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ON THE NERVOUS AND HORMONAL CONTROL OF MELANOPHORES and their differential reactions IN THE CATFISH *ICTALURUS MELAS* (RAFINESQUE). ~~AND THEIR DIFFERENTIAL REACTIONS.~~

by

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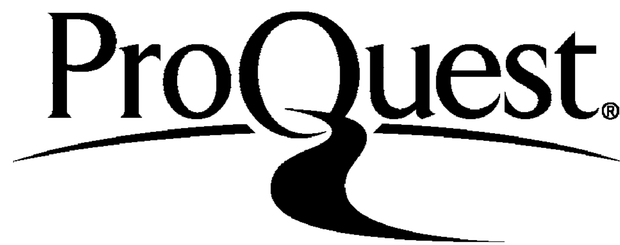

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ABSTRACT

The melanophores in the catfish I. melas are arranged in 3 layers, one epidermal and two dermal. The former are the smallest, and also differ from those in the dermis in their shape and in their reaction-times in response to background change.

Hypophysectomy impairs the ability of the animal to adapt to a black background but has apparently no effect on white adaptation. It severely affects the dispersion of pigment in the epidermal melanophores in comparison to the lower dermal ones. Equilibration on different greys and in darkness of hypophysectomised specimens indicates that the pituitary gland plays no role in the initial phases of dispersion or in adaptation to darkness. The removal of the pituitary also appears to accelerate the initial phases of black-to-white adaptation.

The nerve fibres controlling the aggregation of pigment in I. melas follow in principle the same path as in Phoxinus. The equilibration to a white background following anterior spinal section requires 6-8 weeks. All melanophore layers of white-adapted chromatically spinal fish fully disperse their pigment in about 24 hr on transfer to a black background. On reversal the white-equilibrated condition is obtained in 7-10 days, epidermal melanophores being slowest to achieve

full dispersion and being the first to aggregate it. Hypophysectomised spinal fish remain at an intermediate tint on all backgrounds and after enucleation.

Observations suggest that the pituitary contributes to the dispersion of pigment, following transection of the anterior spinal cord.

White background adaptation is impaired after interrupting the hypothalamo-hypophyseal tract. It is inferred that in *I. melas*, as in elasmobranchs and amphibians, the central control on the pars intermedia is of an inhibitory nature.

Pinealectomy does not affect the ability of the animals to adapt to illuminated white or black backgrounds. The equilibrium MI's of white/black adapted and of enucleated pinealectomised animals in darkness are strikingly similar to those of unoperated specimens.

Noradrenaline and adrenaline cause pigment aggregation in isolated skin. Adrenergic blocking and anti-adrenaline substances cause a slight dispersion in dermal melanophores and moderate dispersion in epidermal melanophores. Acetylcholine has only a slight dispersion effect.

The results of the present investigation can be explained on the assumption of one hormone and mononeuronic control of melanophores in *Ictalurus*.

It is suggested that the epidermal melanophores differ from the dermals in having different threshold levels of response to MSH and to noradrenaline.

Finally, it is concluded that I. melas in its chromatic physiology occupies a position between the teleost species in which the melanophores are predominantly neurally controlled (e.g. Phoxinus) and those in which their co-ordination is mainly hormonal (e.g. Anguilla).

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I. INTRODUCTION

1. The effectors of colour change

In teleosts and other cold blooded vertebrates the colour changes in response to background are brought about by pigment-containing cells, the chromatophores. These are usually branched cells with a fixed cell wall. Dispersion of pigment from the centre into the branches or aggregation from the branches into the centre result in changing the colour of the skin of the animal (the so-called physiological colour changes).

The distribution of the chromatophores in the skin varies from species to species. They may be evenly distributed so as to give a uniform colour to the integument (e.g. *Anguilla*) or be arranged in groups to form a particular colour pattern (e.g. *Phoxinus*).

Of the several kinds of colour cells, those containing the dark brown or black pigment, melanin, are the melanophores. The principal variations in the shade and the colour of the skin, i.e. darkening and blanching, are due to dispersion and aggregation of melanin in them. The melanophores may be distinguished as epidermal or dermal in accordance with their

situation in the integument. *Phoxinus* (Healey, 1951), *Ameiurus* (*Ictalurus*) (Parker, 1940, 1948) and *Anguilla* (Neill, 1940) have both forms of melanophores. In *Fundulus*, however, there are only dermal melanophores (Parker, 1948). Of the two forms of melanophores, the epidermal melanophores are the smaller in size. Epidermal and dermal melanophores may also show differences in their reactions as seen in *Parasilurus* (Matsushita, 1938) and in *Phoxinus* (Healey, 1954). In *Parasilurus* epidermal melanophores on a black background disperse their melanin contents more rapidly than the dermal ones. In *Phoxinus* there is a tendency on the part of the dermal melanophores to be slightly more dispersed than those in the overlying epidermis. The responses of the epidermal melanophores have so far been recorded in only *Parasilurus* (Matsushita, 1938), *Anguilla* (Neill, 1940) and *Phoxinus* (Healey,1954).

2. The estimation of colour changes

Physiological colour changes have been estimated by many methods. (1) Earlier investigators of this field expressed these changes qualitatively in terms of the macroscopic appearance of the animal, such as "palish", "pale", "darkish" and "dark" (Parker, 1940). These terms are vague and inexact and are useless for the purpose of comparison. (2) These changes have also been measured photoelectrically. This method, like the first one, is unreliable, as both of these fail to take into account the previous history of the animal which, by increasing

or reducing the number of melanophores (the so-called morphological changes), may so considerably alter the shade of the skin so that the values of the absorbed and the reflected light provide no dependable indication of the degree of pigment dispersion of the individual chromatophores. Knowledge of the latter is essential for any investigation of their co-ordinating mechanisms. (3) A more reliable and simple method is the use of the melanophore index devised by Hogben & Slome (1931) in which the emphasis is laid on the condition of dispersion of pigment in the melanophores. This is assessed microscopically and the shortcomings of the first two methods are thus avoided. Although the index values are purely arbitrary and therefore, bear no linear mathematical relationship to one another, the method is in general very satisfactory and has been widely employed for measuring colour changes. Observing the conditions of melanophores in the teleosts which change colour rapidly presents many technical difficulties, as even slight handling may interfere with the normal responses. This difficulty, however, has been overcome by numerous techniques in which handling of the animal is avoided (Hogben & Landgrebe, 1940; Healey, 1950, unpublished; modified by Gray, 1956).

3. Influence of light

Colour changes are influenced by various physical factors such as temperature, humidity and light. Of these

the most important is light. The responses to light are generally distinguished as primary and secondary responses. Primary colour change response involves dispersion of melanophore pigment in light and its aggregation in darkness. It occurs through routes other than the eyes, more commonly by direct action of light on melanophores or through a reflex that involves a receptor other than the eye and subsequent nervous or endocrine regulation of the pigment cell. This response is particularly distinct in blind fishes. Secondary or visual responses operate through the eyes for background adaptation. They involve dispersion of pigment on a light-absorbing background and its aggregation on a light-reflecting surface.

The light-sensitivity of the melanophores in vertebrates is not so surprising, for most of these cells originate from the neural crest (Raven, 1936, cf. Burgers, 1966) which consists of the same material as the neural plate from which the light-perceptive tissues of the eyes and the pineal develop.

4. Co-ordination of chromatic responses

(i) Slow and fast colour changes

Investigation of the time required for the completion of the cycle of melanphore responses in either direction, i.e. from a completely aggregated to a fully dispersed condition or *vice versa*, can indicate the nature of the mechanism co-ordinating these responses. Thus, these changes on the basis of time

relationship may be distinguished as slow or fast (Hogben, 1924). If the melanophores are under humoral control colour change is relatively slow. If it is nerually co-ordinated it is fast. These changes can also indicate the part played by nerves and the contribution of hormones when both are participating in the colour change. A complete cycle of colour response involves (i) the time taken for the stimulus to act on the receptor (ii) the time taken for the transmission of the message to the chromatophores (iii) the time taken by the chromatophore to execute its response. If the mechanism is purely nervous, the sequence of events after the impulse has reached the nervous system may be (a) transmission of the nervous impulse along the nerve tracts concerned to synapses (b) transmission from each synapse to the neuro-affector junction (c) execution of the response by the effector. The time taken for completion of the neurally co-ordinated cycle of colour change response apart from the time taken by the chromatophore to execute its response may be less than a minute (Waring, 1942). Since the melanophores are known to react rather slowly to drugs and to the pigment dispersing hormone of the pituitary, the maximum time value to a purely neurally controlled response may be assigned to 90 min (Waring, 1942). A total time of 2 hr. or more shows that some other mechanism is also involved e.g. (1) the reflex liberation of a hormone (2) the distribution of the hormone via the blood to the pigmentary effector (Waring, 1942).

(ii) Hormonal control

In those few species of elsmobranchs and amphibians so far examined the colour changes are exclusively controlled by hormones and thus are relatively very slow. In teleosts, on the other hand, a nervous mechanism is to a varying degree superimposed upon the archaic humoral control (Waring, 1963). In some species, e.g. Anguilla (Neill, 1940), the co-ordination appears to be predominantly hormonal. In others e.g. Macropodus (Umrath & Walcher, 1951) this appears to be under direct nervous control.

(a) The role of the pituitary in the dispersion of pigment.

The most important endocrine gland regulating the chromatic responses is the pituitary. It is mostly associated with darkening of an animal or dispersion of melanophore pigment, brought about by B-substance ("Intermedin"/ MSH) released from the pars intermedia of the pituitary. This has been borne out from observations on hypophysectomised animals. Hypophysectomised elasmobranchs and amphibians have been found to remain permanently pale on any background (Lundstrom & Bard, 1932; Hogben & Winton, 1923; Parker, 1948; Pickford, 1957). Among teleosts full dispersion of dermal melanophores on a black background was not obtainable after hypophysectomy in Ameiurus (Osborn, 1938; Parker, 1940), Parasilurus (Enami, 1939), Phoxinus (Healey, 1940, 1948), Anguilla (Waring, 1940). The darkening of chromatically spinal pale Phoxinus (its melanophores having been released from the central aggregating

influences) on a black background was reported to be very slow (Healey, 1940, 1951). The faded caudal bands consisting of denervated melanophores in pale *Ameiurus* and pale *Parasilurus* were reported by Parker (1934a) and Matsushita (1938) respectively to take a longer time in darkening than the innervated melanophores of the rest of the animal. (Please also refer to Section VI, pp.90-92).

(c) The pituitary and the aggregation of the pigment. It has also been suggested that the pituitary gland produces a W-substance (paling hormone or MCH) (Hewer, 1926; Hogben & Slome, 1931, 1936; Waring, 1940, 1942, 1963; Healey, 1940, 1948, 1951; Pickford, 1957; Kent, 1959). This hormone is assumed to originate in the anterior lobe complex in elasmo--branchs, anterior lobe in teleosts, and pars tuberalis in amphibians. It has also been suggested that it is formed in some other gland which is directly controlled by the pituitary. Thus anterior lobectomy was reported to result in permanent full dispersion of dermal melanophores in *Scyllium* (Waring, 1963), *Xenopus* (Hogben & Slome, 1931), *Rana pipiens* (Steggerda & Soderwall, 1939 cf. Pickford, 1957) and in *Anguilla* (Waring, 1940). Removal of the anterior lobe in *Phoxinus* resulted in the impairment of white background adaptation (Healey, 1940, 1948). According to Enami (1955) in *Parasilurus* MCH originates in the hypothalamus (nucleus lateralis tuberis) and is stored

in the meso-adenohypophysis.

The concept of MCH in teleosts in particular has been supported by Pickford (1957). The inferences regarding the existence of this hormone were made (1) from the results of injecting pituitary preparations from various sources into the teleosts (see references above), (2) from the observations reported by different authors that many teleosts were unable to maintain white background adaptation following hypophysectomy. Based on the results of pituitary injections, she distinguished two groups of teleosts. Group I includes species such as Ameiurus which are only sensitive to MSH, as the injection of pituitary material from any source always results in dispersion of pigment of the recipient. Group II includes species which exhibit more sensitivity to MCH than to MSH. She divides this group into two sub-groups. Sub-group IIa contains species that show little or no response to MSH e.g. Fundulus, Macropodus, Pleuronectes. Sub-group IIb comprises teleosts e.g. Parasilurus, Salmo, Gobius, which are sensitive to both MSH and MCH and show either response i.e. dispersion or aggregation of pigment.

Pickford also argued that the sensitivity of the fishes of the Group I to MSH only does not imply that they lack MCH. On the other hand, MCH participates in the normal chromatics of these fishes. According to her, in these species the presence of MCH can not be demonstrated "under ordinary experimental conditions" as it is masked by the opposing

effects of minute traces of MSH in the pituitary fractions. This would imply that MCH, in contrast to MSH, is a very weak hormone. If so, how could it override or act antagonistically to a stronger MSH in regulating normal colour change responses? Pickford considered the lack of response to MSH of the Group IIa animals as being due to the presence of an active pigment dispersing nervous system in them. It may be pointed out in this connection that the existence of this system in teleosts has not been established so far.

The data which led Pickford to believe in the existence of a pigment-aggregating hormone are conflicting. For instance (1) Fundulus appears to lack MSH because hypophysectomy does not affect black background adaptation (Abramowitz, 1937; Kleinholz, 1935; Fries, 1943) yet (i) the injection of its own pituitary material causes dispersion of the denervated melanophores, (ii) injection of blood from a pale fish into a hypophysectomised frog was reported to result in dispersion of pigment of the latter. The observations (i) and (ii) are inconsistent with the results of hypophysectomy in Fundulus, or vice versa. (2) Similarly many reports on Phoxinus phoxinus are conflicting and the conclusions derived from them must be treated with reserve. For instance (i) pituitary extracts from various sources injected into intact fish were reported to cause dispersion by Abolin (1925), Giersberg (1930, 1932), Osterhage (1932), Peczenick (1933), Reidinger (1952, in pale fish and in isolated skin); whereas, according to Hewer (1926),

Stutinsky (1934, 1935), Smith & Smith (1934), Astwood & Geschickter (1936), Healey (1940, 1948) and Kent (1960) pituitary extracts not all from the same sources caused aggregation of pigment, while Kabelitz (1941) reported these had no effect. (ii) Hypophysectomised Phoxinus was reported by Healey (1940, 1948) to be unable to maintain permanent black adaptation, suggesting thereby that MSH was involved for dispersion of pigment on a black background. At the same time, injection of its own pituitary material resulted in aggregation of the melanophores suggesting that the nervous system alone cannot maintain full dispersion or aggregation without the support of the hormonal control. (3) In Ameiurus nebulosus, according to Pickford, MCH is present, as administration of its pituitary extract into a MCH-sensitive fish such as Phoxinus causes aggregation of pigment in the latter, yet white background adaptation in hypophysectomised Ameiurus was reported to remain unaffected (Abramowitz, 1936a; Osborn, 1938).

Some objections to the very existence of MCH have been raised:- (1) While there are many reports about the dispersion of melanophores of a pale fish on receiving blood from a dark fish (Parker, 1948), not even a single instance has been reported in which administration of blood of a white-adapted fish into a black-adapted fish had led to aggregation of pigment in the latter. (2) Similarly administration of the

pars tuberalis extract from an amphibian into any MCH-sensitive fish has never been demonstrated to induce melanophore concentration in the latter. (3) Whereas fractination of pituitary material in various species, including teleosts (e.g. Gadus (Burgers, 1963)) has always been reported to yield only MSH (various forms), in only two cases, namely, Cyprinus carpio and Parasilurus asotus (Imai, 1958) has MCH been claimed to have been separated as well.

The whole situation regarding the relative roles of B- and a W-substance is thus far from clear. Kent's (1960) suggestion that one and the same hormone produces opposing effects in different species (e.g. aggregation in some cases like Phoxinus, and dispersion in others like Ameiurus), the response depending upon the constitution of the responding cell may be a clue to the solution of the problem.

The time relations of adaptation from an illuminated background to darkness in a few amphibian and teleost species have been interpreted by Hogben & Slome (1931, 1936), Hogben & Landgrebe (1940), Neill (1940), Waring (1940, 1942, 1963) and Healey (1951) to support the existence of W-substance. These authors found that equilibration of melanophores of intact animals in darkness on transference from an illuminated white or black background involved longer time periods than those involved in adaptation from white-to-black adaptation,

or from black-to-white. They explain these results on the assumption of an antagonism between B- and W-substances (bihumoral theory of colour change). However, according to Kent (1959) illuminated backgrounds and darkness represent entirely different physiological situations. The former involving stimulation or inhibition of pituitary gland through the eyes whereas in the latter case there is a complete cessation of any form of stimulation or inhibition of the gland as the eyes are not involved. Hence the comparison of the two situations appears irrelevant.

Finally the concept of W-substance in elasmobranchs and amphibians has largely been discredited by the works of Mellinger (1963), Jørgensen & Larsen (1960), Etkin (1941, 1943, 1962a, 1962b), Guardabassi (1961) and Kastin & Ross (1965). There is overwhelming evidence indicating an inhibitory central control of the release of MSH from the pars intermedia. It has been shown that any interruptions of the hypothalamo-hypophyseal innervation leads to hypersecretion of MSH and hypertrophy of the lobe resulting in persistent darkening of the operated animal irrespective of the background although the pars tuberalis, i.e. the assumed site of the release of W-substance, remains intact (Guardabassi, 1961). Etkin (1967) thinks that the concept of W-substance should be regarded as of historical interest only. What seems very likely is that during the removal of the anterior lobe or its cauterisation or in partial hypo-

physectomy, the hypothalamus must have been injured, leading to hypersecretion of MSH and permanent darkening which was mistaken by Hogben and his co-workers as an effect of the operation. In none of their work, according to Etkin (1941, 1967), was the pars intermedia examined histologically after the operations, so that the critical data on the effect of the operation on the pars intermedia are not available.

(c) The role of the pineal gland. The pineal has also been suggested to play a part in controlling changes in pigmentation in some lower vertebrates including teleosts. Von Frisch (1911, 1912c) and Scharrer (1928) showed that the pineal and the adjoining brain area is light perceptive in blinded *Phoxinus*, and on being stimulated by light causes dispersion of otherwise aggregated melanophore-pigment. A light-receptive function of the pineal in many other teleosts has also been suggested from the works of Breder & Rasquin (1950), Rasquin (1958) and Holmgren (1959) who have shown that in many cases the integument and other tissues overlying the pineal region are devoid of pigment, thus enabling the light to reach the pineal area. In contrast to these, no such function of the pineal or the pineal region of the brain in *Ameiurus* is indicated from the reported observations of Wykes (1938) and Parker (1940).

There are many indications that the pineal is involved in causing aggregation of pigment in darkness, at least in some lampreys and amphibians particularly in their larval forms. For instance, pinealectomy results in abolition of

blanching in ammocoetes of Lampetra (Young, 1935), Geotria (Eddy & Strahan, 1968), tadpoles of Xenopus (Bagnara, 1960) and Ambystoma larvae (Brick, 1962). On the basis of his findings of the effects of cautery of the pineal on pigment in Xenopus larvae, Bagnara (1960 - 1966) formulated a hypothesis attributing an endocrine role in addition to its being photoreceptive. He claimed that it was darkness and not light that stimulated it to release melatonin (a pigment-aggregating factor isolated from beef pineal by Lerner et al in 1958) which caused aggregation of pigment in darkness. Charlton (1966) extended this hypothesis to adult Xenopus and other amphibians and further suggested that the pineal and its hormone also participate in mediating white background adaptation. However, although there are suggestions that melatonin could be synthesised by the pineal in Xenopus, it has not so far been isolated from the pineal of any amphibian species. Moreover, the effects of pinealectomy on the pigmentation, unlike those of hypophysectomy, are not long lasting (Kelly & Johnson, 1962; Bagnara, 1965; Charlton, 1966).

Recently, however, melatonin has been indentified in the pineal of juvenile Oncorhynchus (Salmonidae) by Fenwick (1970). He suggests that it is concerned with the inhibition of gonadal development in young fish (as is assumed in some mammals and birds cf. Wurtman et al, 1968). Fenwick, however, significantly omitted its possible bearing on the pigmentation.

Thus, in short, the role of the pineal as endocrine

gland in the aggregation of pigment remains an open question. (For fuller treatment, please refer to Section IX, pp.153-158.

(iii) Nervous control of melanophores.

Nervous control of colour changes was for the first time demonstrated by Brücke (1852). He observed that section of the spinal cord or peripheral nerves in the chameleon resulted in the dispersion of pigment. In teleosts the influence of the nervous system on melanophores was first established by Pouchet (1876). He found that in the turbot *Scophthalamus* (= *Rhombus*) *maximus* cutting of the autonomic chain or section of a spinal nerve below the point where it received a ramus, resulted in the dispersion of melanophore pigment in the part of the body supplied by these nerves. Section of the trigeminal nerve caused the dispersion of melanophores of the head region only. He also found that these dispersed melanophores lost their ability to respond to background changes. Section of the spinal cord in the mid-body region had no effect on the melanophores. Pouchet's work thus demonstrated that the nerve fibres controlling the melanophores are autonomic. Von Frisch (1911) established the course of nerve fibres controlling the aggregation of melanophores in *Phoxinus*. He concluded that these fibres, originating in the medulla, pass along the spinal cord. At the level of the 15th vertabra they emerge and pass into the autonomic chain where they run backward and forward and supply the integumentary melanophores of the body region through the spinal nerves via rami, and the head region by the trigeminal

nerve. Von Frisch found that section of the spinal cord resulted in the dispersion of melanophore pigment only when it was cut anterior to the level of the 15th vertebra. He also noted that section of the autonomic chain anterior to that level resulted in dispersion of the melanophores of the anterior body region, whereas section posterior to the 15th vertebra led to the dispersion of pigment in the posterior part. Electrical stimulation of the medulla in a fish in which the spinal cord was sectioned anterior to the 15th vertebra so that all its melanophores were in a dispersed state did not produce any change in them. Stimulation of the medulla or spinal cord anterior to vertebra 15 in an intact fish resulted in the aggregation of all of its melanophores.

Eberth (1893), Ballowitz (1893) and Eberth & Bunge (1895) provided histological evidence for the innervation of melanophores in several teleost species. They demonstrated that nerve fibres from the spinal nerves supplied the melanophores. A single fibre or many fibres may end in a pigment cell or a single fibre may branch and supply many cells. Similar observations were reported by Wyman (1924) in Fundulus and by Whitear (1952) in Phoxinus. The nerve supply of melanophores has recently been studied by electron microscope. Fujii (1966a), Fujii & Fujii (1966) demonstrated the presence of bundles of unmyelinated axons near the melanophore processes in Chasmicthys. Similar observations have

been made by Bikle et al (1966) in Fundulus, by Fujii (1966b) in Lebistes and in Tautogolabrus (Jacobowitz & Laties, 1968).

Evidence for nervous control of melanophores is also provided by the time-graphs of white-to-black background adaptation and reversal. Quick changes accomplished in less than 2 hr. (already discussed on p. 4-5), as reported in Fundulus (cf. Parker, 1948), Lebistes (Neill, 1940), Gasterosteus (Hogben & Landgrebe, 1940), Phoxinus (Healey, 1951), and Macropodus (Umrath & Walcher, 1951), indicate that these are primarily neurally co-ordinated.

(a) Single or double innervation. There are two views regarding the neural control of melanophores. The first is that the control is mononeuric i.e. only one type of fibre: aggregating fibres supply the melanophores and their stimulation results in the aggregation of pigment. Thus the active phase of melanophores is their concentration and the resting phase is their dispersion, i.e. the tonic influence of the nervous system maintains the aggregation of pigment and release from this influence results in it dispersion. This view was tentatively put forward by von Frisch (1911) in the absence of any evidence for an antagonistic nerve fibre system.

The second view, dineuronic control, originally envisaged by Bert (1875) for the chameleon, has been advocated by Parker (1948) and his school. This postulates the presence of melanophore dispersing fibres in addition to the aggregating ones. This view was based upon the classical concept of antagonistic components of the autonomic system in mammals.

Thus the aggregating fibres are assumed to be sympathetic and the dispersing fibres parasympathetic.

(b) Evidence for double innervation from the results of section of melanophore nerve fibres and their subsequent regeneration. The evidence for the second view mainly rests on Parker's interpretation of the effects of nerve section on the melanophores. According to him, injury or sectioning of chromatic nerve fibres causes a long-lasting stimulation of dispersing fibres and not of aggregating fibres. From the cut ends of these dispersing fibres there is a prolonged discharge of a dispersing neurohumor which induces the melanophores distal to the cut to disperse their pigment in an otherwise pale fish. Parker reached this conclusion from a series of experiments on Fundulus and on Ameiurus involving cutting of radial nerves in the tails of these fishes. When in a white-adapted fish a bundle of these nerves is cut, within a few minutes an elongated darkened area is formed between the cut and the fin margin. This dark area with dispersed pigment is known as a "caudal band". Parker emphasized that it was caused by the action of dispersing neurohumor released from the stumps of the dispersing fibres stimulated by the cut end and not by any circulatory disturbances resulting from the cut. The dark band gradually faded away if the background of the fish was not changed and in 2-3 days was indistinguishable from the rest of the tail. This disappearance of the band was attributed to the action of an aggregating neurohumor invading from the innervated

melanophores surrounding the band, implying that the discharge of the dispersing neurohumor ceases by that time. If a white-adapted fish with a faded band was retransferred to a black background the band followed the tint of the fish at a slower rate and subsequently became indistinguishable from the rest of the body. On reversal of the background the band tint again lagged behind the rest of the fish in paling (Parker, 1934a, 1934c).

The technique of formation of caudal bands was first applied by Wyman (1924) in experiments on Fundulus. Unlike Parker, he had interpreted the formation of the dark band as resulting from the release of the melanophores from the aggregating influence of the nervous system.

Parker (1934c) reported that after the fading of a caudal band (primary band) in a white-adapted Fundulus a new transverse cut made within the old band but slightly distal to it induced the formation of a second band (secondary band) between the new cut and the margin of the tail. From this observation Parker concluded that the part of the chromatic apparatus severed from the central nervous system by the first cut was not paralysed but was capable of full activity and was stimulated by the second cut. In an attempt to show that the impulses were continuously flowing out of the cut ends of the nerves Parker (1934c) applied a local cold block to a dark band midway between the cut ends of the nerves and the distal margin of the band. He found that the part of

the dark band distal to the block became pale whereas the part between the cut and the block remained unaffected. On removing the cold block the faded part of the band became dark again, indicating that the application of cold block had interrupted the flow of the impulses. Parker (1934a) induced two new bands flanking the upper and lower sides of a faded caudal band in white-adapted *Ameiurus* and found that the melanophores along the margins of the faded band slowly became dispersed. Parker attributed this result to the diffusion of dispersing neurohumor into the margins of the faded band from the new bands.

Mills in Parker's laboratory (1932) observed the reactions of the melanophores on the edges of a faded caudal band in white-adapted *Fundulus* to background reversals. She found that in background changes the limits of the band did not exactly coincide. Moreover, some of the melanophores on the edge of band could expand but could not fully contract, while others were capable of full contraction but were unable to expand. Parker (1948) interpreted these results as supporting double innervation. He claimed that nerve sectioning had deprived some of these melanophores of aggregating fibres but not of dispersing fibres while in others the aggregating fibres were left intact and dispersing fibres had been cut.

Mills also observed that the caudal band melanophores on

transfer to a white background did not respond simultaneously. Aggregation of pigment started in the melanophores situated on the periphery of the band and was followed by a gradual concentration in the inner melanophores. She explained these results on the assumption that from the innervated melanophores surrounding the band pigment-aggregating substances were released and that these gradually diffused into the band. To show that these substances were not carried by the blood stream, she isolated the tail of the animal in which previously a caudal band had been induced. On electrically stimulating the melanophore nerves distal to the band she found an effect similar to that observed in the living fish on a white background.

Abramowitz (1936b)induced caudal bands in black-adapted Fundulus and left them as such for two or more weeks. After this period the cut nerves begin to regenerate from their proximal stumps. He subjected these fish to background reversals and to electrical stimulation and observed that (1) some melanophores in the area of the caudal band were capable of full aggregation but were unable to expand; (2) some were capable of full pigment dispersion but incapable of full concentration; (3) others reacted normally by fully dispersing as well as fully aggregating the pigment; (4) others responded neither by dispersion nor by aggregation of their pigment. These responses of melanophores with regenerating nerve connections that were accomplished in

one direction but not in the other could only be explained on the assumption that the aggregation and dispersion of pigment was brought about by two separate sets of nerve fibres.

Gray (1956) induced caudal bands in white-adapted _Phoxinus_ and observed that in a newly formed band the marginal melanophores showed an asymmetrical dispersion. The processes of those melanophores facing the innervated malanophores were more aggregated than their processes directed towards the dispersed melanophores of the band. In the fading of the band not only did the marginal melanophores aggregate ahead of the rest of the melanophores but the outwardly directed processes of the individual melanophores aggregated before the inner ones. These observations on _Phoxinus_ thus confirm Mill's results on _Fundulus_. Gray also chromatically spinal sectioned white-adapted _Phoxinus_ in which earlier a caudal band was induced and then allowed to fade away. He noted that in less than 1 hr. following the operation the melanophores of the faded band became dispersed, the dispersion starting at the margin of the faded band and gradually proceeding inwards. This result also appears to support Parker's interpretation of the results of melanophores nerve section.

Healey (1967) noticed that white- or black-adapted spinal sectioned _Phoxinus_ when subjected to background reversals about 26 weeks following the operation changed their

colour at a fast rate which was entirely different from the slow hormonal colour change typical of the spinal fish. This increase in the rate of colour change indicated that the chromatic fibres were regenerating in operated animals. He subsequently found progressive increases in this rate and in ca. 45 weeks the rates of colour change in general were similar to that of unoperated animals. This implied that the functional neural control in them had been fully re-established by that time. However, in some animals these rates were not found to be fast in both directions i.e. white-to-black or black-to-white. The rate in one direction was fast and was similar to that of unoperated fish whereas in the other it remained slow, indicating hormonal control. These observations also suggest that two separate and active neural mechanisms control the colour changes in *Phoxinus*, one responsible for aggregation of pigment and the other for dispersion.

(c) Some alternative explanations for the effects of melanophore nerve sectioning and other criticisms of Parker's view.

Although the results of many experiments cited above support Parker's assumption of the pigment dipersing fibres, his interpretation of the dispersion of pigment following melanophore nerve section as being caused by a sustained injury discharge in the dispersing fibres and not in the aggregating fibres has never been generally accepted (Sand, 1935; Waring, 1942, 1963; Healey, 1954; Gray, 1956; Young, 1962; Barrington, 1963; Pye, 1964; Scott, 1965; Fujii, 1969).

Thus the initial dispersion of melanophore pigment in a denervated caudal band is generally attributed to release from a tonic aggregating influence of the nervous system. Numerous other suggestions have been offered to explain this phenomenon. Some of these are:- (1) The melanophore pigment dispersing hormone MSH passed from the pituitary gland into the blood circulation can be a factor in the initial dispersion of pigment following nerve section (Waring, 1942). This suggestion is supported by (a) Abramowitz's (1937) report regarding the presence of significant amount of intermedin in white-adapted *Fundulus* and, (b) his failure to induce a secondary caudal band in a previously hypophysectomised *Fundulus*.
(2) Circulatory congestion caused by nerve incision could contribute to pigment dispersion: "The concentration of pigment-aggregating substances present in the blood of a white-adapted fish, would be reduced in the denervated area of partial circulatory arrest. The presence of aggregating substances may explain the observation that dark caudal bands resulting from larger and more drastic cuts require several days for fading on a white background." (Scott, 1965).
(3) That some non-nervous factor is responsible for causing dispersion was suggested by Osborn (1939) who observed some secondary darkening in flat-fish as long as 4 weeks after an earlier and more central cut in the tail fin had been made. Reactivation of the dispersing fibres was unlikely because in all probability only degenerated nerves were present in

the area involved. (4) Removal of central control by nerve section might result in some inherent dispersing mechanism coming into play and at the same time rendering the melanophores refractory to aggregating neurohumor for a certain period. Later on, the melanophores may lose their refractoriness to diffusing neurohumors and may even become hypersensitive(cf. Gray, 1956). The observations (a) that the mammalian autonomic effectors become hypersensitive to chemical stimulating agents some time after their nerve sectioning (Cannon & Rosenblueth, 1937) and (b) that there is increased sensitivity of denervated melanophores to adrenaline in the caudal fin melanophores in Ameiurus, (Parker, 1941a, 1942) in the scale melanophores of Tautoga (Smith, 1941) and in the melanophores in the caudal fin of Chasmichthys (Fujii, 1958) appear to support the suggestion. (5) Very recently Fujii & Novales (1969) offered a new interpretation of pigment dispersion following nerve section. This explanation, though entirely different from that of Parker, supports the hypothesis of double innervation. They believe that after a (caudal) nerve section the melanophores distal to the cut become free from the influence of nerves but this does not cause dispersion of pigment. The initial dispersion results from the action of a dispersing transmitter stored in the presynaptic structures and leaks or is released by the nerve section. The peripheral store of the transmitter is exhausted within a few hours (3-5 in Chasmichthys (Fujii, 1959a)) whereas the denervated melanophores in a caudal band

remain dispersed for days or weeks (Parker, 1948). This subsequent state of dispersion on a white background is assumed to be maintained by passive Brownian movement of the pigment granules, as the melanophores remain free from the influences of pigment-aggregating and pigment-dispersing transmitters. The transmitter molecules invading the denervated region are assumed to be inactivated by the enzymes (cholinesterase and catechol-O-methyl transferase). The main difficulties with this explanation are (a) it has yet to be established that the dispersing transmitter, if present, is acetylcholine; (b) it does not explain why a cut should not also cause the release of the aggregating transmitter.

Parker's observation of the formation of a dark caudal band by section of caudal fin nerves is generally acknowledged. However, his claims regarding the formation of a secondary caudal band in the area of a previously faded primary band in a white-adapted fish and the conclusions made therefrom remain controversial. Formation of secondary caudal bands was not obtained by Osborn (1938) and Wykes (1938) in _Ameiurus_; Vilter (1938, 1939) in _Gobius_ ; Adelmann & Butcher (1937) in _Fundulus_; Scott (1965) in _Scophthalamus_; and Fujii & Novales (1969) in _Chasmichthys_. Similarly, blocking the conduction of impulses by local application of a cold block, i.e. fading of a caudal band distal to the cold block, have been questioned by Scott (1965) in _Scophthalmus_ and by Fujii & Novales (1969)

in Chasmichthys.

(d) Attempted selective stimulation of dispersing and aggregating nerve fibres. Parker & Rosenblueth (1941) claimed to have selectively stimulated pigment-aggregating and pigment-dispersing fibres in Ameiurus by changing the frequency, duration and intensity of repetitive pulses. They reported that stimulation of caudal fin nerves at frequencies of 15-25 per second with pulses lasting 4-8 milliseconds at 8 volts resulted in blanching of the tail in 15-20 min., whereas repetitive darkening could be obtained at frequencies 1-2 per second with long pulses lasting 0.3 to 0.5 per second in 5-10 min. Although they regarded their observations as supporting the concept of double innervation this report was very superficial, no one seems to have succeeded in confirming these results in Ameiurus or in any other species (Waring, 1963; Fujii, 1969). A very obvious weakness of their claim lies in the fact that they used intact and live animals in their experiments. The results can therefore not be relied upon, since they could be due to extra-neural factors rather than to their stimulation of the melanophore nerves.

(e) Giersberg and von Gelei's attempts to demonstrate dispersing fibres, and their criticism. In an attempt to demonstrate the presence of pigment-dispersing nerve fibres (presumed to be cholinergic) Giersberg (1930) injected ergotamine and acetylcholine into Phoxinus. He then electrically stimulated the spinal cord and found that the fish darkened. He inter-

preted this effect as the result of potentiation of dispersing fibres by acetylcholine together with suppression of the action of aggregating fibres (presumably adrenergic) by the ergotamine.

Von Gelei (1942) claimed to have traced the path of the dispersing fibres in Phoxinus. He combined Giersberg's approach with section of the autonomic chain at different levels and electrically stimulated the medulla. The fish became dark anterior to the level of the cut. He concluded that the darkening resulted from the stimulation of cholinergic dispersing fibres. These dispersing fibres according to von Gelei leave the spinal cord at the level of 1st or 2nd vertebra, enter the autonomic chain and run there in a posterior direction, supplying the skin melanophores through the spinal nerves. Healey (1954), on the other hand, found no difference in the chromatic behaviour of spinal Phoxinus in which the spinal cord was severed either posterior to the 1st vertebra (interrupting both von Frisch's aggregating fibres and von Gelei's postulated dispersing fibres) or anterior to the 15th vertebra (severing the aggregating fibres only). Healey thus concluded that if von Gelei's dispersing fibres really existed they played no significant part in the colour changes of Phoxinus. Healey also got inconclusive results by injecting ergotamine into the fish and then electrically stimulating the medulla. Moreover, von Gelei never stated if he had

interrupted the blood circulation of his experimental fish, as electrical stimulation of such animals may have resulted in the stimulation of chromaffin tissues to release adrenaline. The dispersion of pigment might have been caused by the reversal of the effects of adrenaline. Ergotamine is known to reverse the effects of adrenaline in mammals (Dale, 1906).

Pye (1964) repeated von Gelei's experiments on Phoxinus and observed that the paling of untreated animals and darkening of the animals treated with ergotamine in response to electrical stimulation could be evoked from an electrode placed at any level or even in a small isolated section of spinal cord. He therefore suggested that the electrical stimulation of the cord at any point might raise the general level of excitation in the sympathetic chain and so influence chromatic efferent fibres emerging at all levels. Thus he also failed to confirm von Gelei's conclusions regarding the path of chromatic fibres in Phoxinus.

In another experiment Pye (1964) injected regitin (an adrenergic blocking agent) dissolved in minnow pituitary extract (presumably containing MCH), into a pale Phoxinus. The fish showed no dispersion of pigment presumably due to the effect of the pituitary extract. Subsequent electrical stimulation of the ophthalmic branch of the trigeminal nerve running in the orbit of the animal evoked no response in its melanophores. This experiment also does not support von Gelei's conclusions.

Healey & Ross (1966) injected various adrenergic blocking agents and anti-adrenaline substances into white-adapted Phoxinus. Electrical stimulation of the medulla or spinal cord in each case (excepting ergotamine) failed to cause dispersion of pigment. Assuming that the dispersing effects of electrical stimulation, if any, might have been masked by adrenergic blockers, they injected bretylium (an anti-adrenaline agent capable of evoking considerable darkening of pale fish) and plaice pituitary extract into a pale Phoxinus. Electrical stimulation in this case also failed to cause dispersion of pigment. Thus these experiments provide no evidence for dispersing chromatic fibres.

(f) Interpretations for double innervation from electron micrographs. Evidence for double innervation has recently been suggested from the electron microscopy of melanophore nerve fibres. According to Fujii & Novales (1969) in Lebistes in the nervous elements around the melanophores two different sized synaptic vesicles were found. Small sized vesicles of ca. 500 Å (=5000 nanometers) diameter are interpreted as the "packets" of dispersing transmitter assumed to be acetylcholine by these authors. Such vesicles have also been described in few other species such as Chasmichthys (Fujii, 1966a; Fujii & Fujii, 1966) and in Fundulus (Bikle et al, 1966). Larger granulated vesicles with diameter ca. 1000 Å (= 10,000 nanometers) have been regarded as the storage sites of the aggregating transmitter, assumed to be adrenaline or noradrenaline (see also pages 25 and 26).

(g) Experiments with various drugs. Since Dale, Loewi and their co-workers demonstrated that chemical transmission is involved at nerve endings, many drugs such as acetlycholine, adrenaline and ergotamine which are assumed to be associated with the transmitters, have been extensively used on teleosts. The interest has centred around the question whether the autonomic control of melanophores is of only one type or as in mammals is of two antagonistic types. The drugs with their known effects on the autonomic system or on the end organs supplied by it in mammals have been administered to teleost fishes by injecting into the intact animals, or by immersing intact animals, isolated scales or skin into the drug solutions and their effects on the melanophores studied. The effects of someof these drugs are briefly reviewed in the following tables 1-4:-

Table (i)

Effects of sympathomimetic substances on teleost melanophores.
(* in vivo, + in vitro)

Drugs	Effects	Species and source
Adrenaline	aggregation	Abudefduf* (Rasquin, 1958); Ameiurus (Bray, 1918*+; Bacq, 1933 ; Parker, 1934a*; Abramowitz, 1936a*;Wykes, 1938*; Rasquin, 1958*); Bathygobius*, Carapus* (Rasquin, 1958); Carassius+ (Iwata et al, 1959a, 1959b); Chasmichthys+ (Fujii, 1960, 1961); Cyprinodon*, Epinephalus* (Rasquin, 1958); Fundulus (Spaeth, 1916+; Spaeth & Barbour, 1917+; Wyman, 1924*; Smith, 1931 cf. Parker, 1948; Parker, 1934c*; Abbot, 1968*+); Gadus+ (Fänge, 1962); Gambusia+ (Ueda, 1955); Labrus+ (Scheline, 1963); Lebistes+ (Fange, 1962); Lophopsetta* (Osborn, 1939);

Drugs	Effects	Species and source
		Macropodus (Reidinger & Umrath, 1952); Opsanus* (Rasquin, 1958); Oryzias+ (Ueda, 1955); Paralichthys* (Osborn, 1939); Phoxinus (Abolin, 1925; Giersberge, 1930*; Smith, 1931; Reidinger, 1952; Pye, 1964+; Healey & Ross, 1966*; Grove, 1969a*); Pseudopleuronectes*(Osborn, 1939); Pseudorasbora+ (Ueda, 1955); Rhodeus+ (Umrath, 1957); Salmo (Gianferrari, 1922 cf. Parker, 1948; Robertson, 1951*+); Scophthalamus (Scott et al, 1962; Scott, 1965*); Strongylura (Rasquin, 1958*).
	incomplete aggregation	Phoxinus+ (Healey & Ross, 1966).
	aggregation in iris and no effect in dermis or epidermis	Chaetodon*; Eucinostomus*; Gerres*; Haemulon*; Iridio*; Lutianus*; Pomocentrus*; Sparisoma *; Sphyraena*; Thalassoma* (Rasquin, 1958).
	no effect on epidermal melanophores	Abudefduf* (Rasquin, 1958).
	no effect	Angelichthys*; Pomacanthus*; Acanthurus*; (Rasquin, 1958)
	aggregation followed by dispersion	Oryzias+ (Watanabe et al, 1962a)
	dispersion	Parasilurus (Enami, 1940); Chaetodipterus* (Breder & Rasquin, 1955).
Noradrenaline	aggregation	Chasmichthys+ (Fujii, 1961); Fundulus*+ (Abbot, 1968); Gadus* (Fänge, 1962); Gambusia+ (Ueda, 1955); Labrus+ (Scheline, 1963); Lebistes+ (Fänge, 1962); Phoxinus (Pye, 1964+; Healey & Ross, 1966*; Grove, 1969a*); Rhodeus+(Umrath, 1957); Scopthalamus (Scott et al, 1962*, Scott, 1965*).

Drugs	Effects	Species and source
	incomplete aggregation	Phoxinus+ (Healey & Ross, 1966).
Isoprenaline	aggregation	Fundulus*+ (Abbot, 1968); Phoxinus (Healey & Ross, 1966*+; Grove, 1969a*); Scophthalamus (Scott et al, 1962, Scott, 1965*).
Dopamine	aggregation	Fundulus*+ (Abbot, 1968); Phoxinus* (Healey & Ross, 1966); Scophthalamus* (Scott, 1965).
Ephedrine	aggregation	Aequidens, Corydoras (Turner & Carl, 1955); Fundulus* (Abbot, 1968); Gadus+, Lebistes+ (Fänge, 1962), Phoxinus (Healey & Ross, 1966*; Grove, 1969a*); Salmo*+ (Robertson, 1951).
Tyramine	aggregation	Fundulus (Barbour & Spaeth, 1917+); Labrus+ (Scheline, 1963); Phoxinus* (Healey & Ross, 1966; Grove, 1969a*).
Amphetamine	aggregation	Fundulus* (Abbot, 1968); Phoxinus* (Healey & Ross, 1966; Grove, 1969a).

Table (ii)

Effects of adrenergic blocking and anti-adrenaline substances

Drugs	Effects	Species and source
Ergotamine	aggregation	Ameiurus* (Bacq, 1933 -denervated melanophores); Chasmichthys+ (Fujii, 1961); Phoxinus+ (Pye, 1964; Healey & Ross, 1966-slight effect); Salmo*+ (Robertson, 1951-slight effect).
	aggregation followed by dispersion	Fundulus (Spaeth & Barbour, 1917*+; Wyman, 1924*; Smith, 1931*).
	dispersion	Ameiurus* (innervated melanophores - Bacq, 1933; Parker, 1941b); Chasmichthys+ (Fujii, 1961); Scophthalamus* (Scott, 1965).

Drugs	Effects	Species and source
	dispersion followed by aggregation	Phoxinus (Giersberg, 1930; von Gelei, 1942; Pye, 1964; Healey & Ross, 1966-slight dispersion followed by aggregation in white-adapted fish whereas no initial change in black-adapted animals which became pale later on).
	no effect	Ameiurus (Parker, 1941b*, denervated melanophores).
Ergotamine followed by adrenaline	dispersion	Chasmichthys+ (Fujii, 1961); Fundulus+ (Spaeth & Barbour, 1917); Phoxinus* (Healey & Ross, 1966-some dispersion)
	no effect	Phoxinus*(Giersberg, 1930; Pye, 1964); Fundulus* Wyman (1924)
Hydergine	dispersion	Fundulus* (Abbot, 1968); Phoxinus* (Healey & Ross, 1966).
	no effect	Fundulus+ (Abbot, 1968).
Hydergine followed by adrenaline	aggregation	Fundulus*+ (Abbot, 1968); Phoxinus* (Healey & Ross, 1966).
Dibenamine	dispersion	Chasmichthys+ (Fujii, 1961); Fundulus*+ (Abbot, 1968; Oryzias+ (Watanabe et al, 1962b); Phoxinus (Healey & Ross, 1966*+ some dispersion; Grove, 1969a*); Scophthalamus* (Scott, 1965).
Dibenamine followed by adrenaline	aggregation	Fundulus* (Abbot, 1968)
	no effect	Chasmichthys+ (Fujii, 1961); Phoxinus+ (Healey & Ross, 1966).
Dibenzyline Dibenyline/ Phenoxybenzamine	dispersion	Labrus*+ (Scheline, 1963); Phoxinus*(Healey & Ross, 1966)
	no effect	Fundulus* (Abbot, 1968).
Dibenzyline followed by adrenaline	no change	Labrus+ (Scheline, 1963-temporary and partial aggregation there-after redispersion); Phoxinus* (Healey & Ross, 1966).

Drugs	Effects	Species and source
Regitin (Phentolamine)	dispersion	Fundulus*+ (Abbot, 1968); Phoxinus (Pye, 1964*, Healey & Ross, 1966* slight dispersion+; Grove, 1969a*).
Regitin followed by adrenaline/ noradrenaline	aggregation	Phoxinus (Pye, 1964*; Healey & Ross, 1966*; Grove, 1969a*).
Yohimbine	dispersion	Fundulus* (Abbot, 1968); Phoxinus (Healey & Ross, 1966* moderate dispersion+; Grove, 1969a*).
Yohimbine followed by adrenaline/ noradrenaline	no change	Phoxinus* (Healey & Ross, 1966 no change in white-adapted fish, slight aggregation in black-adapted animals).
	aggregation	Phoxinus* (Grove, 1969a - only in white-adapted fish)
Bretylium	dispersion	Fundulus* (Abbot, 1968); Phoxinus (Healey & Ross, 1966* some dispersion+; Grove, 1969a* dispersion followed by aggregation in white-adapted fish).
	no effect	Fundulus+ (Abbot, 1968).
Bretylium followed by adrenaline	aggregation	Fundulus* (Abbpt, 1968-dispersion later on); Phoxinus*+ (Healey & Ross, 1966).
Guanethidine	dispersion	Phoxinus (Healey & Ross, 1966* slight aggregation+; Grove, 1969a* slight dispersion in white-adapted fish followed by aggregation, initial aggregation in black-adapted fish dispersion later on).
	aggregation	Fundulus* (Abbot, 1968).

Drugs	Effects	Species and source
Reserpine	dispersion	Betta, Brachydanio, Corydoras (Turner & Carl, 1955 by immersing in dilute solution); Fundulus (Abbot, 1968* very slow effect); Labrus+ (Scheline, 1963); Macropodus (Turner & Carl, 1955 as above); Phoxinus (Healey & Ross, 1966*+; Grove, 1969a*- dispersion followed by aggregation in white-adapted animals); Trichogaster (Turner & Carl,1955).
Reserpine	no effect	Fundulus+ (Abbot, 1968)
Reserpine followed by adrenaline	temporary and partial aggregation	Phoxinus* (Healey & Ross, 1966, slight aggregation+).
	aggregation	Labrus+ (Scheline, 1963).

Table (iii)

Effects of parasympathomimetic

agents, and atropine, and hexamethonium.

Acetylcholine	dispersion	Ameiurus* (Parker, 1931, 1934b, 1940); Hoplias (Mendes, 1942 cf. Parker, 1948); Fundulus (Parker, 1934b; Green, 1968); Macropodus (Reidinger & Umrath, 1952); Oryzias (Ando, 1960; Watanabe et al+, 1962b-slight dispersion); Phoxinus+ (Healey & Ross, 1966-some dispersion); Rhodeus+ (Umrath, 1957); Salmo*+ (Robertson, 1951).
	aggregation	Carassius (Beauvallet, 1938 cf. Parker, 1948); Fundulus (Bogdanovitch, 1937 cf. Parker, 1948); Parasilurus (Enami, 1955); Scophthalamus* (Scott, 1965).

Drugs	Effects	Species and source
	no effect	Fundulus (Barbour & Spaeth, 1917+; Abbot, 1968*+); Phoxinus (Healey & Ross, 1966*); Rhodeus (Wunder, 1931 cf. Parker, 1948); Scorpaena (Smith & Smith, 1934 cf. Parker, 1948); Scophthalamus* (Scott, 1965)
Acetylcholine + eserine	dispersion	Ameiurus* (Parker, 1934b, 1940)
	no effect	Oryzias+ (Watanabe et al, 1962b- no potentiation of acetylcholine effect); Phoxinus*+ (Healey & Ross, 1966; Grove, 1969b* slight aggregation in black-adapted fish)
Eserine	dispersion	Fundulus+ (Barbour & Spaeth, 1917), Oryzias+ (Watanabe et al, 1962b); Phoxinus (Abolin, 1926); Salmo*+ (Robertson, 1951); Scorpaena (Smith & Smith, 1934);
Eserine	no effect	Fundulus*+ (Abbot, 1968); Phoxinus*+ (Healey & Ross, 1966)
	aggregation	Scophthalamus* (Scott, 1965 slight aggregation in black-adapted fish)
Pilocarpine	dispersion	Fundulus (Barbour & Spaeth, 1917+; Smith, 1931); Oryzias+ (Watanable et al, 1962b); Phoxinus* (Abolin, 1925; Giersberg, 1930); Rhodeus (Umrath, 1957); Salmo* (Robertson, 1951)
	no effect	Phoxinus* (Grove, 1969b)
Atropine	dispersion	Carassius+ (Watanable, 1960); Chasmichthys+ (Fujii, 1960); Fundulus (Barbour & Spaeth, 1917+; Wyman, 1924*; Smith, 1931*; Abbot, 1968* persistent dispersion); Macropodus+ (Reidinger & Umrath, 1952); Oryzias+ (Watanabe et al, 1962b slight dispersion); Phoxinus (Abolin, 1925, 1926; Pye, 1964+; Healey & Ross, 1966*+; Grove, 1969b* slight dispersion); Rhodeus+ (Umrath, 1957); Salmo*+ (Robertson, 1951)

Drugs	Effects	Species and source
	no effect	Fundulus+ (Abbot, 1968)
Hexametho-nium	dispersion	Fundulus* (Wilber, 1960; Abbot, 1968); Phoxinus (Healey & Ross, 1966* slight dispersion+; Grove, 1969b*).
	no effect	Fundulus+ (Abbot, 1968).

Table (iv)

Effects of serotonin and melatonin

Serotonin (5HT)	aggregation	Fundulus*+ (Abbot, 1968); Labrus+ (Scheline, 1963); Scophthalamus* (Scott, 1965).
	no effect	Chasmichthys+ (Fujii, 1961); Phoxinus* (Healey & Ross, 1966).
Melatonin	aggregation	Chasmichthys+ (Fujii, 1961); Phoxinus* (Healey & Ross, 1966); Carassius (Boyles, 1969 cf. Fujii, 1969).
	no effect	Carassius (Etoh, 1961 cf. Fujii, 1969); Fundulus (Mori, 1961 cf. Fujii, 1969; Abbot, 1968*; Fain & Hadley, 1966+).

(h) Conclusions from experiments with drugs. To sum up, the responses of the teleost chromatic nervous system to sympathomimetic amines are in general consistent with the action of these substances on adrenergic mechanism in mammals. These results, together with those produced by reserpine, support the view that the nerve fibres controlling the aggregation of melanophore pigment are adrenergic and the transmitter involved is noradrenaline or adrenaline. However, the results of the experiments with various

adrenergic blockers and anti-adrenaline substances do not appear to lend full support to this view. As already reviewed in the preceding pages, ergotamine and many other adrenergic blocking agents such as dibenzyline, guanethidine and bretylium (in low concentration) have been reported to yield conflicting results and in some cases data are too meagre to permit any definite conclusions to be made from them.

Thus the comparisons of drug effects in mammals and teleosts must be treated with caution, since in the latter the sites of action of most drugs still remain to be determined (Healey & Ross, 1966). In this connection, similarities of effects of different drugs have been recorded in mammals on the one hand and elasmobranchs and amphibians on the other, though the bulk of the evidence indicates that melanophores are not under direct nervous control in the very few elasmobranchs and amphibians that have been studied (Waring, 1942, 1963). Von Euler & Fänge (1961) demonstrated the presence of adrenaline and noradrenaline in various organs including nerves in a few lower vertebrates including the teleost Gadus but believed that true adrenergic fibres with a transmitter action as found in mammals are a late development in the evolution of the autonomic nervous system. They also took the view that the only adrenergic mechanismsin fish are those mediated by various types of catecholamine-containing cells.

In contrast to the plentiful evidence suggesting that the pigment-aggregating mechanism is adrenergic in nature, the evidence from the results of acetylcholine and other parasympathomimetic substances for the existence of a pigment-dispersing cholinergic mechanism is almost negative. The pharmocological work of Burnstock (1958) has shown the absence of antagonistic control of the teleostean gut. Earlier conclusions similar to those of Burnstock were arrived at by Young (1931, 1933b). According to Barrington (1963) it is doubtful if antagonistic components of the autonomic system exist at the level of the evolution of fishes.

II. SOME OBSERVATIONS ON PREVIOUS WORK ON THE CATFISH A. NEBULOSUS (LE SUEUR)

1. Colour change reactions

Extensive work has been done on the colour change and its control in the catfish *Ameiurus* (=*Ictalurus*) *nebulosus* (Le Sueur) by Parker, (1934a, 1934b, 1940, 1941b, 1948), Abramowitz (1936a), Osborn (1938) and Wykes (1938). However, conflicting observations have been recorded by these workers and in some cases by the same author on the colour change reactions. Some of these observations concern (i) the rate of the colour change (Parker, 1934a; Abramowitz, 1936a); (ii) the colour of the animal equilibrated in darkness (Parker, 1936a; Abramowitz, 1936a; Odiorne, 1937; Osborn, 1938); (iii) the body tint of blinded fish adapted to darkness (Parker, 1934a,

1940; Abramowitz, 1936a; Wykes, 1938); (iv) the effects of hypophysectomy on the ability of the animal to background adaptation and the reactions of the operated animals to background changes (Parker, 1934a, 1940; Abramowitz, 1936a; Osborn, 1938); (v) the reactions of the denervated melanophores of the caudal band to background reversals (Parker, 1934a; Abramowitz, 1936a); (vi) the rate of darkening of a previously faded caudal band of a white-adapted fish on transfer to a black background (Parker, 1934a, 1948); (vii) the colour of denervated part of tail fin in darkness (Abramowitz, 1936a; Osborn, 1938); (viii) the formation of a dark caudal band in a white-adapted hypophysectomised fish (Abramowitz, 1936a; Osborn, 1938); (ix) the formation of a secondary caudal band distal to a primary faded band in a white-adapted animal (Parker, 1934a;Osborn, 1938); (x) the effects of electrical stimulation of melanophore nerves (Abramowitz, 1936a; Osborn, 1938; Wykes, 1938; Parker & Rosenblueth, 1941); (xi) the effects of ergotamine on innervated and denervated melanophores of the tail fin (Bacq, 1933; Parker, 1941b). (Detailed references to the above works are made in Sections IV to X where-ever the melanophore reactions in _I. melas_ are compared with those of _A. nebulosus_).

The above works mostly concern the gross macroscopic appearance of the experimental animal and references to the state of melanophores (unspecified layer of dermal melanophores)

if included, lack precision. Moreover, in most cases the previous history of the fish, the illumination intensity, the temperature, and the background history of the animal - all factors affecting the chromatic behaviour - were disregarded.

2. The control of the colour change

The disagreement among the various authors also exists regarding the relative roles of nerves and hormones in the control of the colour changes of *A.nebulosus*. It is of interest to review the conclusions of different authors in this regard.

(i) *Parker's conclusions*. According to Parker (1934a) the *darkening* of the catfish or the dispersion of its melanophores on transfer to an illuminated black background is primarily brought about by the activity of the melanophore-dispersing fibres and the contribution of the pituitary gland is negligible. In 1940 there was a shift in his views and he reported that the chief agent in darkening the fish was the pituitary neurohumor - intermedin, the nervous system playing a secondary role. Parker (1940) also concluded that the discharge of intermedin on an illuminated black background is exclusively initiated through the integumentary photoreceptors and that the eyes are in no way concerned with the stimulation of the gland. In contradiction to these conclusions Parker (1948), while discussing the organisation

of the melanophore system in _A. nebulosus_, observed that the eye, in addition to the dermal photo-receptors, excited the pituitary to discharge intermedin ("Reflex arc No.2, p.165).

Parker (1934a) had concluded that the _paling_ of _Ameiurus_ is solely brought about by the stimulation of concentrating nerve fibres which work antagonistically to the dispersing nerve fibres. Later on (1940) he regarded the paling as a result of either (i) a competition between intermedin and adrenaline released by the concentrating nerve fibres, or (ii), more probably, some inhibitory influence of the eyes on the release of intermedin from the pituitary, and by the activity of the concentrating nerve fibres. Parker (1940) also observed that the removal of the pituitary gland affected the capacity of the operated fish to adapt itself to a white background, as a hypophysectomised fish never became fully pale, thus implying that the pituitary gland, in addition to intermedin, also released a paling hormone.

It is thus evident that his conclusions regarding darkening of catfish on a black background and paling on a white background are contradictory.

According to Parker (1940) the melanophores in a fish in darkness are in a resting stage and this stage may be anywhere between complete aggregation to full dispersion of pigment.

Lastly it may be interesting to describe one of his experiments that led him to conclude that the stimulation of

the pituitary on an illuminated black background to discharge intermedin was exclusively initiated through the dermal photo-receptors and that the eyes had nothing to do with it though he later changed his mind about this. He (1940) completely transected the brain of his experimental animals just in front of the cerebellum (no histological verification), thus severing all connections between the eyes, pituitary gland and the nervous connections between the two, on the one hand, and the hind brain and the spinal cord on the other. The animals that survived the operation did not darken on an illuminated black background, although, according to Parker, the eyes, the pituitary and the nerve connections linking them were 'intact'.

(ii) Abramowitz's views. Abramowitz (1936a) agreed with Parker's (1934a) conclusions that the colour change reactions in *A. nebulosus* are predominantly neurally co-ordinated and that the pituitary plays an auxiliary role in the dispersion of the melanophores. However, his interpretations regarding the paling of intact and blinded (otherwise intact) animals in darkness are entirely different from those of Parker (1934a), since he regarded these reactions, as well as the darkening of a blinded animal in light, as solely mediated by the nervous system. Abramowitz never lent support to Parker's concept of the dual and antagonistic nature of the nervous mechanism controlling the melanophores in *A. nebulosus* (although he (1936b) did lend support to this idea in *Fundulus*) but he did

not explain how the nervous system brings about pigment-dispersion.

Abramowitz's conclusion that the pituitary gland plays a minor role in the dispersion of melanophores in *Ameiurus* is questionable. He never stated how far the dispersion of melanophores is affected after the pituitary gland is removed. He appears to have neglected to take into account the state of the melanophores and his observations concern only the gross appearance of the experimental animals. A correct evaluation of the role of the pituitary in the dispersion of melanophores cannot be made from the appearance of an animal. Furthermore, many of his observations tend to contradict his own conclusion that the role of the pituitary is insignificant. For instance he observed (i) that the hypophysectomised catfish unlike the unoperated animals were 'not enough' black on a black background; (ii) that in many black-adapted hypophysectomised animals, the tail bands remained completely pale unlike those in the unoperated animals; (iii) that the colour of hypophysectomised blinded fish in light was not as extreme as that observed in unoperated animals; and lastly, the extent of hypophysectomy in the operated animals appears not to have been verified histologically. These results would seem to indicate that the role of the pituitary is significant.

(iii) Osborn's conclusions. According to Osborn (1938) the control of melanophores in A. nebulosus is both neural and hormonal. He regarded paling of fish as the result of the combined action of concentrating fibres and an adrenaline-like hormone. He neither elaborated on the site of origin of the latter nor did he produce any evidence for its presence. He only quoted Abramowitz (1936a)who had suggested that "excitment pallor" demonstrated in denervated caudal bands in Ameiurus, could have resulted from an adrenaline-like substance. He appears to have made no distinction between the paling in darkness of (a) black-adapted intact fish, (b) blinded but otherwise intact animals kept in light, (c) hypophysectomised individuals adapted to backgrounds "darker than neutral", hypophysectomised and (d) blinded fish kept in light, on the one hand, and paling of (a) black-adapted intact animals and (b) black-adapted hypophysectomised fish on transfer to a white background, on the other. Thus he does not offer any explanations to this similarity of melanophore response in two entirely different environmental and physiological situations.

The dispersion of malanophores on a black background, according to Osborn, maybe accomplished by 3 means, namely (i) the pituitary hormone intermedin, (ii) possible dispersing nerve fibres, (iii) 'inherent partial expansion of unstimulated melanophores'. Of these 3 agencies, the most important is intermedin, as according to Osborn, the melanophores in hypophysectomised catfish on a black background were capable

of only '1/3' expansion - a conclusion very different from those of Parker (1934a) and Abramowitz (1936a). Osborn was not convinced of the existence of dispersing fibres. He thought that the formation of a black caudal band in a white-adapted fish could be more logically explained partly as a result of the interruption of concentrating fibres and partly by the action of the pituitary's dispersing principle. However, he never demonstrated any evidence for his assumption about the role of the pituitary hormone. He only quoted Abramowitz (1937) who had reported the presence of a significant amount of intermedin in the blood of white-adapted Fundulus.

Osborn also expressed scepticism about Parker's (1934) claim regarding the formation of a secondary caudal band distal to a faded primary band in white-adapted catfish.

(iv) Wykes's views. According to Wykes (1938) the aggregation of melanophores in A. nebulosus results from the tonic influence of the central nervous system and their dispersion is primarily brought about by their release from this influence. Like Parker (1934a) and Abramowitz (1936a) she appears to have believed that the pituitary gland plays only a small part in the dispersion of the melanophores although she had observed that the average diameters of the dermal melanophores in the blinded hypophysectomised animals (71.8 µ) were considerably smaller than those in the blinded intact animals (114.6 µ) under illumination.

III. THE AIMS OF THE PRESENT INVESTIGATION

The present investigation was designed to record the colour changes in the catfish *Ictalurus melas* (Rafinesque) and to attempt to find out the relative roles of endocrine glands and the nervous system in the control of these changes. Such information was necessary not only in order to compare the chromatic system of this fish with those of other species but also to attempt to clarify the position regarding the chromatic behaviour of *A. nebulosus* (Le Sueur) (Section II, pp.40-42).

Having obtained the basic information concerning the colour change reactions in intact animals, the effects of (i) hypophysectomy, (ii) transecting the chromatic fibres and the spinal cord, (iii) transection of the anterior spinal cord followed by hypophysectomy, and (iv) pinealectomy on the melanophores of white- and black-adapted animals and of enucleated specimens have been examined with a view to clarifying the roles of the pituitary, nervous system and the pineal in regulating the responses of the melanophores in *I. melas*. The effects of interrupting the hypothalamo-hypophyseal tract on the melanophores in white-adapted specimens have been observed with the object of finding some indication of the nature of the central control on the pars intermedia function.

Finally, the effect of some drugs with known effects on the mammalian autonomic system on the melanophores of isolated skin pieces have been observed to endeavour to gain some insight into the type of the nervous mechanism controlling the pigmentary movements.

The melanophore reactions throughout the series were recorded from the same site and all the experimental animals were subjected to constantly uniform environmental conditions of temperature and illumination.

IV. MATERIALS AND METHODS

1. General experimental procedure

The fish were kept in white rectangular porcelain tanks (60 x 45 x 25 cm) at uncontrolled room temperature in slow moving aerated water. They were fed upon finely minced ox heart about 3 times a week. After about 4 weeks they were transferred to glass aquaria (35 x 23 x 22½ cm) painted glossy white on their exterior, the water level being ca. 15 cm. They were continuously illuminated from above by the general ceiling lighting and the temperature of the water was maintained at 20± 1°C. The animals were used in experiments usually after about 1 week. For black adaptation they were kept for 48 hr. in laboratory aquaria painted glossy black on the outside after the preliminary 4 weeks in the white tanks. The fish ranged between 55-85 mm in length (average length

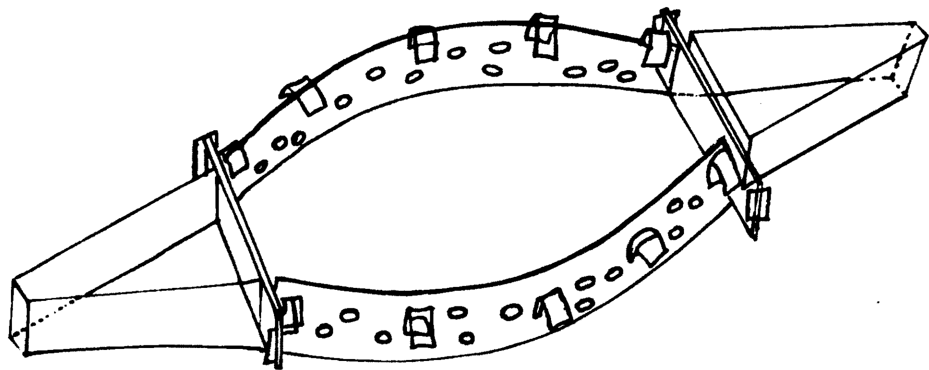
70 mm). About 4 weeks' previous adaptation on a white background was found helpful in observing the MI of the experimental animals as it resulted in quantitative reduction in the number of melanophores and their melanin content (Odiorne, 1937; Osborn, 1941) without affecting their physiology. Longer stay on a white background, however, has a more severe effect on the population of epidermal melanophores than those of the dermal ones. Long adaptation on a black background, on the other hand, stimulates relatively greater multiplication of the epidermal melanophores than of the rest with the result that the former hinder the correct estimation of the MI of the underlying melanophores. The colour change reactions of each fish were tested macroscopically before being used for an experiment. The fish were given a bath in 3-4% Na Cl solution for ca. 3 min every day to protect them from infection by the fungus Saprolegnia.

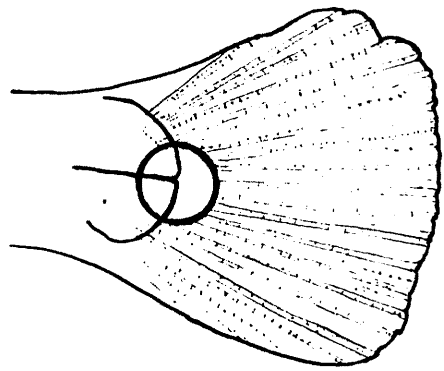
2. Apparatus for observing the colour change reactions

Colour change reactions of the experimental animals were observed in "cells" made from clear perspex (Fig.1 p.51). Each cell was somewhat spindle-shaped with a broad and round middle part and 2 tapering ends. The average size of a cell was 23.7 x 8.0 x 3.1 cm, the gap between the tapering ends measuring ca. 0.7 cm. The sides were perforated to allow free flow of water. The tapering ends were cut off

Fig. 1 A cell with sliding doors (ca. x $\frac{1}{2}$).

Fig. 2 The tail fin of *Ictalurus melas* (ca. x 5). The encircled area represents the site (2 mm in diameter) where the MI's were recorded.





from the main body of the cell by vertically fitting sliding doors of clear perspex to prevent the fish from lodging its head in them. The open top of the cell was covered by transparent nylon net, stretched and fastened to its sides by perspex hooks, so that the efforts if any on the part of the animal to escape were foiled. The nylon net did not interfere with the light falling either on the animal or on the background. The fish did not appear to be disturbed by confinement in the cell and reacted normally macroscopically to changes in the background. After the initial white or black background adaptation in laboratory aquaria, the fish were kept on the same background in the cell for at least 30 min.

The tests for background changes were made in rectangular trays of tin-plate painted glossy white or black inside and outside, measuring ca. 50 x 30 x 10 cm, filled with slow-moving aerated water at 20 $\pm$ 1°C., and illuminated by a 40-W bulb at a height of about 75 cm. For observation, each cell with the fish inside it was quickly lifted out of the water with the sliding doors removed, so that the tail of the fish would slip out of either of the tapering ends for examination under microscope. The tail was dipped in a tray of clear perspex filled with water and fixed to the stage of the microscope. The whole observation was completed within 5-7 seconds.

The pigmentary responses in darkness were recorded with the same apparatus in a dark room with matt black walls. The microscope was kept in a black-painted light-proof wooden enclosure kept ca. 1 meter away from the trays containing the fish in cells, so that light would not be reflected out of the enclosure during the time of recording the observations.

3. The site for observing pigmentary changes

The observations on the reactions of melanophores throughout the series of experiments were always recorded from the same site in the tail, namely the circular area around the centre of the vascular arc (Fig.2, p.51), its diameter being 2 mm. The magnification used was x 100.

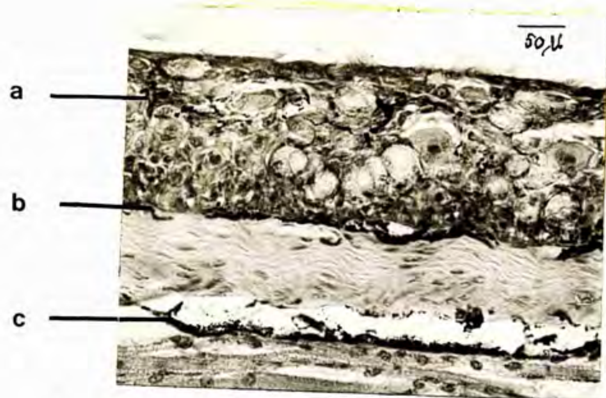
4. The chromatophores of the catfish

Abbreviations used:-
edm = epidermal melanophores
udm = upper dermal melanophores
ldm = lower dermal melanophores

All chromatophores in the skin of the catfish *I. melas* are melanophores scattered throughout the dorsal and lateral surfaces and fins and extending between the fin rays. They do not form any pattern and occur in 3 fairly well-defined layers. These are clearly distinguishable in transverse section (Fig.3, p.54), as well as in whole mounts of the skin and under most conditions in the skin of the living animal by focussing the microscope up or down (Figs. 4a-c, p.54). The top layer lies in the epidermis, and the next two layers in

Fig.3 (Top) Photomicrograph of section of skin of _I. melas_ showing layers of (a) epidermal, (b) upper dermal and (c) lower dermal melanophores. (Fixed liquid nitrogen and Bouin. Haematoxylin and Eosin).

Fig. 4 Three photomicrographs taken from a whole mount of skin showing (a) epidermal, (b) upper dermal and (c) lower dermal melanophores by gradually lowering the objective of the microscope. The skin was removed from a white-adapted fish killed by plunging into liquid nitrogen and fixed in Bouin's fluid.


50µ
a
b
c

20 µ
a

b

c

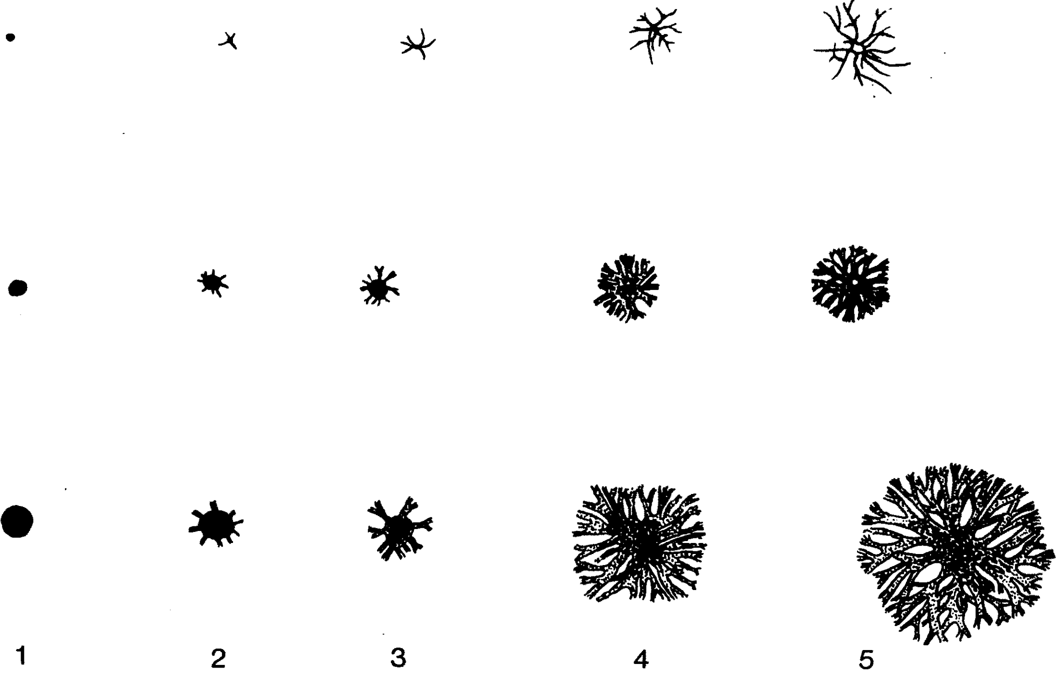
Fig. 5 Photomicrograph of the apparent network formed by the fully dispersed epidermal melanophores in a 24-hr black-adapted *I.melas*. (Skin preparation as for Fig.4).

Fig. 6 Photomicrographs of fully dispersed (a) upper dermal and (b) lower dermal melanophores in a 24-hr black-adapted *I.melas*. (Skin preparation as for Fig.4).

a

b

Fig. 7 The melanophore index.
Top row: epidermal,
middle row: upper dermal,
bottom row: lower dermal
melanophores.
(Modified from Hogben &
Slome, 1931; Healey, 1951).


1
2
3
4
5

the upper and the lower parts of the dermis respectively. The epidermal melanophores are the smallest and the lower dermal ones are the largest in size. (Parker (1934a,1940, 1948) only mentions dermal melanophores in A. nebulosus without reference to layers). In the fully aggregated state of their pigment the average diameters of the epidermal (edm), upper dermal (udm) and the lower dermal melanophores (ldm) are 8.4, 25.3 and 50.0 microns respectively. In the fully dispersed state the distance between the tips of opposite branches of the respective melanophores average 103.4, 90.3 and 157.5 microns (see Table 1, p. 58).

The pattern of dispersion of edm is markedly different from that of the other melanophores. Many long and fine processes arise from the central nuclear mass to ramify in all directions. The thin and fine processes of the neighbouring edm appear to join with one another (Fig.5, p.55) indicating a possible continuity as suggested by Fujii & Fujii (1966). The processes of udm and ldm, on the other hand, are thick and short and are mostly confined to one plane only i.e. parallel to the skin surface, and the branches of the adjoining udm and ldm generally remain separate from one another (Fig. 6a,b, p.55). Moreover, these melanophores in the fully dispersed state occupy more or less a circular area. The branching at all levels appears to be dichotomous. Odiorne (1937) described a similar branching of the dermal melanophores

in A. nebulosus but did not record the presence of the deeper layer of dermal melanophores. All melanophores in I. melas in general resemble those described in the African siluroid fishes Clarias and Synodontis by Lutfy (1961).

5. The assessment of the changes in melanophores

The estimation of the degree of pigment dispersion was made by the use of a melanophore index as devised by Hogben & Slome (1931) and modified in the present case to agree with the pattern of dispersion in the catfish (Fig. 7, p.56).

Table 1. The average diameters or distances (in microns) between the tips of opposite branches of melanophores at different MI values (15 melanophores)

Type of melanophore	Melanophore index 1	2	3	4	5
Epidermal (edm)	8.4 ±2.10	23.2 ± 6.89	44.3 ± 4.66	60.9 ±3.50	103.4 ±19.83
Upper dermal (udm)	25.3 ±3.73	38.9 ±5.48	45.8 ±6.18	70.0 ±3.83	90.3 ±9.10
Lower dermal (ldm)	50.1 ±7.58	64.5 ±6.30	76.0 ±8.40	109.7 ±6.65	157.5 ±17.50

The average dimensions of edm, udm and ldm at different MI values are shown in Table 1 above. These measurements and the drawings for the MI were made from the skin mounts of the fish previously plunged into liquid nitrogen (Temp. -195.8°C.) and then transferred to 10% formalin or Bouin's fluid for permanent fixation. (Liquid nitrogen was found to

be a satisfactory agent for killing the fish instantaneously with only a slight change in the state of the melanophores. It often split open the skull and ruptured the belly but this was of no consequence so far as the skin preparations were concerned). The skin pieces were always taken from the site where the MI readings were taken in the living material.

It may be emphasized again that the MI values only indicate the differences in the chromatic behaviour since the MI scale is arbitrary and the figures have no linear relationships.

V. MELANOPHORE RESPONSES OF INTACT AND EYELESS ICTALURUS MELAS (RAFINESQUE)

1. Introduction

Observations on the rates of colour changes as well as other experiments indicate that the relative degree of nervous and hormonal control of melanophores in teleosts is variable. In *Macropodus opercularis*, according to Umrath & Walcher (1951), the colour changes in response to black/white illuminated background reversal are very rapid and are stated by them to be mediated entirely through the nervous system. In *Phoxinus phoxinus* (Healey, 1940, 1951), *Gasterosteus aculeatus* (Hogben & Landgrebe, 1940) and *Lebistes reticulatus* (Neill, 1940), the nervous system is known to play a prominent part

and is responsible for considerable and rapid colour changes but the pituitary is concerned with slow attainment of final equilibrium. In *Anguilla anguilla* (Neil, 1940), on the other hand, the nervous system plays only a smallpart and the colour changes are relatively slow and predominantly hormonally controlled (p.6).

In addition to the varying roles of the nervous and endocrine systems, the melanophores themselves vary in their reactions. Thus, Abolin (1925) found that the melanophores of *Phoxinus phoxinus* did not all show the same degree of pigment dispersion when the animal was subjected to the actions of various hormone preparations. Healey (1951, 1954, 1967) found that this fish develops a marked irregular pattern of lighter and darker regions during colour changes in response to black/white background reversal and after equilibration to various shades of grey. This pattern is the result of differential reactions of groups of larger (macro-) melanophores and smaller (micro-) melanophores. Finally, in this fish there is a tendency for the dermal melanophores in any region to be slightly more dispersed than those in the overlying epidermis. Matsushita (1938) found that when white-adapted specimens of *Parasilurus asotus* were transferred to a black background the epidermal melanophores dispersed pigment twice as fast as the dermals. On the other hand, in *Anguilla anguilla* the pigment in the epidermal melanophores disperses

more slowly and aggregates more quickly (Neill, 1940) (p. 2).

The rate of colour change of the catfish Ameiurus (=Ictalurus) nebulosus has received little attention. The observations of Parker (1934a, 1940) and Abramowitz (1936a) indicate that A. nebulosus does not resemble Macropodus, Phoxinus or Anguilla with regard to the relative importance of neural and hormonal control of melanophores.

In the present section, firstly, the colour change reactions of white- and black-adapted I.melas were determined in relation to time and in terms of melanophore index. The reactions of all layers of melanophores were recorded in this way. Having done so, secondly, the melanophore responses of this species were compared with those of the known teleosts in order to make a preliminary assessment of the parts played by the nervous and endocrine mechanisms in controlling these reactions in I. melas.

2. Materials and methods

These have already been described in general in Section IV. For recording the melanophore reactions of normal fish, two sets each of more than 15 animals were observed. One set comprised white-adapted animals and the other equilibrated on a black background for 48 hr. In each set MI's of the same individuals were recorded at successive time intervals till the completion of the experiment. The

two sets were observed both ways, i.e. from white-to-black adaptation/black-to-white adaptation and the reverse with the other set.

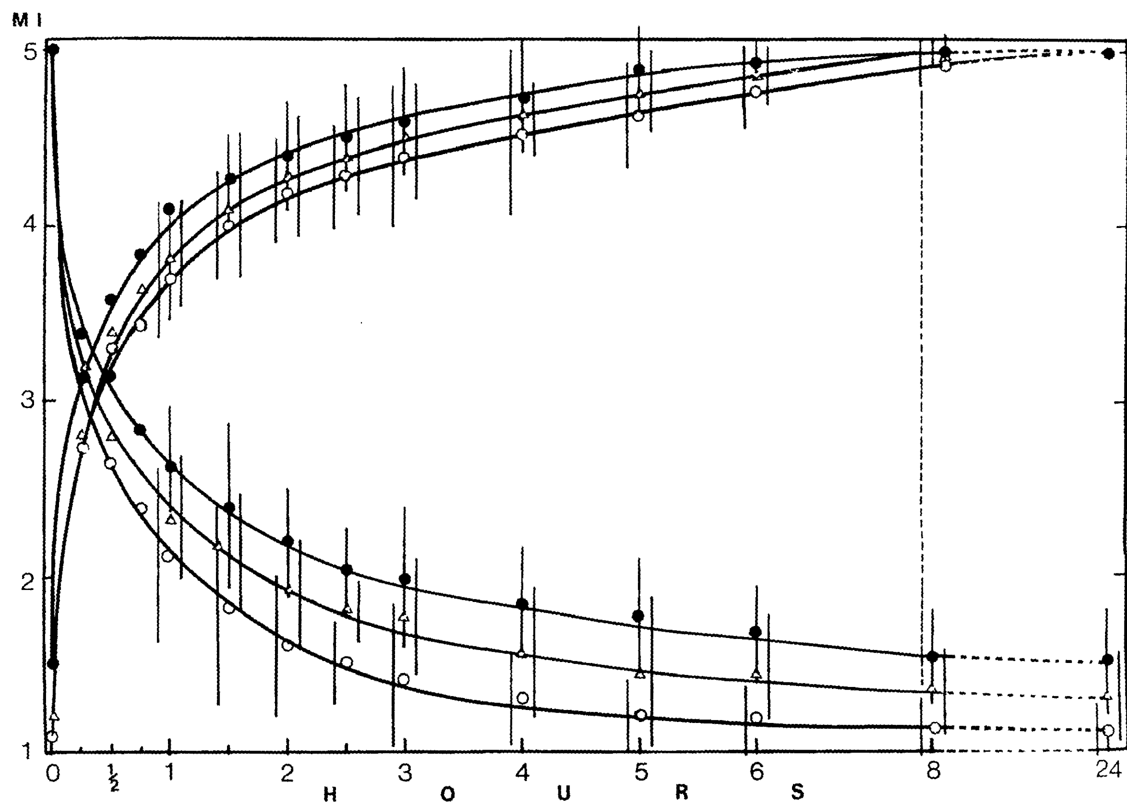
3. Results

(i) Chromatic responses of intact fish.

(a) Adaptation to white/black illuminated backgrounds. The capacity of background adaptation in this fish is considerable. A normal or intact fish takes ca. 5 hr. for near-adaptation on a white/black illuminated background and reversal. For complete adaptation 6-8 hr. are required. A white-adapted fish is yellowish brown in appearance but a black-adapted fish is coal-black and is indistinct from its background. The changes in body colour are mainly related to the degree of dispersion or aggregation of pigment in the two layers of dermal melanophores. However, the role of the epidermal melanophores is also significant, especially in the black background adaptation when they form a network.

(b) Reactions of melanophores of different layers to background reversals. The MI's of ldm, udm, and edm of white-adapted catfish (more than 15 animals) averaged 1.50, 1.26 and 1.10 respectively (see Fig.8, p.63, Table 2 in Appendix). On transfer to a black background the melanophore pigment started dispersing, changing the body-tint from yellowish brown to greyish brown. The dispersion in the first 30 min. was very quick, ldm attaining a MI 3.61, the increase in dispersion

Fig. 8 Responses of melanophores of *I. melas* transferred from equilibrium on an illuminated white background to an illuminated black background and reversal after 24 hr. Each point with standard deviations as vertical bars is the mean of 15 fish. The SD's for the first $\frac{3}{4}$ hr have been omitted for the sake of clarity. Temp 20 $\pm 1^{\circ}$C. Overhead illumination 40 W lamp at 75 cm. Open circles = epidermal melanophores, open triangles = upper dermal, and closed circles = lower dermal melanophores. (The SD's for epidermal melanophores are shifted to the left and for upper dermals to the right).


MI
5
4
3
2
1
0
½
1
2
3
4
5
6
8
24
H O U R S

being equivalent to 2.11 on MI scale; in the next 30 min. the process became slower, the increase being 0.50 MI; subsequently the rate fell further till full dispersion (MI 5) had been accomplished. Of the 3 layers, pigment in the edm was always less dispersed than in the dermal melanophores and the edm were the slowest to achieve full dispersion.

On reversal to a white background the pigment in all melanophores began to aggregate. Comparison with black adaptation shows that white adaptation in its initial stages was slower as far as the ldm were concerned (Fig. 8, p.63 Table 2 in Appendix), their MI in the first 30 min. falling from 5.0 to ca. 3.2, a decrease of 1.8 MI. The rate of aggregation was slightly slower in the next hour, the average fall in MI being 1.4. After 1½ hr. the rate became still slower. The edm showed a marked difference in comparison to ldm. They concentrated pigment more rapidly initially, showing a steep fall to MI ca. 2.7 in the first 30 min., a decrease of ca. 2.3 MI; in the next hour their rate of aggregation was slower than that of ldm; after that the aggregation was slow and gradual, until full adaptation was reached. On the 3 layers the edm were first to achieve equilibrium to a white background.

The time graphs of 48 hr. black-adapted animals in black-to-white transition and reversal were essentially similar

to those of the white-adapted animals in the above transitions (Table 3 in Appendix).

The responses of melanophores of different layers at different time intervals during the transitions white-to-black and black-to-white are shown in Figs. 9 to 18 on pages 66 to 75).

MI values of edm and ldm show statistically significant differences by the t-test (i) on a white background, (ii) during the transition from white-to-black, excepting near the completion of the adaptation, i.e. after ca. 5 hr., (iii) throughout the transition from black-to-white background adaptation. The differences in the index values of udm and ldm are generally statistically insignificant. The only apparent difference between them is that the udm are smaller than the ldm.

Thus in their behaviour as well as in their histology there is a pronounced difference between the epidermal and the two layers of the dermal melanophores, the latter being more or less alike in their reactions and in their pattern of dispersion.

Parker (1934a) reported that A. nebulosus (at an unspecified temperature and with an unspecified background history) required 15-24 hr. for adaptation to a black background and 24-36 for white adaptation. Repeated reversal of backgrounds resulted in a shortening of the time, namely,

Figs. 9 to 13 show the dispersion of pigment in epidermal, upper dermal and lower dermal melanophores in white-to-black transition. These figures like Figs. 4-6 are photomicrographs taken from the whole mounts of skin of white-adapted *I.melas* transferred to an illuminated black background and killed by plunging them in liquid nitrogen at various time intervals and fixed in 10% formalin).

Fig. 9 shows (a) epidermal, (b) upper dermal, (c) lower dermal melanophores after 20 min on a black background and (d) general picture of all melanophore layers.

a

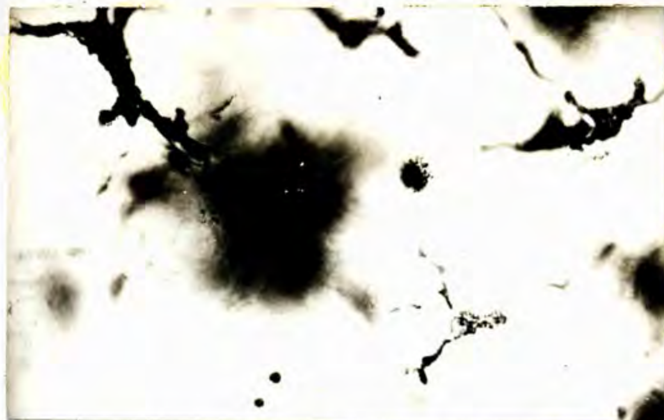
b

c

d

Fig.10 shows (a) epidermal, (b) upper dermal, (c) lower dermal melanophores after $\frac{3}{4}$ hr on a black background and (d) general view of all melanophores. (Fixed liquid nitrogen and formalin).

a

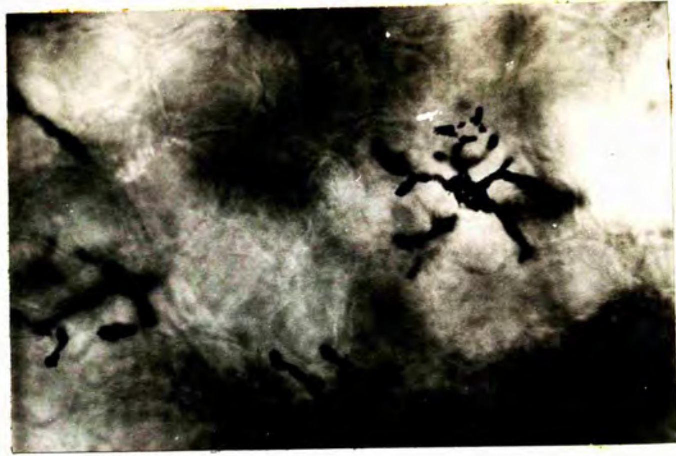


b

c

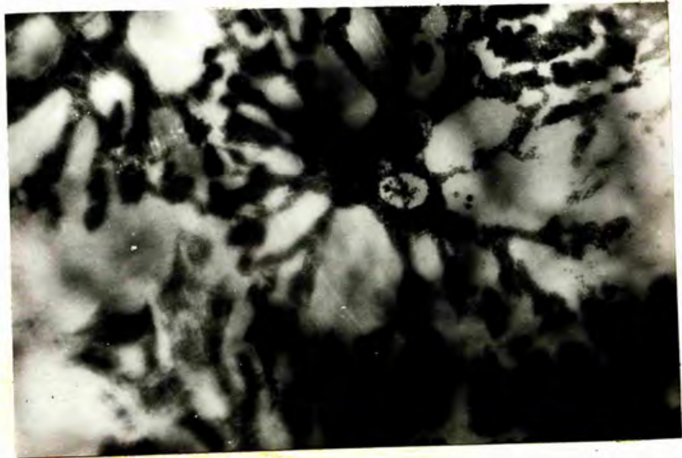
d

Fig.11 shows (a) epidermal, (b) upper dermal and (c) lower dermal melanophores after $1\frac{1}{2}$ hr on a black background. (Fixed liquid nitrogen and formalin).



a

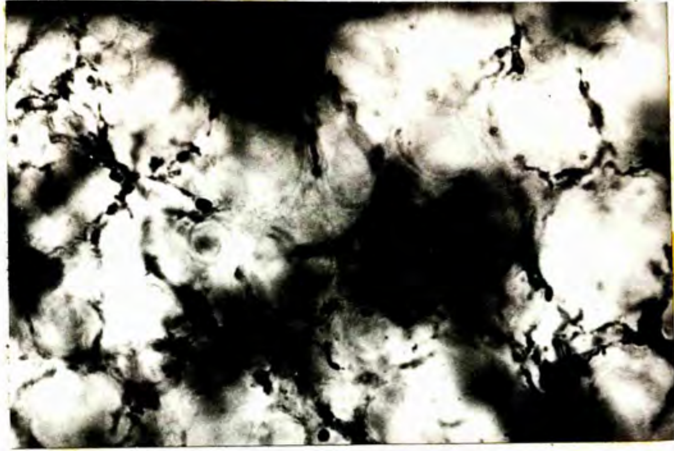
b



c

Fig. 12 shows (a) epidermal, (b) upper dermal and (c) lower dermal melanophores after $2\frac{1}{2}$ hr on black background. (Fixed liquid nitrogen and formalin).

a



b

12

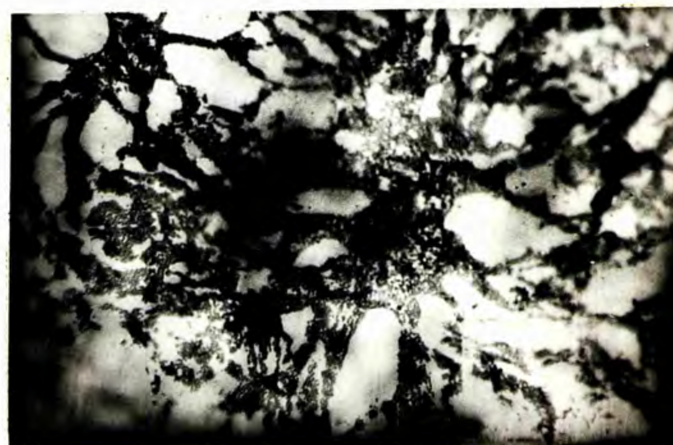
c

Fig. 13 shows (a) epidermal, (b) upper dermal and (c) lower dermal melanophores after 6 hr on a black background. (Fixed liquid nitrogen and formalin).

a

b

c

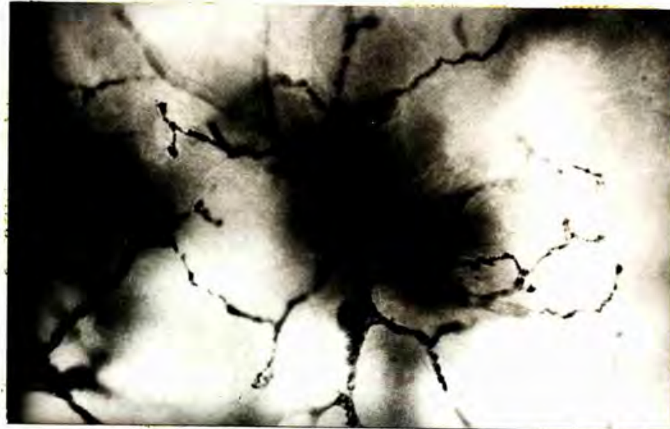


13

Figs. 14-18 show the aggregation of pigment in different melanophore layers in black-to-white transition, the animals being previously 24-hr black adapted. (Fixation method as for preparations shown in Figs. 9-13, p.66).

Fig. 14 shows (a) epidermal, (b) upper dermal, (c) lower dermal melanophores after 15 min on a white background and (d) general view of all melanophore layers.

a



b

c

d

Fig 14

1

Fig. 15 shows (a) epidermal, (b) upper dermal, (c) lower dermal melanophores after $\frac{3}{4}$ hr on a white background and (d) general view of all melanophore layers. (Fixed liquid nitrogen and formalin).

a

b

c

d

Fig. 16 shows (a) epidermal, (b) upper dermal and (c) lower dermal melanophores after $1\frac{1}{2}$ hr on a white background and (d) general view of all melanophore layers. (Fixed liquid nitrogen and formalin).

a

b.

c.

d.

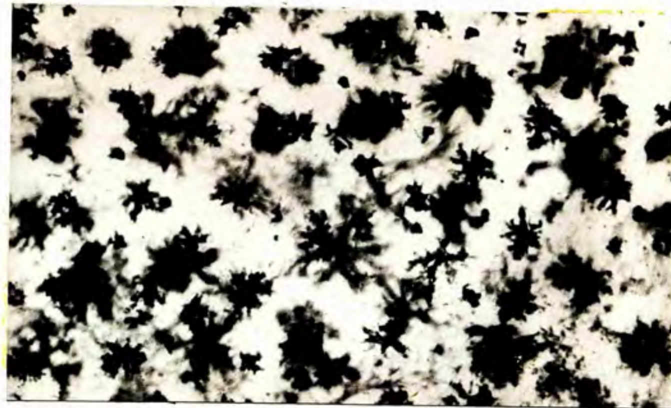


Fig. 17 shows (a) epidermal, (b) upper dermal, (c) lower dermal melanophores after 3 hr on a white background and (d) general view of all melanophore layers. (Fixed liquid nitrogen and formalin).

a

b

c

d

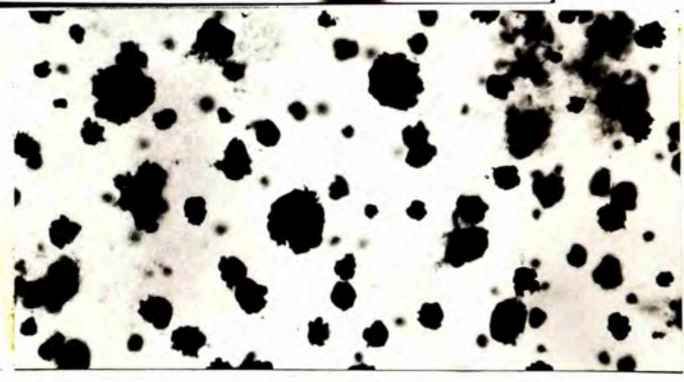
Fig. 18 shows (a) epidermal, (b) upper dermal, (c) lower dermal melanophores after 6 hr on white background and (d) gives overall picture of all melanophore layers. (Fixed liquid nitrogen and formalin).

a.

b.

c.

d.



from white to black in 1 hr. and from black to white in about 3½ hr. Later (1943, 1948) he referred to shorter times required for colour changes. He gave no information about the changes occurring in the body-tint or in the melanophores at different time-intervals during the transition from one background to another. According to Abramowitz (1936a) a white-adapted catfish turned dark in 3 hr. on transfer to a black background and a black-adapted one became pale in 35 hr. on a white background. Neither of these observations agrees with those on I. melas. Regarding the epidermal melanophores, Parker (1934a) stated that they reacted like the dermal ones in darkening and in paling. This, again, is not the case with I. melas.

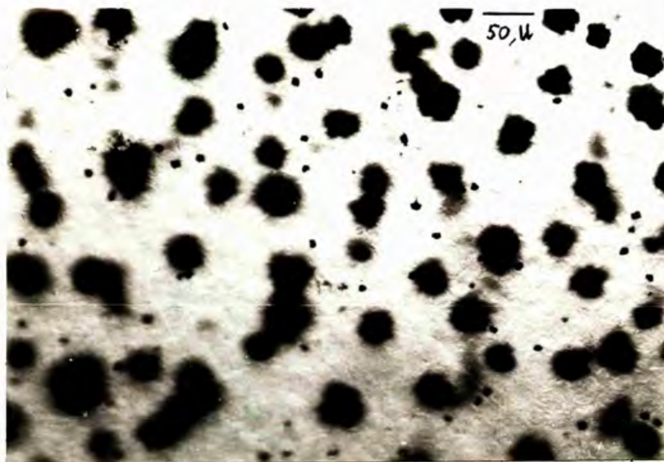
In the Japanese catfish Parasilurus asotus, Matsushita (1938) observed differential reactions of epidermal and the dermal melanophores in response to a black background, as seen in I. melas. In both these species the initial rate of dispersion of the dermal melanophores is almost identical (MI 3.6-3.7 in 30 min.) but later on it decreases in Ictalurus. However, the rate of aggregation in both species is similar. On the other hand, the epidermal melanophores in Parasilurus unlike those of Ictalurus disperse their pigment much more rapidly than the dermal ones. No data regarding the rate of aggregation of epidermal melanophores in Parasilurus are available.

<u>(c) Differential reactions of melanophores of different layers on equilibration to various greys.</u> A marked difference in the dispersion of pigment in epidermal and dermal melanophores was observed on adaptation of previously white-adapted fish to various greys, derived from the Ostwald White-Grey-Black series (Healey, 1967). Table 4 given below summarizes the results when 3-4 fish were equilibrated to 3 shades of grey, DOI 2 (28% reflection), 4 (11% reflection), and 6 (4.5% reflection) (Figs.19-21, p. 78-79) for 48 hr. and then killed in liquid nitrogen, fixed in Bouin's fluid and cleared in methyl benzoate. The difference in dispersion of melanophores of different layers conforms with the differential rates of dispersion in response to a black background (DOI 8, reflection 1.8%) on transfer from a white background (DOI 0, reflection 71%). Since the equilibrium MI of edm on white and grey (DOI 2) backgrounds is almost the same, the threshold of their response to a light-absorbing background appears to be comparatively higher than in either udm or ldm.

Table 4. Mean MI's of (3-4) previously white-adapted I. melas equilibrated to different greys (DOI's 2, 4 and 6). 20 ±1°C. 40 W at 75 cm.

DOI and reflection of the background	Mean MI's		
	edm	udm	ldm
2 (28% reflection)	1.20 ±0.19	1.50 ±0.20	1.80 ±0.25
4 (11% reflection)	1.90 ±0.24	2.50 ±0.32	2.80 ±0.42
6 (4.5% reflection)	2.90 ±0.30	3.35 ±0.30	3.75 ±0.45

Fig. 19 Photomicrographs showing dispersion of pigment in (a) epidermal, (b) upper and lower dermal melanophores after 48 hr adaptation to a background of 28% reflection (DOI 2) (Fixed liquid nitrogen and Bouin).



a

b

Fig. 20 Photomicrographs showing dispersion of pigment in (a) epidermal and (b) upper and lower dermal melanophores after 48 hr on a background of 11% reflection (DOl 4). (Fixed liquid nitrogen and Bouin).

Fig. 21 Photomicrograph showing dispersion of pigment in different melanophore layers after 48 hr adaptation to a background of 4.5% reflection (DOl 6). (Fixed liquid nitrogen and Bouin).

a

b

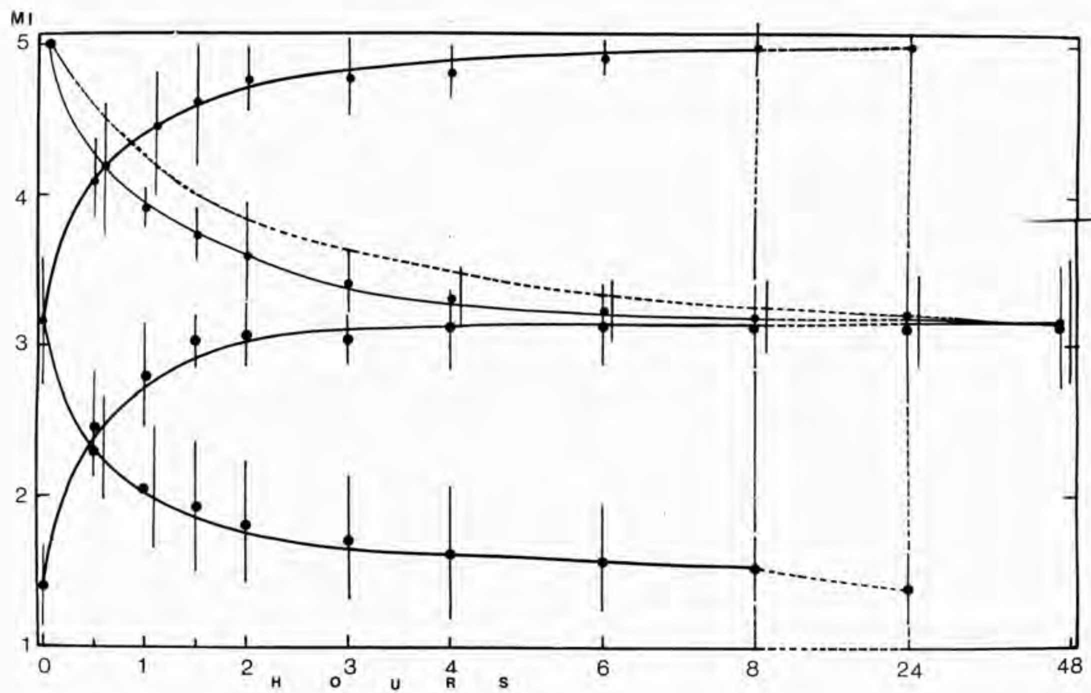
(d) Responses to darkness. The responses of black- and white-adapted catfish, 2 groups of 8 animals each, to total darkness were studied under similar conditions. Of the two classes of dermal melanophores, only the indices of the ldm were recorded at successive time-intervals, as these were found more convenient to read (Fig.22, p.81). These melanophores equilibrated in darkness at MI 3.15-3.17 in about 6 hr. in white- and 8 hr. in black-adapted fish; and for reversal about 8 hr. in both cases. The responses of edm were erratic and therefore were not included (Tables 5 and 6 in Appendix).

Regarding the reactions of A. nebulosus to darkness, conflicting data are available (Bray, 1918; Parker, 1934a, 1940; Abramowitz, 1936a; Osborn, 1938). Osborn's observations agree with those on I. melas. He reported that A. nebulosus took 5-10 hr. or less for adaptation to darkness, a period similar to that required by I. melas.

(ii) Chromatic responses of blinded fish.

(a) Reactions to darkness and light. The optic nerves of 6 white-adapted animals were cut just behind the eyeball. The fish were then replaced in white tanks (close overhead illumination was not switched on, as the laboratory was constantly illuminated). Immediately after blinding the fish lost the background response and all the melanophores started to disperse. Variations in the dispersion responses of

Fig. 22 Responses of lower dermal melanophores of *I. melas* transferred from equilibrium on illuminated white/black background to darkness and reversal after 7 days. Each point with SD's is the mean of 8 animals. Broken curve represents the responses of a group of 6 blinded animals transferred from equilibration on an illuminated background to darkness. 20 ± 1°C. 40 W at 75 cm.


MI
5
4
3
2
1
0
1
2
3
4
6
8
24
48
H O U R S

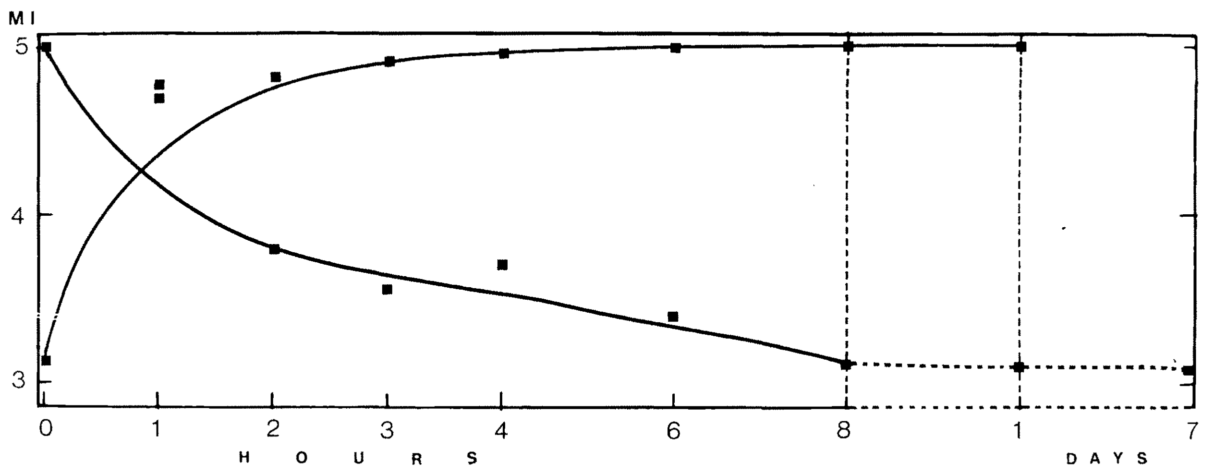
different individuals were found. In some fish the melanophores became fully dispersed within a few hours but generally all fish became permanently coal-black within 24 hr. of enucleation.

After about a fortnight the fish were transferred to total darkness. In about 24 hr. (Fig.23, p.83, Table 7 in Appendix) the pigment in ldm became aggregated to MI 3.2. No further significant change was observed in 7 days. Thus in intact as well as in eyeless specimens the equilibrium MI of ldm was almost similar. On return to light ldm became fully dispersed in ca. 24 hours.

In the blinded fish, unlike the intact animals, extremely irregular behaviour of edm in darkness was not observed. After about 6 hr. in darkness their average MI was 2.82, in 24 hr. 3.1 and in 7 days 2.85 (Table 7).

Blinded _A. nebulosus_ is mostly reported to become pale in darkness (Abramowitz, 1936a; Osborn, 1938; Wykes, 1938). Parker (1934a) reported such a fish as being pale but later on (1940) he reported that blinding had no effect on the tint of the fish previously white- or black-adapted. These observations do not agree with those on _I. melas_. Blinded _Ameiurus_ in light was described as being dark or very dark (von Heusen, 1917; Bray, 1918; Pearson, 1930; Abramowitz, 1936a; Odiorne, 1937; Wykes, 1938; Parker, 1940). These observations are similar to those on _I.melas_.

Fig. 23 Responses of lower dermal melanophores of blinded *I.melas* transferred from equilibrium on an illuminated background to darkness and reversal after 7 days. Each point is the mean of 6 fish. $20 \pm 1^{o}C$. 40 W at 75 cm.


MI
5
4
3
0
1
2
3
4
6
8
1
7
H O U R S
D A Y S

(iii) Primary responses. It was not possible to make reliable estimations of the direct response of melanophores to light, different values being obtained in different attempts. For instance, the average response from a group of white-adapted fish was found to be 0.17 on the MI scale and from a group of black-adapted fish 0.63. A group of blinded fish gave a high value of about 2.12 MI. The cause of this discrepancy may be the unusual delay in reading the MI. The fish which had been in darkness for a week became very excited on switching on the light and some delay occurred in getting them in a proper position to read the MI. (Tables 5-7 in Appendix).

4. Discussion and conclusions

In the white-to-black background response ldm in *I. melas* reach an MI of about 3.2 within 15 min. On the other hand, *Lebistes*, *Gasterosteus*, and *Phoxinus* require less than 5 min. for the same change (Table 8, p.85). While the initial dispersion responses of all these fish appear to be predominantly neurally co-ordinated (Hogben & Landgrebe, 1940; Neill, 1940), subsequently, the rate of dispersion in *Ictalurus* lags behind that of the former group of fishes, i.e. the MI 4.10 obtained between 15 to 30 min. in the former case and in 1 hr. in *Ictalurus*. Full dispersion of melanophores in the former group of fishes is accomplished in 1½ to 2 hr. and in *Ictalurus* in 6 to 8 hr. These observations indicate that

Table 8. Comparison of melanophore responses of I. melas with some other teleosts in white-to-black background adaptation.

Species	Author	Temp.	MI on White Adaptation	From 1.0-3.0	Shift of melanophore index in relation to time 3.1-3.5	3.6-3.7	3.8-4.0	4.1-4.2	4.3-4.4.	(dermal melanophores) 4.5	4.6	4.7-4.8	4.9	5.0
Gasterosteus	Hogben & Landgrebe (1940)		1.2			3.6 5 min		4.1 15 min	4.3 30 min		1 hr	4.7 2 hr		
Phoxinus	Healey (1951)	12°C	1.25	2.4 3 min	3.3 5 min		3.8 10 min 4.0 15 min		4.3 30 min		$\frac{3}{4}$ hr	4.7 2 hr		
Ictalurus		20° ±1 C	1.50		3.5 15 min	3.6 30 min	3.8 $\frac{3}{4}$ hr	4.1 1 hr	4.3 $1\frac{1}{2}$ hr	$2\frac{1}{2}$ hr	3 hr	4.7 4 hr	5 hr	8 hr
Anguilla	Neill (1940)	8.3° ±0.8 C	1.2			3.6 $2\frac{1}{2}$ hr	3.8 5 hr 4.0 24 hr			84 hr	120 hr			
										epidermal melanophores				
Parasilurus	Matsushita (1938)		1.0							$\frac{3}{4}$ hr		1.0 hr		1-1¼ hr
Ictalurus			1.10	2.7 15 min	3.5 $\frac{3}{4}$ hr		4.0 $1\frac{1}{2}$ hr	4.2 2 hr	4.3 $2\frac{1}{2}$ hr	4 hr	5 hr	4.8 6 hr		8 hr
Anguilla			1.2	2.5 5 hr	3.5 120 hr		3.8 13 days					4.7 20 days		

the nervous co-ordinating mechanism in *Ictalurus* is less dominant and that more participation of the pituitary is required in order to reach full pigment dispersion. A comparison shows that the initial dispersion responses in *Ictalurus* are 5 to 6 times faster than those of *Anguilla* in which the mechanism of dispersion of melanophores is presumed to be predominantly or entirely hormonally controlled.

When the rate of aggregation of ldm (in black-to-white response) is taken into consideration *Gasterosteus* , *Phoxinus* and *Lebistes* bear some resemblance to each other in that the melanophores become almost fully aggregated (MI 1.5) in 7 to 45 min. Table 9, p.87), showing that the co-ordinating mechanism is entirely or predominantly neural. In distinction, in *Ictalurus* and *Parasilurus* the initial fast neurally controlled reactions result in relatively little paling and subsequent hormonally controlledconcentration requires a much longer time.

Regarding the reactions of the edm in species other than *Parasilurus*, data are available from only one species, *Anguilla* (Neill, 1940). As in *Ictalurus*, in *Anguilla* the epidermal melanophores are slow to disperse. The rate of aggregation in *Anguilla*, again, like *Ictalurus* is faster than that of the dermal ones. A similar situation exists in the reactions of *Xenopus* (Neill, 1940).

Table 9. Comparison of melanophore responses of I. melas with some other teleosts in black-to-white adaptation.

Species	Author	MI on Black Adaptation	Shift of melanophore index in relation to time									
			dermal melanophores									
			From 5.0-3.3	3.2-3.1	3.0-2.8	2.7-2.6	2.5-2.4	2.3-2.2	2.1-2.0	1.9-1.7	1.6-1.5	1.4-1.2
Gasterosteus	Hogben & Landgrebe (1940)	5.0 ?				2.7 5 min				1.8 15 min	30 min	1.3 1 hr 1.2 2 hr
Phoxinus	Healey (1951)	5.0 ?			3.0 3 min		2.5 5 min	10 min		30 min	$\frac{3}{4}$ hr	2 hr
Parasilurus	Matsushita (1938)	5.0			3.0 30 min	1 hr		$1\frac{1}{2}$ hr			3 hr	4 hr
Ictalurus		5.0	3.7 15 min	3.2 30 min	2.9 $\frac{3}{4}$ hr	1 hr	$1\frac{1}{2}$ hr		2.1 2 hr	1.8 3 hr 1.7 4 hr	8 hr	
Anguilla	Neill (1940)	5.0	3.8 5 hr	3.2 36 hr			2.5 13 days			1.8 20 days		
			epidermal melanophores									
Ictalurus		5.0		15 min	30 min		2.5 $\frac{3}{4}$ hr 2.36 1 hr			1.96 $1\frac{1}{2}$ hr	1.6 2 hr	1.4 $2\frac{1}{2}$ hr 1.2 4 hr
Anguilla	Neill (1940)	5.0	3.5 5 hr 3.4 $7\frac{1}{2}$ hr		2.9 24-120 hr				13 days		20 days	

Observations on the responses of intact as well as eyeless fishes to darkness show much diversity. However, no case of fully dark body-tint or fully dispersed state of melanophores has been reported. Intact I. melas in its behaviour in darkness resembles A. nebulosus, Parasilurus and Anguilla in showing an intermediate body-tint and melanophore equilibration in the range of MI 3.00 to 3.50. Eyeless Ictalurus in darkness shows a similar response to Parasilurus.

As to the control of melanophores in darkness of both intact and eyeless animals, the situation is obscure. (1) The suggestion that the aggregating influence of the nervous system is not removed in darkness (Abramowitz, 1936a; Wykes, 1938) is not borne out by the protracted nature of the time relation required in adaptation to darkness. (2) The proposition that darkness directly stimulates the pineal to release melatonin which then aggregates the pigment by overriding the effects of MSH (Bagnara, 1960, 1965) also seems questionable on many grounds ((i) although melatonin has been identified from the pineal of the pacific salmon Oncorhynchus tshawytscha by Fenwick (1970), his findings indicate that melatonin may be involved in gonodal changes rather than playing the role of a melanophore contracting principle, and (ii) melatonin is regarded as a fast-acting agent, causing complete blanching of Xenopus larva in 30 min.

(Bagnara, 1960, 1965). The observation that the melanophores of some white-adapted fish, including *Ictalurus*, on transfer to darkness show some dispersion after a considerable time has suggested that MSH is involved in these responses and that there is a gradual build-up of the hormone to an effective level. But how the pituitary is stimulated to release a particular concentration of MSH and how this level is maintained is open to conjecture. Parker (1940, 1948) attempted to explain the situation by supposing that in darkness, at least in blinded fish, the melanophores are in a passive state and this passive state may be anywhere between full dispersion or full concentration (p.43).

In contrast to the behaviour in darkness, the species of fishes that have been blinded mostly exhibit darkening on transfer to light, though the range of pigment dispersion varies among species. In extreme cases, e.g. *Ictalurus* and *Parasilurus*, where maximum dispersion occurs, the response appears to be co-ordinated through a reflex mechanism involving photoreceptors, possibly dermal or pineal.

To sum up, comparison of the time relations of white-to-black and black-to-white background adaptations in *I.melas* with those of the other known teleosts indicates that *I.melas* in its chromatic physiology does not belong to the class of fishes (e.g. *Macropodus*) in which the complete

melanophore responses are very rapid. It also cannot be included in the group (e.g. Lebistes, Gasterosteus, and Phoxinus) in which the control of melanophores is predominantly neural but requires the participtation of the pituitary gland for full dispersion and complete aggregation. Neither can it be grouped with Anguilla in which the chromatic responses are very slow, taking days or weeks to complete, their control being predominantly if not exclusively humoral. Ictalurus appears to occupy a position between Lebistes, Gasterosteus and Phoxinus on the one hand and Anguilla on the other.

VI. EFFECTS OF HYPOPHYSECTOMY ON THE REACTIONS OF MELANOPHORES

1. Introduction

Among the elasmobranchs and the amphibians the removal of the pituitary has universally been reported to result in permanent blanching of the operated animals (Hogben & Slome, 1923; Lundstrom & Bard, 1932; Parker, 1948; Pickford & Atz, 1957). In the teleostean fishes, on the other hand, such a response is rare (Pickford & Atz, 1957) and diverse results have been reported (p.9).

Giersberg (1932) observed that the destruction of the pituitary in Phoxinus phoxinus prevented normal dispersion of the lipophores on yellow or red backgrounds. Matthews (1933) found that the removal of the hypophysis in Fundulus heteroclitus did not effect the responses of the melanophores

to changesin background and to total darkness. Parker (1934a) and Abramowitz (1936a) reported that hypophysectomised *Ameiurus nebulosus* were only slightly less dark than unoperated controls on a black background. Osborn (1938), on the other hand, observed that the dispersion of melanophore pigment in hypophysectomised *A. nebulosus* was "1/3" that of the normal fish whereas the response to a white background remained unaffected. Waring (1940) found that in completely hypophysectomised *Anguilla anguilla* the melanophores equilibrated at MI 3.5 and 1.8 on black and white backgrounds respectively. Healey (1940, 1948) noted that permanent white and black background adaptations were impaired in *Phoxinus phoxinus* following the removal of the pituitary. Thibault & Thibault (1947) reported that the melanophores in hypophysectomised catfish (species ?) remained fully aggregated. Chavin (1956) observed that the lipophores of xanthic goldfish *Carassius auratus* became fully concentrated after the removal of the pituitary. Pickford (1957), in contrast to Matthew's observations, found that hypophysectomised *Fundulus heteroclitus* on a black background became markedly paler than controls (pp.6-13).

With the exception of Enami's work (1939) there is no record in which the responses of more than one type of melanophore of a hypophysectomised fish were investigated.

He found that in hypophysectomised Parasilurus asotus the epidermal and the dermal melanophores responded differently to background changes. On a black background the dermal melanophores equilibrated at MI 3.0 whereas the epidermal ones remained fully contracted. On a white background the dermal melanophores aggregated their pigment at a slower rate than that of the epidermal ones.

Injection of pituitary extracts from various sources into intact or operated teleost fishes has provided voluminous but conflicting information regarding the effects on the melanophores (cf. Pickford, 1957). Many of the conclusions reached in this way must therefore be regarded with reserve (pp. 8-11).

The present section is mostly concerned with the responses of melanophores of hypophysectomised I. melas to background changes compared with those of intact fish in order to assess the role of the pituitary gland in the colour changes of this species.

2. Material and method

(i) Hypophysectomy. The fishes used for hypophysectomy were 70 to 85 mm long. The pituitary gland was removed under anaesthesia with MS 222 Sandoz. Immersion in a 0.0125% solution in tapwater immobilized a catfish in 2-3 min. The fish was then secured on its back in the groove of a

specially designed operation tray (von Frisch & Stetter, 1932; Healey, 1940, 1948) by means of rubber bands stretched across it. The groove enclosing the fish was lined with moist filter paper. Water was constantly dripped into the mouth of the fish by means of a glass tube and was drained off along the groove, thus also keeping the body of the fish wet all the time during the operation. The operation was performed under a Nikon stereoscopic binocular (magnification x 20) with adjustable twin lighting. The head of the fish was slightly raised by a pad of moistened filter paper.

An inverted U-shaped cut was made in the skin of the lower jaw along the outline of the underlying basihyal. A cut was then made along the margin of the basihyal which exposed the floor of the mouth cavity. 3 hooks made from bent nickel-plated pins were inserted, two anteriorly and one posteriorly, in the basihyal and the tongue. The hooks were connected by the thread to lead blocks. By gently moving out the latter the wound was stretched to form a gap wide enough to permit manipulation on the roof of the mouth cavity (Fig. 24, p.94). A longitudinal cut was made in the mucous membrane covering the roof of the mouth cavity by means of a small scalpel to expose the parasphenoid bone. The outline of the pituitary and the associated brain parts are visible through the bone. At this stage a fine jet of Ringer solution was used to remove blood from the site of the operation. 4 very small holes were made around the

Fig. 24 Photograph showing the general arrangement for the operation of hypophysectomy.

Fig 21

outline of the pituitary by means of a finely sharpened dental drill. With experience one could feel when the drill was about to pierce the bottom of the bone and so avoid damage to the surrounding brain. The piece of bone was then gently pulled out with a watch-maker's forceps. The pituitary in the catfish lies in a depression between the inferior lobes (Fig.25, p.96). It was gently sucked with a pipette so that it was raised. The pituitary-stalk underneath was pinched off with fine forceps and the pituitary was removed. The cut piece of the bone was replaced. In some cases it was accepted and grew into the rest of the parasphenoid. The cut mucous membrane was usually not stitched. When the hooks were removed the tongue and the basihyal came back to their normal position and the wound was closed with fine nylon. In a successful operation no damage was done to the neighbouring blood-vessels. The whole operation could be carried out in 5-7 min. The fish recovered rapidly on return to an aquarium tank after the operation. They were twice daily given a bath in 3-4% NaCl solution with a few grains of potassium dichromate added to it to protect them from infection by the fungus Saprolegnia. Complete healing of the wound and the equilibration of the melanophores required about 10 days. Experimental observations were generally made 2-3 weeks after the operation.

Fig. 25 Photographs showing (a) the approach to the pituitary and (b) the exposed pituitary gland.

a

b

Two sets of animals were utilized for experimental observations, one comprising white-adapted animals which were replaced on a white background after the operation and the other consisting of 48 hr.-black-adapted animals which were placed back on the black background after hypophysectomy. After the operation the fish were handled as little as possible for some time in order to avoid any damage to the wound. About 70% of the operated animals lived for many weeks after the operation. Reactions have been taken into account of only those animals which lived right through to the completion of the experiment. On completion the extent of hypophysectomy was histologically verified (Fig. 26 in Appendix). The same experimental procedure was adopted for controls, their hypothalamic region being exposed without touching the pituitary.

3. Observations and results

(i) The equilibration of melanophores after the operation.

The immediate effect of the removal of the hypophysis from a white-adapted fish was some dispersion of its melanophores (Table 10, p.98), presumably following the release of some MSH and partly due to the effect of anaesthesia. No contraction of melanophores was observed in black-adapted specimens immediately after the operation. In both cases the equilibration of melanophores was generally obtained within 4-10 days.

Table 10. Equilibration of melanophores on illuminated white and black backgrounds after hypophysectomy

Time after the operation	White background			Black background		
	Average MI's					
	edm	udm	ldm	edm	udm	ldm
Immediately after	1.50	2.05	2.98 (8) *	5.00	5.00	5.00 (15)
1 day	1.00	1.56	2.01 (8)	2.80	3.76	4.52 (8)
4 days	1.00	1.53	1.82 (8)	2.50	2.84	4.08 (13)
10 days	1.00	1.48	1.85 (7)	2.40	3.00	3.62 (15)

*The number of animals shown in parentheses.
Edm = epidermal melanophores, udm = upper dermal, and ldm = lower dermal melanophores.

Conflicting data are available regarding the time taken by A. nebulosus for equilibration on white/black background after hypophysectomy. Veil (1937) reported it became fully pale 3 days after the operation. Osborn (1938) observed that it required ca. 90 hr. for equilibration on a black background, whereas, Parker (1940) reported that it took ca. 5 days. Black-adapted Anguilla anguilla according to Waring (1940) required 5-13 days for equilibration on a black background after hypophysectomy.

(ii) Degeneration of melanophores and fading of melanin pigment in the hypophysectomised animals. Within about a fortnight after hypophysectomy degeneration of melanophores into numerous minute fragments began in the fish kept on a white background. Such degeneration, however, was not commonly noted on a black background. Moreover, a marked fading of the pigment, especially in the lower dermal melanophores, was also observed in white as well as black-adapted hypophysectomised individuals.

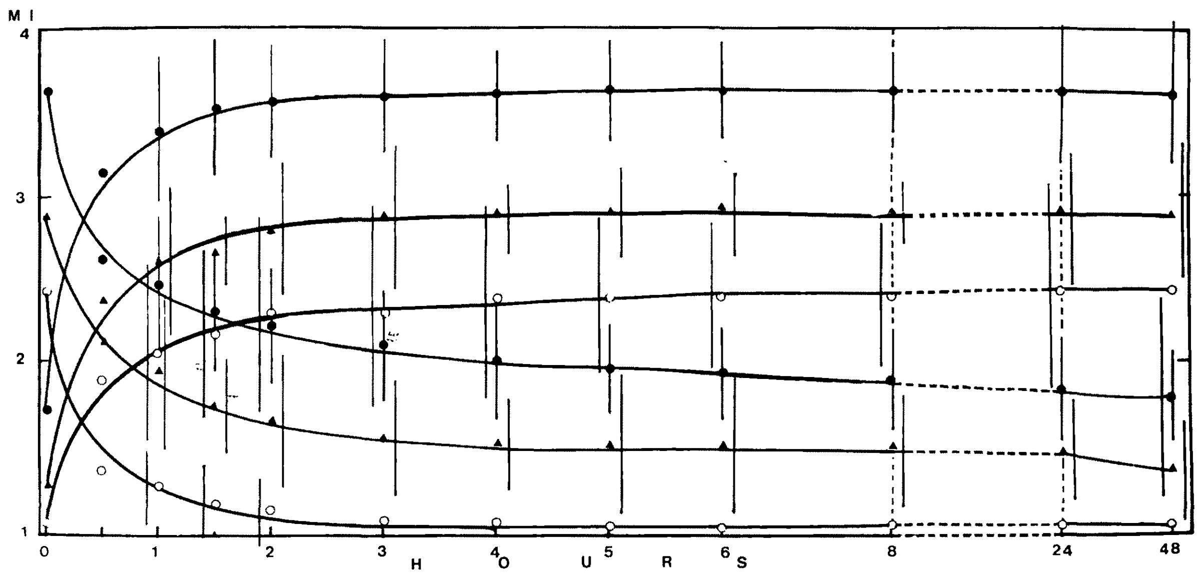
The degeneration of melanophores was earlier reported by Osborn (1941) in A. melas and Thibault & Thibault (1947) in catfish (species ?). The fading and degeneration of melanin in the larger melanophores in hypophysectomised Fundulus heteroclitus was noted by Kosto et al (1959). The degeneration of melanophores in hypophysectomised Carassius auratus on a dark background was reported by Castrejon & Flores

(1955) (cf. Pickford & Atz, 1957).

(iii) Chromatic responses of hypophysectomised catfish to black and white backgrounds and reversal. The MI's of the three classes of melanophores, the lower dermal melanophore (ldm) the upper dermal (udm)and the epidermal (edm) of a white-adapted hypophysectomised *I. melas* averaged 1.74, 1.25 and 1.00 respectively. The mean index values of the ldm of operated fish were slightly higher than those of the unoperated fish but these values as well as those of the udm and edm show no statistically significant difference by the *t*-test. (It may be added here that complete aggregation of melanophores of all layers on a white background was noted in hypophysectomised animals which survived over 2 months).

On transfer to an illuminated black background all the layers of melanophores of a white-adapted animal reacted quickly by dispersing their pigment. The divergence in the reactions of the different layers of melanophores became markedly wider. In 30 min. MI's of ldm, udm and edm reached 3.24, 2.40 and 1.90 respectively. After this initial rapid dispersion there was very little further change in melanophore index, this being at the most 0.50. After about 4 hr. there was little further change in the degree of adaptation, the MI's of ldm, udm and edm averaging 3.63, 2.90 and 2.40 respectively (Fig. 27, p.101) (Table 11 in Appendix).

Fig. 27 Melanophore responses of hypophysectomised *I.melas* from equilibrium on an illuminated white background to an illuminated black background and reversal after 48 hr. Each point with SD's as vertical bars is the mean of 15 animals. SD's for the first 30 min not shown. Temp. 20 ± 1°C. Overhead illumination 40 W at 75 cm. Open circles=epidermal melanophores, closed triangles= upper dermal, closed circles= lower dermal melanophores.


M I
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48
H O U R S

After 48 hr. the fish were transferred back to a white background to which their melanophores reacted rapidly. In 30 min. MI's of ldm, udm and edm averaged 2.62, 2.15 and 1.32, the fall in MI being 1.01, 0.75 and 1.10 respectively. In 3 hr. the respective mean MI's of ldm, udm and edm were 2.10, 1.56 and 1.00, showing a further decrease in MI of 0.50 for ldm and udm and 0.32 for edm. While the epidermal melanophores (edm) became fully aggregated in 3 hr, the lower (ldm) and upper (udm) dermal ones showed a slow and gradual aggregation for more than 48 hr, although the range of this aggregation was quite narrow, being 0.3 to 0.4 on the MI scale (Table 11 in Appendix).

Almost similar responses of the melanophores of black-adapted hypophysectomised I. melas were observed on transfer to a white background and reversal. The MI's of ldm, udm and edm of these fish averaged 3.67, 3.05 and 2.44 respectively, on a black background. After 48 hr. on a white background their ldm and udm equilibrated to MI's 1.80 and 1.30 respectively (Table 12 in Appendix). The melanophore responses of controls to background changes were observed to be essentially similar to those of intact fish.

One fact which is clearly borne out by the above observations is that the ability of adaptation to a black background in I. melas is largely impaired as a result of

the removal of the hypophysis, whereas the ability of adaptation to a white background appears to remain almost unaffected. Another interesting feature of melanophore responses to a black background is the varying effect of hypophysectomy on the dispersion of pigment in the different melanophores layers (Fig.28, p. 104). Of the three layers the deepest (ldm) seems to be the least affected and the melanophores of this layer exhibit an MI of ca. 3.65, whereas the superficial layer (edm) is severely affected, the melanophores remaining more or less at MI 2.4. The effect of hypophysectomy on the middle layer (udm) seems also to be severe but less so than in the case of epidermal melanophores.

The responses of the melanophores (in an unspecified dermal layer) of hypophysectomised A. nebulosus to background adaptation were observed by Parker (1934a,1940), Veil (1937), Abramowitz (1936a) and Osborn (1938). Veil and Abramowitz confined their observations to the macroscopic appearance of the fish whereas Parker and Osborn also recorded the average diameters of the dermal melanophores. Parker (1940) stated that a white-adapted fish had a "palish" body-tint with the diameters of its dermal melanophores ranging from 50-60 microns (45 μ in an unoperated fish). On a black background its body shade was "intermediate" and he gave two different measure-

Fig. 28 Three photomicrographs taken from a whole-mount of the skin of the tail fin of a black-adapted hypophysectomised I.melas showing (a) epidermal, (b) upper dermal and (c) lower dermal melanophores by gradually lowering the objective of the microscope. (The fish was killed about 3 months after hypophysectomy. Fixed liquid nitrogen and Bouin).

a

b

c

ments of its melanophores 70 μ (p.238) and about 100 μ (p. 246) in the same publication (about 140 μ in unoperated fish). Veil stated that the hypophysectomised catfish remained completely pale irrespective of the background. Osborn reported that it maintained white adaptation following hypophysectomy (at 12°C.) (the same diameter of 45 μ of the dermal melanophores in the operated animals and controls) and that the melanophores of the hypophysectomised fish equilibrated to a black background never exceeded 70 μ (120-130 μ in unoperated fish) after as long as black adaptation for one month. Thus, so far as the state of the dermal melanophores on white/black background adaptation is concerned, the observations made on hypophysectomised _I. melas_ are in general agreement with those of Parker (1940) on _A. nebulosus_.

Abramowitz (1936a) recorded the time taken by hypophysectomised _A. nebulosus_ to adapt to background reversals at an unspecified room temperature. According to him white-adapted catfish on transfer to a black background became dark in 3 hr. and the black-adapted ones paled in 30 min. to 8 hr. after transference to a white background. These observations appear to be not very different from those on _I. melas_. But Abramowitz's conclusion that the pituitary

is not indispensible for colour change in *A. nebulosus* appears to be incompatible with the observations made on *I. melas* in the present investigation. The conclusions of Parker (1940) and Osborn (1938) appear to agree with the latter.

The melanophore responses of hypophysectomised fishes to background changes in relation to time and in terms of melanophore index (Hogben & Slome, 1931) have been worked out in only two teleost species: *Parasilurus asotus* (Enami, 1939) and *Anguilla anguilla* (Waring, 1940). The equilibrium MI of dermal melanophores on a black background after transfer from a white background in *I. melas* resembles *Anguilla* (3.5) more than *Parasilurus* (3.0), although the time required to reach this equilibrium is different in *Ictalurus* (ca. 4 hr.) and *Anguilla* (30-45 min at 10-15°C, shift of MI from 1.8 to 2.7).

The reactions of the epidermal melanophores in hypophysectomised fishes have been so far only recorded in *Parasilurus* (Enami, 1939) in which they were reported to remain fully aggregated on a black background (pp.91-92).

(iv) Comparison of the rates of dispersion and aggregation of melanophore pigment of intact and hypophysectomised animals.

Comparison of the time relations of the rates of dispersion of melanophores of unoperated white-adapted fish

with those of hypophysectomised fish reveals that the ldm in intact animals on transfer to a black background reach MI 3.6 in 30 min. whereas the same MI in hypophysectomised fish is attained in 3 hr. (the dispersion of ldm in 30 min. in hypophysectomised fish is MI 3.24, a statistically insignificant difference); the udm in intact fish reach MI 2.8 in 15 min whereas in a hypophysectomised fish they reach the maximum MI of 2.4 in ca. 4 hr. Thus the lack of pituitary not only limits the degree of dispersion of the edm and udm but also relatively prolongs the time to reach it.

Comparison of the rates of aggregation of the melanophore pigment of black-adapted intact catfish on transference to a white background with that of a hypophysectomised fish, on the contrary, gives rather a different picture. In an intact black-adapted fish the index changes from 5.0 to 3.7 for the ldm in 15 min. on transfer to a white background. This index is the starting point for the aggregation responses of ldm in a hypophysectomised fish. A shift in MI of ldm from 3.7 to 2.6 in an intact fish is reached in $\frac{3}{4}$ hr. and in a hypophysectomised fish in 30 min; the udm of an unoperated animal exhibit a shift from MI 3.1 to 2.14 in 1 hr. whereas approximately the same shift in a hypophysectomised animal takes only 30 min; the edm in an intact fish aggre-

gate from MI 2.5 to 1.3 in about $2\frac{3}{4}$ hr. whereas nearly the same change in a hypophysectomised fish is completed in only 30 min. Thus the removal of the pituitary or the absence of MSH, in sharp contrast to retarding the rate of dispersion of melanophores, seems to accelerate the initial pace of aggregation of all types of melanophores.

From the above comparisons it appears that of all the classes of the integumentary melanophores in *I. melas* the responses of the epidermals are perhaps mainly under the influence of MSH, as the removal of the pituitary results in (1) largely limiting their dispersion and (2) retarding the pace of their dispersion. Further the release from the influence of the pituitary also seems to result in (3) quickening their rate of aggregation.

(v) Effects of long-time adaptation to changed backgrounds.

In two more groups of hypophysectomised fishes, one group white-adapted and the other black-adapted, the responses of the ldm to background reversals for comparatively longer time periods were observed. The objective was to find out if long-time adaptation (more than 48 hr.) to the changed background would affect the extent of dispersion or concentration of these melanophores. The data obtained from these observations were essentially similar to those shown in Table 12 (in Appendix). A long period of black-adaptation (5 days

or more) did not induce any further dispersion of ldm beyond MI 3.7.

(vi) Melanophore reactions to different shades of grey.

4 hypophysectomised and an equal number of unoperated fish previously white-adapted for about 4 weeks were equilibrated for 48 hr. in three grey perspex tanks corresponding to Derived Ostwald Index 2 (28% reflection), 4 (11% reflection) and 6 (4.5% reflection) (Healey, 1967). The mean MI's recorded are summarized in Table 13 (p.110). (The mean index values of unoperated living animals are slightly lower than those observed from the skin preparations of the fixed animals. However, the differences between them are statistically insignificant excepting for the values of the ldm at D O I 2, where slight additional concentration of pigment appears to have been caused by the fixative - liquid nitrogen). Comparison of MI's of hypophysectomised and intact fish shows a statistically significant difference only in the values of the edm at D O I.'s 4 and 6.

Since there is no significant difference in MI's of operated as well as unoperated fish adapted to D.O I 6, a background of a low value of reflectivity (4.5%), it appears that the pituitary gland comes into action when the reflectivity of the background falls below 4.5% value. Furthermore, the relatively greater aggregation of pigment in the edm of

Table 13. Mean MI's of hypophysectomised and unoperated I. melas (4 animals) equilibrated to different greys (DOI's 2, 4 and 6) 20 ± 1°C. 40 W at 75 cm.

DOI and reflection of the background	Average MI's of operated animals			Average MI's of unoperated animals		
	edm	udm	ldm	edm	udm	ldm
2 28% reflection	1.00 ±0.00	1.95 ±0.10	2.37 ±0.12	1.30 ±0.25	2.00 ±0.14	2.30 ±0.21
4 11% reflection	1.62 ±0.41	2.50 ±0.18	3.00 ±0.10	2.17 ±0.30	2.77 ±0.19	3.15 ±0.22
6 4.5% reflection	1.63 ±0.33	2.70 ±0.25	3.47 ±0.33	2.93 ±0.50	3.13 ±0.60	3.70 ±0.15

hypophysectomised fish adapted to different greys supports the conclusions already made (p.108).

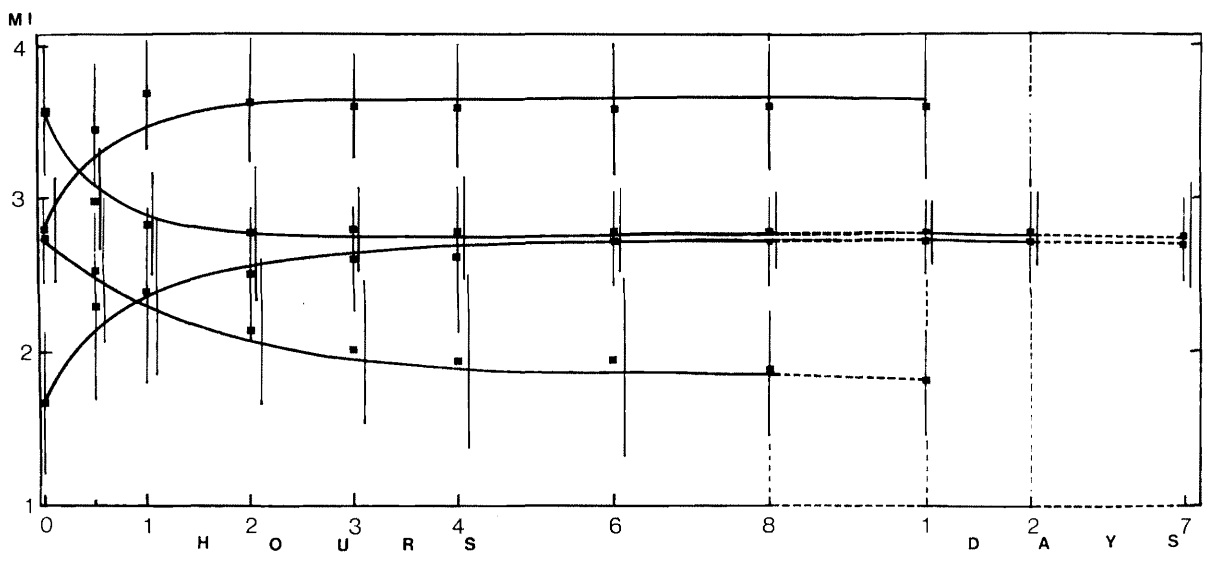
<u>(vii) Responses to darkness.</u>

The responses of ldm and edm of the two groups of white- and black-adapted hypophysectomised fish to darkness and reversal were recorded under similar conditions to those described for the responses to white and black backgrounds. The ldm in white- and black-adapted *I. melas* reach a state of equilibration in darkness in about 6 hr. (6-8 hr. in unoperated fish) at MI 2.74-2.77 (3.15 in unoperated fish) and the reversal to white and black illuminated backgrounds is accomplished in about 4-6 hr. (about 8 hr. in unoperated fish) (Fig. 29, p. 112 Tables 14, 15 in Appendix). However, a slow and gradual aggregation of these melanophores continues on a white background for more than 24 hr. as already noted (p.102).

The responses of edm of white- and black-adapted hypophysectomised animals to darkness and reversal were found to be quite uniform unlike those of intact animals. These melanophores of white- and black-adapted animals equilibrate in darkness at MI 1.28 - 1.32 in 2 and 6 hr. respectively and their reversal to illuminated white and black backgrounds require 4 and 8 hr. respectively (Tables 14,15)

Comparison of the reactions of ldm of hypophysectomised

Fig. 29 The responses of lower dermal melanophores of hypophysectomised *I.melas* transferred from equilibrium on illuminated white/black background to total darkness and reversal after 7 days. Each point with SD's as vertical bars is the mean of 8 animals. 20 $\pm$ 1°C. 40 W at 75 cm.



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animals with those of intact ones indicates that (1) in both cases the equilibration in darkness is achieved in almost the same time and (2) removal of the pituitary does not result in any significant change in their state of equilibration in darkness (MI being only 0.4 higher in unoperated animals, statistically insignificant difference). It seems therefore that the pituitary hormones play an insignificant role in regulating the responses of ldm to darkness.

Hypophysectomised A. nebulosus previously adapted to a "neutral" background was reported to become generally lighter in colour in darkness than the "intermediate" controls (Osborn, 1938). On the other hand, Parker (1940) reported that they did not undergo any change in darkness on transfer from a white or a black background. Thus the observations on I. melas appear to be in agreement with those of Osborn on A. nebulosus.

(viii) Melanophore reactions of blinded hypophysectomised animals to light.

The melanophores of white-adapted hypophysectomised I. melas (only 3 animals observed) on enucleation dispersed their pigment in light like the blinded unoperated animals (p. 82). The MI's of ldm, udm and edm averaged 3.5, 2.8 and 2.2 respectively 24 hr. after blinding. Thus the extent of their dispersion is similar to that of intact hypophysectomised animals adapted to an illuminated black background.

Dispersion of dermal melanophores (unspecified layer) or darkening of blinded hypophysectomised A. nebulous was reported by Abramowitz (total colour only) (1936a), Osborn (1938), Wykes (1938) and Parker (1940). Osborn also stated that the melanophores of such a fish were of the size of those of hypophysectomised fish which had been black-adapted for a long time.

(ix) The effects of injecting crude pituitary extract.

The pituitary glands from 2 white-adapted anaesthetised fish (white-adapted for about 4 weeks), average size 7 cm, were taken out, crushed and ground with glass-wool and then mixed with 4 ml of Ringer at room temperature (20°C). After the glass-wool had settled down (1) 0.3 ml each of supernatant was injected intraperitoneally into 3 white-adapted hypophysectomised fish (hypophysectomised more than one month previously and of the same size); (2) the same volume of Ringer was injected into 2 white-adapted hypophysectomised fish, serving as controls; (3) the same volume of pituitary extract was also injected into 3 white unoperated fish (average size 6.7 cm); (4) the same volume of Ringer was injected into 2 white-adapted unoperated animals. After the injection all these fish were kept on a white background. The results are summarized in Table 16,(p.115). These indicate that hypophysectomised I. melas, like Anguilla

Table 16. Mean MI's of white-adapted hypophysectomised and unoperated I. melas (2-3 animals) after injecting crude pituitary extracts from white-adapted I. melas and Ringer

Fish	Material injected	Average MI's before injection		MI's 1 hr. after injection		MI's after 2 hr.		MI's after 3 hr.	
		edm	ldm	edm	ldm	edm	ldm	edm	ldm
Hypophy-sectomised	Pituitary extract 0.3 ml	1.0	1.4	3.2	3.7	3.5	4.0	2.0	2.7
Hypophy-sectomised	Ringer 0.3 ml	1.0	1.5	1.0	2.0	1.0	1.5	1.0	1.5
Unoperated	Pituitary extract 0.3 ml	1.0	1.2	1.0	2.0	1.5	2.2.	1.2	1.8
Unoperated	Ringer 0.3 ml	1.0	1.2	1.0	1.4	1.0	1.2	1.0	1.2

(Waring, 1940), is very sensitive to pituitary extract as compared to unoperated fish.

Similar results were reported by Osborn (1938) in hypophysectomised _A. nebulosus_ but here the darkening effect lasted much longer (8 hr.), as much more concentrated extracts (2-4 glands) were injected into a fish. The dispersion of melanophores in intact _A. nebulosus_ by the injection of its pituitary was reported by Healey (1948) which may again be related to the strength of the material (equivalent to 1 gland in a fish, cf. Pickford & Atz, 1957) whereas in the present investigation a slight dispersion effect on the melanophores of unoperated fish appears to be due to much dilution of the material (about 1/7th gland).

4. Discussion and conclusions

The results of the present investigation indicate the importance of the pituitary gland in the black background adaptation of _Ictalurus melas_ and in the dispersion responses of all its integumentary melanophores. These results are in generalagreement with those of Osborn (1938) and Parker (1940) on _Ameiurus nebulosus_. These workers, however, only refer to dermal melanophores and do not specify any layer.

The presence of the dispersing factor intermedin or MSH in the catfish had already been demonstrated by Kent (1960) following a standard technique (Pickford & Atz, 1957) of injecting the pituitary material of the catfish into an

intact pale frog. This was also confirmed from the results of injecting catfish pituitary extract into hypophysectomised fish in the present investigation. Evidence for the humoral contribution in the chromatic responses of this species has already been furnished (pp.84-90) by a comparison of the time relations of these responses with those teleost species in which these responses are regarded as being mainly neurally controlled and in those in which their co-ordination is primarily hormonal.

The fact that the ability of black background adaptation is only limited in hypophysectomised _I. melas_ and is not totally abolished, as seen in elasmobranchs and amphibians, shows that it is not solely controlled by the pituitary.

Comparison of the time-graphs of the melanophore changes during white-to-black background adaptation of unoperated and operated animals shows that the pituitary is not of significance in the initiation of the process of the dispersion of the pigment, as was also observed by Abramowitz (1936a) and Wykes (1938) in _A. nebulosus_. Since the extent of dispersion on adaptation to DOI 2 (28% reflection), 4 (11% reflection) and 6 (4.5% reflection) is more or less similar in the operated and unoperated fish (Table 13, p.110), it appears that not only the initiation of dispersion but also the initial phases of dispersion responses are independent of the influence of the pituitary. It seems that the pituitary

begins to release MSH as the reflectivity of the background becomes much reduced (the critical point being apparently below the level of 4.5% reflectivity) or as the ratio of direct to reflected light increases, as pointed by many earlier investigators (cf. Parker, 1948).

The limited dispersion of the melanophores of hypophysectomised animals on a black background can be explained as either (1) a result of their release from a central aggregating influence or (2) a consequence of the stimulation of dispersing chromatic nerves. (For fuller treatment please see pp.143-146.)

The varying reactions of the different layers of melanophores in hypophysectomised *I. melas*, namely the least dispersion of the edm and the greatest dispersion of the ldm, indicate relatively more share of MSH in the dispersion of the former. A somewhat similar situation exists in *Parasilurus* (Enami, 1939). This conclusion is also supported by the observations of the reactions to background changes of intact animals (relative slower rate of dispersion of edm on transfer from a white to a black background) (P. 64).

The inability of eyeless hypophysectomised fish to disperse their melanophore pigment fully in light, unlike the eyeless but otherwise intact fish, also points to the importance of the pituitary for full dispersion of the melanophores.

Regarding the role of the pituitary gland in white background adaptation, the observations that the ability of the fish remains unimpaired after the removal of the pituitary indicates the absence of an aggregating hormone. The mean MI values of the ldm in 4-6 weeks' white-adapted hypophysectomised animals are slightly higher than those of unoperated animals and controls but this difference is statistically insignificant. Moreover, this slightly higher value is not permanent, as these melanophores in white-adapted Ictalurus which lived for several months after the operation were observed to become completely concentrated (MI 1.0) like those in unoperated animals, (p. 91).

The initial faster rates of aggregation of all types of melanophores observed in black-adapted hypophysectomised fish on transfer to a white background compared with those of unoperated animals could be due to the absence of MSH in the former group. On the other hand, the comparatively slower rate of aggregation of the dermal melanophores in hypophysectomised animals after about 1½ hr. white adaptation suggests that there is something lacking in them. It could be suggested that there might be a paling hormone from the pituitary or the secretion of some other gland under the influence of the pituitary or a possible interaction between MSH and some neurosecretion. Since in darkness (1) the

equilibrium of the ldm in the operated as well as unoperated animals is obtained in almost the same time and (2) the mean MI of the latter is only slightly higher than that of the former group (MI 0.42), it would appear that the pituitary is contributing very little in regulating the responses to darkness (p.113). Thus the data regarding the equilibrated states of melanophores on a white background and in darkness in general do not indicate that any hypothetical MCH is playing any role in the chromatic physiology of I.melas

VII. THE RESPONSES OF MELANOPHORES AFTER ANTERIOR SPINAL SECTION AND AFTER HYPOPHYSECTOMY FOLLOWING ANTERIOR SPINAL SECTION

1. Introduction

The part played by the nervous system in co-ordinating the responses of the integumentary melanophores of teleost fishes is variable in different species. In Macropodus, according to Umrath & Walcher (1951), the control of melanophores is entirely nervous, whereas

in *Anguilla* the nervous system plays an insignificant role (Neill, 1940)(pp.6, 59-60). Agreement is unanimous that at least the aggregation of pigment in melanophores is controlled by nerves. Thus the electrical stimulation of nerve fibres causes concentration of pigment in the melanophores which they innervate; cutting of these nerve fibres always results in the dispersion of pigment. Aggregation of melanophores has long been considered to be associated with the release of an adrenaline-like substance. This view has recently been supported by the results of experiments with adrenergic blocking agents in *Labrus* (Scheline, 1963), *Scophthalamus* (Scott, 1965), *Phoxinus* (Healey & Ross, 1966) and *Fundulus* (Fujii & Novales, 1968).

In many investigations on different species the results have suggested a dineuronic control of melanophores i.e. the presence of pigment-dispersing nerve fibres which act antagonistically to the aggregating fibres. These species include *Phoxinus* (von Gelei, 1942; Gray, 1956; Healey, 1967; Grove, 1969b); *Ameiurus* (Parker, 1934, 1948); *Fundulus* (Mills, 1932; Abramowitz, 1936; Parker, 1948);

Parasilurus (Matsushita, 1938); *Pterophyllum* (Tomita, 1940) and *Macropodus* (Umrath & Walcher, 1951). Experiments with acetylcholine and other parasympathomimetic substances have given conflicting results and have provided no satisfactory evidence that cholinergic mechanisms, as proposed by Parker (1948), are concerned in the dispersion of pigment in melanophores (Scott, 1965; Healey & Ross, 1966; Abbot, 1968). Positive evidence for the existence of pigment-dispersing nerve fibres has not been provided (Waring, 1942, 1963; Healey, 1954; Barrington, 1963; Pye, 1964; Scott, 1965). Watanabe *et al* (1962a) have suggested that, if they do exist, they may also be adrenergic. (These matters are more fully discussed on pp. 17-40.

The path of the nerve fibres controlling the aggregation of melanophore pigment in *Phoxinus* was traced by von Frisch (1911) who showed that these fibres pass from a centre in the medulla along the spinal cord. At about the level of

the 15th vertebra they emerge and enter the sympathetic chain, reaching the skin melanophores through the spinal nerves and the trigeminal nerve. Von Frisch's results indicated that the tonic action of these fibres, resulting in the aggregation of pigment, is controlled by a centre in the medulla. If these fibres are interrupted by spinal section anterior to vertebra 15, all the melanophores are separated from the medullary region and the whole fish become very dark (pp.15-16). Healey, (1940, 1948, 1951, 1954) further found that such a chromatically spinal Phoxinus kept on a white background in an upright position gradually pales, reaching a steady state in about 10 days. After that it can slowly respond to background changes, indicating the control of colour change by a hormonal mechanism.

Von Frisch's findings of the pigmento-motor fibres and their course in Phoxinus are generally accepted for other teleost species. Specific information about the path of the fibres is available for Crenilabrus (von Frisch, 1912b); Trigla (von Frisch, 1912a), Pleuronectes (Schaefer, 1921) and Fundulus (Adelmann & Butcher, 1937).

In the present work the approximate level of exit of the chromatic fibres from the spinal cord was determined and the spinal cord of the fish was sectioned anterior to that level. After the operated fish had equilibrated on

white and black backgrounds their responses to background reversals were observed and compared with those of (i) intact and (ii) hypophysectomised specimens in order to assess the relative parts played by the nervous system and the pituitary in the colour change of this species. In addition, the effects of hypophysectomy on the melanophores of spinal-sectioned animals were observed.

2. Material and method

The fish, 6.0 to 8.0 cm. long, after spinal section were subjected to the same experimental conditions as described for the intact fish (pp49-53) excepting that during background changes they were kept in rectangular clear perspex containers ($10\frac{1}{2}$ x 7 x $7\frac{1}{2}$ cm) which had numerous perforations in their walls to allow circulation of constantly aerated water and at the time of observation, they were lifted out of these. The MI's were recorded from the same site in the tail, i.e. around the centre of the vascular arc.

(i) The path of the chromatic fibres in the spinal cord of I. melas.

The chromatic fibres in *I. melas* appear to leave the spinal cord at about the level of the 12th vertebra: as (i) sectioning of the cord posterior to vertabra 12 generally

produced no change in the skin colour and (ii) cutting of the sympathetic chain behind that level resulted in darkening of the body posterior to the cut. Section of the anterior region of the cord i.e. anterior to the 6th vertebra was found impracticable as the first 4 vertebrae are fused with one another to form a hard "complex vertebra", the 5th being firmly anchored to it. The spine of the complex vertebra projects into the median dorsal fin so that any efforts to cut it could cause severe damage to many structures.

(ii) Spinal sectioning.

The operation was performed under anaesthesia (0.0125% MS222 Sandoz in tap water) in an operation tray (Von Frisch & Stetter, 1932; Healey, 1940, 1948) provided with inlet and outlet tubes. The tray was filled up with paraffin wax and had a groove in which the fish was secured by rubber bands. Water was constantly dripped into the mouth of the fish. A 4 mm longitudinal cut was made on the dorsal surface slightly to the right of the median fin and the spinal cord was exposed. A piece about 1-2 mm long was cut at the level of the 7/8th vertebra and then removed with the aid of a dental drill. After suturing the wound the fish were replaced in white/black-painted glass aquaria. The operated fish usually maintained their normal upright position

owing to their flat ventral surface and broad and flattened pectoral fins.

Two sets of animals were used for experimental observations, one of animals previously white-adapted for 3-4 weeks which were replaced on a white background after the operation; the other consisted of white-adapted individuals which were black-adapted for 2 days before the operation and replaced after it on a black background and kept there for 6-8 weeks. On completion of the subsequent experiments the site of spinal section was checked. The fish included in this paper had their spinal cord sectioned between the 7th and 10th vertebrae. In controls a cut was made in the same location without exposing the spinal cord. In the case of hypophysectomised animals, the success of the operation was verified histologically (Fig. 30 in Appendix).

3. Observations and results

(i) The immediate effect of cutting the spinal cord anterior to vertebra 12.

In most cases all the melanophores of previously white-adapted animals (MI 1.0-1.5) were observed to become fully dispersed (MI 5.0) immediately after the operation. In some cases, however, they became fully dispersed only after the animal had resumed active breathing in the aquarium. (Anaesthesia of the intact fish only produced a relatively slight darkening to ca. MI 2.0 and 2.67 for epidermal and lower dermal melanophores respectively).

(ii) The equilibration of melanophores on illuminated backgrounds after the operation.

The responses of the melanophores to an illuminated white background following spinal section are summarised in Table 17 (p.128). These show that (i) the equilibration of melanophores was a slow process generally requiring 6-8 weeks; (ii) during the first week following the operation there was relatively little change, the pigment staying nearly fully dispersed, especially in the lower dermal melanophores (ldm); (iii) the pigment assumed an intermediate stage of dispersion (ca. MI 3.5) in about 3 weeks but that the rate of aggregation thereafter became much slower; (iv) the pigment in the epidermal melanophores (edm) was generally less dispersed than in those of the upper dermal (udm) and lower dermal melanophores (ldm) and reached equilibration in a relatively shorter time than in the latter two layers. The equilibration on a white background exhibited marked variations in different individuals.

The melanophores in the black-adapted fish after the operation remained fully dispersed throughout.

In *Phoxinus phoxinus* (Healey, 1951), unlike *I. melas*, the melanophores equilibrate on a white background in a much shorter time (10 days), the pigment from full dispersion becoming approximately semi-aggregated in only 4 days (the same condition being arrived at in ca. 7 weeks in *I. melas*), whereas on a black background the pigment does not remain

Table 17. Equilibration of melanophores of I. melas on an illuminated white background following sectioning of the spinal cord anterior to the level of vertebra 12. Temp. 20 ± 1°C. Illumination general ceiling lighting.

Time following the operation	Mean MI's edm	Mean MI's ldm		Time following the operation	Mean MI's edm	Mean MI's ldm	
Immediately after	5.00	5.00		19 days	3.00 ±0.85	3.80 ±0.54	(10)
2 days	4.60 ±0.70	4.88 ±0.17	(15)*	22 days	2.92 ±0.84	3.62 ±0.82	(11)
4 days	4.36 ±0.78	4.53 ±0.54	(17)	26 days	2.64 ±0.84	3.40 ±0.66	(14)
6 days	4.43 ±0.67	4.72 ±0.37	(14)	30 days	2.47 ±0.75	3.31 ±0.78	(12)
8 days	4.07 ±0.80	4.46 ±0.60	(10)	35 days	2.20 ±0.78	3.07 ±0.62	(12)
10 days	3.67 ±0.76	4.17 ±0.59	(15)	40 days	1.68 ±0.64	2.47 ±0.45	(16)
15 days	3.30 ±0.76	4.00 ±0.47	(10)	45 days	1.57 ±0.40	2.23 ±0.49	(16)
				52 days	1.42 ±0.38	2.12 ±0.61	(20)

*The number of animals shown in parentheses.

Edm = epidermal, ldm = lower dermal melanophores. The MI's of upper dermal melanophores are not shown in the Table. Those were generally slightly higher than those of edm.

fully dispersed (MI 4.5).

(iii) Degeneration of melanophores in operated animals.

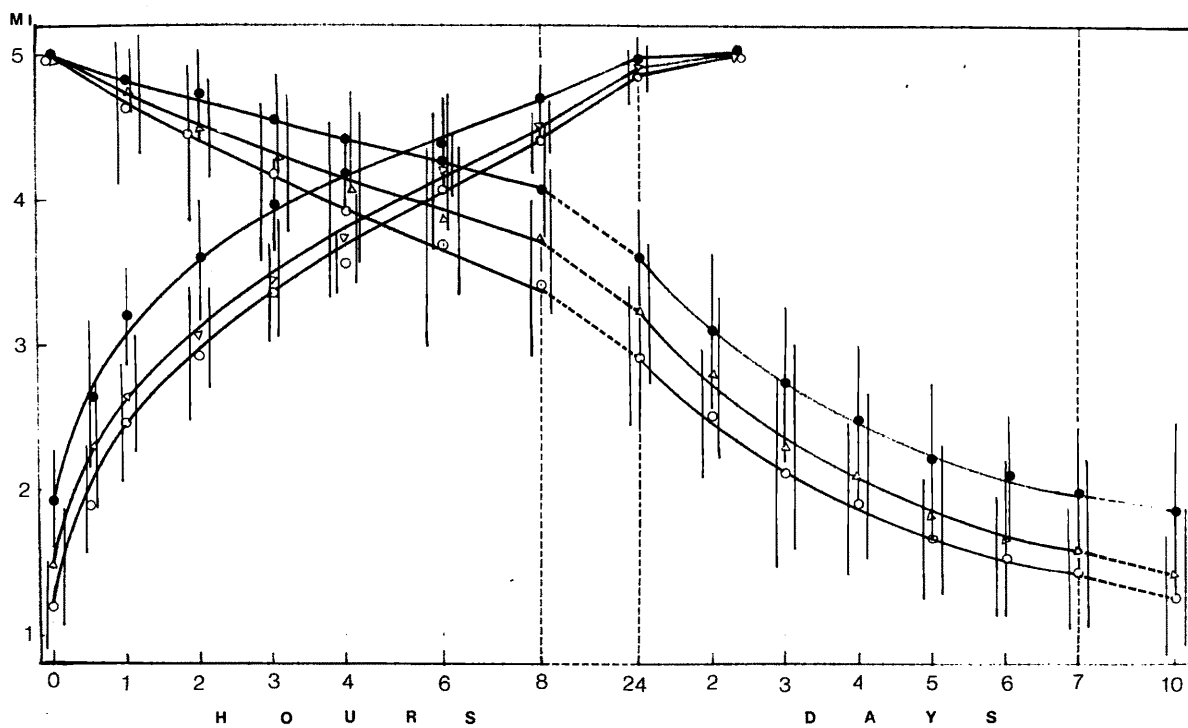
Spinal section, like hypophysectomy, resulted in the degeneration of melanophores, often noticed a few weeks after the operation. However, the degeneration was much less marked than that in hypophysectomised specimens.

(iv) Melanophore responses of white- and black-adapted chromatically spinal animals to background reversals.

The melanophore indices of chromatically spinal animals equilibrated on an illuminated white background for 8-10 weeks following the operation averaged 1.93, 1.48 and 1.21 for ldm, udm and edm respectively (the values of ldm show statistically significant difference by the *t*-test from the similar values of unoperated animals but the other melanophores do not). On transfer to a black illuminated background all melanophores dispersed their pigment slowly, the adaptation to the changed background being accomplished in 24 hr. (Fig. 31, p.130, Table 18 in Appendix). The different melanophore layers showed differential dispersion, the edm usually lagging behind the udm and ldm.

The reversal, i.e. black-to-white adaptation was a very slow process requiring 7-10 days (Fig. 31, p 130), the different melanophore layers again reacting differentially, the edm being more aggregated than the udm and ldm at given time intervals. Wide variations in the responses of different individuals were noted during this phase of colour change, the melanophores in many cases remaining

Fig. 31 Melanophore responses of chromatically spinal *I.melas* transferred from equilibrium on an illuminated white background to an illuminated black background and reversal after 48 hr. Each point with SD's as vertical bars is the mean of 15 animals. Temp. 20 ± 1°C. Overhead illumination 40 W at 75 cm. Open circles = epidermal, open triangles = upper dermal and closed circles = lower dermal melanophores.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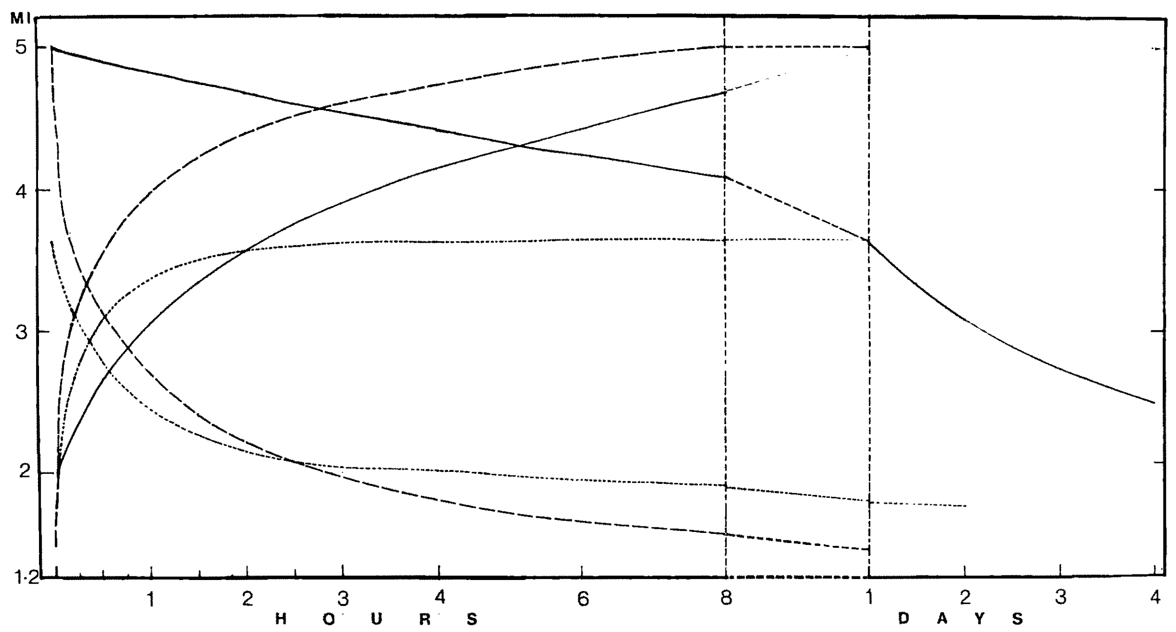
fully dispersed for many hours after transfer to a white background. If such fish were once again placed on a black background they became fully dark in less than 24 hr. The reactions of white-adapted controls were similar to those of intact animals (as described on pp. 62-64).

The black-adapted chromatically spinal animals during black-to-white transition and reversal reacted almost in the same way as the white-adapted specimens. The resulting plots give a figure in no way different from Fig. 31, p. 130, excepting that in them slightly higher index values than those of white-adapted animals were recorded during the first 24 hr. of the transition (Table 19 in Appendix). This could be the result of their long stay on a black background, as controls also showed such higher MI values incomparison to 48 hr. black-adapted animals (Fig.31b in Appendix).

(v) Comparison of the rates of dispersion and aggregation of melanophores of spinal with those of intact and hypophysectomised fish.

The rates of dispersion of ldm during white-to-black transition in chromatically spinal fish are somewhat slower than the rates in intact fish, full dispersion being obtained in something over 8 hr, whereas in the latter it is fast initially, becoming gradual later on and complete in 6-8 hr. (Fig. 32, p.132). The different melanophore

Fig. 32 Comparison of the time-graphs of lower dermal melanophores of white-adapted chromatically spinal *I.melas* (represented by continuous lines) with those of white adapted intact (broken lines) and hypophysectomised animals (dotted lines) during the transition white-to-black and reversal. 20 ± 1°C. 40 W at 75 cm. (15 animals each).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layers in both cases show differential reactions, the edm being slow to disperse. In comparison with hypophysectomised fish (Fig. 32, p.132) the rate of dispersion in spinal fish is again slow. However, in the former the removal of the pituitary limits the extent of dispersion (pp.102-103) whereas in the spinal fish the total separation of integumentary melanophores from the CNS in no way hinders their full dispersion.

In distinction, the time relations of black-to-white transition in spinal specimens are markedly different from those of both unoperated and hypophysectomised but otherwise intact individuals. The rate of aggregation of pigment is very slow in the spinal animals but in intact animals it is fast initially and slower later on, the adaptation being accomplished in 7-10 days in the former and 6-8 hr. in the latter (Fig. 31, p.130). In spinal fish, in contrast to hypophysectomised fish the rate is again very slow. (Fig. 32, p. 132). In all cases different melanophore layers react differently, the edm being the first to complete aggregation, (Fig.8,p.63; 27,p.101;31,130).

(vi) Comparison with *Phoxinus*.

The melanophores (epidermal and dermal together) in white-adapted chromatically spinal *Phoxinus* on transfer to a black background reach equilibrium (MI ca. 4.25) in about 50 hr. (at 12°C) (Healey, 1951, 1967), whereas

in chromatically spinal Ictalurus equilibrium (MI 5) is achieved in 24 hr, i.e. the rate of dispersion in the former is significantly slower than in the latter (change in MI from 1.7 to 2.6 in Phoxinus in 5 hr. from 1.9 to 2.6 (ldm) in Ictalurus in about 30 min; MI 4.0 in the former in 34 hr, 3 hr. in the latter). The edm in spinal Phoxinus disperse much more slowly than those of spinal Ictalurus (shift of MI from ca. 1.7 to 2.5 in 5 hr. in Phoxinus, from 1.2 to 2.5 in 1 hr. in Ictalurus). They both have a slower rate of dispersion than the dermal melanophores.

Black-to-white transition in black-adapted spinal Phoxinus, like Ictalurus, requires a longer period than white-to-black adaptation (MI 1.25 in 4 days in Phoxinus, 1.87 (ldm) in ca. 10 days in Ictalurus). The edm in both cases show a faster aggregation than the dermal melanophores, although the rate is much faster in Phoxinus (shift in MI from 5.0 to 1.9 obtained in 1 day) than in Ictalurus (a similar change in 4 days).

(vii) Melanophore responses in spinal Ictalurus to different greys.

Chromatically spinal catfish (4 animals each) white-adapted for about 8 weeks after the operation were equilibrated on 3 different greys - DOI's 2, 4 and 6 (% reflection 28, 11 and 4.5 respectively) (Healey, 1967) for 48 hr. to find out whether there were any changes in responses reported already for intact (Table 4, p. 77) and hypophysectomised animals (Table 13, p.110). The results are summarized

in Table 20, p.136). The index values of udm and ldm (not edm) of spinal catfish equilibrated to DOI's 4 and 6 show statistically significant differences from the values obtained from the same melanophore layers in intact animals. The results generally agree with those obtained from hypophysectomised animals and appear to support the conclusions already arrived at (p.109) that the active role of the pituitary in the dispersion of melanophore pigment seems to commence when the reflectivity of the background falls below ca. 4.5%.

(viii) Equilibration in darkness.

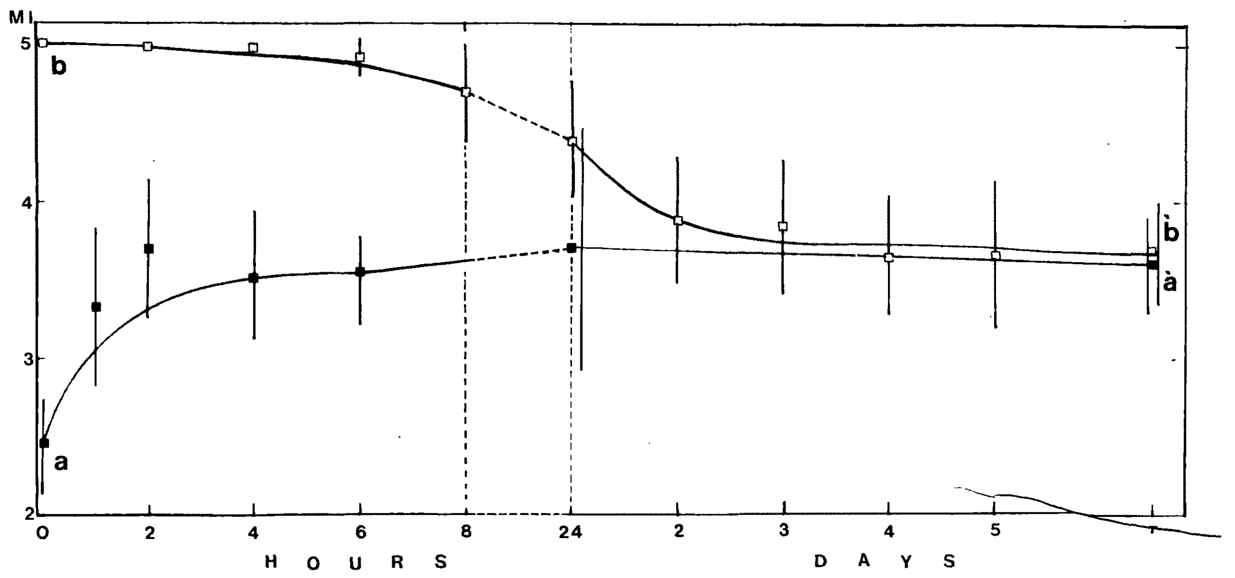
The ldm of 6 white- and 7 black-adapted chromatically spinal animals reached equilibrium in total darkness in 3-4 days at MI ca. 3.7 (Fig. 33, p.137, Tables 21, 22 in Appendix). On transfer to illuminated white and black backgrounds the index values of white-adapted animals averaged 2.37 in 2 days and those of black-adapted animals reached a mean MI of 5.0 in 1 day. The reactions of edm appeared to be erratic, as in intact animals (p. 80 , Tables 5, 6 in Appendix) their MI's averaged 2.75 in white-adapted fish after 7 days in darkness and 3.1 in black-adapted after 10 days in darkness. The difference between the two values is, however, statistically insignificant.

Table 20. Mean MI's of white-adapted chromatically spinal I. melas (4 animals each) equilibrated to different greys (DOI's 2, 4 and 6). Temp. 20 + 1 °C. Illumination 40 W at 75 cm

DOI and reflection of the background	Mean MI's		
	edm	udm	ldm
2,28% reflection	1.57 ±0.34	1.87 ±0.16	2.25 ±0.12
4,11% reflection	1.84 ±0.36	1.92 ±0.22	2.59 ±0.16
6, 4.5% reflection	2.31 ±0.46	2.45 ±0.18	2.84 ±0.30

Edm = epidermal, udm = upper dermal, ldm = lower dermal melanophores.

Fig. 33 The equilibration of lower dermal melanophores of chromatically spinal I. melas on transfer from illuminated white/black backgrounds to darkness. Each point on the curve aá is the mean of 6 animals previously equilibrated for about 6 weeks on an illuminated white background after spinal sectioning and on curve bb́ of 7 animals previously equilibrated on an illuminated black background for about the same period. SD's shown as vertical bars. Temp. 20 ± 1°C. Overhead illumination 40 W at 75 cm.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In spinal fish, in comparison to intact and hypophysectomised fish, the equilibrium MI in darkness is higher and the transition from illuminated white or black background to darkness requires a longer time. These results are explainable as a consequence of a rise or fall in the level of a single darkening hormone MSH rather than an antagonism between MSH and a hypothetical MCH or W-substance.

A rapid primary response averaging 0.28 on the MI scale was recorded from the batch of black-adapted spinal specimens which had been in darkness for 10 days when they were first illuminated (Table 22 in Appendix).

(ix) Melanophore reactions of blinded spinal animals in light.

4 chromatically spinal catfish which had been on an illuminated white background for about 14 weeks following the operation, were enucleated by removing their eyes. Their melanophores, originally almost fully aggregated (MI 1.0-1.3) commenced to disperse following enucleation and had become fully dispersed in 1-2 days on an illuminated background.

(x) Effects of hypophysectomy on the melanophores of spinal fish equilibrated on white or black backgrounds.

The pituitary glands of chromatically spinal *Ictalurus* (14 animals in all) which had been on illuminated white or black backgrounds for 10-15 weeks following spinal section

were removed in order to find the effect of the two operations on the responses of melanophores. In white-adapted specimens there was some dispersion of pigment following hypophysectomy (MI of ldm averaged 3.2 24 hr. after the operation), whereas in the black-adapted specimens some aggregation of pigment was noted in the corresponding period (mean MI of ldm 3.8). In both cases the MI generally became stable (1.0, 1.8 and 2.9 for edm, udm and ldm respectively) in about 1 week. In a few cases a further slow aggregation of melanophores was observed in the next 4-6 weeks with udm becoming fully aggregated and ldm remaining semi-aggregated (ca. MI 2.0).

Hypophysectomised spinal <u>Phoxinus</u>, unlike <u>Ictalurus</u>, were observed to remain very dark on any background (Healey, 1940, 1948, 1951). However, according to Gray (1955) they became pale after a few days. This lack of agreement may have resulted from the great number of melanophores in the fish observed by Healey.

<u>(xi) Effects of hypophysectomy on the melanophores of blinded spinal fish</u>.

Blinded spinal animals (3) which had been on an illuminated background for about 2 weeks following enucleation, were hypophysectomised and the resultant changes in them observed. Their melanophores before the removal of the

gland were in a fully dispersed state. 24 hr. after hypophysectomy their indices averaged 3.0 (edm), 4.0 (udm) and 4.2 (ldm); after 3 days 2.0, 3.0 and 4.0; after 8 days 1.2, 2.7 and 3.4. Thus the blinded spinal animals, unlike black-adapted intact animals and blinded otherwise intact animals, were incapable of maintaining full dispersion of pigment in light after the pituitary had been removed.

(xii) Immobility of melanophore pigment in hypophysectomised spinal fish.

Hypophysectomised spinal animals appeared to be inert chromatically, as their melanophores showed no response to any background changes. Even enucleation (of 6 animals) did not cause any change in their pigmentation (excepting for slight transitory dispersion probably due to anaesthesia). In darkness, however, slight aggregation (= ca. 0.6 MI) of udm and ldm was noted (3 animals). No such aggregation was observed in an enucleated specimen (Fig.34 in Appendix).

(xiii) Effect of hypophysectomy 24 hours following spinal section.

In order to ascertain whether the pituitary gland is in any way associated with the dispersion of pigment following spinal sectioning anterior to vertebra 12, 5 animals that were previously white-adapted for about 4 months were hypophysectomised 24 hr. following spinal section

and then left on a white background for equilibration. Their MI's were recorded at different time intervals like those described in Table 17, (p.128). The results are summarized in Table 23 (p.142). Comparison of these results with those in Table 17 shows that at least in this species MSH is apparently involved in (i) the dispersion of pigment following spinal sectioning and (ii) the subsequent maintenance of the dispersed state of pigment. In 2 more animals that were spinal sectioned about 2 weeks earlier and were being equilibrated on a white background, the pituitary glands were removed. Their mean MI's before the removal of the gland were 3.7 (edm) and 4.1 (ldm) and 8 days after the operation 1.0 and 2.75. Thus hypophysectomy appeared to accelerate the aggregation of their melanophores on a white background in comparison to the spinal-sectioned animals with intact pituitary gland.

Many attempts were made to carry out (i) hypophysectomy and spinal section simultaneously and (ii) spinal section of previously white-adapted hypophysectomised animals. However, these had to be abandoned because of the very high rate of mortality in the experimental animals.

Table 23. Effects of hypophysectomy on the melanophores of white-adapted I. melas 24 hr after sectioning of the spinal cord anterior to the level of vertebra 12.

Time following operation	Mean MI's (5 animals)	
	edm	ldm
1 day after spinal section	4.63 ±0.72	4.81 ±0.36
1 day after hypophysectomy	3.14 ±0.79	4.06 ±0.37
2 days after hypophysectomy	2.10 ±0.63	3.80 ±0.51
5 days after hypophysectomy	1.62 ±0.38	3.32 ±0.23
9 days after hypophysectomy	1.24 ±0.39	3.22 ±0.46
15 days after hypophysectomy	1.00 ±0.00	3.00 ±0.00
		(3 animals)

Edm = epidermal, ldm = lower dermal melanophores.

4. Discussion and conclusions

The results of the present investigation indicate that the pituitary is one of the contributory factors in the dispersion of melanophore pigment that follows the interruption of the pigmento-motor tract by sectioning the spinal cord in *I. melas* and is concerned with the subsequent maintenance of the dispersed state of pigment (Tables 17 and 23). This conclusion is also supported from the demonstration of MSH in the blood of white-adapted intact *Fundulus* by Abramowitz (1937), and is also in agreement with Osborn's reported failure (1938) to obtain the formation of a dark caudal band in white-adapted hypophysectomised *A, nebulosus*. It is, however, incompatible with the explanations offered by either Parker (1948) or Fujii and Novales (1969) for the dispersion of pigment following section of melanophore nerves. Gray's observation (1956) that hypophysectomy failed to inhibit the dispersion of melanophores following section of the nerve fibresin the tail fin of white-adapted *Phoxinus* is not incompatible with the above inference from *Ictalurus*, because in *Phoxinus* (in distinction to *Ictalurus*) the very role of the pituitary in the dispersion of pigment is controversial (Healey, 1951; Kent, 1960).

The comparatively long time required by the melanophores in the operated animals to attain equilibrium on an illuminated white background appears to be due to (i) their becoming sensitized to circulating MSH, and/or (ii) a slow destruction/excretion of the hormone, and (iii) possibly some after-effect of the shock of the drastic operation of spinal section. Once the animals recovered from these effects, they became capable of reacting to background changes, these subsequent reactions being apparently solely co-ordinated by the pituitary gland.

Whereas the time relations of black-to-white and white-to-black transitions are almost of the same order in the intact animals (Fig. 32, p.132) these are fundamentally altered by the sectioning of the anterior spinal cord, white-to-black transition becoming 7-10 times faster than the reverse process (Fig. 32, p.132). It might be suggested that in the former transition not only MSH but also melanophore-dispersing fibres may be involved, implying that these fibres may be following a path different from that of the aggregating fibres and that the spinal section may not be affecting them. However, the fact that this faster dispersion of pigment is still relatively gradual in the chromatically spinal animals during white-to-black adaptation as well as the immobility and unresponsiveness of the pigment in the hypophysectomised spinal animals (p.140) to background changes would appear

to exclude any involvement of a nervous mechanism in the co-ordination of dispersion of melanophores in the spinal animals. Another explanation is that in the absence of antagonistic activity of melanophore-aggregating nerve fibres, MSH from the pituitary is capable of causing full dispersion of pigment in spinal animals within the relatively shorter time of 24 hr. (The importance of the pituitary in full dispersion of pigment in this species has already been shown (Section VI).

Longer duration of black-to-white transition in reaching an equilibrium on a white background than in the case of the reverse process in the chromatically spinal specimens indicates that in the absence of an aggregating neurohumor there is a slow excretion/destruction of MSH accumulated while the animals were on a black background rather than a formation of a pituitary aggregating hormone (MCH). (i) This observation, i.e. slower rate of aggregation of melanophores in spinal animals, together with many other results such as (ii) the comparisons of time relations of the equilibration of melanophores from an illuminated background to darkness in (a) intact animals and (b) of hypophysectomised animals (pp.111,113) and (c) in spinal-sectioned animals (Fig. 33, p.137), (iii) the equilibrium MI's of (a) intact animals (b) and of hypophysectomised animals in darkness (pp. 111, 113), and finally (iv) the apparent ability of hypophysectomised catfish to adapt to a white background (Fig. 27, p. 101)

indicate that no hypothetical MCH or W-substance is involved in the melanophore responses of either intact or chromatically spinal *I. melas.*

Dispersion of melanophores following enucleation appears to result in the release of the pars intermedia from a central inhibitory control resulting in an uncontrolled secretion of MSH (Section VIII). The results of blinding of intact (pp. 80, 82), hypophysectomised (p.113), spinal (p.138) and hypophysectomised spinal catfish (pp.139-140) are consistent with this assumption. Somewhat similar conclusions have already been arrived at by Wykes (1938) and Parker (1940) on *A. nebulosus*.

Finally, the results of the present investigation do not indicate that any active pigment-dispersing nervous mechanism is involved in the colour changes of the catfish *I.melas* (pp.129-131; 140-142). They rather support von Frisch's (1911) conclusions that dispersion results from the release of melanophores from a central aggregating influence. In this species this dispersion is also apparently augmented by MSH from the pituitary.

VIII. EXPERIMENTAL EVIDENCE FOR THE NATURE OF THE CENTRAL CONTROL OF THE PARS INTERMEDIA FUNCTION

1. Introduction

It is generally agreed that the colour changes in elasmobranchs and amphibians are regulated by the pars intermedia (PI) of the pituitary gland which itself is under the inhibitory control of the brain. Thus any interruption of the hypothalamo-hypophyseal innervations or transplantation of the gland, i.e. its release from the central control, leads to its hypertrophy and uncontrolled liberation of MSH. Consequently pigment in the skin melanophores becomes either permanently dispersed or remains so for a long time and the ability of the animal to adapt to a white background is totally impaired (Mellinger, 1963; Etkin, 1941, 1943, 1962, 1967; Jørgensen & Larsen, 1960, 1963, 1967; Guardabassi, 1961; Jørgensen, 1968), (pp. 12-13).

The nature of the central control of the PI (meta-adenohypophysis - Pickford & Atz, 1957) function in teleosts is, however, obscure. In the only case so far reported, i.e. *Poecilia formosa* (Olivereau & Ball, 1966), the PI, in

contrast to the situation in amphibians and elasmobranchs, was found to be markedly atrophied in ectopic pituitary transplants. This indicates that in this species the brain has a stimulatory control on PI function. However, the role of MSH in the pigmentation in Poecilia has not been experimentally established (Olivereau & Ball, 1966).

In the present investigation the effects of denervation of the PI on the melanophores in I. melas, (in which MSH plays an important role in the colour changes - Section VI) are reported with a view to exploring the nature of the innervation controlling PI function.

2. Material and method

Denervation of the PI was effected by making ca. 4 mm deep cuts and lesions by means of a dental drill and a fine probing needle in the exposed hypothalamo-hypophyseal region in between the optic chiasma and the pituitary; in some cases lesions were also made around the gland. In most cases thin rectangular pieces of black plastic (about 2.0 x 0.7 mm) were vertically inserted into the lesions to prevent the reestablishment of vascular and nervous connections. In all, 20 animals (average length 7.0 cm) were operated. Out of these 9 did not survive beyond 14 days (1st casualty after 5 days),

5 lived 27-38 days, 3 50-62 days and 3 were killed after 143 days. The fish previously were white-adapted for 3-4 weeks, their mean MI before the operation being 1.4, and were replaced in a white background after the operation. In controls the hypothalamic region was only exposed.

3. Results and discussion

p.150

The results are summarised in Table 24. These show that (i) the immediate effect of interrupting the hypothalamo-hypophyseal tract on the melanophores of white-adapted animals was full dispersion of pigment (MI 5.0); (ii) 1-3 days after the operation there was a considerable aggregation of pigment and the MI became relatively stable (3.2 - 3.3); in the next 2 months no significant change in the melanophores was detected and white background adaptation of the animals remained impaired; (iii) the animals retained their ability to adapt to a black background; (iv) about 2 months after the denervation of PI the fish appeared gradually to regain their ability to adapt to a white background and in about 4 months they were able to aggregate melanophore pigment almost completely (Table24). In controls white/black adaptation was unaffected.

Table 24. The state of melanophores in I. melas on illuminated white and black backgrounds at various time intervals following the denervation of the pars intermedia.

Time (days)	Background	Mean MI's of lower dermal melanophores	Time (days)	Background	Mean MI's of lower dermal melanophores
Immediately after	White	5.00 (10)*	55	White	3.38 -0.35 (6)*
2	White	3.77 -0.50 (7)	71	White	2.60 -0.24 (3)
3	White	3.33 -0.71 (7)	95	White	2.40 -0.43 (3)
6-8	White	3.20 -0.34 (12)	108	White	2.30 -0.47 (3)
19	White	3.32 -0.45 (8)		Transferred to black	
	Transferred to black		109	Black	5.00 (3)
20	Black	5.00 (7)	120	Returned to white	5.00 (3)
38	Returned to white	5.00 (7)	121	White	1.73 -0.21 (3)
39	White	3.60 -0.36 (7)	130	White	1.23 -0.20 (3)
46	White	3.20 -0.49 (6)			

* Number of animals shown in parenthesis. Illumination general ceiling lighting. Temperature 20 $\pm 1^{o}C$. Melanophore index (MI) readings were recorded from the tail around the vascular arc. Epidermal melanophores always showed slightly less dispersion of pigment than the dermal melanophores on a white background.

Full dispersion of melanophores immediately following the disruption of the hypothalamo-hypophyseal innervation (result i) was presumably caused by abrupt and excessive release of MSH stored in the PI of white-adapted animals.

The state of partial but significant dispersion of pigment which lasted for several months on a white background could be interpreted in many different ways, e.g. (a) it could be due to the interruption of stimulatory control of the anterior lobe of the pituitary which is supposed to secrete W-hormone (Hogben & Slome, 1931; Hogben & Landgrebe, 1940; Healey, 1951; Pickford & Atz, 1957; Waring, 1963). But white background adaptation in this species (Sections VI and VII) and in *A. nebulosus* (Abramowitz, 1936a; Osborn, 1938) does not depend on the pituitary and appears to be controlled by the aggregating nerves; (b) the nature of the nerves controlling PI function in *Ictalurus*, as in *Poecilia*, could be only stimulatory but this does not explain result (iii); (c) the possibility of the control being excitatory as well as inhibitory also does not seem to offer an entirely satisfactory explanation of results (i) to (iii); (d) finally the assumption that PI function is regulated by an inhibitory innervation appears to provide a simple and logical explanation to result (ii). The uncontrolled release

of MSH resulting from the denervation of PI is unable to maintain full dispersion of melanophores on a white background because of the activity of the aggregating nerves. Result (iii) is also explainable by this conclusion: MSH released by the denervation is capable of fully dispersing the melanophore pigment on a black background due to the lack of the aggregating activity of melanophore nerves.

The regaining of the ability of white background adaptation by the operated animals (result iv) could be due to a gradual regeneration of the cut nerves after about 2¼ months, leading ultimately to the reestablishment of the central control on the PI. The slow reestablishment of the control requiring several months indicates that the PI in *I. melas* is controlled by direct nervous action (and not by neuro-secretion) as reported in the amphibians *Xenopus*, *Bufo*, and *Ambystoma* (Jørgensen & Larsen, 1963). Extensive digitation of the neurohypophysis into the PI in teleosts in general (Pickford & Atz, 1957; Dodd & Kerr, 1963) also appears to support this conclusion.

Histological changes following denervation of the PI have not been studied as yet. However, the interruption of the hypothalamo-hypophyseal tract by plastic barriers has been verified by cutting serial sagittal sections of

on the heads of 10 animals, 8 of which had lived for at least 14 days following the operation. These include the last 3 animals (Table 24, p.150) that were killed and fixed after 143 days. (Fig. 35 in Appendix).

These results can only be regarded as tentative and further work with histological study is necessary.

IX. THE ROLE OF THE PINEAL IN THE COLOUR CHANGE

1. Introduction

Many reports suggest that the pineal organ in lower vertebrates in certain situations such as on being exposed to light or total darkness, causes changes in pigment cells. Von Frisch (1911, 1912c) found that the pineal part of the brain and not the pineal alone, is photo-receptive in blinded Phoxinus and its stimulation by light resulted in the dispersion of melanophores. Scharrer (1928) following von Frisch's work confirmed these results. Young (1935) noted that the diurnal rhythm of colour change in the ammocoetes of Lampetra was interrupted and was disturbed in the adult specimens following the removal of the pineal. Wykes (1938) reported that the destruction of the pineal and a part of the roof of diencephalon in blinded Ameiurus did not in any way affect the dispersion of pigment. She, however, did not specify the effect of the operation on the aggregation of pigment in darkness. Her conclusions were confirmed by

Parker (1940). Simonnet *et al* (1952, 1954 cf. Burgers & van Oordt, 1962) reported that adult specimens of the frog (*R. esculanta*) became considerably darker following pinealectomy. Hoar (1955) found that the effect of destroying the pineal in juvenile *Oncorhynchus* was not pronounced on the pigmentation, the operated fish being darker than control but lighter than the blinded fish. Rasquin (1958) found that pinealectomy in the teleost *Astyanax* had no observable effect on its melanophores. Bagnara (1960, 1963) observed that the melanophores of the body region in *Xenopus* larvae after cautery of the pineal area were unable to contract in darkness, whereas those in the tail region were not affected by the operation and continued to disperse in darkness. Brick (1962) reported that pinealectomy did not cause much dispersion of melanophore-pigment in *Ambystoma* larvae, and the extent of its dispersion was similar in operated and unoperated specimens, the only difference being that the pigment in the former was unable to aggregate in darkness. He further observed that pinealectomy did not cause any dispersion of pigment in hypophysectomised larvae. Charlton (1966) reported that larvae and adult *Xenopus* generally became dark after epiphysectomy and did not show any colour change in darkness or in light. However, the effect of epiphysectomy generally did not last beyond 2 weeks. Eddy & Strahan (1968) noted that whereas hypophysectomy abolished the diurnal rhythm in *Geotria*

ammocoetes and metamorphosing Mordacia, pinealectomy abolished the rhythm only in Geotria.

These observations and many other data such as (1) neurophysiological evidence indicating that the pineal organs in the lower vertebrates are capable of producing neural responses to darkness or to photic stimulation (Dodt, 1963 in Salmo; Dodt & Jacobson, 1963 in a frog cf. Wurtman et al 1968); (2) morphological studies with the light and electron microscope showing that the sac-like pineal organs of lower vertebrates are composed of sensory and nerve cells (Holmgren, 1969 in Osmerus), the former i.e. the sensory cells having protruding apices bearing resemblance to retinal elements especially to cones (Breucker & Horstmann, 1965 in Salmo; Osksche & Kirschstein, 1967 in Phoxinus cf. Wurtman et al, 1968; Rüdeberg, 1968 in Sardina and Mugil) strongly substantiate the concept that the pineal organs in lower vertebrates are photo-receptive (cf. Kelly, 1962; Ariëns Kappers, 1965 cf. Wurtman et al, 1968).

The possibility that the pineal may be influencing pigmentation by producing a pigment-aggregating factor, was first suggested from the works of McCord & Allen (1917) who reported that a crude acetone extract of bovine pineal glands fed or injected into frog tadpoles caused a swift but temporary lightening of their skin. A similar effect was observed when the tadpoles were allowed to swim in water containing some of the pineal extract. Bors & Ralston (1951

cf. Bagnara, 1965) observed that the pineal extracts of pig and man induced contraction in larval and adult Xenopus. Later Lerner *et al* (1958) isolated this factor from beef pineals and named it melatonin(N-acetyl-5-methoxytryptamine). Since then the pineal in the lower vertebrates, as in mammals, has been presumed by many investigators to function as an endocrine gland, producing a pigment-aggregating factor, in addition to responding to changes in illumination.

Bagnara (1960, 1963, 1965, 1966) postulated a hypothesis to explain the body blanching of Xenopus larvae in darkness by assuming that darkness stimulated the pineal to release into blood circulation melatonin in minute quantities which caused a rapid aggregation of pigment in the body region (and not in the tail) in ca. 30 min. by overriding MSH. He further assumed that the dispersion of melanophores on return to light was caused by MSH as the pineal then ceased to release any more melatonin and the hormone already present in circulation was gradually destroyed or rendered ineffective. The temporal sequence - ca. 60 min or more of this dispersion response being consistent with a humoral effect. Bagnara thus explained the results of epiphysectomy on this presumed endocrine function of the pineal. Bagnara (1963) proposed that his hypothesis concerning darkness-induced blanching by the pineal was equally applicable to other lower vertebrates including teleosts, but the main

weakness of his hypothesis is that melatonin has yet to be isolated from the pineal of *Xenopus* or any other amphibian. It also does not offer any satisfactory explanations of Brick's and many other observations.

Fenwick (1970) identified melatonin in the pineal of *Oncorhynchus* by thin layer chromatography and fluorometry demonstrating thereby the endocrine nature and function of the pineal in this teleost. He reported that the level of melatonin found in the pineals of sexually immature young specimens was significantly greater (about x 6) than that in the adult specimens, and suggested that the higher level of the compound in immature specimens may be concerned with the inhibition of maturation of gonads by acting on certain hypothalamic centres that regulate the release of gonadotrophins. Fenwick also administered melatonin to male and female goldfish *(Carassius)* which were at the same time exposed to increased daylight. He noted that the injection of melatonin inhibited the increase in gonad size which should have resulted from the increased exposure of the animals to light. The weight of the gonads, however, was not affected by melatonin treatment. Gonadal function was also not arrested. Thus Fenwick's work suggests some measure of functional similarity between the pineal of fish and some mammals and birds (e.g. pineal extract or melatonin blocks some of the stimulatory effects of light on the rat gonads; the exposure of male or female rats to continuous darkness causes a moderate but significant retardation of sexual

development and similar decrease in the size of the adult reproductive organs, these effects being counteracted by pinealectomy) - for details and references see Wurtman, Axelrod & Kelly (1968). Fenwick stated nothing regarding the effect of melatonin administration on pigmentation in Carassius. Neither did he mention any differences in the pigmentation of immature and adult Oncorhynchus. It may be pointed out that the melatonin administered into Carassius was of mammalian source.

Whereas melatonin is generally known to cause aggregation of pigment in amphibians relatively little information is available about its effects on teleost melanophores. It causes aggregation in Chasmichthys (Fujii, 1961; Fujii concluded that it acts directly on the melanophores as he claimed it to be effective on denervated melanophores as well), and in Phoxinus (Healey & Ross, 1966). In Carassius (Etoh, 1961 cf. Fujii, 1969) and in Fundulus (Mori, 1961 cf. Fujii, 1969; Abbot, 1968) it failed to cause aggregation. (Table iv, p.38)

In the present section (i) the melanophore responses of white- and black-adapted epiphysectomised I. melas to background reversals and to darkness and of (ii) blinded epiphysectomised specimens to illumination and to darkness are described and these are compared with the similar responses of intact fish (Section V) in order to attempt to find out the role of the pineal organ in the colour change of this species.

2. Material and method

The operated animals were subjected to the same experimental conditions as described for intact unoperated specimens in Section IV and V.

(i) Epiphysectomy.

The pineal in _I. melas_ appears to be a well-developed disc-like compact vesicle somewhat flattened dorsally, convex ventrally. It lies above the middle and distal part of the cerebral lobe as a translucent body underneath the parietal bone, not clearly distinguishable from the latter (Holmgren, 1969, confirmed it personally). The skin covering the parietal bone is uniformly pigmented as in _A. nebulosus_ (Rasquin, 1958). In sagittal sections it appears to have a central lumen with many tubular diverticulae radiating out from it, (Fig. 36a, p.234 in Appendix). For pinealectomy the integument covering the parietal bone in an anaesthetised fish was cut mid-dorsally with a fine scalpal; the cut being 4-5 mm long, extending slightly beyond the anterior and the posterior margins of the bone. The cut skin was pulled out laterally by means of hooks, (Fig. 37, p.160). Having exposed the parietal bone in this way, two minute holes were made on each of its lateral boundaries by means of a fine dental drill (the skull is not bony beyond the mid-anterior or mid-posterior boundaries of the bone). The

Fig. 37 Photographs showing (a) the exposed pineal region and (b) the same region after pinealectomy. The removed bone with pineal attached to it is visible on the body of the animal just below the wound as indicated by an arrow.

a

b

bone with the attached pineal was then lifted up and removed by means of watchmaker's forceps. The exposed brain surface was gently sucked with a pipette to remove the torn or detached part of the pineal and its stalk since the latter, being transparent, was not visible, (Fig.37, p.160). The cut skin was stitched with nylon. The operated fish were replaced in their respective tanks (illuminated white or black). They resumed active swimming soon after the effect of anaesthesia had passed off. The cut skin generally healed within about a week.

Pinealectomy did not appear to make any longtime impact on the melanophores of the operated animals. There was only slight and transitory dispersion of pigment, lasting less than an hour, presumably due to anaesthesia. The pigment around the cut skin (in white-adapted individuals) remained dispersed for many days, becoming aggregated with the healing of the cut, (a normal event after any cuts). The removal of the pineal in many cases was verified histologically (Fig.36b, p.234 in Appendix) by cutting serial sagittal sections of the heads of operated animals, and in others by dissection and by cutting the head mid-dorsally by a sharp razor blade and examining the relevant parts of the skull under a microscope. There were no signs of regeneration of the organ in the operated animals, even as long as 14 months after the operation.

Pinealectomised animals that had been on their respective illuminated backgrounds before and after the operation were subjected to background reversal tests generally 2-3 weeks following the operation. 7 white-adapted and 12 black-adapted operated specimens were used in these tests in the same manner as described in Sections V and VI.

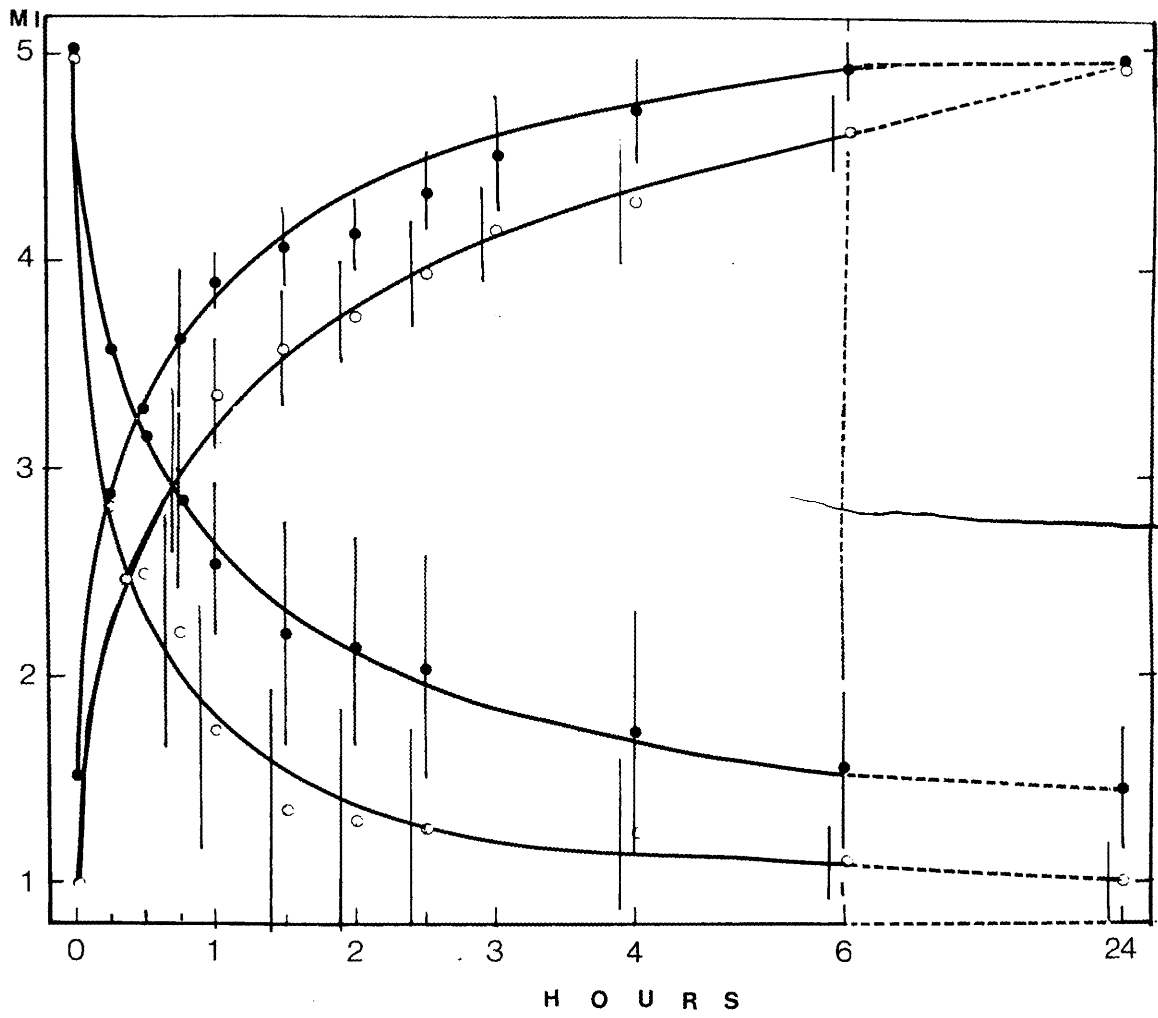
3. Observations and results

(i) Chromatic responses of pinealectomised catfish to black/white illuminated backgrounds and reversal, and their comparison with those of unoperated animals.

The ability of pinealectomised animals to adapt to illuminated white or black backgrounds appeared to remain unaffected. The melanophores (ldm and edm having mean MI's 1.53 and 1.00 respectively) on transfer to an illuminated black background (Fig.38, p.163 and Table 25 in Appendix) and on reversal to a white background after 24 hr. exhibited responses indistinguishable from those described for normal intact animals (Fig.8, p.63 and Table 2 in Appendix). Similarly, black-adapted pinealectomised catfish on transfer to an illuminated white background and reversal (Table 26 in Appemdix) reacted in the same way as the intact black-adapted animals (Table 3 in Appendix).

Equilibration of white-adapted epiphysectomised animals on different greys was not investigated in view of the close similarity of melanophore responses of operated animals to those of the unoperated ones during white-to-black transition.

Fig. 38 Melanophore responses of pinealectomised *I. melas* transferred from equilibrium on an illuminated white background to an illuminated black background and reversal after 24 hr. Each point with SD's as vertical bars is the mean of 7 animals. Temp. 20 ± 1°C. Overhead illumination 40 W at 75 cm. Open circles = epidermal, closed circles = lower dermal melanophores.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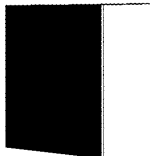
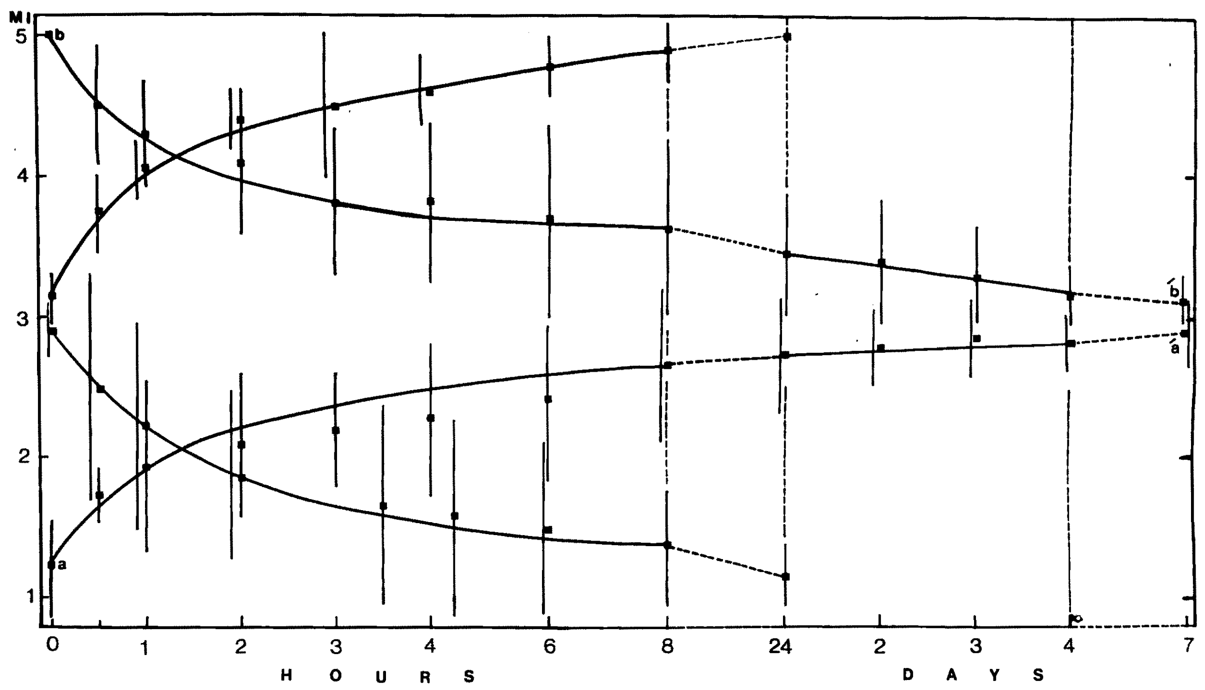


(ii) Responses of operated animals to darkness and their comparison with those of unoperated animals.

6 white-adapted pinealectomised animals 3-4 weeks following the operation were placed in darkness. Their ldm took ca. 3-4 days for equilibration to darkness (Fig.39, p.165, Table 27 in Appendix) with equilibrium MI averaging ca. 2.9. In contrast to this, unoperated white-adapted fish attained equilibrium to darkness in 6-8 hr. with their MI averaging 3.15 (Fig.22, p. 79, Table 5 in Appendix). However, the equilibrium MI values in both cases, as well as MI values after 8 hr. or a longer stay in darkness, do not show statistically significant differences. Reversal to an illuminated white background was obtained in ca. 8 hr. i.e. a similar time to that required by the unoperated animals.

Black-adapted operated animals, like the white-adapted ones, took about the same time (4 days) to attain equilibrium to darkness, the mean equilibrium index value being 3.1. Comparison of this value with the similar value in intact animals after 8 hr. (Tables 6, 28 in Appendix) or longer shows no statistically significant differences. Reversal to an illuminated black background after 7 days stay in darkness appeared to have taken a longer time in operated animals (24 hr.) in comparison with the unoperated animals (8 hr.). However, mean MI's of ldm in both groups after 8 hr. or less on an illuminated black background do not show any significant differences.

Fig. 39 The responses of lower dermal melanophores of pinealectomised *I. melas* transferred from equilibrium on an illuminated white/black background to total darkness and reversal after 7 days. Each point with SD's as vertical bars is the mean of 6 animals. Temp. 20 $\pm 1^{o}$C. 40 W at 75 cm.



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The edm in pinealectomised (white- and black-adapted) specimens (Tables 27 and 28 in Appendix), unlike those in unoperated animals (Tables 5 and 6 in Appendix), generally did not show any erratic behaviour. In white-adapted fish after transference to darkness, they became slightly dispersed and appeared to attain equilibrium to darkness in about 8 hr., the mean MI being 1.5 (1.9 in unoperated specimens for the same period). In black adapted operated fish the edm on transfer to darkness continued to show aggregation of pigment as long as the animals remained in darkness (mean MI after 7 days being 1.33), unlike the change observed in intact black—adapted specimens where they appeared to reach a state of equilibrium in ca. 8 hr, the mean MI being ca. 2.76. (Possibly they would have reached the same MI as originally white-adapted fish if they had been left for a longer time).

(iii) Responses of enucleated pinealectomised catfish to light and darkness and their comparison with unoperated blinded animals.

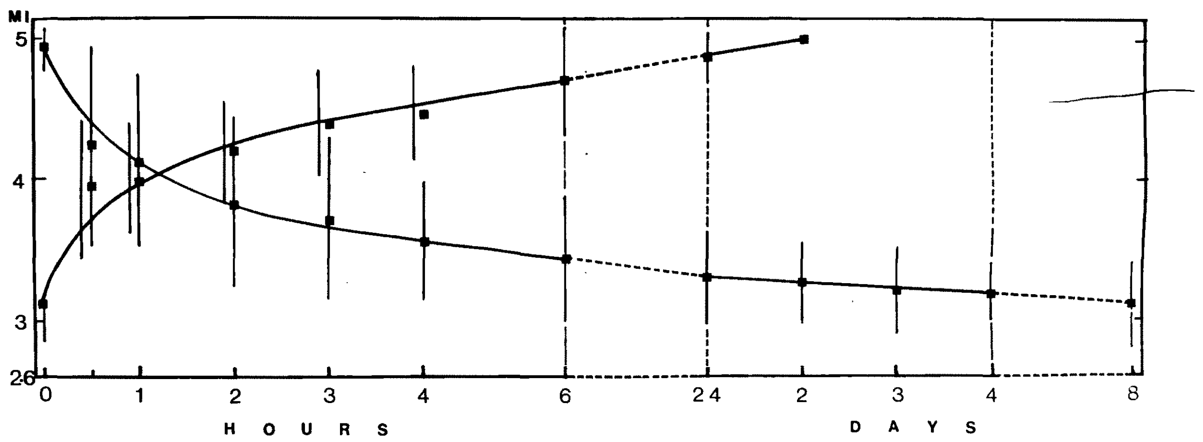
The melanophore reactions of 8 pinealectomised blinded fish to darkness and to illumination were observed. All of them were previously white-adapted. Of these 5 were enucleated 10 days following the operation and 3 about 4 months after pinealectomy. After enucleation they were replaced in white tanks (illuminated by general ceiling

lighting). As in the case of unoperated white-adapted fish (Section V, p.80-82), their melanophore pigment dispersed following enucleation. The dispersion in many cases appeared to be slow. They were subjected to darkness about 2 weeks after enucleation. At that time their MI's averaged ca. 4.9 (ldm and edm). Their reactions to darkness and reversal to illuminated background after 8 days are shown in Fig. 40, p.168, Table 29 in Appendix.

The mean equilibrium MI of ldm (ca. 3.2) of pinealectomised and blinded animals in darkness is similar to that of blinded otherwise intact animals (Fig. 23 Table 7 in Appendix). However, whereas the operated animals required ca. 4 days to reach a state of equilibrium in darkness, the unoperated fish reached it in 24 hr. At the same time the mean MI's in both cases after 1 day in darkness do not show any statistically significant difference and, excepting for the first 1 hr. after transfer to darkness, the MI's of the two groups are very much alike.

On reversal to illumination, the ldm-pigment in the operated fish became nearly fully dispersed (MI ca. 4.9) in 24 hr. (MI 5.0 in unoperated fish). However, the comparison of this transition in pinealectomised fish with that of unoperated fish shows that in the former the rate of dispersion was slower than in the latter.

Fig. 40 Responses of lower dermal melanophores of blinded pinealectomised *I. melas* transferred from equilibrium on an illuminated background to darkness and reversal after 8 days. Each point with SD's as vertical bars in the mean of 8 animals. 20 ±1°C. 40 W at 75 cm.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The edm in pinealectomised blinded specimens on transfer to darkness, like those in black-adapted pinealectomised animals, continued to aggregate pigment and after 8 days their mean MI was observed to be 1.85 (2.85 in the case of unoperated fish after 7 days). On reversal to illumination they gradually dispersed their pigment, reaching the fully dispersed state in 2 days (3 hr. in unoperated fish), (Table 29 in Appendix).

4. Discussion and conclusions

It is relevant firstly to examine the question whether the pineal plays any role in regulating the aggregation of pigment on a white background, as suggested by Charlton (1966). So far as the teleosts are concerned there is no such indication either from the present investigation or from any previous work. On the contrary, there is overwhelming evidence for a widespread presence of aggregating nerve fibres responsible for melanophore aggregation in these fish. Regarding the amphibians, the co-ordination of background colour response by the pituitary is well documented and there appears no justification in implicating other factors or glands without any solid and convincing evidence. Charlton's (1966) conclusion that white background adaptation in *Xenopus* is mediated by the pineal which, on being stimulated through the eyes, releases a whitening hormone or melatonin is based on his results of epiphysectomy

in this species. This alone can not be accepted as any positive evidence for Charlton's conclusion unless (i) it is substantiated by the isolation of a pigment-aggregating factor from the pineal and/or (ii) the pineal extract from Xenopus or any other amphibian source has been demonstrated to cause aggregation of melanophores in epiphysectomised specimens and in intact black-adapted specimens. Another major weakness of his suggestion is that the effect of pinealectomy on the melanophores of his experimental animals was observed to be only short-lived ("reduced to 2 weeks or more" - Charlton, 1966). Although the recovery of the normal chromatic behaviour was attributed to the possibility of regeneration of the pineal in the operated specimens, it tends to suggest that organs other than the pineal may be involved in the aggregation of pigment, as has been rightly pointed out by Wurtman et al (1968). Charlton's reported failure (1968) to locate serotonin, a precursor of melatonin in the pineal of Xenopus, also casts doubt on his postulated views. Thus the role of the pineal as an endocrine gland in co-ordinating background colour responses in Xenopus or any other species remains only speculative.

There are, however, indications that the pineal, at least in some species, is concerned with the regulation of diurnal rhythm of colour change and affects the reactions of melanophores to darkness. But any suggestion that the

pineal in all lower vertebrates controls these responses by synthesising and releasing melatonin (Bagnara, 1963, 1965, 1966) is questionable. The results of epiphysectomy in the adult *Lampetra* (Young, 1935) and in metamorphosing *Mordacia* (Eddy & Strahan, 1968) do not support this concept. The results of the present investigation also do not support it. The equilibration of melanophores to darkness in pinealectomised eyeless and in pinealectomised but otherwise intact fish required a longer time than that in unoperated specimens. While this protracted time relation in the operated animals may be interpreted in favour of the pineal-induced response, overall comparison of the similar responses in the operated and unoperated animals (p.165) does not appear to justify this inference and consequently no significance may be attached to it. It could rather be due to individual variations than to any other cause. Furthermore, another difficulty would arise in offering an explanation for the dispersion of pigment in white-adapted operated as well as in unoperated animals in darkness. Lack of results on photo-sensitivity in *Ictalurus* could probably be due to dense pigmentation affecting the light impinging on it (Breder & Rasquin, 1950; Rasquin, 1958 in *Astyanax*). Another possibility is that other brain tissues in the pineal region may be taking part in the pigmentary reaction to light, as postulated by von Frisch (1911) for *Phoxinus*. The results of the present work are consistent with Rasquin's (1958) observations on the effects of pinealectomy on the

on the pigmentation in Astyanax and confirm Wyke's (1938) and Parker's (1940) conclusions on the effects of pinealectomy in A. nebulosus.

The isolation of melatonin from the pineal of Oncorhynchus tshawytscha does lend support to the possibility that the pineal may be an endocrine gland in some teleosts and lower vertebrates but no generalizations that it could be an endocrine gland in all cases or that it is the only site of melatonin synthesis and release (Quay, 1965) are justifiable at this stage from this isolated instance. Moreover, Fenwick did not state its impact on the pigment in this species. The only available information is from Hoar's work (1955) (on juveniles of O. nerka) which does not appear to show that pinealectomy had any pronounced effect on the skin colour of the operated animals. That melatonin is a potent pigment-aggregating factor in all teleosts is not demonstrated from the observations of Etoh (1961), Mori (1961), Abbot (1968) and Fain & Hadley (1966).

The results of the experiments involving implantation of pineal also do not appear to support the above views. For instance, implantation of Atherina pineals into Cyprinodon did not produce any change in the melanophores of the latter (Rasquin, 1958); Kelly (1962) reported that transplantation of developing pineals from the newt (Tricha) larvae into the tail fin of host larvae failed to produce any consistent change in the melanophores of the latter

(blanching of variable intensity lasting 4-5 days, sometimes altogether absent) in spite of continued development of the pineal graft. He also reported that in some cases aggregation also resulted when brain tissue other than pineal was transplanted; Eddy & Strahan (1968) found that implantation of pineal from a pale Geotria ammocote (MI ca. 2.2) into dark ammocoete kept in light (MI5) caused a temporary (lasting ca. 2 hr.) aggregation of pigment (MI 2-3.5) around the transplanted area of skin (3-4 mm diameter).

Many other situations are also inconsistent with the assumption that the blanching of the amphibian larvae in darkness is brought by the stimulation of the pineal to release melatonin. Some of these are: (1) failure of the melanophores of the tail region in Xenopus larvae to aggregate in darkness even though melatonin is assumed to be present in the blood circulation and is supposed to counteract the effects of MSH on the pigment (cf. Bagnara, 1965); (2) darkening of amphibian larvae kept in darkness for a long time (cf. Bagnara, 1965); (3) in an experiment specially designed to test pigment response to pinealectomy on the newt Tricha and salamander Ambystoma larvae, Kelly & Johnson (1962, cf. Kelly, 1962) observed that the blanching response of the operated larvae to darkness was only temporarily abolished (5-10 days) and larvae returned to normal pigment behaviour in about 30 days despite pineal loss; (4) dispersion

of pigment in *Xenopus* larvae kept in melatonin solution for a long time, although the solution was demonstrated to be potent as it caused aggregation in untreated controls (Burgers & van Oordt, 1962; Charlton, 1966). In fact, the results of many of the experiments that have been attributed to the direct effect of melatonin or the pineal, could be explained by assuming that the pineal plays an indirect role, i.e. by inhibiting MSH output of the pituitary as was earlier suggested by Young (1935) and later on by Brick (1962). Brick's experiments and other observations, for example that administration of pineal extract to hypophysectomised frogs did not cause blanching of the skin as it does in normal animals (Simonnet *et al*, 1954, cf. Burgers & van Oordt, 1962), reinforce this suggestion.

Lastly it is significant to note that most of the conclusions indicating the control of blanching response by the pineal have been arrived at from the observations on larval and juvenile forms rather than in the adult animals. Fenwick's reported finding of a significantly greater level of melatonin from juvenile *Oncorhynchus* than in the adult appears to be pertinent to the whole problem. It seems very likely that the primary role of the pineal in young forms may be to cause inhibition of gonadal development and the effects on the pigmentation of these forms, especially in darkness may be only side effects of the whole phenomenon. After all, the adaptive value of blanching in

darkness is debatable.

In short, the role of the pineal as an endocrine gland in the pigmentary effector system of lower vertebrates is still an open question.

X. THE EFFECTS OF CERTAIN DRUGS ON THE MELANOPHORES IN ISOLATED SKIN

1. Introduction

Various drugs with known effects on the mammalian autonomic system have been administered to many different teleosts in an effort to find out whether the nervous control of their melanophores is only adrenergic or whether it also involves cholingergic dispersing system (pp. 31-40). Much of this pharmocological work has been carried out on two species, namely *Fundulus* (Spaeth & Barbour, 1917; Abbot, 1968) and *Phoxinus* (Healey & Ross, 1966; Grove, 1969a, 1969b); whereas very little is known about the drug effects in *Ameiurus*. Adrenaline in *Ameiurus* was reported to cause aggregation of pigment *in vivo* as well as *in vitro* (Bray, 1918; Bacq, 1933; Parker, 1934a, 1940, 1948; Abramowitz, 1936a; Wykes, 1938; Rasquin, 1958). According to Parker (1934b, 1940), injection of acetycholine preceded by eserine resulted in darkening of otherwise pale fish. The only other information about a drug effect in this catfish relates to ergotamine which, according to Bacq (1933), in intact white-adapted fish caused dispersion of innervated

melanophores and aggregation of otherwise dispersed denervated melanophores of the caudal band. On the other hand, Parker, (1941b) reported that in such a fish the melanophores showed some dispersion of pigment whereas the denervated melanophores of the caudal band remained unaffected.

In the absence of any information of the effects of various sympathomimetic, adrenergic blocking and parasympathomimetic agents in I. melas, an attempt was made to observe the influence of some of the above and other drugs on the melanophores of this species. This work was of only a preliminary nature, as it involved relatively few drugs which were administered only in vitro.

2. Materials and methods

White-adapted fish, 60-80 mm long, were used in the drug experiments. Before removing their skin, they were killed instantaneously by crushing the brain with coarse forceps. Each animal was then immersed in a dish filled with Ringer at room temperature varying from 18.7-20.5^{o}C. The strip of skin above the lateral line was cut dorso-laterally under a binocular and it was gently removed by fine forceps. It was then cut into numerous small fragments, each of which was transferred to a watch glass containing freshly prepared Ringer. The whole procedure, from killing of the animal to the transfer of its skin pieces into watch glasses, was completed in ca. 30-45 min.

A fresh stock solution of each drug of concentration

10^{-1}-10^{-2}M was prepared by dissolving it in Ringer and further necessary dilutions were made from it. Ordinary Ringer in the watch glass containing a skin fragment was replaced by the drug-containing Ringer. 8 to 10 skin pieces were observed in each case under a microscope (magnification x 100) at 30-45 min. intervals. Changes in melanophore-pigment were assessed and recorded by the melanophore index (Fig. 7, p.56). The effects of 18 drugs in all were observed. For convenience and for comparison, the effects of various drugs have been arbitrarily regarded as: (i) negligible when the shift of mean MI (for 8-10 skin pieces) was less than 0.20; (ii) slight, when the range of change was between 0.20 to 0.25 MI; (iii) some, when it fell between 0.26 to 0.35, (iv) moderate, when it was between 0.36 to 0.50; (v) distinct if the shift of mean MI was in the range 0.51-0.90; (vi) and lastly pronounced if it was above 0.90 MI. (This arrangement is only indicative of the magnitude of the effectiveness of the drug and has no statistical significance). It is relevant to state here that the effects of various drugs generally appeared to wane on return of the skin fragments to Ringer and the melanophore pigment recovered its original state in varying time intervals, generally between 30 to 90 min. Controls were kept in Ringer throughout the duration of the experiments and did not show any changes.

3. Results and discussion

(i) Equilibration of melanophores in Ringer.

On being immersed in Ringer in 15-30 min. the melanophores in isolated skin fragments assumed a semi-dispersed condition and remained so for many hours, 6-8 or more. Equilibrium MI's usually ranged between 1.0 to 1.5 for epidermal melanophores and 2.7 to 3.1 for dermal melanophores (upper and lower dermals together), their mean MI's being 1.49 and 2.89 respectively.

(ii) Effects of some sympathomimetic agents.

Noradrenaline bitartrate and adrenaline bitartrate were observed to be equally effective in causing significant aggregation of pigment both in epidermal and dermal melanophores in isolated skin. The former appeared to be relatively more effective at lower concentrations such as 10^{-5}M than adrenaline of similar concentration (Table 30, p. 180). The minimum effective concentration of noradrenaline appeared to be between 10^{-6} and 10^{-7}M, as reported by Scheline (1963) for *Labrus*. Of all the drugs used, the action of these two was most rapid and most pronounced. Their action on melanophores in *I. melas* is consistent with the generally accepted conclusion that an adrenergic or a similar mechanism is involved in the aggregation of pigment (pp. 38-40).

Isoprenaline sulphate (which in mammals is known to act on beta-adrenergic receptors) had a moderate aggregating effect on dermal melanophores (Table 30). It has been

reported to cause marked paling of black-adapted intact *Phoxinus* by Healey & Ross (1966) and by Grove (1969a) (Table i p. 31). *Tyramine* hydrochloride (the only non-catechol amine used) had some aggregating effect in *Ictalurus* as was observed by Healey & Ross (1966) and Grove (1969a) in *Phoxinus*. It was also reported to cause aggregation of pigment in isolated scales of *Fundulus* (Barbour & Spaeth, 1917; Abbot, 1968) and *Labrus* (Scheline, 1963). This effect may be attributed to the release of noradrenaline from the adrenergic nerve terminals as was suggested by Scheline.

(iii) Effects of adrenergic blocking agents.

Ergotamine (Femergin - Sandoz) caused a distinct aggregation of pigment in *Ictalurus* (Table 30, p.180). This result does not agree with the observations made by Bacq (1933) and Parker (1941b) on *A. nebulosus*. However, the present observation is in agreement with the similar observations in *Salmo* (Robertson, 1951), *Chasmichthys* (Fujii, 1961) and *Phoxinus* (Healey & Ross, 1966). Moreover, in the present case a distinct reversal of the noradrenaline effect was also noted in ergot-treated skin (Table 31, p.182). Results similar to this were reported in isolated preparations of *Fundulus* (Spaeth & Barbour, 1917), *Chasmichthys* (Fujii, 1961). Administration of adrenaline into ergot-treated white adapted *Phoxinus* caused some dispersion of pigment (Healey

Table 30. Effects of drugs on the melanophores in isolated pieces of Ictalurus skin

Class of drug	Drug and dose		Mean melanophore indices: Before adding the drug		After adding the drug: 30-45 min.		After 60-90 min.		Maximum change 90-120 min.	
			edm	dm	edm	dm	edm	dm	edm	dm
Sympatho-mimetic agents	Adrenaline,	10^{-5}M	1.48	3.05	1.00	1.73	1.00	1.80	-0.48	-1.32
	Noradrenaline,	10^{-5}M	1.55	3.19	1.00	1.68	1.00	1.78	-0.55	-1.51
	Noradrenaline,	10^{-6}M		2.92		1.70		1.84		-1.22
	Isoprenaline,	10^{-3}M	1.20	2.68	1.00	2.34	1.00	2.22	-0.20	-0.46
	Tyramine,	10^{-3}M	1.20	2.64	1.00	2.40	1.00	2.34	-0.20	-0.30
Adrenergic blocking agents	Ergotamine,	2×10^{-3}M	1.00	2.40	1.00	1.72	1.00	1.73		-0.68
	Regitin,	10^{-3}M	1.04	2.50	1.75	2.47	1.70	2.62	+0.71	+0.12
	Yohimbine,	10^{-4}M	1.87	3.00	1.90	3.21	2.17	3.25	+0.40	+0.26
	Piperoxane,	10^{-2}M	1.17	2.63			1.71	2.78	+0.54	+0.15
Anti-adrenaline agents	Bretylium,	10^{-3}M	1.00	2.72			1.20	2.74	+0.20	+0.02
	Guanethidine,	10^{-3}M	1.00	2.32			1.73	2.50	+0.73	+0.18
	Reserpine,	10^{-3}M	1.00	2.55	1.00	2.57	1.00	2.70		+0.17
Parasym-pathomimetic agents	Acetylcholine	10^{-2}M	1.56	3.32			1.80	3.54	+0.24	+0.22
	Eserine,	10^{-3}M	1.70	3.11			2.10	3.38	+0.40	+0.27
	*Eserine + Acetylcholine,	10^{-3}M	1.92	3.20	(after eserine)		2.13 (after acetylcholine)	3.27	+0.21	+0.07
			1.62	3.19	1.90	3.46	1.74	3.57	+0.12	+0.38
	Pilocarpine	10^{-4}M	1.00	3.17			1.30	3.48	+0.30	+0.31
Cholinergic blocking agents	Atropine	10^{-3}M	1.28	3.04			1.57	3.31	+0.29	+0.27
Ganglion blocking agents	Hexamethonium	10^{-3}M	1.00	2.50			1.00	2.73		+0.23

Class of drug	Drug and dose		Before adding the drug		Mean melanophore indices: After adding the drug				Max.change 90-120 min.	
					30-45 min.		After 60-90 min.			
			edm	dm	edm	dm	edm	dm	edm	dm
Miscellaneous	Serotonin	10^{-3}M	1.14	2.76	1.00	2.66	1.00	2.44	-0.14	-0.32
	Melatonin	10^{-3}M	1.50	3.17	1.33 (20 min.)	2.18	1.33	2.23	-0.17	-0.99

Edm = epidermal, dm = dermal melanophores (mostly the upper dermal melanophores as the lower dermal ones generally remained attached to the muscular tissue underlying the skin.) Mean MI's for 8-10 pieces of skin. Room temp. 18.7-20.5°C.

Table 31. Effect of noradrenaline on melanophores of isolated skin pieces of Ictalurus pre-treated with some adrenergic blocking and anti-adrenaline substances

Dose and drug		Mean melanophore indices: In Ringer		In drug		In Noradrenaline 10^{-4}M		Maximum change	
		edm	dm	edm	dm	edm	dm	edm	dm
Ergotamine	2 x 10^{-3}M	1.00	2.40	1.00	1.73	1.07	2.30	+0.07	+0.57*
Regitin	10^{-3}M	1.04	2.50	1.70	2.62	1.00 (10^{-5}M)	2.07	-0.70	-0.55
Yohimbine	10^{-4}M	1.46	3.00	1.87	3.13	1.75	2.83	-0.12	-0.30
Piperoxane	10^{-2}M	1.17	2.63	1.71	2.78	1.42	2.73	-0.29	-0.05
Bretylium	10^{-3}M	1.00	2.72	1.20	2.74	1.00	2.54	-0.20	-0.20
Guanethidine	10^{-3}M	1.00	2.32	1.73	2.50	1.28	1.91	-0.45	-0.59
Reserpine	10^{-3}M	1.00	2.53	1.00	2.70	1.00	2.13		-0.57
Reserpine	10^{-2}M	1.00	2.55	1.00	2.58	1.00	2.05		-0.53

(*Melanophores in isolated skin pre-treated with noradrenaline, also dispersed pigment on being immersed in ergotamine, extent of dispersion being 0.50 and 0.63 for edm and dm respectively.)

Edm = epidermal, dm = dermal melanophores (mostly upper dermal ones). Mean MI's for 8-10 pieces of skin.

Table 32. Effects of noradrenaline on melanophores of isolated skin pieces of Ictalurus pre-treated with parasympathomimetic and other agents.

Drugs and dose		Mean melanophore indices							
		In Ringer		In drug		In noradrenaline 10^{-5}M		Maximum change	
		edm	dm	edm	dm	edm	dm	edm	dm
Eserine	10^{-3}M	1.70	3.24	2.10	3.30	1.30	2.20	-0.80	-1.10
Acetylcholine	10^{-3}M	1.92	3.20	2.13	3.27	1.00	1.95	-1.13	-.132
+ Eserine	10^{-3}M								
Atropine	10^{-3}M	1.28	3.04	1.57	3.31	1.00	1.96	-0.57	-1.35
Hexamethonium	10^{-2}M					(10^{-4}M)			
		1.00	2.33	1.25	2.51	1.22	2.38	-0.03	-0.13
Pilocarpine	10^{-4}M	1.35	3.30	1.65	3.60	1.05	1.78	-0.60	-1.82

Edm = epidermal, dm = dermal melanophores. Mean MI's for 8-10 pieces of skin.
Room temperature 18.7-20.5°C.

& Ross, 1966). In contrast to this, Pye, (1964) found no such reversal of the adrenaline effect in _Phoxinus_ either _in vivo_ or _in vitro_ (Table ii, p.34).

Regitin (= phentolamine) mesylate (CIBA) which in mammals causes moderately effective but transient alpha-adrenergic blockade, appeared to have a negligible effect on dermal melanophores (Table 30, p. 180). This observation, however, agrees with the result in _Phoxinus_ (Healey & Ross, 1966) but not with that in _Fundulus_ (Abbot, 1968). In contrast to its effect on isolated skin, it was reported to cause considerable darkening of intact white-adapted _Phoxinus_ (Pye, 1964; Healey & Ross, 1966; Grove, 1969a). This indicates that the site of its action lies outside the melanophores. Noradrenaline was distinctively effective in causing aggregation of pigment in regitin treated skin in _Ictalurus_ (Table 31, p.182), as was also noted in _Phoxinus_ _in vivo_ (Pye, 1964; Healey & Ross, 1966) and _in vitro_ (Healey & Ross, 1966) (Table (ii) p.33-36).

Yohimbine hydrochloride in mammals is known to produce a competative alpha-adrenergic blockade of limited duration (cf. Innes & Nickerson, 1965). It had a slight dispersion effect on dermal melanophores (Table 30, p.180) in _Ictalurus_. On the other hand, in _Phoxinus_ it caused a moderate dispersion _in vitro_ and had a marked effect _in vivo_ (Healey & Ross, 1966; Grove, 1969a). Noradrenaline caused some aggregation of pigment

in skin pretreated with yohimbine in Ictalurus (Table 31, p.182), whereas in Phoxinus it produced a negligible effect (Table ii, pp. 33-36).

Piperoxane hydrochloride (May & Baker) like regitin appeared to have a negligible effect on the dermal melanophores in Ictalurus (Table 30, p.180). This result agrees with the similar observation in Phoxinus (Healey & Ross, 1966). However, it caused considerable darkening in intact white-adapted Phoxinus (Healey & Ross, 1966; Grove, 1969a). In mammals piperoxane and related compounds cause transient blockade of the responses to circulating adrenergic mediators more readily than to those resulting from sympathetic nerve activity (cf. Innes & Nickerson, 1965). This probably explains the lack of response in isolated preparations. Post-treatment of skin with noradrenaline appeared to have a negligible effect on melanophores already treated with piperoxane (Table 31, p. 182), whereas in Phoxinus it caused some aggregation of pigment.

(iv) Anti-adrenaline substances.

Bretylium tosylate had no effect and guanethidine sulphate (CIBA) a negligible effect on dermal melanophores in Ictalurus indicating that site of their action was probably post- or pre-ganglionic. In Phoxinus bretylium had a moderate dispersion effect and guanethidine caused negligible aggregation in isolated melanophores, (Healey & Ross, 1966). In

Fundulus (Abbot, 1968), as in Ictalurus, bretylium produced no change in pigment in isolated melanophores. In intact Phoxinus bretylium and guanethidine (irrespective of potency of dose) caused moderate to considerable darkening (Healey & Ross, 1966; Grove, 1969a). On the contrary, in Fundulus (Abbot, 1968) bretylium in lower concentration was reported to cause aggregation and in high concentration dispersion of pigment while guanethidine caused aggregation only. Noradrenaline appeared to be only slightly effective in bretylium-treated skin and moderately effective in melanophores previously treated with guanethidine. On the contrary, in Phoxinus adrenaline was remarkably effective in reversing the effects of both drugs *in vivo* as well as *in vitro* (Healey & Ross, 1966). In Fundulus noradrenaline was only temporarily effective in reversing the effects of bretylium (Abbot, 1968). Thus the situation regarding the effect of bretylium on melanophores is by no means clear.

The results of treatment of isolated melanophores with reserpine (Koch-Light) in Ictalurus are entirely different from the results in Phoxinus i.e. negligible dispersion of pigment in the former and a pronounced dispersion (of ca. 1.2 MI) in the latter. The lack of reserpine effect in the present case perhaps was due to the difficulty of dissolving the drug in Ringer in which it appeared to form a milky suspension. However, in Fundulus, like Ictalurus, it did not cause any change in the melanophores of isolated scales

(Abbot, 1968). Reserpine-treated skin on being immersed in noradrenaline showed considerable aggregation of pigment. This result appears to support the suggested cause of lack of reaction as reserpine is also known to reduce "uptake" of catecholamines *in vitro* as well as *in vivo*, (cf. Nickerson, 1965). In *Phoxinus* adrenaline could only negligibly reverse reserpine effects both *in vitro* and *in vivo* (Healey & Ross, 1966)

(v) Reactions of epidermal melanophores to adrenergic blocking and anti-adrenaline agents.

Whereas adrenergic blocking agents and anti-adrenaline substances generally caused little or slight change in dermal melanophores in *Ictalurus* skin (an exception being ergotamine) these agents generally appeared to cause moderate to distinct dispersion in epidermal melanophores (Table 30, p.180) though their responses were widely variable. Perhaps these melanophores have a lower threshold response to these drugs than the dermal ones. Another possibility which is suggested from their faster rates of aggregation in black-to-white background adaptation in intact and in hypophysectomised animals (Figs. 8, p.63 and 27, p.101 respectively) is that these melanophores are more abundantly or more profusely supplied by aggregating nerve-fibres than the dermal melanophores. Only investigations on the innervation of melanophores in this species could elucidate this question.

(vi) Effects of parasympathomimetic agents.

Regarding the effect of acetylcholine hydrochloride (Roche) on the melanophores in teleosts conflicting information is available (Table (iii), p. 36-37). In the present case the dispersion effect of acetylcholine (10^{-2}M) was only slight on dermal as well as on epidermal melanophores (Table 30, p.180). Acetylcholine and eserine together had a negligible to slight effect. Pretreatment with eserine caused some dispersion and after the addition of acetylcholine the effect was again negligible. These results agree with those reported on *Oryzias* (Watanable *et al*,1962b), *Phoxinus* (Healey & Ross, 1966). Noradrenaline was distinctly effective on melanophores previously treated with acetylcholine and eserine (Table 32, p.183). Eserine (phytostigmine) sulphate (Burroughs & Wellcome) itself had some effect on isolated melanophores. Post-treatment with noradrenaline caused distinct pigment aggregation. These observations are thus apparently inconsistent with Parker's (1934b, 1940) conclusion that the dispersion of pigment in *Ictalurus* and other teleosts is caused by dispersing cholinergic nerve fibres and that the transmitter concerned is acetylcholine.

Pilocarpine nitrate in *Ictalurus*, like in other teleosts (Table (iii), p.36-37), caused some dispersion of pigment. Noradrenaline reversed this effect. Pilocarpine administered into intact white-adapted *Phoxinus* failed to produce any darkening effect (Grove, 1969a). Thus some dispersion noted

in the present case could be due to its direct effect on melanophores. In mammals too it is generally assumed to produce its cholinomimetic effects extensively by a direct action on the autonomic effector cells (cf. Koelle, 1965).

(vii) Ganglion blocking agent.

Hexamethonium bromide (May & Baker) appeared to cause only a slight dispersion of pigment in Ictalurus (Table 30, p. 180). In Phoxinus its effect is considerable (Healey & Ross, 1966) while in Fundulus it produced no change (Abbot, 1968). Apparently the changes resulting in the pigment in isolated skin of Ictalurus and Phoxinus may be due to its direct effect, as its administration into intact Phoxinus and Fundulus resulted in some darkening. Grove (1969a) suggested that hexamethonium might be acting antagonistically to nervously co-ordinated background responses in Phoxinus.

(viii) Cholinergic blocking agent.

Atropine sulphate which should cause aggregation of pigment, on the contrary, caused some dispersion in Ictalurus as in Phoxinus and many other teleosts, (Table iii, p.37), presumably by its direct action on melanophores of isolated preparations (Watanabe, 1960).

(ix) Miscellaneous substances.

5-hydroxytryptamine (Serotonin) (May & Baker) produced considerable aggregation of pigment in the present case

(Table 30, p.181). This result agrees with the similar observations on Labrus (Scheline, 1963), Fundulus (Abbot, 1968) and in amphibians in general (cf. Fänge, 1962). It is, however, inconsistent with the results in Chasmichthys (Fujii, 1961) and Phoxinus (Healey & Ross, 1966). It possibly could be exerting a direct effect on melanophores (Table iv, p.38).

Melatonin (Koch-Light) appeared to cause a marked and rapid aggregation of melanophores in Ictalurus (Table 30, p.181), as in Chasmichthys (Fujii, 1961) (Table iv, p.38). Its effect on melanophores also appears to be direct. This assumption is also supported from the results of the observations on background responses of pinealectomised specimens described in Section IX p.162. Its results are known in few other cases (Table iv, p.38) and these appear to be conflicting.

Finally, the dermal melanophores in Ictalurus like those in Phoxinus on equilibration in physiological saline, do not show either complete dispersion or complete aggregation of pigment and tend to remain in an intermediate state, the mean MI being ca.2.9. In distinction to this, the isolated epidermal melanophores appear to remain almost fully aggregated, their mean MI being ca.1.5. These observations appear to support Healey & Ross's (1966) conclusion that some noradrenaline is available to the melanophores in isolated skin. However, the results of experiments with reserpine in the present case do not appear to reinforce this conclusion.

The effects of administering reserpine in intact animals could help in clearing the situation.

It is relevant to point out here that the equilibrium mean MI of isolated melanophores in Ringer is very similar to the mean MI's of all categories of intact and operated animals (with or without eyes) equilibrated to darkness. It is also the mean MI of the animals devoid of pituitary and with severed chromatic tract (p.139). Does it mean that this state of pigment in this species, at least, is the state of unstimulation or inactivity of melanophores? Only further investigation in this direction can help in solving this riddle.

4. Conclusions

The results of the present attempt with certain sympathomimetic agents, adrenergic blockers, anti-adrenaline agents and atropine, appear to support the conclusion already arrived at (Section VII p. 146) that the nervous control of melanophores in *I. melas* is only adrenergic. However, the results obtained from the drug effects on the pigment in isolated skin fragments are only tentative and these need verification and confirmation from the effects of these and other drugs on administration into intact or unoperated as well as operated animals on the lines of the work carried out on *Phoxinus* by Healey & Ross (1966) and Grove (1969a, 1969b).

Only then a more reliable and true picture of the drug effects in this species could emerge and more solid interpretations could be made.

The tests with acetylcholine and eserine in the present work have apparently provided no evidence for a cholinergic dispersing mechanism in *I. melas*.

Finally it is relevant to emphasize again that any conclusions from the interpretation of drug effects in teleosts by their comparison in mammals must be treated with reserve as has rightly been pointed out by many authors including Waring (1963), Pye (1964) and Healey & Ross (1966).

XI. DISCUSSION AND CONCLUSIONS

The respective roles of hormones and nerves in controlling melanophore responses in *I. melas* have already been discussed in detail on pages 84-90, 116-120, 143-147, and 169-175.

Nevertheless, it would be pertinent briefly to discuss the results of the present investigation from the point of view of two issues viz. the controversal existence of (1) melanophore concentrating hormone (MCH) in the pituitary and (2) pigment-dispersing nerve fibres. It also seems necessary (3) to consider why the epidermal melanophores differ in their reactions from the dermal melanophores.

Relatively very few results of the present work support belief in the existence of MCH in *Ictalurus*. These results include (1) the rate of pigment aggregation becoming compara-

tively very slow after about 1½ hr. during black-to-white transition in intact animals (Fig.8, p.63, Tables 5 and 6 in Appendix); (2) the pigment in dermal melanophores after an initial faster rate of aggregation continuing to show slight and gradual aggregation for ca. 48 hr. in hypophysectomised specimens during black-to-white background adaptation (Fig. 27, p.101, Tables 11 and 12 in Appendix); (3) The relatively longer period required by white-adapted chromatically spinal animals to equilibrate in darkness (3-4 days) than that required in white-to-black adaptation (about 24 hr.)(Fig.31,p.130).

However, alternative explanations could be offered for the results outlined above. In result (1) after the initial faster rate of aggregation, presumably due to the activity of aggregating nerve fibres, a subsequent slackening of the rate may very likely be due to a passive and slow inactivation or destruction of the residue MSH - the main bulk of which having already been rendered inactive by the antagonistic action of noradrenaline or other pigment-aggregating transmitter. (Competative antagonism between noradrenaline and MSH was demonstrated by Novales & Novales (1965) _in vitro_ in _Rana pipiens_). An effort has already been made to explain result (2) on page 119 . Moreover, the range of this aggregation i.e. the difference in MI values of upper and lower dermal melanophores after 3 hr. on a white background up to 48 hr. (Tables 11, 12 in Appendix) is very narrow and is statistically insignificant. Hence no importance may be attached to this result. In result (3) the equilibration of lower dermal

melanophores in chromatically spinal white-adapted catfish on transfer to darkness appears to require a longer time than in white-to-black transition, but it also may be noted that the equilibration of black-adapted spinal animals to darkness, on the other hand, requires a shorter time than that in black-to-white transition (Fig. 31, p. 130 , Fig.33, p.137 , Tables 18, 19 and 22 in Appendix). Thus these results do not fully support the bihumoral concept. As already stated (p.138) the assumption of decrease/increase in the level of single hormone (MSH) appears to offer a more reasonable explanation of the two situations. This assumption is also consistent with the time-relation of black-to-white background adaptation in chromatically spinal animals.

Notwithstanding the results 1-3 stated above, many other observations already enumerated on pp. 145-6, appear conclusively to deny any assumptions for the role of a hypothetical MCH in white background adaptation or in the aggregation of melanophore pigment in this species. Of course this is not the final word, the problem can be further pursued by fractionation of pituitary material and the administration of the fractions to *Ictalurus* melanophores.

The present work gives no indication that any active nervous mechanism is involved in the dispersion of pigment (p.146), and thus does not support Parker's views to this effect. However, the initial faster rate of dispersion in

white-to-black transition in intact animals (Fig.8, p. 63, Tables 2 and 3 in Appendix) could be interpreted as evidence for pigment dispersing fibres although it could be alternatively explained by assuming that, in addition to the action of MSH, the release of melanophores from the central aggregating influence may be contributing to this initial rapid rate of dispersion. Many other results such as (i) the impairment of the ability of black background adaptation in hypophysectomised individuals(pp.102-103);(2) there being no apparent loss or impairment of the ability of chromatically spinal fish to adapt to a black background (p. 129); (3) the rate of pigment dispersion in spinal fish in white-to-black background adaptation (Fig. 31, p. 130) being slower than in intact animals (p. 131); (4) the unresponsiveness of hypophysectomised spinal specimens to a black or white background (p. 140) all, of course, demonstrate the importance of the pituitary in the dispersion of pigment, but at the same time also appear to deny the existence of an active dispersing nervous mechanism. Similarly (5) the effects of hypophysectomy following anterior spinal cord section (p. 141) and (6) the results of the experiments with acetylcholine and acetylcholine in combination with eserine (Table 30, p.180) again appear to show rather a passive role of nerves regarding dispersion of melanophore pigment. Histological and electro-physiological studies on the innervation of melanophores, together with

pharmacological work could possibly provide more conclusive evidence.

Observations on the reactions of epidermal melanophores in general appear to suggest that the relationship between MSH and the aggregating neurohumor in their control is possibly slightly different from that of the dermal melanophores, particularly the lower dermal ones. (1) A generally slower rate of pigment dispersion in epidermal melanophores during white-to-black transition and (2) a relatively more aggregated condition of pigment in them than in the dermal melanophores in hypophysectomised animals adapted to a black background indicate that pigment dispersion in epidermal melanophores depends more on MSH than on the nerves (p.108). However, in that case one may expect that (i) in chromatically spinal white-adapted fish these melanophores (a) should be faster than the dermal melanophores in dispersing their pigment in white-to-black transition and (b) should be slower in aggregating in black-to-white transition; and (ii) in animals with an interrupted hypothalamo-hypophyseal tract they should presumably be more dispersed than the lower dermal ones. This is not so (see Fig. 31, p. 130, Table 24, p. 150)and therefore suggests that these melanophores, though being dependent on MSH for dispersion, have at the same time a higher threshold level of response or SAR (structure-activity-relationship). The results of injection of pituitary extract into the white-adapted hypophysectomised animals (Table 16,

p. 115) are consistent with this suggestion.

In the aggregation of pigment, the aggregating fibres appear to exert a relatively greater influence on the epidermal melanophores than on the dermal ones as is suggested from (i) their faster rate of aggregation in (a) intact and (b) hypophysectomised animals in black-to-white transition (Fig. 8, p. 63) and (Fig. 27, p. 101), and (ii) by their being apparently more reactive to adrenergic blocking agents and anti-adrenaline substances than the dermal melanophores (Table 30 , p. 180). Alternatively, the epidermal melanophores, in distinction to MSH, may have a lower threshold value of response or SAR than the dermal melanophores to the aggregating neurohumor. A relatively slower rate of aggregation of epidermal pigment in chromatically spinal animals in black-to-white transition is consistent with this assumption. McGuire & Möller's (1966) observation that epidermal melanophores, in contrast to dermal melanophores, in isolated skin of *Rana pipiens* pre-treated with MSH exhibited no paling response to noradrenaline, melatonin and acetylcholine - also favours the above suggestion, especially since the epidermal melanophores in the catfish like those in the frog appear to be morphologically different from the dermal melanophores (Fig. 5, p.55). Incidentally, there appears to be a striking similarity in the epidermal melanophores of *I. melas* and *R. pipiens*. The above quoted authors also believe that the epidermal melanophores of frogs bear a close

morphological resemblance to the epidermal melanocytes in mammals)

The situation is by no means clear when the reactions of epidermal melanophores in white/black-adapted animals on transfer to darkness, are considered (Table 33, p.199). Their reactions, in contrast to those of lower dermal melanophores, appeared to be generally erratic, especially so in intact animals and in chromatically spinal animals (Table 33). However, when the transitions white-to-darkness and black-to-darkness are considered separately, the situation becomes less confusing. That (i) in white-to-darkness transition, these melanophores generally remain aggregated in darkness (Table 33, p. 199) (an exception being the chromatically spinal white-adapted animals); (ii) in black-to-darkness transition (excepting in chromatically spinal animals) and (iii) in enucleated animals (Table 33) these melanophores not only show aggregation of pigment in darkness but also appear to exhibit faster rate of aggregation than that in dermal melanophores - these observations appear to confirm conclusions already arrived at (p.113) that thepituitary gland does not actively participate in co-ordinating melanophore responses to darkness. At the same time, it tends to suggest that in darkness, at least in epidermal melanophores, the tonic aggregating influence of the CNS is not removed. However, this suggestion does not seem to be applicable to the lower dermal melanophores, which in darkness assume an intermediate condition between full dispersion to complete aggregation (see also pp.190, 191).

Table 33. Showing comparison of the rates of aggregation /dispersion of epidermal melanophores with those of dermal melanophores in different transitions

Transition	Intact	Hypophysectomised	Chromatically spinal	Pinealectomised
White-to-black	slower	slower	slower	slower
Black-to-white	faster	faster	faster	faster
White-to-darkness	slower but erratic, mean MI 1.90 after 7 days	slower, mean MI 1.25 after 2 hr.	slightly faster but erratic, mean MI 2.75 after 7 days	slower, mean MI 1.50 after 8 hr.
Darkness-to-white	slower	slower	slower	slower
Black-to-darkness	erratic but faster, mean MI 2.74 after 7 days	faster, mean MI 1.32 after 6 hr.	faster, mean MI 3.1 after 10 days	faster, mean MI 1.33 after 7 days
Darkness-to-black	slightly faster	faster	faster	faster
Illuminated background-to-darkness*	faster, mean MI 2.85 after 7 days	not observed	not observed	faster, mean MI 1.85 after 8 days
Darkness-to-illuminated background*	faster	not observed	not observed	faster

* Blinded animals.

Thus behaviour and control of epidermal melanophores definitely requires further investigation on the lines already suggested for melanophores in general (p. 191).

Finally, the present work in general confirms von Frisch's (1911) conclusion, that pigment dispersion results from the release of melanophores from the central aggregating control, and this in this species is augmented by MSH. (The suggestions of Fujii & Novales have already been discussed, (pp. 25-26)). It is not consistent with the views held by Parker (p. 42) on the control of melanophores in _A_. _nebulosus_.

XII. SUMMARY

New contributions to the field of study are denoted thus:*

Section IV

1. General experimental treatment of the catfish is described (pp. 49-50).

2.* An apparatus for observing colour change reactions was designed (pp. 50-52).

3. The site for recording the pigmentary changes is described (p. 53).

4.* The melanophores in the skin of _I_. _melas_ are arranged in 3 layers, one layer being in the epidermis, and two in the dermis. The epidermal melanophores are the smallest and those of the lower dermis are the largest. The shape of epidermal melanophores is markedly different from that of the other melanophores (pp. 53-58)

5. A melanophore index for the assessment of changes in

melanophore-pigment was devised (pp. 56-58).

6. Average dimensions of melanophores at different MI values are shown (p. 58).

Section V.

7.* An intact fish takes about 5 hr. for near-adaptation on white/black illuminated backgrounds. Complete adaptation after black/white background reversal requires 6-8 hr., the process being initially fast but slower later (p. 62).

8.* On black/white background reversal the pigment in epidermal melanophores disperses more slowly and becomes aggregated more rapidly than that of the upper and lower dermal melanophores (pp.62-64).

9.* On adaptation to different shades of grey the epidermal melanophores show less pigment dispersion than those of the dermis (p.77).

10.* The intact as well as the blinded catfish equilibrate at almost the same MI in darkness in 6-8 hr. and in 8-24 hr. respectively. The pigment in the melanophores of illuminated blinded fish is fully dispersed (pp. 80-83).

11.* Comparison with the time-relations of other species of fishes shows that I. melas in its chromatic physiology occupies a position between the species in which the melanophores are predominantly neurally controlled and those in which they are mainly hormonally co-ordinated (pp. 84-90).

Section VI

12. The technique of hypophysectomy is described (pp.92-96).

13. The equilibration of melanophores on white/black illuminated background following hypophysectomy requires 4-10 days (pp. 97-98).

14. Hypophysectomy results in degeneration of melanophores and fading of pigment (p.99).

15. Hypophysectomy considerably impairs the ability of I. melas to adapt to a black background but apparently has no effect on adaptation to a white background (pp.100-101).

16.* The removal of the pituitary affects the dispersion of different layers of integumentary melanophores to a different degree when the animal is on an illuminated black background. Of the 3 layers the deepest layer (lower dermal melanophores) is the least affected, the mean MI of the melanophores being 3.65, whereas the superficial layer (epidermal melanophores) is severely affected, the melanophores remaining at a mean MI 2.4. The upper dermal melanophores have a mean MI 2.9 (p.103).

17.* Hypophysectomy not only limits the degree of pigment dispersion on a black background but also prolongs the time required by a previously white-adapted fish to reach it. On the other hand, the initial rate of aggregation of pigment on a white background appears to be accelerated (pp. 107-108).

18.* In hypophysectomised animals the adaptation from white-to-black and reversal takes ca. 4 hr. within the limits now possible (no's 15 and 16) (p.101).

19.* Comparison of hypophysectomised with intact fish indicates that in the latter the initiation of the dispersion response of melanophores as well as the initial phases of dispersion are independent of the pituitary (p.106).

20.* Of all the layers of melanophores, the epidermal melanophores appear to be mainly under the influence of the pituitary for their dispersion (p.108).

21.* The melanophores in hypophysectomised catfish react differently when the animal is on backgrounds of different greys and these reactions are independent of the pituitary (pp. 109-110).

22. The pituitary gland appears to play an insignificant role in regulating the responses of melanophores to darkness as (a) the melanophores in hypophysectomised animals equilibrate in ca. the same time as in intact animals and (b) the equilibrium mean MI in the former shows a statistically insignificant difference from that in the latter (p.113).

23. The pigment in the melanophores of blinded hypophysectomised animals is dispersed in light, the extent of dispersion being similar to that observed in hypophysectomised black-adapted animals with eyes intact (p.113).

24. Injection of pituitary extract of white-adapted catfish into hypophysectomised catfish causes dispersion of pigment (pp. 114-115).

Section VII

25.* The pigmento-motor fibres controlling the aggregation of integumentary melanophores in Ictalurus follow essentially the same path as in Phoxinus (pp.124-125).

26. The equilibration of melanophores following anterior spinal section on a white background generally requires 6-8 weeks (pp. 127-128).

27.* The pituitary appears to contribute to the dispersion of the pigment following the transection of the anterior spinal cord as well as to the subsequent maintenance of dispersion (pp. 140-142).

28.* All melanophore layers of chromatically spinal fish equilibrated on an illuminated white background (mean MI's ca. 1.9, 1.5 and 1.2 for lower dermal, upper dermal and epidermal melanophores respectively) fully disperse pigment in ca. 24 hr. on transfer to a black background. On reversal the white-equilibrated condition is obtained (pp. 129-131).

29.* In both transitions (No. 28) the different melanophore layers exhibit differential responses, the epidermal melanophores being slower to disperse pigment and faster to aggregate it than the upper and lower dermal ones (p.131).

30.* The pituitary appears to be active in the dispersion of pigment after the reflection of the background falls below 4.5% (pp. 134-135).

31.* No pituitary aggregating hormone (MCH) is apparently participating in the pigment movements of either the spinal or intact fish (pp. 135-138).

32.* Melanophores of hypophysectomised-spinal fish show no reaction to illuminated background, remaining at mean MI's ca. 3.0, 2.0 and 1.0 for lower dermal, upper dermal and epidermal melanophores respectively. Even enucleation does not cause any change in them (pp. 138-140).

33.* There are no indications that any active pigment-dispersing nervous mechanism is involved in the melanophore responses of _I. melas_, (pp. 129-131, 141-142).

Section VIII

34. A method to interrupting the hypothalamo-hypophyseal tract or the denervating of the pars intermedia is described (pp. 148-149).

35.* The melanophores in white-adapted catfish on denervation of the pars intermedia generally become fully dispersed and the fish loses the ability to adapt to a white background (pp. 149-152).

36.* There is a gradual aggregation of pigment in the operated fish on a white background, and slow recovery of its ability to adapt to a white background, black background adaptation, however, remaining unaffected, (pp. 149-152).

37.* About 4 months after the operation, the operated fish appear to be capable of aggregating melanophore-pigment on a white background, presumably due to regeneration and re-establishment of the central control on the pars intermedia (p. 152).

38.* The results suggest that in I. melas, as in elasmobranchs and amphibians, the central control on the pars intermedia function is of an inhibitory nature (p.152).

Section IX

39. A method for extirpation of the pineal is described (pp. 158-160).

40.* There are no indications that the pineal plays any significant role in the pigmentary effector system of the catfish I. melas (pp. 162-169).

41.* Pinealectomy does not in any way affect the ability of the operated animals to adapt to illuminated white or black backgrounds (pp. 162-163).

42.* The melanophores in pinealectomised blinded specimens as well as in pinealectomised but otherwise intact specimens require a longer time than in unoperated animals to attain equilibration to darkness. However, there is close similarity in the equilibrium MI's (of ldm) in the two groups and the significance of this time difference is not known (pp. 164-166).

43.* The dispersion of melanophores in the blinded specimens kept in illumination is not affected by pinealectomy and their ability to aggregate pigment in darkness remains unimpaired (pp. 166-169).

44.* Epidermal melanophores in white-adapted pinealectomised animals show slight dispersion in darkness, whereas those in black-adapted pinealectomised and enucleated pinealectomised specimens show a gradual aggregation throughout their stay in darkness (pp. 166-169).

45. The suggestions that the darkness-induced blanching response in lower vertebrates is under the direct control of the pineal and its assumed hormone and that the pineal also controls white background adaptation and the evidence cited in support of these suggestions have been examined (pp. 169-175).

Section X

46* The epidermal melanophores in isolated skin preparations in *I. melas* on being equilibrated in Ringer generally remain almost fully aggregated whereas the dermal melanophores assume an intermediate state, their mean MI's being ca. 1.5 and 2.9 respectively (p.178).

47. The catechol amines noradrenaline and adrenaline cause pronounced aggregation of pigment. Sympathomimetic amines - isoprenaline and tyramine cause some aggregation (pp. 178-180).

48.* Ergotamine appears to produce a distinct aggregating effect and reverse the noradrenaline effect (pp. 179-182).

49.* Of the adrenergic blocking agents and anti-adrenaline substances, bretylium apparently causes no change, regitin, guanethidine, and reserpine produce negligible dispersion,

while yohimbine appears to have a slight dispersing effect on dermal melanophores. In distinction, all these agents excepting bretylium apparently cause moderate to distinct dispersion of pigment in epidermal melanophores. Dispersing effects of all these agents are variably reversed by noradrenaline (pp. 180, 182, 187).

50.* Hexamethonium and atropine have some dispersion effect (pp. 180, 184, 189).

51.* Acetylcholine itself causes slight dispersion of pigment but in combination with eserine has little effect. Eserine and pilocarpine cause some dispersion which is distinctly reversed by noradrenaline (pp. 183, 188).

52.* Serotonin causes moderate and melatonin appears to cause marked aggregation of dermal melanophores (pp. 181,189,190).

53. The results in general are consistent with the conclusion that the nervous control of melanophores in _I. melas_ is only adrenergic and apparently have not provided any evidence that pigment dispersion involves cholinergic fibres (p. 183, 188).

54. The results obtained with drugs are only tentative and need confirmation from the effects of administration of these drugs in intact animals (p. 192).

Section XI.

55.* The results of the present investigation can be explained on the assumption of one hormone and mononeuronic innervation of the melanophores (pp. 191-200).

56.* Since the relationship of the pituitary and aggregating nerve fibres in the control of epidermal melanophores is apparently not the same as in the control of the dermal melanophores it is suggested that the epidermal melanophores differ from the dermal melanophores in having different threshold levels of response to MSH and noradrenaline, (pp. 196-200).

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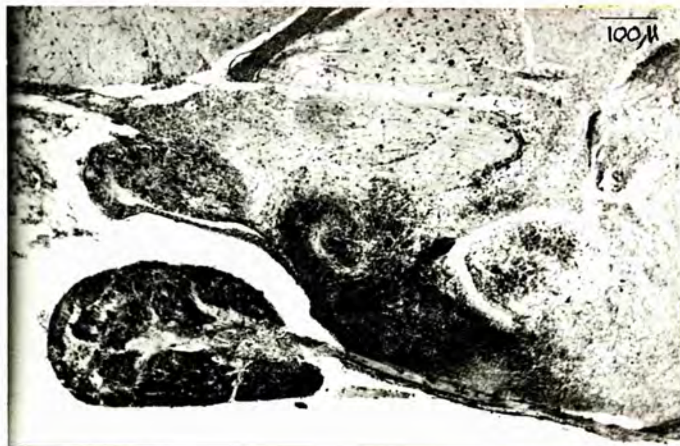
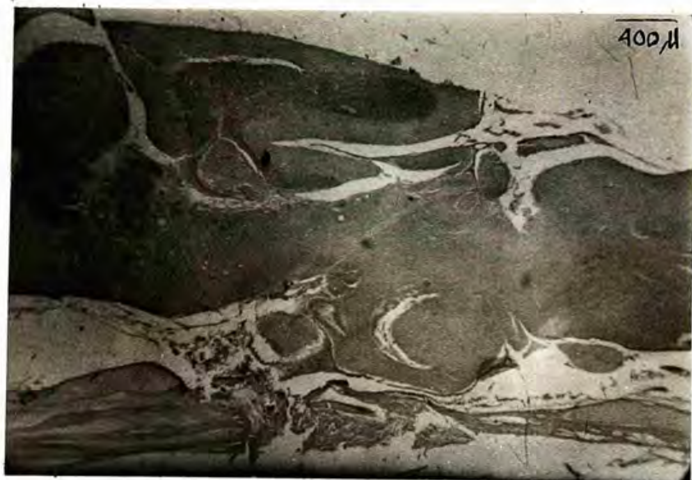
A P P E N D I X

Fig. 26 Photomicrographs showing (a) intact pituitary in *I. melas* and (b) the extent of removal of the pituitary in an experimental fish which lived for 21 days after the operation. (Fixed Bouin).



a



b

Fig. 30 Photomicrograph showing the extent of removal of the pituitary in a hypophysectomised spinal fish. The fish was hypophysectomised 24 hr following anterior spinal section and was killed 40 days after hypophysectomy. (Fixed hot water temp. ca. 70°C, and Bouin).

Fig. 34 Photomicrograph of the skin of blinded spinal hypophysectomised fish showing general condition of melanophores. The fish previously being white-adapted was spinal sectioned and kept on a white background for 48 days. Thereafter it was hypophysectomised and blinded 40 days after hypophysectomy. After 3 days on an illuminated background it was killed. (Fixed hot water temp. ca. 70°C. Bouin).

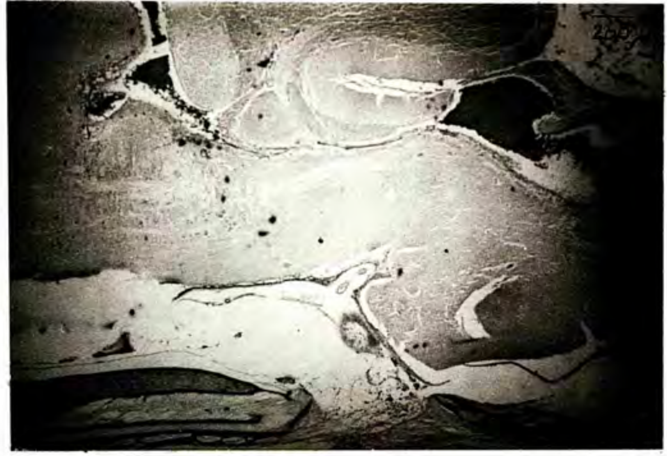


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Fig. 31b. Melanophore responses of 4-6 month black-adapted unoperated intact *I. melas* on transfer to an illuminated white background and reversal after 24 hr. Each point with SD's as vertical bars is the mean of 6 animals. 20 $\pm 1^{o}$C. 40 W at 75 cm. Open circles = epidermal, open triangles = upper dermal and solid circles = lower dermal melanophores.

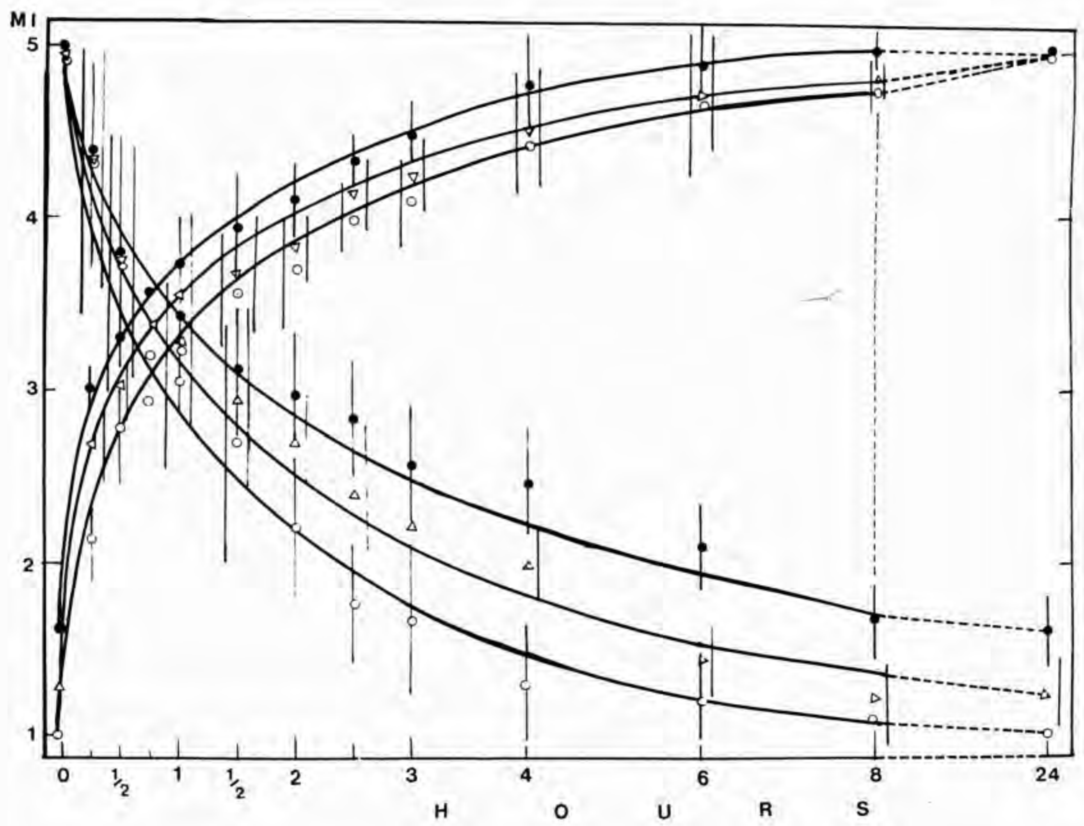

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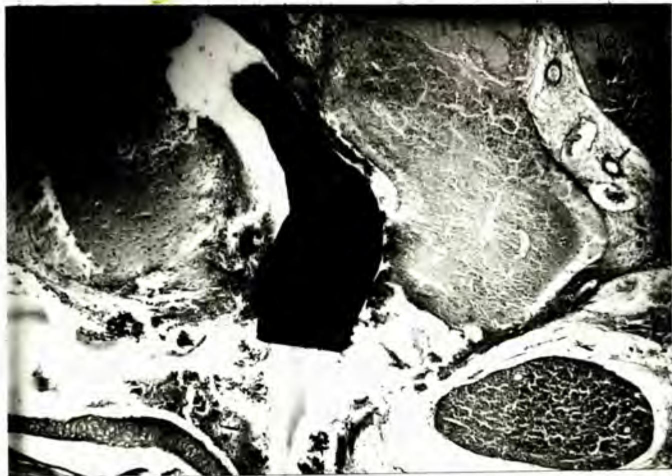
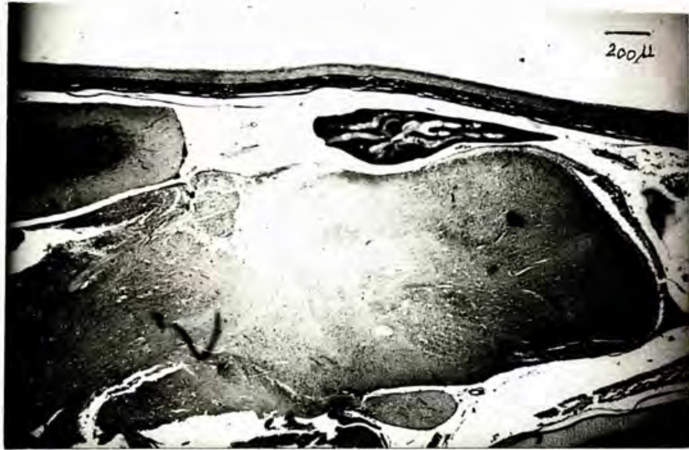
Fig. 35 Photomicrographs showing interruption of hypothalamo-hypophyseal tract by plastic barrier in white-adapted *I.melas*. Fig. 35(a) is the sagittal section of the head of the experimental fish which lived for 50 days after the operation. Fish (Fig. 35(b) lived for 16 days following the operation. (Fixed Bouin. PAS and lead Haematoxylin).

a

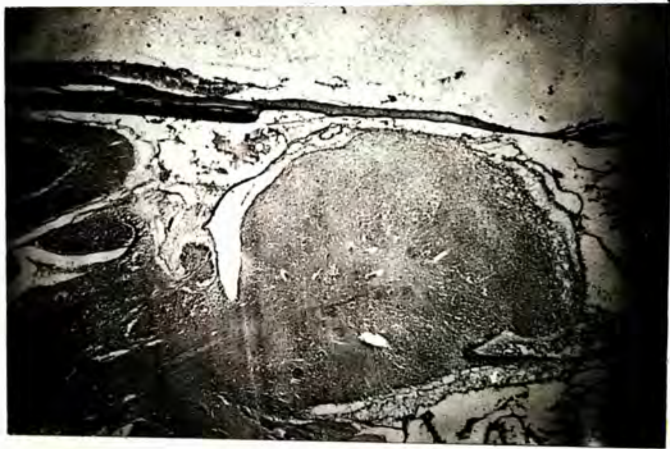


b

Fig.36 Photomicrographs showing (a) intact pineal in *I.melas* and (b) the extent of removal of the pineal in an experimental fish that died about 3 months following the operation (Fixed Bouin).



a



b

Table 2. Mean MI's with SD's of I. melas transferred from equilibrium on an illuminated white background to an illuminated black background and reversal after 24 hr. (more than 15 animals). Temp. 20±1°C. Overhead illumination 40 W lamp at 75 cm. Ldm = lower dermal, udm = upper dermal, edm = epidermal melanophores.

Transition	Time	Mean MI's		
		ldm	udm	edm
White-to-black	0 hr	1.50 ±0.28	1.26 ±0.29	1.10 ±0.14
	15 min	3.15 ±0.29	2.81 ±0.39	2.74 ±0.50
	30 min	3.61 ±0.31	3.40 ±0.42	3.31 ±0.43
	$\frac{3}{4}$ hr	3.82 ±0.20	3.60 ±0.39	3.50 ±0.46
	1 hr	4.10 ±0.26	3.83 ±0.38	3.70 ±0.40
	$1\frac{1}{2}$ hr	4.28 ±0.29	4.16 ±0.38	4.00 ±0.28
	2 hr	4.40 ±0.29	4.30 ±0.36	4.20 ±0.28
	$2\frac{1}{2}$ hr	4.50 ±0.30	4.37 ±0.35	4.30 ±0.33
	3 hr	4.60 ±0.29	4.50 ±0.33	4.40 ±0.39
	4 hr	4.74 ±0.23	4.62 ±0.29	4.54 ±0.32
	5 hr	4.90 ±0.12	4.80 ±0.18	4.63 ±0.31
	6 hr	4.94 ±0.13	4.88 ±0.13	4.77 ±0.17
	8 hr	5.00	4.94 ±0.10	4.94 ±0.15
	24 hr	5.00	5.00	5.00
Reversal	(0 hr)			
	15 min	3.40 ±0.30	3.22 ±0.34	3.19 ±0.47
	30 min	3.16 ±0.42	2.80 ±0.50	2.66 ±0.53
	$\frac{3}{4}$ hr	2.85 ±0.50	2.60 ±0.50	2.40 ±0.60
	1 hr	2.66 ±0.36	2.32 ±0.37	2.13 ±0.50
	$1\frac{1}{2}$ hr	2.40 ±0.49	2.16 ±0.38	1.83 ±0.56
	2 hr	2.21 ±0.35	1.90 ±0.30	1.61 ±0.42
	$2\frac{1}{2}$ hr	2.05 ±0.22	1.80 ±0.14	1.50 ±0.15
	3 hr	2.00 ±0.43	1.77 ±0.34	1.45 ±0.41
	4 hr	1.83 ±0.35	1.55 ±0.38	1.30 ±0.25
	5 hr	1.77 ±0.34	1.46 ±0.40	1.20 ±0.20
	6 hr	1.70 ±0.28	1.48 ±0.36	1.20 ±0.20
	8 hr	1.55 ±0.28	1.35 ±0.24	1.15 ±0.18
	24 hr	1.47 ±0.30	1.30 ±0.24	1.10 ±0.17

Table 3. Mean MI's with SD's of *I. melas* transferred from equilibrium on an illuminated black background to an illuminated white background and reversal after 24 hr (15 animals). Temp. 20 ±1°C. Overhead illumination 40 W lamp at 75 cm. Ldm = lower dermal, udm = upper dermal, edm = epidermal melanophores.

Transition	Time	Mean MI's ldm	udm	edm
Black-to-white	0 hr	5.00	5.00	5.00
	15 min	3.70 ±0.27	3.50 ±0.30	3.14 ±0.45
	30 min	3.20 ±0.38	3.10 ±0.40	2.82 ±0.40
	¾ hr	2.90 ±0.35	2.60 ±0.45	2.52 ±0.48
	1 hr	2.62 ±0.33	2.33 ±0.34	2.36 ±0.43
	1½ hr	2.40 ±0.40	2.14 ±0.40	1.96 ±0.35
	2 hr	2.12 ±0.24	1.80 ±0.35	1.60 ±0.35
	2½ hr	1.95 ±0.30	1.67 ±0.28	1.42 ±0.32
	3 hr	1.83 ±0.30	1.57 ±0.30	1.30 ±0.27
	4 hr	1.70 ±0.21	1.46 ±0.29	1.24 ±0.30
	5 hr	1.68 ±0.24	1.40 ±0.37	1.08 ±0.30
	6 hr	1.57 ±0.21	1.32 ±0.20	1.10 ±0.10
	8 hr	1.48 ±0.12	1.25 ±0.10	1.10 ±0.15
	24 hr	1.40 ±0.28	1.20 ±0.22	1.10 ±0.19
Reversal	(0 hr)			
	15 min	3.33 ±0.42	2.90 ±0.46	2.76 ±0.42
	30 min	3.71 ±0.35	3.33 ±0.37	3.30 ±0.43
	¾ hr	3.93 ±0.42	3.54 ±0.37	3.40 ±0.41
	1 hr	4.06 ±0.29	3.76 ±0.37	3.57 ±0.45
	1½ hr	4.22 ±0.34	4.00 ±0.30	3.87 ±0.24
	2 hr	4.33 ±0.23	4.18 ±0.20	4.10 ±0.27
	2½ hr	4.52 ±0.20	4.35 ±0.17	4.16 ±0.22
	3 hr	4.60 ±0.24	4.47 ±0.30	4.32 ±0.26
	4 hr	4.80 ±0.17	4.62 ±0.27	4.38 ±0.29
	5 hr	4.90 ±0.17	4.80 ±0.25	4.65 ±0.25
	6 hr	4.94 ±0.12	4.92 ±0.12	4.74 ±0.15
	8 hr	5.00 ±0.12	4.98 ±0.12	4.94 ±0.15
	24 hr	5.00	5.00	5.00

Table 5. Mean MI's with SD's of I. melas transferred from equilibrium on an illuminated white background to darkness and reverse after 7 days (8 animals). Temp. 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	Mean MI's edm
White-to-darkness	0 hr	1.45 ±0.27	1.00
	30 min	2.48 ±0.34	1.24 ±0.39
	1 hr	2.81 ±0.34	1.46 ±0.63
	1½ hr	3.03 ±0.10	1.91 ±0.77
	2 hr	3.10 ±0.17	1.90 ±0.78
	3 hr	3.06 ±0.10	1.50 ±0.63
	4 hr	3.16 ±0.26	1.65 ±0.59
	6 hr	3.16 ±0.28	1.91 ±0.66
	8 hr	3.15 ±0.28	1.90 ±0.94
	24 hr	3.15 ±0.55	2.57 ±0.71
	2 day	3.17 ±0.24	2.48 ±0.68
	7 day	3.15 ±0.45	1.90 ±0.95
Reversal	(0 hr)		
On exposure to light	1-5 min	3.32 ±0.37	2.92 ±0.38
	½ hr	2.31 ±0.36	1.40 ±0.35
	1 hr	2.08 ±0.42	1.40 ±0.31
	1½ hr	1.95 ±0.45	1.35 ±0.30
	2 hr	1.86 ±0.41	1.40 ±0.39
	3 hr	1.72 ±0.40	1.26 ±0.30
	4 hr	1.66 ±0.45	1.30 ±0.22
	6 hr	1.57 ±0.33	1.22 ±0.18
	8 hr	1.52 ±0.22	1.15 ±0.20
	24 hr	1.40 ±0.23	1.00

Table 6. Mean MI's with SD's of I. melas transferred from equilibrium on an illuminated background to darkness and reversal after 7 days (10 animals). 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores.

Transition	Time	Mean MI's ldm	Mean MI's edm
Black-to-darkness	0 hr	5.00	5.00
	½ hr	4.10 ±0.26	3.50 ±0.45
	1 hr	3.90 ±0.10	3.38 ±0.65
	1½ hr	3.77 ±0.17	3.23 ±0.52
	2 hr	3.61 ±0.39	3.22 ±0.49
	3 hr	3.42 ±0.20	2.85 ±0.64
	4 hr	3.33 ±0.20	2.90 ±0.47
	6 hr	3.26 ±0.20	2.95 ±0.46
	8 hr	3.20 ±0.24	2.78 ±0.52
	24 hr	3.17 ±0.30	2.64 ±0.70
	7 days	3.17 ±0.44	2.74 ±0.67
Reversal	0 hr		
On exposure to light	1-5 min	3.78 ±0.36	3.34 ±0.59
	½ hr	4.16 ±0.43	3.82 ±0.66
	1 hr	4.40 ±0.42	4.18 ±0.40
	1½ hr	4.58 ±0.43	4.42 ±0.45
	2 hr	4.66 ±0.20	4.44 ±0.30
	3 hr	4.77 ±0.25	4.62 ±0.20
	4 hr	4.81 ±0.16	4.67 ±0.23
	6 hr	4.90 ±0.11	4.72 ±0.20
	8 hr	4.98 ±0.16	4.90 ±0.15
	24 hr	5.00	5.00

Table 7. Mean MI's with SD's of blinded I. melas from equilibration on an illuminated background to darkness and reversal after 7 days (6 animals) 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	edm
On transfer to darkness	0 hr	5.00	5.00
	1 hr	4.70 ±0.52	4.60 ±0.56
	2 hr	3.86 ±0.69	3.83 ±0.24
	4 hr	3.73 ±0.61	3.83 ±0.23
	6 hr	3.40 ±0.42	2.82 ±0.54
	24 hr	3.22 ±0.53	3.10 ±0.41
	7 days	3.20 ±0.32	2.85 ±0.37
Reversal on exposure to light	0 hr		
	1-5 min	4.32 ±0.68	3.66 ±0.84
	1 hr	4.76 ±0.48	4.50 ±0.78
	2 hr	4.83 ±0.37	4.94 ±0.24
	3 hr	4.91 ±0.18	5.00
	4 hr	4.94 ±0.15	5.00
	6 hr	4.96 ±0.17	5.00
	24 hr	5.00	5.00

Table 11. Mean MI's with SD's of hypophysectomised I. melas transferred from equilibrium on an illuminated white background to an illuminated black background and reversal after 48 hr. (15 animals). 20 ±1°C. 40 W at 75 cm. Ldm= lower dermal, udm = upper dermal, edm = epidermal melanophores.

Transition	Time	Mean MI's ldm	udm	edm
White-to-black	0 hr	1.74 ±0.30	1.25 ±0.31	1.00 ±0.00
	30 min	3.24 ±0.47	2.38 ±0.51	1.90 ±0.50
	1 hr	3.40 ±0.44	2.57 ±0.46	2.05 ±0.56
	1½ hr	3.52 ±0.41	2.65 ±0.12	2.15 ±0.57
	2 hr	3.55 ±0.38	2.80 ±0.44	2.28 ±0.64
	3 hr	3.58 ±0.36	2.87 ±0.45	2.31 ±0.61
	4 hr	3.63 ±0.33	2.90 ±0.20	2.40 ±0.56
	6 hr	3.63 ±0.31	2.89 ±0.26	2.40 ±0.52
	8 hr	3.63 ±0.33	2.90 ±0.22	2.40 ±0.48
	24 hr	3.63 ±0.40	2.90 ±0.40	2.42 ±0.66
	48 hr	3.63 ±0.42	2.90 ±0.40	2.42 ±0.66
Reversal	(0 hr)			
	30 min	2.62 ±0.38	2.15 ±0.46	1.32 ±0.27
	1 hr	2.48 ±0.42	1.95 ±0.49	1.25 ±0.24
	1½ hr	2.31 ±0.38	1.73 ±0.29	1.15 ±0.25
	2 hr	2.20 ±0.36	1.65 ±0.40	1.10 ±0.25
	3 hr	2.10 ±0.34	1.56 ±0.37	1.00 ±0.20
	4 hr	2.02 ±0.37	1.50 ±0.35	1.00 ±0.20
	6 hr	1.95 ±0.26	1.47 ±0.40	1.00 ±0.20
	8 hr	1.92 ±0.15	1.42 ±0.40	1.00
	24 hr	1.84 ±0.25	1.40 ±0.31	1.00
	48 hr	1.78 ±0.33	1.35 ±0.32	1.00

Table 12. Mean MI's with SD's of hypophysectomised I. melas transferred from equilibrium on an illumunated black background to an illuminated white background and reversal after 48 hr (15 animals) 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, udm = upper dermal, edm = epidermal melanophores

Transition	Time	ldm	udm	edm
			Mean MI's	
Black-to-White	0 hr	3.67 ±0.46	3.05 ±0.70	2.44 ±0.58
	30 min	2.70 ±0.46	2.30 ±0.52	1.42 ±0.44
	1 hr	2.43 ±0.53	2.10 ±0.43	1.24 ±0.25
	1½ hr	2.34 ±0.49	1.84 ±0.45	1.20 ±0.29
	2 hr	2.24 ±0.45	1.77 ±0.43	1.14 ±0.26
	3 hr	2.18 ±0.41	1.57 ±0.46	1.07 ±0.24
	4 hr	2.12 ±0.42	1.53 ±0.42	1.00
	6 hr	2.10 ±0.40	1.51 ±0.39	1.00
	8 hr	2.00 ±0.11	1.42 ±0.36	1.00
	24 hr	1.87 ±0.35	1.35 ±0.28	1.00
	48 hr	1.80 ±0.25	1.30 ±0.25	1.00
Reversal	(0 hr)			
	30 min	3.15 ±0.45	2.33 ±0.33	2.00 ±0.44
	1 hr	3.26 ±0.42	2.57 ±0.55	2.17 ±0.67
	1½ hr	3.30 ±0.51	2.60 ±0.50	2.26 ±0.61
	2 hr	3.37 ±0.42	2.72 ±0.48	2.28 ±0.61
	3 hr	3.48 ±0.37	2.86 ±0.58	2.35 ±0.63
	4 hr	3.55 ±0.35	2.97 ±0.55	2.38 ±0.58
	6 hr	3.62 ±0.39	3.05 ±0.56	2.40 ±0.64
	8 hr	3.62 ±0.33	3.05 ±0.43	2.42 ±0.57
	24 hr	3.62 ±0.43	3.05 ±0.60	2.42 ±0.47
	48 hr	3.62 ±0.41	3.05 ±0.56	2.42 ±0.50

Table 14. Mean MI's with SD's/ for ldm of hypophysectomised I.melas transferred from equilibrium on an illuminated white background to darkness and reversal after 7 days (8 animals) 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal melanophores, edm = epidermal melanophores.

Transition	Time	Mean MI's ldm	edm
White-to-Darkness	0 hr	1.67 ±0.48	1.00
	30 min	2.30 ±0.68	1.33
	1 hr	2.38 ±0.64	1.28
	2 hr	2.50 ±0.43	1.28
	3 hr	2.60 ±0.39	1.25
	4 hr	2.60 ±0.46	1.25
	6 hr	2.75 ±0.30	1.25
	8 hr	2.74 ±0.28	1.25
	24 hr	2.75 ±0.29	1.29
	48 hr	2.74 ±0.30	1.25
	7 days	2.74 ±0.28	1.25
Reversal On exposure to light	(0 hr)		
	1-5 min	2.90	
	30 min	2.54 ±0.50	1.25
	1 hr	2.34 ±0.48	1.22
	2 hr	2.16 ±0.54	1.21
	3 hr	2.03 ±0.57	1.10
	4 hr	1.93 ±0.64	1.00
	6 hr	1.93 ±0.64	1.00
	8 hr	1.89 ±0.42	1.00
	24 hr	1.82 ±0.39	1.00

Table 15. Mean MI's with SD's for ldm of hypophysectomised I. melas transferred from equilibrium on an illuminated black background to darkness and reversal after 7 days (10 animals). 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	edm
Black-to-Darkness	0 hr	3.58 ±0.43	2.60
	30 min	2.98 ±0.32	2.08
	1 hr	2.82 ±0.33	1.61
	2 hr	2.78 ±0.41	1.50
	3 hr	2.80 ±0.28	
	4 hr	2.77 ±0.32	1.40
	6 hr	2.75 ±0.27	1.32
	8 hr	2.75 ±0.25	1.35
	24 hr	2.75 ±0.20	1.35
	48 hr	2.77 ±0.24	1.35
	7 days	2.77 ±0.32	1.32
Reversal on exposure to light	(0 hr)		
	1-5 min	3.30	2.49
	30 min	3.45 ±0.48	2.80
	1 hr	3.67 ±0.40	3.01
	2 hr	3.61 ±0.37	2.90
	3 hr	3.60 ±0.38	
	4 hr	3.58 ±0.41	2.90
	6 hr	3.58 ±0.44	2.80
	8 hr	3.58 ±0.42	2.60
	24 hr	3.60 ±0.48	2.62

Table 18. Mean MI's with SD's of chromatically spinal *I. melas* transferred from equilibrium on an illuminated white background to an illuminated black background and reversal after 48 hr (15 animals). 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, udm = upper dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	udm	edm
White-to-Black	0 hr	1.93 ±0.37	1.48 ±0.40	1.21 ±0.30
	30 min	2.66 ±0.50	2.27 ±0.40	1.91 ±0.40
	1 hr	3.22 ±0.34	2.68 ±0.34	2.48 ±0.47
	2 hr	3.60 ±0.42	3.07 ±0.35	2.94 ±0.43
	3 hr	3.98 ±0.30	3.47 ±0.42	3.37 ±0.33
	4 hr	4.20 ±0.24	3.74 ±0.31	3.57 ±0.20
	6 hr	4.40 ±0.29	4.23 ±0.25	4.16 ±0.47
	8 hr	4.70 ±0.20	4.50 ±0.18	4.45 ±0.23
	24 hr	5.00 ±0.15	4.95 ±0.16	4.90 ±0.17
	48 hr	5.00 ±0.15	5.00 ±0,12	5.00 ±0.15
Reversal	(0 hr)			
	1 hr	4.84 ±0.22	4.76 ±0.36	4.58 ±0.51
	2 hr	4.74 ±0.28	4.50 ±0.36	4.39 ±0.59
	3 hr	4.54 ±0.36	4.26 ±0.48	4.15 ±0.62
	4 hr	4.42 ±0.33	4.15 ±0.46	3.92 ±0.63
	6 hr	4.27 ±0.47	3.87 ±0.57	3.70 ±0.66
	8 hr	4.09 ±0.37	3.74 ±0.50	3.45 ±0.54
	24 hr	3.62 ±0.36	3.25 ±0.47	2.96 ±0.47
	48 hr	3.09 ±0.62	2.70 ±0.55	2.56 ±0.45
	3 days	2.74 ±0.58	2.30 ±0.70	2.14 ±0.62
	4 days	2.48 ±0.59	2.11 ±0.68	1.92 ±0.55
	5 days	2.24 ±0.58	1.84 ±0.58	1.70 ±0.46
	6 days	2.12 ±0.59	1.70 ±0.58	1.54 ±0.45
	7 days	2.00 ±0.45	1.59 ±0.56	1.48 ±0.43
	10 days	1.87 ±0.60	1.40 ±0.47	1.27 ±0.45

Table 19. Mean MI's with SD's of chromatically spinal I. melas transferred from equilibrium on an illuminated black background to an illuminated white background and reversal after 10 days (15 animals) 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, udm = upper dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	udm	edm
Black-to-White	0 hr	5.00	5.00	5.00
	1 hr	4.94 ±0.17	4.94 ±0.14	4.94 ±0.14
	2 hr	4.88 ±0.21	4.86 ±0.24	4.84 ±0.28
	3 hr	4.77 ±0.39	4.75 ±0.37	4.74 ±0.40
	4 hr	4.71 ±0.48	4.70 ±0.53	4.65 ±0.49
	6 hr	4.49 ±0.50	4.42 ±0.51	4.38 ±0.61
	8 hr	4.34 ±0.62	4.29 ±0.63	4.21 ±0.72
	24 hr	3.90 ±0.69	3.66 ±0.65	3.46 ±0.74
	48 hr	3.27 ±0.70	2.91 ±0.59	2.58 ±0.75
	3 days	2.86 ±0.80	2.53 ±0.82	2.36 ±0.84
	4 days	2.62 ±0.72	2.18 ±0.78	1.96 ±0.86
	5 days	2.36 ±0.68	1.83 ±0.65	1.72 ±0.60
	6 days	2.18 ±0.69	1.66 ±0.67	1.56 ±0.56
	7 days	2.05 ±0.69	1.57 ±0.73	1.42 ±0.59
	10 days	1.96 ±0.74	1.35 ±0.56	1.28 ±0.51
Reversal	(0 hr)			
	1 hr	3.12 ±0.31	2.97 ±0.46	2.85 ±0.41
	2 hr	3.67 ±0.57	3.41 ±0.49	3.25 ±0.46
	3 hr	3.87 ±0.42	3.65 ±0.38	3.56 ±0.33
	4 hr	4.10 ±0.35	3.90 ±0.33	3.82 ±0.35
	6 hr	4.42 ±0.30	4.30 ±0.22	4.21 ±0.25
	8 hr	4.60 ±0.21	4.46 ±0.24	4.37 ±0.30
	24 hr	4.93 ±0.13	4.90 ±0.18	4.87 ±0.18

Table 21. Mean MI's with SD's of chromatically spinal *I. melas* previously equilibrated on an illuminated white background (for about 6 weeks) on transfer to darkness and reversal after 7 days (6 animals). 20 $\pm 1^{\circ}C$. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's: ldm	Mean MI's: edm
White-to-Darkness	0 hr	2.50 ±0.30	1.80 ±0.31
	1 hr	3.37 ±0.49	2.25 ±0.56
	2 hr	3.70 ±0.43	3.42 ±0.54
	4 hr	3.55 ±0.43	3.05 ±0.20
	6 hr	3.50 ±0.24	3.23 ±0.38
	24 hr	3.73 ±0.77	3.17 ±0.71
	7 days	3.60 ±0.31	2.75 ±0.66
Reversal	(0 hr)		
	1 hr	3.45 ±0.35	3.27 ±0.52
	2 hr	3.35 ±0.15	3.00 ±0.17
	4 hr	3.12 ±0.24	2.35 ±0.42
	6 hr	3.00 ±0.36	2.30 ±0.41
	8 hr	2.95 ±0.37	2.42 ±0.43
	24 hr	2.50 ±0.12	1.67 ±0.40
	48 hr	2.37 ±0.17	1.42 ±0.43

Table 22. Mean MI's with SD's of chromatically spinal I. melas previous equilibrated on an illuminated black background (for 7-8 weeks following the operation) on transfer to darkness and reversal after 10 days (7 animals) 20 ±1°C. 45 W at 75 cm. Ldm = Lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	edm
Black-to-Darkness	0 hr	5.00	5.00
	1 hr		
	4 hr	5.00	5.00
	6 hr	4.92 ±0.10	4.88 ±0.14
	8 hr	4.70 ±0.31	4.20 ±0.76
	24 hr	4.40 ±0.37	4.10 ±0.66
	48 hr	3.90 ±0.42	3.54 ±0.93
	3 days	3.86 ±0.43	3.62 ±0.41
	4 days	3.66 ±0.37	3.20 ±0.40
	5 days	3.66 ±0.45	3.18 ±0.50
	7 days		
	10 days	3.68 ±0.32	3.10 ±0.55
Reversal On exposure to light	(0 hr)		
	1-5 min	3.96	3.70
	1 hr	4.54 ±0.35	4.25 ±0.83
	2 hr	4.75 ±0.43	4.62 ±0.41
	3 hr	4.80 ±0.37	4.75 ±0.41
	4 hr	4.80 ±0.34	4.75 ±0.43
	6 hr	4.87 ±0.21	4.80 ±0.34
	24 hr	5.00	5.00

Table 25. Mean MI's with SD's of pinealectomised I.melas equilibrated on an illuminated white background (2-3 weeks following the operation) on transfer to an illuminated black background and reversal after 24 hr. (7 animals). 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	Mean MI's edm
White-to-Black	0 hr	1.53 ±0.26	1.00
	15 min	2.90 ±0.32	2.20 ±0.22
	30 min	3.32 ±0.42	2.65 ±0.60
	¾ hr	3.65 ±0.35	3.00 ±0.39
	1 hr	3.90 ±0.12	3.40 ±0.23
	1½ hr	4.07 ±0.15	3.57 ±0.27
	2 hr	4.16 ±0.10	3.76 ±0.24
	2½ hr	4.35 ±0.16	3.95 ±0.25
	3 hr	4.52 ±0.28	4.16 ±0.23
	4 hr	4.75 ±0.25	4.32 ±0.29
	6 hr	4.95 ±0.12	4.66 ±0.16
	24 hr	5.00	5.00
Black-to-White	(0 hr)		
	15 min	3.58 ±0.43	2.90 ±0.73
	30 min	3.17 ±0.48	2.50 ±0.79
	¾ hr	2.85 ±0.43	2.23 ±0.62
	1 hr	2.57 ±0.39	1.70 ±0.68
	1½ hr	2.20 ±0.54	1.33 ±0.55
	2 hr	2.17 ±0.51	1.30 ±0.55
	2½ hr	2.03 ±0.58	1.28 ±0.45
	4 hr	1.73 ±0.53	1.25 ±0.38
	6 hr	1.57 ±0.33	1.07 ±0.15
	24 hr	1.46 ±0.28	1.00 ±0.16

Table 26. Mean MI's with SD's of pinealectomised I.melas transferred from equilibrium on an illuminated black background to an illuminated white background and reversal after 24 hr (12 animals). 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores.

Transition	Time	Mean MI's ldm	Mean MI's edm
Black-to-White	0 hr	5.00	5.00
	15 min	3.66 ±0.47	3.26 ±0.62
	30 min	3.37 ±0.61	2.71 ±0.77
	1 hr	3.03 ±0.62	2.40 ±0.87
	1½ hr	2.83 ±0.57	1.87 ±0.83
	2 hr	2.56 ±0.50	1.80 ±0.70
	3 hr	2.51 ±0.60	1.54 ±0.59
	4 hr	2.32 ±0.60	1.37 ±0.50
	6 hr	2.18 ±0.56	1.26 ±0.43
	8 hr	1.90 ±0.57	1.20 ±0.40
	24 hr	1.70 ±0.55	1.12 ±0.22
Reversal	(0 hr)		
	15 min	3.17 ±0.22	2.60 ±0.42
	30 min	3.32 ±0.32	2.84 ±0.47
	¾ hr	3.50 ±0.36	3.12 ±0.48
	1 hr	3.83 ±0.22	3.40 ±0.45
	1½ hr	4.04 ±0.30	3.61 ±0.45
	2 hr	4.30 ±0.41	3.84 ±0.44
	3 hr	4.52 ±0.55	4.07 ±0.38
	4 hr	4.70 ±0.30	4.34 ±0.37
	6 hr	4.90 ±0.18	4.62 ±0.18
	8 hr	4.98 ±0.12	4.87 ±0.16
	24 hr	5.00	5.00

Table 27. Mean MI's with SD's of pinealectomised black adapted I. melas (operated 3-4 weeks earlier), transferred to darkness and reversal after 7 days (6 animals) 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	Mean MI's edm
White-to Darkness	0 hr	1.22 ±0.34	1.00 ±0.00
	30 min	1.74 ±0.21	1.08 ±0.12
	1 hr	1.94 ±0.61	1.23 ±0.33
	2 hr	2.10 ±0.53	1.33 ±0.47
	3 hr	2.20 ±0.42	1.33 ±0.47
	4 hr	2.27 ±0.52	1.33 ±0.47
	6 hr	2.45 ±0.55	1.25 ±0.43
	8 hr	2.68 ±0.56	1.50 ±0.61
	28 hr	2.75 ±0.43	1.50 ±0.43
	48 hr	2.80 ±0.25	1.67 ±0.43
	3 days	2.88 ±0.26	1.55 ±0.36
	4 days	2.83 ±0.20	1.55 ±0.36
	7 days	2.93 ±0.15	1.50 ±0.35
Reversal	0 hr		
	30 min	2.50 ±0.80	1.80 ±0.47
	1 hr	2.25 ±0.77	1.75 ±0.43
	2 hr	1.86 ±0.61	1.38 ±0.25
	3½ hr	1.68 ±0.73	1.12 ±0.22
	4½ hr	1.62 ±0.70	1.05 ±0.20
	6 hr	1.50 ±0.61	1.00 ±0.20
	8 hr	1.36 ±0.40	1.00
	24 hr	1.15 ±0.21	1.00

Table 28. Mean MI's with SD's of pinealectomised black-adapted I. melas (3-4 weeks following operation) transferred to darkness and reversal after 7 days (6 animals). $20 \pm 1^{\circ}C$. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's	
		ldm	edm
Black-to-Darkness	0 hr	5.00	5.00
	30 min	4.50 -0.41	4.10 -0.26
	1 hr	4.30 -0.37	3.97 -0.33
	2 hr	4.13 -0.50	3.73 -0.20
	3 hr	3.80 -0.50	3.30 -0.29
	4 hr	3.83 -0.53	3.17 -0.62
	6 hr	3.70 -0.66	3.13 -0.61
	8 hr	3.63 -0.61	3.23 -0.55
	24 hr	3.46 -0.41	2.57 -0.42
	48 hr	3.40 -0.43	2.57 -0.42
	3 days	3.37 -0.35	2.50 -0.41
	4 days	3.17 -0.20	2.07 -0.48
	7 days	3.07 -0.15	1.33 -0.47
Reversal	0 hr		
	30 min	3.75 -0.25	3.65 -0.37
	1 hr	4.10 -0.22	3.95 -0.25
	2 hr	4.40 -0.20	4.20 -0.20
	3 hr	4.50 -0.51	4.37 -0.18
	4 hr	4.65 -0.22	4.37 -0.18
	6 hr	4.77 -0.20	4.50 -0.24
	8 hr	4.84 -0.20	4.65 -0.30
	24 hr	5.00	5.00

Table 29. Mean MI's with SD's of pinealectomised I.melas transferred from equilibrium on an illuminated background to darkness and reversal after 8 days (8 animals) transferred to darkness about 4 weeks after enucleation. They were previously white-adapted, and were pinealectomised and white-adapted 1-4 months before being blinded, 20 ±1°C. 40 W at 75 cm. Ldm = lower dermal, edm = epidermal melanophores

Transition	Time	Mean MI's ldm	Mean MI's edm
Illuminated background to darkness	0 hr	4.94 -0.12	4.93 -0 17
	30 min	4.24 -0.70	4.00 -0.84
	1 hr	4.13 -0.68	3.67 -0.93
	2 hr	3.83 -0.65	3.18 -0.90
	3 hr	3.73 -0.59	3.20 -0.85
	4 hr	3.54 -0.40	3.14 -0.70
	6 hr	3.46 -0.45	2.82 -0.56
	24 hr	3.32 -0.32	2.40 -0.49
	48 hr	3.30 -0.27	2.80 -0.52
	3 days	3.24 -0.27	2.77 -0.76
	4 days	3.20 -0.20	2.28 -0.64
	8 days	3.10 -0.28	1.85 -0.60
Reversal	0 hr		
	30 min	3.97 -0.46	3.48 -0.70
	1 hr	4.00 -0.39	3.88 -0.23
	2 hr	4.20 -0.38	4.00 -0.53
	3 hr	4.40 -0.36	4.28 -0.41
	4 hr	4.45 -0.34	4.34 -0.61
	6 hr	4.73 -0.36	4.70 -0.23
	24 hr	4.87 -0.16	4.81 -0.30
	48 hr	5.00	5.00