

OBSERVATIONS ON PIGMENTARY CO-ORDINATION IN ELASMOBRANCHS

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(With One Text-figure)

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I. INTRODUCTION

It was first suggested by Lundstrom and Bard (1932), on the basis of results obtained after hypophysectomy in *Mustelus*, that the colour responses of elasmobranchs to background are under the control of pituitary hormones. More recently, Hogben (1936) has made a comparative study of colour control in various common English elasmobranchs. His evidence demonstrates that, in all the species investigated, the degree of dispersion of chromatophore pigment is controlled by the differential secretion of two pituitary hormones. The hormone of the pars neuro-intermedia causes expansion, that of the pars anterior contraction. When the whole pituitary is removed, the chromatophores are completely released from hormonal control and assume a totally contracted condition with a melanophore index 1. In those species in which background responses can be observed, the degree of secretion of these hormones appears to depend upon visual stimulation. Diffuse light, from a white background, stimulates the pars anterior to increased secretion. When, however, the fish is on a black background, most of the light enters the eye from above, stimulating an increased secretion in the pars neuro-intermedia. When, by section of the optic nerves, the source of differential stimulation is removed, the output of the two hormones is equilibrated and the pigment reaches an intermediate degree of dispersion. In animals which had undergone this operation, Hogben observed a slight expansion of the chromatophores on white and contraction on a black background. This may possibly be due to the direct effect of light on the pigment cells. The physiology of colour control in elasmobranchs seems, therefore, to be closely similar to that already elucidated in Amphibia. It is now clearly established that in the latter group no nervous control is involved and, as Hogben in his paper points out, the time relations of elasmobranch colour responses indicate that the same is true here.

It has, however, been suggested (Parker and Porter, 1934) that, while melanophore *expansion* may be controlled by a pituitary hormone, *contraction* is dependent on the discharge of impulses in special "contracting" nerve fibres. It was found that a chisel cut through the fin of *Mustelus*, in such a position that it does not obviously impede the blood supply, results in a stripe of pallor peripherally.

Parker supposes that this is due to a sustained discharge set up in the severed "contracting" fibres. This explanation has been criticized by Hogben (1936), and Sand (1935) points out that hitherto no physiological work has made it necessary to postulate a discharge of impulses in fibres severed from the central nervous system, except the so-called "injury discharge" which is of relatively short duration.

Although much work has been done on the section and stimulation of nerves in the teleost fishes, few such tests have been performed on elasmobranchs. It therefore appears desirable that the possibility of nervous control in this group should be further investigated.

The following experiments were planned with this end in view. They were carried out during November and December 1935, and January 1936, at the Laboratory of the Marine Biological Association. I should like to thank the staff there for their help, and I am most grateful to the Board of Faculties at Oxford for allowing me a grant towards my expenses. I am much indebted to Dr Sand for his help and advice throughout the work.

II. EFFECT OF NERVE SECTION

In those teleosts and reptiles in which colour change has been found to be under nervous control, the chromatophores of a denervated region invariably assume an expanded condition. In the Amphibia, where colour control is hormonal, section of spinal nerves may affect the condition of the chromatophores owing to the fact that severance of the vaso-motor fibres results in concomitant vaso-motor changes. This possibility is, however, excluded in the elasmobranchs, since the vaso-motor fibres are not associated with the spinal nerves but "run out from the sympathetic ganglia on either side of the segmental arteries" (Young, 1933 *a*). According to Parker, section of the spinal nerves should result in a prolonged stimulation of the melanophore contracting fibres.

Six specimens of *Raia brachyura*, *R. maculata* and *Scyllium canicula* were removed from the general tank with their melanophores fully expanded (melanophore index 5). In each case the animal was anaesthetized with 1 per cent. urethane. Four consecutive spinal nerves running to the pectoral fin were then cut just behind the girdle, the wound being subsequently sewn up with catgut. Three individuals of each species were then placed in a white tank and three in a black tank. The fishes were watched closely for melanophore changes, the melanophore index being recorded, after microscopic examination of the edge of the pectoral fin, on the scale used by Hogben and Slome (1931). During the first day very little change could be detected. At the end of the second day following the operation the ray species in the white tank had reached a melanophore index of 3.5, while those in the black tank remained at 5.0. In neither case was the melanophore index of the denervated area in any way different from that of the rest of the body. In the specimens of *S. canicula*, the melanophores all over the body showed considerable variation in degree of expansion. This was so also in the denervated area of the pectoral fin, which could not be differentiated by macro- or microscopic means from the normal skin.

This operation was then carried out on specimens of *Raia brachyura* which had previously reached a melanophore index of 1.5 following hypophysectomy. It seemed possible that this pallor might be due to tonic innervation. No melanophore changes were detected, however, in the denervated area, though the fishes were kept for several days.

III. EFFECT OF NERVE STIMULATION

It has frequently been shown that, in teleosts, stimulation of the spinal nerves results in chromatophore contraction. This effect is due to impulses passing along the autonomic fibres and can be most quickly obtained by placing the electrodes of the stimulating circuit in the haemal canal.

Experiments of this type were carried out on *Raia brachyura*, *Rhina squatina* and *Scyllium catulus* under urethane anaesthesia. In the case of *Raia brachyura*, which responds readily to background, both white and black adapted animals were used. In the specimens of *Rhina* and *Scyllium*, the melanophore index was 5. Electrical stimulation by condenser discharges through a neon lamp was employed, the strength and frequency being controlled by variable resistances.

Each species underwent three experiments. First the spinal nerves to the pectoral fins were stimulated, the electrodes were then placed in the haemal canal, and finally direct stimulation of the skin was tried. In each case the stimulation was maintained for 5 min., its strength and frequency being varied. In no case, however, were any changes in the condition of the melanophores observed, although at suitable intensities of stimulation a clear muscular response was obtained. Since, even in Amphibia, chromatophore contraction has been observed after prolonged faradic stimulation of the skin (Laurens, 1915), the duration of stimulation was increased to 15 min. No chromatophore changes could, however, be detected. The same experiments were then carried out on the plaice, *Pleuronectes platessa*, under identical conditions. Pallor developed each time within 30 sec. The spinal nerves stimulated in *P. platessa* were those running just above the peritoneum of the body cavity.

IV. THE ACTION OF ADRENALINE

Adrenaline has repeatedly been found to act directly on the chromatophores of excised scales in teleosts causing them to contract. Chromatophore contraction results also from the injection of this hormone, though it has never been demonstrated that the secretion of the adrenal medulla plays any part in the control of colour under normal conditions.

Excised skin of *Raia brachyura*, *R. maculata*, *Rhina squatina* and *Scyllium canicula* was placed in a 1:10,000 solution of adrenaline chloride obtained by diluting the 1:1000 solution prepared by Parke, Davis and Co. with elasmobranch Ringer of the type advocated by Young (1933 *b*). Similar pieces of skin were placed in elasmobranch Ringer as controls. After one hour no change could be detected in the melanophores of *Raia* or *Rhina*. Those of *Scyllium canicula* showed a slight

contraction both in adrenaline and in Ringer. The melanophores of teleosts have been shown (Spaeth, 1916) to be extremely sensitive to ionic changes, so that a slight contraction of this order is more easily understood than the extreme insensitivity of the melanophores of *Raia* and *Rhina*.

Scales of *Pleuronectes platessa* and *Ctenolabrus rupestris* were placed in 1 : 10,000 adrenaline chloride for comparison. The melanophores contracted completely within a minute, whereas those placed in teleost Ringer remained fully expanded.

Parker and Porter (1934) observe that the subcutaneous injection of adrenaline in *Mustelus canis* induces a general paling within 30 min. This pallor, however, is "seldom as pronounced as that seen in fish from the white tank". Lundstrom and Bard (1932) also report the fact that *Mustelus* becomes lighter after the injection of adrenaline and are inclined to regard this as a secondary effect due to vascular changes. Young (1933 a) was unable to obtain any melanophore response after adrenaline injection in *Scyllium canicula* and *S. catulus* or *Torpedo ocellata*.

One c.c. of 1 : 10,000 adrenaline chloride, prepared as in the previous experiment, was injected subcutaneously into the pectoral fin of *Raia brachyura* and *Rhina squatina* (melanophore index 4). In both cases a slight degree of general pallor developed, both species reaching an index of 3 at the end of an hour. Control species injected with 1.0 c.c. of elasmobranch Ringer developed a comparable pallor which was, however, strictly local.

Similar adrenaline injections in the plaice caused a much swifter and more complete chromatophore contraction. The difference is so striking as to lend considerable support to the assumption that the adrenaline is having a direct, sympathetico-mimetic effect in the teleost, while in the elasmobranchs it is affecting the colour indirectly, as a result of its vaso-constrictor action.

V. EFFECT OF OBSTRUCTING THE BLOOD SUPPLY

It remains only to show that the condition of the melanophores can be affected by interference with the blood supply, as a theory of hormonal control requires.

It is not easy to obstruct completely the arterial supply to the fins. If the subclavian or iliac arteries are ligatured near their origin, blood still reaches the limbs by means of the segmental arteries. In the case of the pelvic fins, this secondary source may be reduced by ligaturing the aorta at the level of the lienogastric artery. In five specimens of *Raia brachyura*, which were taken from the white tank with a melanophore index 2, the body cavity was opened and the aorta ligatured. The fish were then returned to the black tank.

Within 3 hours of the operation a colour difference could be observed macroscopically between the tail, pelvic fins and posterior spinal region, which remained pale, and the rest of the body, which had become dark. This differentiation became even more marked 7 hours after the operation, when the melanophore index of the posterior region was 2.0, that of the anterior part of the body being 4.5. Gradually, however, the posterior region darkened, so that at the end of 24 hours the two areas

were indistinguishable. It seems, therefore, that the arterial blood must eventually reach the posterior region even after ligation of the aorta.

This would suffice to explain why the reverse experiment (aortal ligation in black adapted animals, which were then placed in the white tank) did not yield comparable results. While the black response can be accomplished in a few hours, the white response is very gradual, taking at least 3 days. Although the fishes were

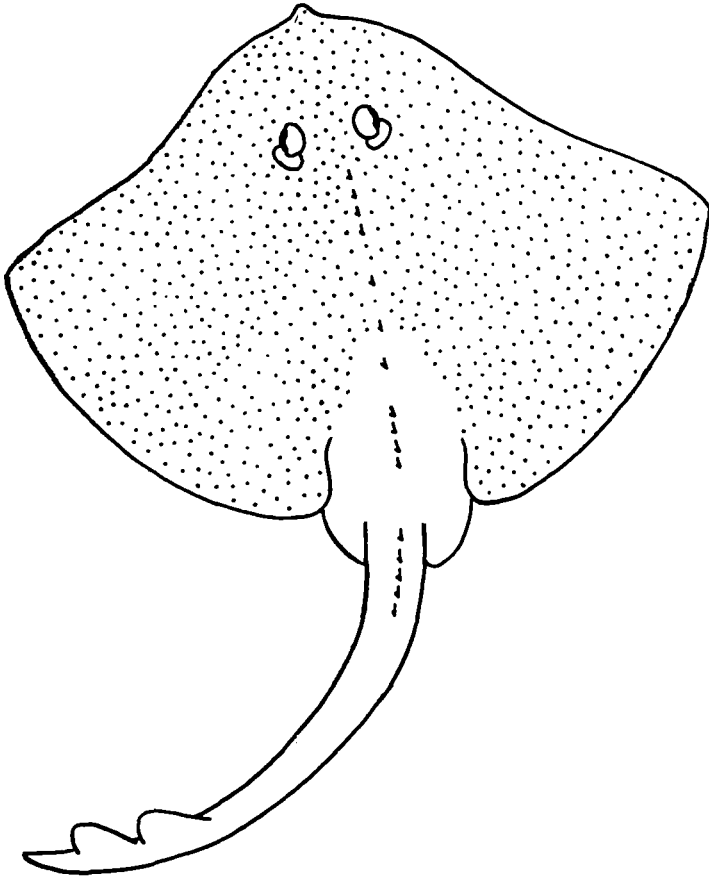


Fig. 1. Showing colour conditions in white-adapted *Raia brachyura* left on a black ground for 7 hours after aortal ligation. Melanophore index in stippled area 4.5, in unstippled area 2.0.

repeatedly examined during this time, no chromatophore differences between the anterior and posterior regions were observed.

Complete pallor can be obtained within a few hours as a result of total hypophysectomy. In this case melanophore contraction is caused not by the pars anterior hormone but by the complete removal of pituitary control.

Specimens of *Raia brachyura*, which were hypophysectomized after ligation of the aorta, became maximally pale anteriorly within 4 or 5 hours. The pelvic fins

and tail, however, remained dark, with a melanophore index of 3.5, so that the two regions could be differentiated macroscopically.

It is clear from these results that, in a region where the circulation is impeded, melanophore changes are temporarily eliminated, though they may be induced by various means in the rest of the body.

VI. CONCLUSION

The evidence provided by these experiments suggests that, in the species investigated, the nerves play no part whatever in the control of the background colour responses. Impulses originating from retinal stimulation, passing to the pituitary by means of the opticus, control the differential output of two pituitary hormones which are carried in the arterial blood to the chromatophore effectors.

Certain results obtained by Hogben (1936) in *Raia brachyura* suggest the probability that light has a further effect on the chromatophores, independent of optic stimulation. No further investigation of this response has yet been made, so that it is impossible to say whether it is called forth by the direct effect of light on the pigment cells or whether it is dependent, as is a similar response in the chameleon (Zoond and Eyre, 1934), upon spinal reflexes.

VII. SUMMARY

1. Section of the spinal nerves to the pectoral fins in *Raia brachyura*, *R. maculata* and *Scyllium canicula* had no effect on the state of the chromatophores in the black adapted fish, nor did it prevent the development of uniform pallor on a white ground.

2. Electrical stimulation of

- (a) spinal nerves to the pectoral fins,
- (b) the haemal canal,
- (c) the skin,

in *Raia brachyura*, *Rhina squatina* and *Scyllium catulus* did not result in any chromatophore changes.

3. Excised skin of *Raia brachyura*, *R. maculata*, *Rhina squatina* and *Scyllium canicula* placed in adrenaline chloride solution showed little or no change in melanophore index during one hour and could not be differentiated from control pieces placed in elasmobranch Ringer.

4. The injection of adrenaline chloride caused a slight paling in *Raia brachyura* and *Rhina squatina*. This may be a secondary effect due to the vaso-constrictor action of this hormone.

5. White adapted specimens of *Raia brachyura*, which were placed on a black ground after ligation of the dorsal aorta, became dark anterior to the point of ligation within 3 hours. The posterior region was still completely pale at this time and did not become fully dark for 24 hours.

Black adapted specimens, hypophysectomized after aortal ligation, showed the reverse condition, the anterior region becoming pale while the tail and pelvic fins remained dark.

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