sex.

However, it is also possible that the colour change in itself has no present biological significance, having neither positive nor negative survival value. The control exercised by the pineal over the pituitary perhaps affects the colour only secondarily, the primary influence being on other and more significant processes, such as blood pressure and respiration rate, which might be expected to show daily fluctuations on the analogy of the temperature and other rhythms of mammals, which incidentally are also controlled from the hypothalamus. Further investigation of the respiration and heart rate of lampreys is required to determine whether there is any basis for such suggestion.

The result of this enquiry into the function of the pineal eye has been, then, to show that, though it is sensitive to light, it plays only a doubtful role in controlling the main motor reactions of the whole animal. On the other hand, it plays a leading part in controlling the colour of the animal, and possibly in regulating other and still more significant functions of the pituitary. Thus even at this early stage of its history the pineal complex is connected not so much with somatic as with visceral functions. It is idle to speculate whether this was its original and only activity, but it is in agreement with what little is known of its functioning in other forms, and is perhaps not unconnected with the peculiar visceral relations of the habenular complex throughout the vertebrate series.

IV. SUMMARY.

1. Illumination of the dorsal region of the head of an ammocoete larva is followed by movements of the animal, but only after exposure for longer periods than are necessary to elicit responses from the tail.

2. Since this reaction persists unaffected after removal of the pineal and paired eyes, it is concluded that it is produced by the direct effect of light on some tissue in the brain.

3. Larval and adult *L. planeri* show very pronounced daily rhythms of colour change, becoming pale at night and dark during the daytime.

4. Continuous artificial illumination of the animals produces maximal darkening and stops the diurnal rhythm.

5. When animals are left in total darkness the diurnal changes usually persist, though diminished in extent; sometimes the melanophores come to rest in the expanded phase.

6. Since section or faradic stimulation of spinal nerves is not followed by local changes in the melanophores, it is concluded that these are not under nervous control.

7. After removal of either the whole pituitary complex or its pars nervosa and intermedia the animals become maximally pale, and remain so indefinitely in spite of changes of illumination.
8. Injection of extracts of mammalian posterior pituitary lobe causes darkening of such hypophysectomised lampreys.

9. Pituitrin was also found to be capable of maintaining the expansion of isolated melanophores.

10. After removal of the pineal complex from ammocoetes the rhythms of colour change were interrupted, the melanophores remaining in the expanded phase under all conditions of illumination. Removal of the pineal of adult *L. planeri* disturbed the colour rhythm, which was then completely abolished if the paired eyes were also removed.

11. Thus the paling of an ammocoete when it passes from light to darkness is probably due to the inhibition of posterior pituitary secretion by nervous impulses set up by the change of illumination of the pineal complex.

REFERENCES.


EXPLANATION OF PLATE I.

Fig. 1. Effect of hypophysectomy on colour of ammocoetes of *L. planeri*. The pituitary had been completely removed from the upper animal three days previously.

Fig. 2. On the left normal adult *L. fluviatilis* and on the right an individual from which the pituitary had been removed four days previously.

Fig. 3. Same animals as in Fig. 2, but photograph taken 1½ hours after the injection of 2½ units of pitressin into the hypophysectomised animal, which is on the left.

Fig. 4. Tails of adult *Lampetra fluviatilis*, showing the pale areas produced by cuts through the fins. Photograph taken 2 days after the operation.
JOINTER OF EXPERIMENTAL BIOLOGY, XII, 3. PLATE I.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

YOUNG — THE PHOTORECEPTORS OF LAMPREYS (pp. 254—270).