THE PHYSIOLOGY OF COLOUR CHANGES IN THE MINNOW, (PHOXINUS PHOXINUS) WITH ~~SPECIAL~~ PARTICULAR REFERENCE TO THE PHARMACOLOGY OF THE MELANOPHORE-CONTROLLING FIBRES

A THESIS

submitted to the University of London

by

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in candidature for the degree of Doctor of Philosophy

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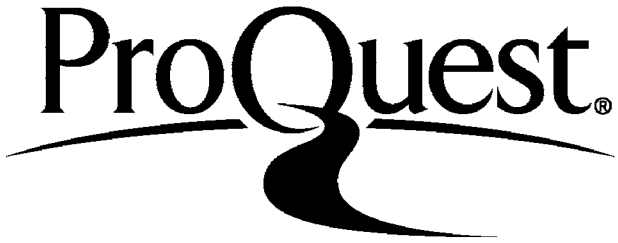


DECLARATION

The work presented in this thesis has not been accepted in substance for any other degree and is not concurrently being submitted in candidature for any other degree.

Signed ..David Grout.....
Candidate

Date ..10th March 1967.....

This is to certify that the work here submitted was carried out by the candidate himself. Due acknowledgement has been made for any assistance received.

Signed ..R. J. Dealey.
Supervisor

Date 13th March 1967

Abstract.

Changes in state of melanophores in the minnow, Phoxinus phoxinus (L)., which are known to be controlled by sympathetic paling fibres and pituitary paling hormone, were recorded macroscopically by comparison of the fish with standard grey papers. The effects of mammalian autonomic drugs on background adaptation were investigated by injections into intact, spinal sectioned and spinal nerve sectioned minnows. Adrenaline and noradrenaline, and to a lesser extent other sympathomimetic amines (ephedrine, tyramine, amphetamine, isoprenaline) were potent paling agents. Alpha-adrenergic blocking agents dispersed the melanophores. There was no evidence for antagonistic beta receptors. Hypotensive agents (reserpine, guanethidine, bretylium) abolished the nervously-coordinated background responses.

Changes in potency of catecholamines after lesions in the chromatic tract, cocaine and chronic treatment with hypotensive drugs suggest that these amines stimulate melanophores directly and are closely related to the peripheral transmitter. Suppresion of the actions of tyramine and amphetamine after cocaine and hypotensives implies that they act indirectly by displacing transmitter from an adrenergic store.

Muscarinic stimulants possesed little, if any, darkening action on the minnow. Muscarinic blocks antagonized fast colour changes and atropine exerted pronounced darkening activity. Nicotinic blockade affected the shade of fish: hexamethonium prevented paling but nicotine was strongly sympathomimetic.

It is concluded that aggregating fibres from the spinal cord pass to the sympathetic chain and are cholinergic. Their postganglionic counterparts are adrenergic and run in the spinal nerves. The adrenergic mediator (adrenaline, noradrenaline or dopamine) is stored at or near the nerve terminals and the indirect actions of sympathomimetic amines at this site are antagonized by cocaine, bretylium, reserpine and probably dibenamine. The adrenergic receptors are considered either as synergistic alpha and beta receptors or as primitive undifferentiated receptors.

There is evidence for an antagonistic set of darkening fibres which accompany the paling fibres to the periphery but they cannot with certainty be said to be cholinergic.

CONTENTS

Section 1.0 INTRODUCTION

Section 1.1 The autonomic nervous system in lower vertebrates

Anatomical, physiological and pharmacological studies have indicated that in mammals the autonomic nervous system acts through the interplay of the sympathetic and parasympathetic outflows (Langley 1921). In general, there has been a tendency to assume that the autonomic nervous system in lower vertebrates resembles this mammalian organisation. However, Goodrich (1927, 1930) pointed out that Langley's physiological criteria were difficult to apply to lower vertebrates, such as Petromyzon, where sympathetic fibres are morphologically different from those of mammals. Nicol (1952) used the terms "cranial", "thoracico-lumbar" and "sacral" outflows from the central nervous system to compare the organisation of the A.N.S. in lower vertebrates.

The studies by Young (1931a, 1933a, 1936) and Burnstock (1958a) on teleost fishes, using electrical stimulation and autonomic drugs, showed that autonomic control of the smooth muscle of the iris and alimentary canal differed from that of mammals. No antagonism between the parasympathetic and sympathetic innervation to

the gut could be demonstrated. In addition, although antagonistic autonomic control of the iris could be demonstrated in Uranoscopus scaber, the roles of the autonomic fibres are the opposite to the situation in mammals (Young, 1931b, 1933a). Recently Östlund and Fänge (1962) and Steen and Kruysse (1964) demonstrated that the blood vessels of the gills of many teleost fish are apparently under the control of ~~the~~ antagonistic adrenergic and cholinergic mechanisms (see sections 1.31 and 1.32). The latter workers were able to show that injected, blood-borne ink particles were diverted either into central lacunae of gill lamellae or into the peripheral respiratory capillaries by parasympathetic and sympathetic drugs respectively.

By far the greatest amount of work on teleost autonomic nervous systems has been carried out in relation to the pigmentary effector system. The chromatophores of many teleost fish are controlled to a greater or lesser extent by nerve fibres of autonomic origin. Under natural or experimental conditions, when fish with this system are subjected to changes in shade or colour of their substratum, obvious changes in the degree of pigment distribution within chromatophores occur, which may be recorded microscopically (Slome and Hogben, 1928 and Fig. 3 p.19) or

macroscopically (Healey, 1948).

Section 1.2 Colour changes in teleost fish

Section 1.21 The eye and fish colour change

The eye has long been known to be the important receptor organ in the adaption of fish to different coloured backgrounds. Von Frisch (1911) carried out experiments which suggested that the retina of the trout was differentiated into lower and upper regions and that these regions mediated the response to dark and light tinted backgrounds respectively. Sumner and Keys (1929) and Sumner (1933) showed that the retinae of Rhomboidichthys and Fundulus were similarly differentiated and that colour changes were dependent on the ratio of intensity of illumination of the upper and lower parts of the visual field. Hogben and Slome (1936) presented Xenopus with tanks in which the sides, top or bottom of the tank could be light or dark. They, too, were able to conclude that the overall tint of the horned toad depended on the illumination of different parts of the retina. Butcher and Adelmann (1937) extended Sumner's observations on Fundulus and the eye of Gasterosteus was studied by Hogben and Landgrebe (1940) (Fig.1; p.17)

Butcher (1937a, b; 1938a, b; 1939) related the dispersion of xanthophores in Fundulus to an action of incident yellow light on localised dorsal cone cells in the retina. He also related melanophore dispersion to the ratio of direct to reflected light reaching the eye.

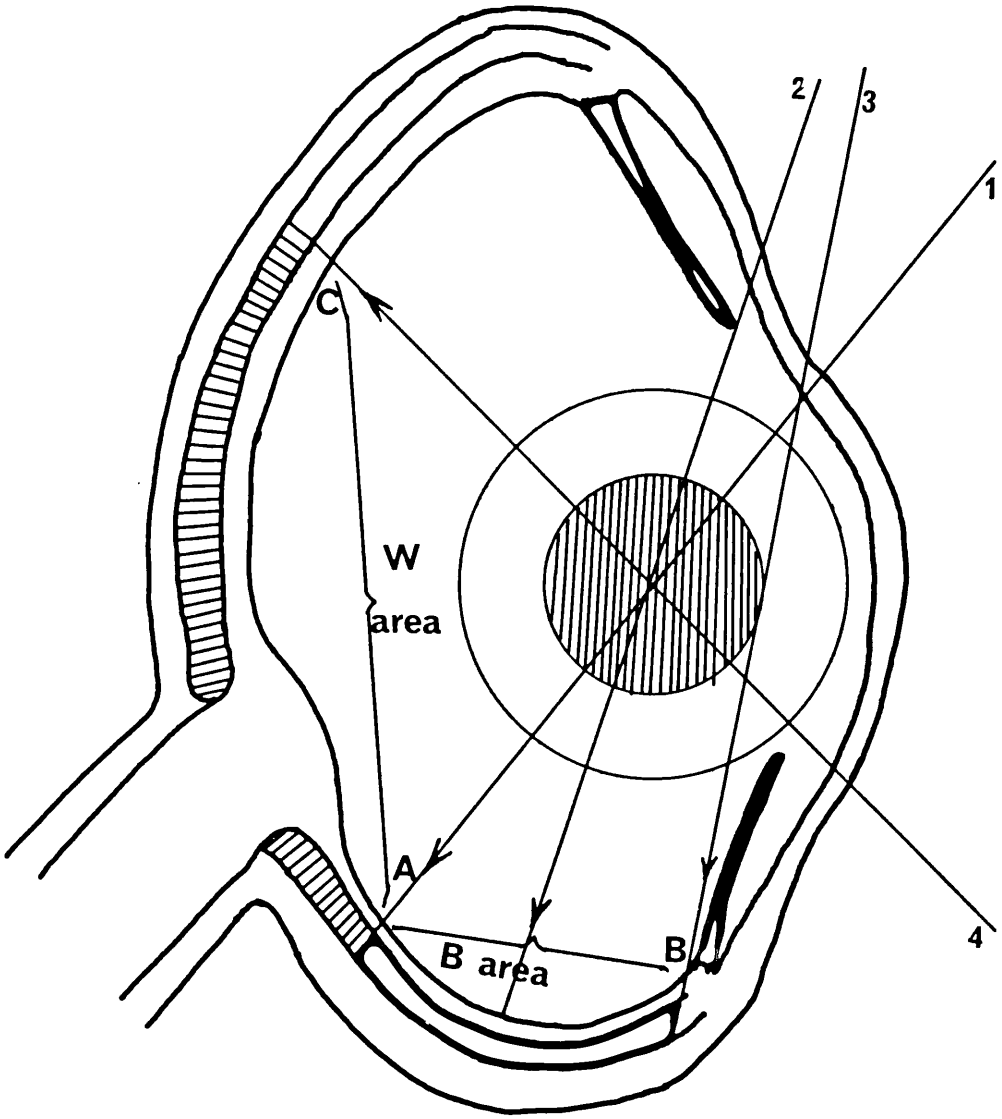
Section 1.22 The Chromatophores

The pigmentary effector cells are classified by the pigments they contain. Melanophores contain black or brown melanin. Chromatophores containing red or yellow pigments soluble in alcohol, ether and other reagents are termed erythrophores and xanthophores. White guanophores containing guanine are also found and many fish have complex chromatosomes containing several pigments (Parker, 1948).

Section 1.23 Nervous components of teleost colour changes

The first observations on teleost colour changes in modern times were made by Stark (1830). He noticed that the shade of Leuciscus (=Phoxinus) phoxinus, Gasterosteus aculeatus, Cobitis barbatula and Perca fluviatilis became dark or pale when the fish were kept on black or white backgrounds. Subsequently Vogt (1842), Bucholz (1863) and von Siebold (1863) described the effector cells, the melanophores, responsible for the shade changes. Brücke (1852) asserted that the complex colour changes of the chamaeleon were controlled by the nervous system and

Fig. 1. The eye of Gasterosteus showing the regions of the retina responsible for paling (W) and darkening (B) when illuminated (after Hogben and Landgrebe, 1940).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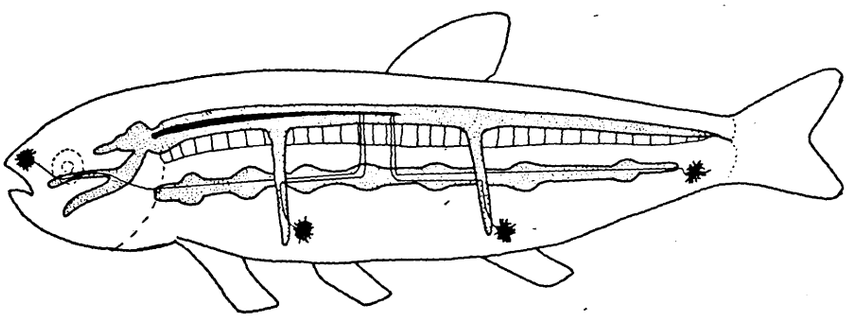
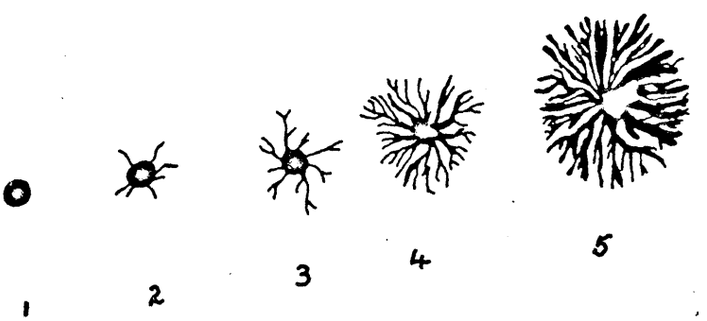
described the abrupt darkening of parts of the body when nerves to that region were severed. Pouchet (1872 et seq.) performed similar experiments on the turbot Scophthalamus (=Rhombus) maximus. He showed that section of the spinal nerves, of the maxillary branch of the trigeminal nerve or of the sympathetic chain in the haemal canal caused dispersion of melanophores distal to the site of the cut. He concluded that nerve fibres running in the sympathetic chain and thence to the skin carried impulses which aggregated the melanophores. Von Frisch (1910, 1911b) traced the path of chromatic fibres from the brain to the melanophores in the minnow, Phoxinus phoxinus L. (=P. laevis Ag.). He showed that the chromatic fibres left the spinal cord in the region of the 15th vertebra and passed along the sympathetic chain anteriorly and posteriorly. The melanophores were innervated by way of the segmental spinal nerves and, in the head region, by the trigeminal nerve (Fig. 2; p.19). Young (1931a) showed that in Uranoscopus scaber, fibres from the sympathetic chain join the segmental spinal nerves by way of grey rami communicantes. Von Frisch showed that electrical stimulation of the medulla oblongata led to pallor of the whole fish. On the other hand, he described overall darkening of the body after electrical stimulation

Fig. 2. The pathways of the melanophore aggregating fibres in the minnow (after von Frisch, 1911b).

Fig. 3. The melanophore index in the minnow (after Healey, 1951).

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of the diencephalon or illumination of the pineal region in the absence of other light stimuli. Section of the chromatic tract at any point led to a dispersion of melanophores thus isolated from the central nervous system. Von Frisch concluded that the paling response of the minnow was controlled from a centre in the medulla which could be inhibited by another centre anterior to it in the brain. He also described the darkening after death which was followed by a pronounced pallor of the whole body. This pallor could be prevented by removal of large sections of the spinal cord near the point of outflow of the sympathetic fibres. He suggested that there might be a spinal paling centre in this region which controlled melanophore aggregation under these conditions.

Wyman (1924a) studied small areas of the tail of Fundulus which had been denervated by a 1-2 mm. cut near the base of the tail. The melanophores whose nervous supply was affected dispersed soon after the cut was made and the region appeared as a dark strip, with discrete margins, from the site of the cut to the end of the fin (Fig. 4a; p.22). Care was taken to ensure that minimal vascular disturbances within this area occurred as a result of the incision. The melanophore dispersion was independent of the background on which the fish were kept

and lasted for several hours. Subsequently, if the fish were kept on a white background, these melanophores slowly aggregated. Those at the margins of the affected area, where normally innervated melanophores were to be found, aggregated first and the more centrally placed melanophores last. If fish with faded caudal bands were then transferred to a black background, the caudal bands darkened much more slowly than adjacent areas. Wyman believed that the first darkening of the caudal bands was due to paralysis of melanophores in this region. Aggregating influences carried from the central nervous system in chromatic tracts were interrupted and melanophore dispersal was attributed to a tendency for relaxed melanophores to disperse. Subsequent slow colour changes in the affected area during background reversal were ascribed to control of melanophores by circulating hormones. A similar interpretation of the dispersal of "relaxing" melanophores has been put forward by Sand (1935), Umrath and Walcher (1951) and Gray (1956a).

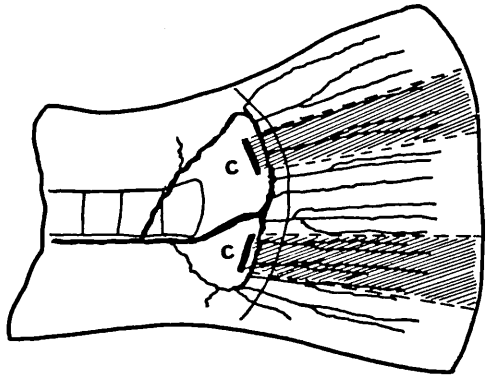
Mills (1932a, b, c, d) repeated Wyman's operations on Fundulus but in addition introduced new lesions into faded caudal bands before the cut chromatic fibre stumps had degenerated. Renewed caudal bands appeared distal to the second lesion (Fig. 4b; p.22). Parker (1934b) showed

Fig. 4. a. Diagram to show the effects of lesions in the tail of white-adapted Fundulus on the caudal melanophores. The parallel arterial and venous systems are shown together. Denervated bands appear as dark stripes. (from Fries, 1931).

b. The effect of a second lesion on melanophores of a faded caudal band in Fundulus (from Parker, 1934c).

c. Diagram to show the failure of melanophore dispersion in a fresh caudal band of Fundulus after the application of a cold block at A (from Parker, 1934c).

d. Diagram to show the effect of flanking a faded caudal band of Ameiurus by fresh bands. Denervated melanophores between the new bands begin to disperse (from Parker, 1934b).



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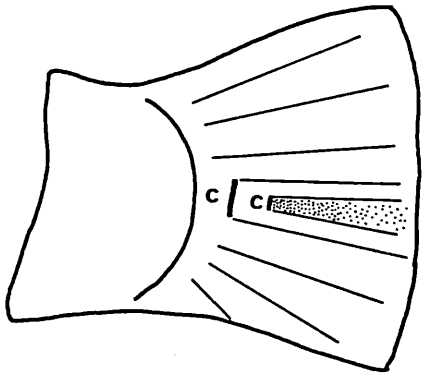

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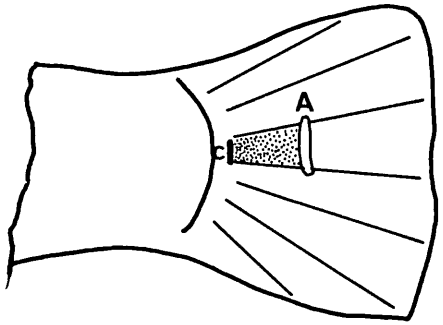

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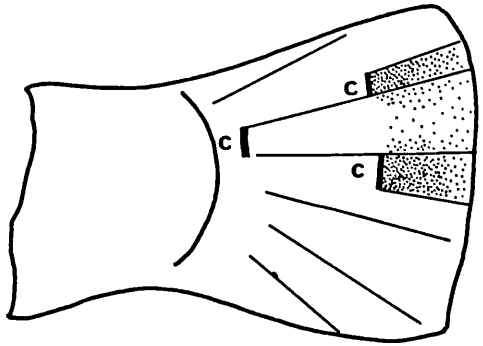

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that such renewal in a caudal band could also be caused by flanking a "faded" band with freshly denervated melanophores (Fig. 4d; p.22). Parker proposed that the dispersion which followed lesions in the chromatic tract was therefore active and was brought about by mechanical stimulation of dispersing fibres. Dispersion of chromatophores after chromatic tract lesions have been described in Holocentrus (Parker, 1937), Macropodus (Dalton and Goodrich, 1937), Parasilurus (Matsushita, 1938), Pterophyllum (Tomita, 1938a, b; 1940), Scophthalamus (Osborn, 1939), Phoxinus (Gray 1955, 1956a), Chasmichthys (Fujii, 1959) and Carassius (Iwata et al., 1959). Fries (1942b) found that caudal bands could only be renewed in 50 - 60% of the animals he studied: (Labrus ossifagus, Pleuronectes platessa and Gobius minutus).

Microscopic observations of the melanophores in caudal bands (Mills, 1932a, b; Abramowitz, 1935, 1936a; Gray, 1955) showed that those nearest the margins responded assymetrically during background reversal. During colour changes evoked by this means or by electrical stimulation some melanophores were seen which could not aggregate or could not disperse their pigment completely. Furthermore, Abramowitz (1936a) elicited caudal bands in Fundulus and then observed the responses of the denervated area to

background reversal. As the cut sections of nerve degenerated over a period of two weeks no fast reactions of melanophores in the band were seen. Thereafter, as the stumps of the chromatic nerves regenerated, more and more melanophores were recruited into the fast adaptation to background reversal. The recruitment began near the site of the cut and moved distally at a rate of about half a millimetre a day.

When teleost melanophores are subjected to local cooling or warming they aggregate or disperse respectively (von Frisch, 1911a; Smith, 1928). Denervated melanophores show smaller changes to these stimuli in the reverse direction. Parker (1934b) described that conduction in the nerves which cause dispersion of melanophores in caudal bands can be blocked by the application of cold. A fine tube, carrying water cooled to a few degrees above 0°C., when placed midway along a newly developed caudal band caused a slow disappearance of the band distal to the site of application (Fig. 4c; p.22). Pye (1964c) repeated the experiments of von Frisch and Smith. He found that section of sensory nerves from the skin of Phoxinus (lateralis X and cutaneous X) did not affect the responses of innervated melanophores to temperature changes. The responses of innervated

melanophores were only reversed by lesions in tracts containing chromatic motor fibres. Pye concluded that the reaction of normal melanophores to temperature changes was unlikely to be a reflex involving sensory fibres which follow the chromatic motor pathway exactly and have a one-to-one relation to these fibres centrally. He believed that melanophores receive a dual innervation of antagonistic fibres running in the chromatic tract and that those responsible for dispersion were blocked by low temperatures and those responsible for aggregation were blocked by high temperatures.

Following injections of ergotamine (see Section 1.3221) into minnows, it has been found that electrical stimulation always causes darkening (Giersberg, 1930; von Gelei, 1942; Pye 1964b). Pye found that this darkening was apparently not due to "reversal" of the effects of catecholamines (Section 1.322) released from the aggregating nerve terminals. He concluded that ergotamine blocked the aggregating fibres and unmasked the activity of dispersing fibres which responded to electrical stimulation. Fujii (1961), however, found that the aggregating effect of adrenaline on Chasmichthys melanophores is reversed by ergotamine.

Parker (1934 et seq.) interpreted the experimental

findings described above as indicative of a dual, antagonistic nerve supply to the melanophores of many teleost fish. The aggregating system is anatomically sympathetic and apparently "adrenergic" (Section 1.32) so Parker suggested that the antagonistic system was parasympathetic and its actions mediated by acetylcholine. Parker was able to extract a lipid-soluble, darkening agent from the skin of black-adapted Ameiurus which, when assayed against leech muscle, contained about 0.08 micrograms of acetylcholine per gram of wet skin. Parker attempted to stimulate the two types of nerve fibre selectively, (Parker and Rosenblueth, 1941). Pye (1964a) discussed the significance of the colour changes elicited in Ameiurus by the latter workers. He suggested that stimulation which caused darkening might have blocked conduction in aggregating tracts. He also pointed out that the rate of darkening in the animals was slow. Slow colour changes are frequently found in eviscerated preparations and are associated with the onset of death (von Frisch, 1911b).

Umrath and Walcher (1951) reinterpreted the observations of Parker in a series of experiments on caudal bands in Macropodus opercularis. They believed that a dual innervation of melanophores occurs in this fish but that stimulation of dispersing fibres by cutting was short

lived. Orthodromic and antidromic impulses in parasympathetic fibres were set up leading to melanophore dispersion on both sides of the cut. Antidromic stimulation was antagonised by intact aggregating fibres but orthodromic dispersion was reinforced by a tendency for the isolated melanophores to disperse in the absence of aggregating innervation. This latter circumstance leads to the appearance of a macroscopic caudal band of unstimulated, dispersed melanophores and does not postulate continued "injury discharge" of dispersing fibres as suggested by Parker. Umrath and Walcher also suggested a novel interpretation of fading in the band. They found that the entry of water into the cut stimulated re-aggregation of the isolated melanophores and concluded that as long as the terminal portions of the aggregating fibres were living they became rhythmically excited in the presence of unphysiological conditions. The formation of new dark bands in faded old bands involved sectioning the aggregating fibre stumps distal to the site of the rhythmical activity. The same workers also suggested that caudal bands elicited AFTER preganglionic sections in the chromatic tract interrupted the path of impulses generated spontaneously in the isolated sympathetic ganglia. The hypotheses of Umrath and Walcher therefore transfer the

main cause of melanophore dispersion after lesions in the chromatic tracts from that suggested by Parker (prolonged injury discharge in dispersing fibres) to that suggested originally by von Frisch and Wyman (separation from central control).

An alternative explanation of "Parker's Effect" (viz. the formation of caudal bands) was suggested by Gray (1956). He proposed that the removal of central nervous control by the cut allows an inherent dispersing mechanism of the melanophore to come into play. Subsequent fading of the band depends on the development of hypersensitivity in the denervated melanophores to neurohumours diffusing into the region from adjacent, unaffected parts.

1.24

Hormonal components of teleost colour changes

Hogben and co-workers (1922 et seq.), following the studies of Adler (1914), Smith (1916) and Allen (1916) on hypophysectomised amphibia, demonstrated the role of the pituitary gland in the colour changes of Rana and Xenopus. Operations on the pituitary and injections of pituitary extracts showed that a melanophore-dispersing hormone was released from the pars intermedia (Hogben and Winton,

1922a, b, c; 1933; Hogben and Slome, 1931). These were based on the times required to reach colour-change equilibria on transferring normal animals from black and white illuminated backgrounds to darkness and on subjecting them to illuminated black and white background reversal. Microscopic observations of the melanophores were made and the times for full equilibration between one experimental environment and another were recorded. An analysis of these times led Hogben and Slome (1931, 1936) to conclude that all stages of melanophore aggregation and dispersion depended on a balance of antagonistic pituitary hormones in the blood. Hogben and Winton (1922) and Hogben and Slome (1931, 1936) reported that lower doses of injected intermedine (dispersing hormone) were required to darken frogs if the pars tuberalis was removed.

Smith (1928) proposed that the slow residual colour changes seen by von Frisch (1911b) in denervated regions of the minnow (Phoxinus) were mediated by circulating hormones similar to those described in amphibia. Injections of teleost pituitary extracts aggregated melanophores (Hewer, 1926) and dispersed erythrophores (Giersberg, 1932; Healey, 1948). Healey showed that the slow colour changes of spinal-sectioned minnows disappeared after hypophysectomy, the animals remaining dark regardless of background tint. Injections of minnow

pituitary extracts made such fishes become pale but darkened Ameiurus and Rana. Kent (1961) was unable to separate the paling and darkening activities of the minnow pituitary by chemical treatment and electrophoresis. Other fish have been found to produce a pituitary hormone; melanophore stimulating hormone (MSH) ("intermedine") which disperses their melanophores:- Ameiurus (Abramowitz 1936b; Parker, 1940), Anguilla (Parker, 1943), Fundulus (Kleinholz, 1935).

Several workers have studied the time relations of the hormonal colour changes of teleosts (Neill, 1940; Healey, 1951). These workers studied Anguilla and spinal Phoxinus respectively, and concluded that in both cases the results could be explained by Hogben's two-hormone hypothesis as applied to amphibia. Kent (1959b) has criticised the logic of the use of time relations in determining the number of hormones involved in colour changes. He pointed out that background reversal in light, in which the pituitary may receive different kinds of stimulation, differs physiologically from transfer to and from darkness. In darkness, the pituitary may receive no stimulation at all (as regards its chromatic functions) and may slowly achieve a resting level of secretion.

Partial hypophysectomy has been attempted in various

fishes. Waring (1942) worked on elasmobranchs and suggested that paling hormones were present in the anterior region of the adenohypophysis whilst darkening hormones were to be found in the neuro-intermediate lobe. Healey (1940) studied the effects of partial hypophysectomy in Phoxinus and concluded that the paling hormone occurred in the anterior portion of the gland. Kent (1959a) worked on the same species but suggested that the paling hormone was synthesized anteriorly and stored posteriorly. Enami (1955) injected aqueous extracts of the pituitary and hypothalamus of Parasilurus into other individuals of the same species and suggested that both aggregating and dispersing principles were present. Imai (1958) was unable to elucidate the chemistry of the aggregating principle. Kent (1961) found no indication of a chemical antagonistic to the aggregating hormone of the minnow when he subjected their pituitaries to Enami's techniques.

Parry and Holliday (1960) found that ablation of the pseudobranch in Salmo trutta, Salmo gairdnerii, Clupeus harengus, Gadus virens and Pleuronectes platessa led to degeneration of the choroid gland and to darkening of the whole body. They suggested that the pseudobranch might be involved in a humoral mechanism responsible for melanophore aggregation. It has been pointed out that

degeneration of the choroid gland may interfere with the functioning of the eyes as receptors (Barrington, 1964).

McCord and Allen (1917) demonstrated that the pineal gland in vertebrates contained a substance with potent melanophore aggregating activity. Recently the active agent, melatonin, has been extracted and identified (Lerner and Case, 1960). In teleosts it has been found that ablation of the pineal leads to permanent darkening of Oncorhynchus (Hoar, 1955). Von Frisch (1911) demonstrated that the epiphysis and adjacent structures of the minnow were involved in body darkening. The role of the pineal gland in teleost colour changes requires clarification.

Section 1.25
Colour changes in the minnow, Phoxinus phoxinus, (L.)

The melanophores in the skin of the minnow disperse their pigment when stimulated directly by local illumination or indirectly when the pineal or ventral region of the retina is illuminated. Minnows placed on illuminated black or white backgrounds adapt rapidly to that background by dispersing or aggregating the melanophore pigment. The initial (nervous) phase of the adaptation is rapid and is

almost complete within 10 - 15 minutes but complete adaptation may not occur until two or more hours have elapsed. This latter phase is due to additional participation of pituitary hormone (Healey, 1951). Lesions in the chromatic tracts (Section 1.23) abolish the fast reactions but slow residual colour changes occur in the denervated regions. Hypophysectomy abolishes even these changes. Immediately after any operation which transects chromatic fibres, a pronounced dispersion of melanophores in the denervated region occurs, regardless of the background (von Frisch, 1911b; Healey, 1948; Gray, 1956). Von Gelei (1942) claimed to have shown that darkening fibres from the central nervous system enter the sympathetic chain in the region of vertebra 2 and innervate the skin by way of the spinal nerves. Von Frisch (1911b) and Healey (1948 et seq.) performed operations which would deprive some regions of the body of paling fibres without affecting the course of von Gelei's postulated fibres to this region. Neither worker was able to observe differences in melanophore reaction between such affected regions and regions to which both supplies were cut. Pye (1964a) found that electrical stimulation of the kind used by von Gelei could stimulate nearby regions of the sympathetic system by way of connecting rami. He concluded that von Gelei's techniques

were not sufficiently precise to map the path of chromatic fibres. Gray (1955, 1956) studied caudal bands in the minnow and Pye (1964a, b, c) severed other portions of the chromatic tract. Both workers suggested that dispersing fibres in the minnow accompany the aggregating fibres described by von Frisch (Fig. 2; p.19).

The chromatic system of the minnow may thus be considered as consisting primarily of a set of sympathetic nerve fibres which rapidly pale the fish. There is some evidence that there may also be an antagonistic system of nerve fibres causing active darkening and following the same peripheral pathways as the paling fibres.

The slow hormonal control of colour changes in the minnow has already been described (viz. pituitary secretions causing aggregation of melanophore pigment and dispersion of the pigment in the non-innervated coloured chromatophores.) There is no direct evidence for a darkening "intermedine"-like hormone.

Section 1.3

The pharmacology of the autonomic nervous system in mammals with reference to the drugs used in the experimental section

Early workers in the field of neurophysiology proposed that nerves stimulated their end organs by releasing

chemicals at the neuroeffector junction (Dubois Reymond, 1855-7; Elliot, 1905). Langley (1905) suggested that the surface of effector cells contained a receptor substance which received the transmitter agent. This combination leads to effector action. Subsequent workers were able to describe the main divisions of the peripheral nervous system not only on anatomical and physiological grounds but also by identification of the chemicals involved in synaptic transmission.

Section 1.31
Cholinergic drugs

Peripheral neurones other than postganglionic sympathetic fibres exert their effects by releasing acetylcholine. Such fibres were termed "cholinergic" by Dale (1934). Injected acetylcholine acts at the peripheral synapses of the parasympathetic system to mimic the action of the postganglionic fibres. The alkaloid muscarine exerts similar effects and Dale (1914) termed this action of acetylcholine "muscarinic". Such actions of acetylcholine and muscarine are blocked by the alkaloid atropine. Injections of acetylcholine in the presence of atropine stimulate both sympathetic and parasympathetic ganglia.

Nicotine fleetingly stimulates these ganglia and then exerts a blockade which prevents acetylcholine exerting an effect at this site. Accordingly Dale distinguished this "nicotinic" action of acetylcholine from the more peripheral muscarinic action (Fig. 5; p.79).

Extracts from mammalian tissues were found to contain choline and Loewi and Navratil (1926) suggested that this choline derived from the metabolism of acetylcholine. Extraction, perfusion and bioassay studies by other workers led to the general acceptance of the role of acetylcholine as a transmitter at sympathetic and parasympathetic ganglia (Kibjakow, 1933; Feldberg et al., 1934 et seq.; Perry and Talesnik, 1953). Similarly, the identification of the peripheral parasympathetic (muscarinic) transmitter as acetylcholine was established (Dixon, 1906, 1907; Hunt and Taveau, 1906, 1909; Dale, 1914; Loewi, 1921 et seq.; Dale and Dudley, 1929; Dale and Feldberg, 1934a, b; Bain, 1932.).

Empirical studies with plant alkaloids made it possible to prevent selectively the nicotinic and muscarinic effects of acetylcholine in vivo. Physostigmine (eserine) had been found to potentiate the actions of small amounts of acetylcholine and to protect it from hydrolysis in blood and tissue fluids (Chang and Gaddum, 1933). Cholinesterase,

the enzyme responsible for the hydrolysis, was isolated by Marnay and Nickerson (1937). The enzyme responsible for acetylcholine synthesis, choline acetylase, was isolated by Machmansohn (1940). The synaptic vesicles seen in nerve terminals in electron micrographs are believed to contain the cholinergic neurotransmitter (de Robertis and co-workers, 1954, 1957).

Section 1.311

Acetylcholine and related cholinesters

Acetylcholine was isolated from ergot by Ewins (1914) and investigated as a pharmacological agent by Dale (1914). The latter worker described the abolition of muscarinic and nicotinic effects of the ester by atropine and nicotine respectively. Various synthetic analogues have been investigated in mammals for acetylcholine-like actions (Noll, 1932; Hunt, 1934; Farber, 1936). The most effective agents have a quarternary ammonium ion acting as a cationic "head" and exert greatest stimulatory activity if the attached groups are methyl. If the chain of the molecule is branched (methachol, bethanechol) nicotinic activity is reduced and muscarinic activity enhanced. Esters of carbamic acid (carbachol, bethanechol) are

resistant to cholinesterase.

The muscarinic esters act directly on effector cells and do not depend on the presence of nerve terminals. Denervation leads to supersensitivity of the end organ, in accordance with Cannon's Law (1939).

Methacholine, synthesized by Hunt and Taveau (1911) is a potent muscarinic agent with low susceptibility to cholinesterase hydrolysis (Simonart, 1932; Starr et al., 1933; Hunt, 1934). Carbachol, synthesised by Kreitmar (1932) resembles methacholine but has strong nicotinic activity (Noll, 1932; Dautreband, 1933; Molitor, 1936). Bethanechol is solely muscarinic but is even more resistant to hydrolysis by cholinesterase than is methacholine (Simonart and Simonart, 1935; Farber, 1936; Molitor, 1936).

Section 1.312

Drugs which inhibit cholinesterase

Stedman et al., (1932) described the destruction of acetylcholine in vivo as enzymatic and referred to the enzyme as cholinesterase. Later workers were able to separate a variety of esterases with differing pH optima and substrate specificities from various mammalian tissues. Nachmansohn (1959) classified the various types of

esterase, cholinesterase and the specific nerve-bound acetylcholinesterase. Drugs which prevent the hydrolysis of acetylcholine and its congeners were classified by Koelle and Gilman (1949) as reversible or irreversible cholinesterase inhibitors. The only anticholinesterase used in the present study was eserine (physostigmine), (Stedman and Barger, 1925) a reversible anticholinesterase which was first used to constrict the iris of man by Arhyll-Robertson (1863). Anderson (1905) showed that this alkaloid could antagonise the action of belladonna on the iris. It was later shown that the parasympathomimetic action of the alkaloid eserine depended on the integrity of the parasympathetic nerves to the iris, (Loewi and Navratil, 1926).

Section 1.313

Miscellaneous parasympathomimetic agents

Pilocarpine was extracted from Pilocarpus by Hardy in 1871 and was found to initiate salivation, sweating and mydriasis even in the absence of a parasympathetic nerve supply (Weber, 1876). This action is blocked by atropine and is therefore muscarinic. Nicotinic actions of pilocarpine have been described by Dale and Laidlaw (1912),

Bacq and Simonart (1938) and Trendelenburg, (1955, 1957). Other potent muscarinic agents, muscarine and arecoline were not available for use in the present work.

Section 1.314

Cholinergic blocking drugs

Section 1.3141

Drugs blocking muscarinic agents and parasympathetic (postganglionic) fibres

Alkaloids from the plant Atropa belladonna block the action of postganglionic cholinergic fibres. The first alkaloid of this group, atropine, was isolated by Mein (1831) and subsequently Bezold and Bloebaum (1867) showed that it blocked the effect of vagal stimulation on the heart. Heidenhain (1872) and Langley (1878) showed that salivation following stimulation of the chorda tympani was also suppressed by atropine.

Atropine and related alkaloids are esters formed from tropic acid and an organic base. The base may be tropine (e.g. atropine) or scopine (e.g. scopolamine). Synthetic analogues have been developed using mandelic acid (e.g. homatropine) or in which the nitrogen of the base is quarternised (e.g. homatropine methyl bromide).

Atropine and homatropine in mammals depress the muscarinic actions of acetylcholine in a highly selective manner. The release of acetylcholine from cholinergic nerve endings is not affected but after blockade sympathetic activity is enhanced. Atropine has been shown to block nicotinic acetylcholine if sufficiently high doses are used (Cahen and Tvede, 1953; Bainbridge and Brown, 1960). There is some evidence that atropine may have a stimulating effect on the receptors that it blocks (Goodman and Gilman, 1955; p. 543) and occasionally, in mammals, atropine has been found to cause vasodilatation by a direct effect on blood vessels. This action has been attributed to a local release of histamine by the alkaloid. Atropine has been shown to antagonise the actions of adrenaline (Hildebrandt, 1920; Backman and Lundberg, 1922; Regniers, 1926 and Bussell, 1940).

Section 1.3142

Drugs blocking preganglionic (sympathetic and parasympathetic) fibres

Many drugs have been found to depress ganglionic transmission which have little chemical similarity with each other and with acetylcholine. Drugs such as nicotine,

atropine and tetraethylammonium all seem to combine with the postsynaptic cholinergic receptors. Quarternary ammonium ions of several kinds, which are related to acetylcholine, have been found to react with these receptors. Acetylcholine itself, and also carbachol, block ganglionic transmission if the doses are sufficiently high. Initial stimulation is followed by a prolonged depolarisation of the postsynaptic membrane (Nicotine 1 and 2, Fig. 5).

Nicotine, isolated from Nicotiana tabacum by Posselt and Riemann (1828), was shown by Langley and Dickinson (1899) first to stimulate and then to block sympathetic ganglia. Paton and Perry (1951a, b; 1953) showed that the initial stimulatory phase was due to depolarisation of the postsynaptic membrane. Other effects of the alkaloid have been described. Loewi (1937) showed that vasoconstriction could be caused by a direct action of nicotine and Burn (1961) suggested that piloerection in the cat tail and vasoconstriction in the rabbit ear are caused by a local release of noradrenaline by nicotine (Nicotine 3; Fig. 5.).

Methonium ions, polymethlene bis-trimethyl ammonium salts, were first synthesised by Barlow and Ing (1948) and Paton and Zaimis (1949). Members of this series of compounds with five or six carbon atoms separating the two nitrogens

proved to be potent ganglion blocking drugs (Fig. 5; p.79). Hexamethonium (C6) is mainly devoid of muscarinic and skeletal muscle-stimulating activity. A weak vasodilatation in sympathectomised limbs has been attributed to a direct action of the drug (Goodman and Gilman, 1955, p. 635). Hexamethonium does not prevent the release of acetylcholine from preganglionic fibres. The block is postsynaptic, does not cause depolarisation and does not affect axonal conduction (Paton and Zaimis, 1951, 1952). In mammals, hexamethonium has been shown to block both sympathetic and parasympathetic ganglia.

Section 1.32

Adrenergic drugs

Dale (1934) introduced the term "adrenergic" to differentiate those nerve fibres which release an adrenaline-like substance at their terminals from those which release other neurohumours. In the mammal, most postganglionic sympathetic fibres are adrenergic. Known exceptions are the eccrine sweat glands of the cat and man (Dale and Feldberg, 1934b) and the sympathetic vasodilator fibres to the hind limb of the dog (Bulbring and Burn, 1935). Recently, it has been suggested that the sweat glands of

the cat receive both adrenergic and cholinergic fibres, both of which initiate secretion (Lloyd 1965).

Elliot (1905) first postulated that the sympathetic nervous system liberated small quantities of adrenaline-like substance which stimulated effector cells. He observed that the actions of injected adrenaline mimicked the effect of sympathetic discharge and that denervated organs could still respond to the amine. Earlier workers had obtained active extracts from the adrenal glands (Oliver and Schäfer, 1895; Abel and Crawford, 1897, 1899; Takamine, 1901) and recorded that the extracts raised the blood pressure of dogs. Barger and Dale (1910) studied a wide range of amines to test for adrenaline-like action and to determine the molecular structure necessary for such action. They found that strongest sympathomimetic activity occurred in amines with a phenyl ring; a two carbon side-chain and a terminal amine group (Table 1; p.49). Hydroxylation of the beta carbon enhanced this activity but terminal methylation of the amine group increased inhibitory activity on certain organs.

Cannon and co-workers extended the study of the adrenergic transmitter in the cat. They found that, although injections of adrenaline both stimulated the heart and inhibited the intestine, the mediator liberated from the

hepatic nerve stimulated the nictitating membrane but failed to inhibit the non-pregnant uterus. Cannon and Rosenblueth (1933, 1937) added further observations to support the previous suggestions that the adrenergic transmitter, "sympathin" had fewer inhibitory actions than adrenaline. The earlier work of Barger and Dale (1910) had suggested that primary amines were less effective inhibitory agents on smooth muscle and several workers therefore suggested that the adrenergic mediator was noradrenaline (Bacq, 1934; Cannon and Rosenblueth, 1937; Greer et al., 1938; Raab, 1943; Bacq and Fischer, 1947). Extracts of sympathetic nerves were shown to contain both adrenaline and noradrenaline but both amines disappeared as the nerves degenerated (Cannon and Lissak, 1939); von Euler, 1946a, b; 1948, 1951; von Euler and Purkhold, 1951; Goodall, 1951). Von Euler and Hillarp (1956) showed that the mediator was stored in granules similar to those isolated from the adrenal medulla by Blaschko et al. (1955) and Hillarp et al. (1953, 1954). From these studies it is generally accepted that the principal neurotransmitter in mammalian sympathetic nerves is noradrenaline. Synthesis of the catecholamines in sympathetic nerves is similar to that found in adrenal medullary tissue (von Euler, 1958b; Goodall and Kirshner, 1958). Dopamine, an intermediate in

the synthesis of adrenaline and noradrenaline, has been found in relatively high concentrations in sympathetic nerves (Carlsson et al., 1958; von Euler and Lishajko, 1958) and has been suggested as a transmitter by Schümann (1959).

Metabolism of the catecholamines is slower than that of acetylcholine. Bacq (1949), Blaschko (1952) and Burn (1952) reviewed the pathways which led to removal of the sympathetic transmitter from the neuroeffector synapse. These mechanisms involved deamination and conjugation of the active molecules. Axelrod (1960) reviewed the evidence for another pathway which metabolises the adrenergic transmitter by O-methylation. Potter and Axelrod (1962, 1963) described a model for the adrenergic nerve terminal (see Section 1.324 and also Fig. 6; p.79) in which they indicated that catecholamines in the "bound" store are deaminated but those in the "available" store are subjected to O-methylation. Veldstra (1956) had previously proposed that the sympathetic transmitter may be absorbed into adjacent tissues from the synapse and only subsequently metabolised.

Section 1.321

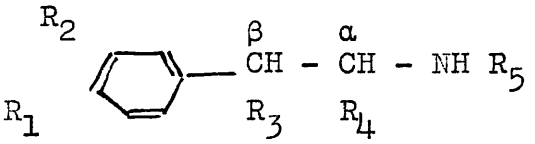
Sympathomimetic amines

The early studies of Barger and Dale (1910) on a wide variety of amines led these workers to conclude that greatest sympathomimetic activity was found in those amines which bore close structural similarity with naturally occurring catecholamines. They showed that the phenyl ring should carry hydroxy groups on the 3 and 4 carbon atoms and that the beta carbon atom of the side chain should also be hydroxylated. Frölich and Loewi (1910) showed that the response to injected adrenaline was potentiated by cocaine and Tainter and Chang (1927) and Tainter (1931, 1933) extended this work by testing the effect of cocaine on sympathomimetic amines. The latter workers showed that noradrenaline was also potentiated by cocaine but the actions of tyramine were antagonised. Burn and Tainter (1931) found that, after denervation, the actions of adrenaline and noradrenaline were again potentiated. This potentiation was greater than that caused by pretreatment with cocaine (Burn and Hutcheon, 1949). Fleckenstein and co-workers (1953, 1955) studied the effects of a variety of amines on denervated organs and on organs treated with cocaine and concluded that three main classes

of sympathomimetic amines occur. The first group consists of amines which are believed to act directly on the effector cell. Such amines are potentiated by cocaine and by denervation (Group A, Table 1). Amines in this group generally possessed hydroxyl groups at carbon 3, 4 and beta (Table 1). The second group of amines were described as "indirect". Their actions are strongly antagonised by denervation and by cocaine (Group B). Typically such amines are phenylethylamines with one or no phenyl hydroxyl groups (Table 1). Finally, a third group was described with "mixed" actions and which are generally neither potentiated nor antagonised by cocaine and denervation. Phenylethanolamines such as ephedrine (Table 1) are typical of this group of amines (Group C). Burn and Rand (1958) presented a similar classification of sympathomimetic amines based on activity found before and after treatment with the alkaloid reserpine (Sections 1.3231 and 1.326). Innes and Kosterlitz (1954) and Trendelenburg (1963) concluded that the different actions of sympathomimetic amines <u>in vivo</u> depended on the occurrence of OH groups at key sites in the molecules and on the optical isomerism of the amine tested. Table 1 represents the molecular structures of several sympathomimetic amines used in Section 3 and indicates their type of action as found in mammals.

TABLE 1

Structural formulae of sympathomimetic amines



DRUG	R_1	R_2	R_3	R_4	R_5	Group
betaphenyl-ethylamine	H	H	H	H	H	B
tyramine	OH	H	H	H	H	B
noradrenaline	OH	OH	OH	H	H	A
adrenaline	OH	OH	OH	H	CH_3	A
isoprenaline	OH	OH	OH	H	$CH_3(CH_2)_2$	A
amphetamine	H	H	H	CH_3	H	B
ephedrine	H	H	OH	CH_3	CH_3	C

Section 1.3211

Tyramine

Barger and Dale (1910) described the sympathomimetic activity of tyramine and Tainter and Chang (1927) and Burn and Tainter (1931) showed that doses of cocaine which potentiate the action of adrenaline or noradrenaline antagonise the actions of tyramine. This antagonism by

cocaine is surmountable as sufficiently large doses of tyramine will overcome it. Burn (1932a) showed that the action of tyramine may depend on the presence of catecholamines in or near the tissues it excites and the same worker (1932b) and Burn and Tainter (1931) showed that denervated tissues could not respond to the amine. Burn and Rand (1957, 1958a, b), Burn (1961) and Moore and Moran (1962) depleted the adrenergic stores (Section 1.324) with reserpine as well as by denervation and were able to abolish the actions of tyramine. It is generally believed that tyramine can displace adrenaline and noradrenaline *in vivo* from chromaffin granules of the adrenal medulla, sympathetic nerve endings and other stores (Lockett and Eakins, 1960a, b; Schümann, 1960, 1961a, b; Schümann and Weigman, 1960; Burn and Burn, 1961; Carlsson and Hillarp, 1961; von Euler and Lishajko, 1960b; 1961b; Haag *et al.*, 1961; Lindmar and Muscholl, 1961; Schümann and Philippu, 1962; Stjarne, 1961; Axelrod *et al.*, 1962; Chidsey *et al.*, Mueller and Shideman, 1962; Weiner *et al.*, 1962).

Schumann and Weigmann (1960) showed that cocaine did not prevent the release of catecholamines from isolated chromaffin granules and concluded that the antagonism occurred at the cell membrane.

Section 1.3212

Ephedrine

Ephedrine, isolated from the plant Ephedra by Yamanashi in 1885, was reported to have an adrenaline-like action on the eye by Miura (1887). Takahashi and Miura (1888) proposed that the drug acted by stimulating the sympathetic fibres to the eye. The actions of the drug on the cardiovascular system were described as resembling those of adrenaline by Chen and Schmidt (1924, 1930). According to Goodman and Gilman (1955) injections of ephedrine differ from adrenaline in that they are less potent, are not reversed after ergot alkaloids (See Section 1.3221) and continued treatment causes gradual decrease in response (tachyphylaxis). Tainter (1931, 1933) reported that the actions of ephedrine were antagonised by cocaine and Fleckenstein and co-workers (1953, 1955) classified the amine as having both direct and indirect actions. Thus, after denervation (see Section 1.326) or pretreatment with cocaine or reserpine, the responses of various organs to the alkaloid are reduced but not abolished. Burn and Rand (1958) and Bejrablaya et al., (1958) consider that the residual activity following the pretreatments of the test organs is due to the presence of a beta-OH group in the ephedrine molecule which enables it

to stimulate adrenergic receptors. Gaddum and Kwiatkowski (1938) and Gaddum (1938) had previously suggested that ephedrine acted in a similar way to eserine in cholinergic systems. They proposed that the alkaloid interfered with enzymatic breakdown of catecholamines. Trendelenberg (1963) reviewed the evidence which suggests that ephedrine not only has a tyramine-like action on stores of catecholamines but also has a direct stimulatory action on adrenergic receptors (Ephedrine 1&2, Fig. 5; p.79). Goodman and Gilman (1955) describe central stimulatory activity of the alkaloid on the vasomotor centre and cerebral cortex.

Section 1.3213

Amphetamine

The vasopressor activity of amphetamine in mammals was shown to be only 1/100 to 1/200 as potent as that of adrenaline (Alles, 1933). Alles and Prinzmetal (1933) showed that the drug caused bronchodilatation and central respiratory stimulation. Tainter (1933) reported that the vasopressor actions were antagonised by cocaine and that continued injections of amphetamine by itself led to tachyphylaxis. According to Goodman and Gilman (1955)

treatment with ergot alkaloids does not reverse the action of amphetamine as it does with adrenaline. The actions of amphetamine are antagonised or abolished by reserpine, cocaine or denervation (Trendelenberg, 1963). Amphetamine acts indirectly in mammals to release noradrenaline from the adrenergic store (Fig. 5; p.79).

Section 1.3214

Isopropylnoradrenaline

Konzett (1940), Lands et al.,(1947), Lands (1949) and Barcroft and Konzett (1949) studied a series of catecholamines with substitutions on the amine group to investigate their enhanced inhibitory actions. Isopropylnoradrenaline (isoprenaline) has actions which resemble those of adrenaline (but not noradrenaline) after pretreatment with alpha adrenergic blocking drugs. Vasodilatation, bronchodilatation, inhibition of the alimentary canal and uterine muscle and also cardiac stimulation follow treatment with isoprenaline (Barcroft and Swan, 1953; Cobbold et al., 1960). Isoprenaline is a more potent stimulator of adrenergic beta receptors (see Section 1.325; Fig. 5,p.79) than adrenaline (Ahlquist, 1948; Youmans et al., 1955; Green and Kepchar, 1959).

Section 1.322

Adrenergic Blocking Agents

Dale (1905, 1906) and Barger and Dale (1907) observed that preparations of ergot of rye prevented the pressor response to injected adrenaline and to sympathetic stimulation. This blockade unmasked an inhibitory property of adrenaline on vascular smooth muscle, referred to as "adrenaline reversal" (Dale 1913). Subsequent workers investigated a variety of chemical agents which similarly antagonised the effects of circulating catecholamines and sympathomimetic amines. These agents, which compete with catecholamines at alpha adrenergic receptors were less able to block sympathetic nervous system activity that the effects of circulating catecholamines, (Nickerson, 1949).

Section 1.3221

Ergot alkaloids

The studies of Dale were continued by Sollman and Brown (1905) and Stoll and Hofmann (1943a, b). The active alkaloids of ergot, both l- and d-isomers, were identified and investigated for individual pharmacological activity by

Rothlin (1946). These alkaloid molecules are all based on an indole moiety built into a tetracyclic ring system, called ergoline. The pharmacological actions of these natural alkaloids include central depression of the vasomotor centre (Barcroft et al., 1951; Konzett and Rothlin, 1953) and stimulation of central emetic and mid-brain sympathetic centres. Peripheral stimulation of smooth muscle occurs and the actions of noradrenaline, adrenaline and 5-hydroxytryptamine are antagonised (Rothlin, 1946). Stoll and Hofmann (1943) demonstrated that saturation of the 9-10 double bond in the ergoline moiety led to a change in activity of the natural alkaloids. The direct stimulation of smooth muscle and the central stimulation of sympathetic centres becomes reduced without affecting neurohumoral antagonism or the inhibition of the vasomotor centre (Rothlin, 1946; de Vleeschouwer, 1949; Barcroft et al., 1951; Hofmann, 1961).

The antiadrenergic effects of the alkaloids, whether natural or dihydrogenated, are exerted competitively at the alpha adrenergic receptor. Brown and Dale (1935) showed that the stimulatory action of ergometrine on smooth muscle was blocked by adrenergic blocking agents (see Sections 1.3223 and 1.3225) and Innes (1962) showed that the receptors for the direct stimulation of the uterus by

ergotamine are the same as those for adrenaline. The dihydroergoalkaloids are more able to antagonise the inhibitory effects of adrenaline on the rabbit intestine (Rothlin, 1946) than its inhibitory effects on the uterus. They are able to block the stimulatory effects of the natural alkaloids. Both natural and dihydrogenated ergot alkaloids are active only against alpha adrenergic receptors (Section 1.325 and Fig. 5; p.79).

Section 1.3222

Benzodioxanes

Fourneau and Bovet (1933) and Bacq and Fredericq (1935a, b) demonstrated the antagonism of methylbenzodioxanes to circulating adrenaline. Prosympal (883F) was described as also antagonising the sympathetic nerves whilst piperoxane (933F) was active mainly against circulating adrenaline. The blockade is transient and competitive and Nickerson (1949) stated that only stimulatory adrenergic actions were antagonised. De Vleeschouwer (1935) showed that 1 mg/kg piperoxane antagonised the effects of circulating adrenaline on blood pressure and led to adrenaline reversal. Piperoxane and its congeners only block alpha adrenergic receptors (Fig. 5; p.79).

Goodman and Gilman (1955) include among the many side effects of piperoxane central vagal and sympathetic stimulation and direct stimulation of uterine and bronchial smooth muscle.

Section 1.3223

Imidazolines

Antiadrenaline activity and adrenaline reversal were reported for phentolamine by Meier et al., (1949). Priscol, a related substance, had been found to have a similar action on circulating adrenaline (Meyer, 1941; Chess and Yonkman, 1945, 1946). Phentolamine was found to exert a blockade of sympathetic nerve impulses (Trapold et al., 1950; Gross et al., 1951) and this competitive blockade was found to occur at the adrenergic receptor (Gottstein and Hille, 1955). Phentolamine does not prevent inhibitory actions of adrenaline on blood vessels, bronchi and the non-pregnant cat uterus. It is therefore believed that the drug only blocks alpha adrenergic receptors (Fig. 5; p.79). Goodman and Gilman (1955, p. 579) describe the diverse direct stimulatory and inhibitory actions of imidazoline drugs on a wide variety of smooth muscles and secretory glands which are unrelated to adrenergic blocking activity.

Section 1.3224

Yohimbine

The adrenergic blocking activity of yohimbine was reported by Raymond-Hamet (1925), Yonkmann et al., (1944) and reviewed by Nickerson (1949). The blockade is labile and of short duration, is limited to alpha adrenergic receptors and is competitive. The effects of circulating catecholamines are more easily antagonised than is sympathetic nerve activity. Wischhusen (1933) described a direct, stimulatory action of the alkaloid on isolated uterine muscle.

Section 1.3225

Beta-haloalkylamines

Unlike most of the drugs which antagonise catecholamines and sympathomimetic amines at the alpha adrenergic receptor, the beta-haloalkylamines exert a prolonged, specific blockade which is equally active against both circulating amines and sympathetic nerve activity (Nickerson and Goodman, 1947, 1948; Acheson et al., 1949; De Vleeschouwer, 1947; Hecht and Anderson, 1947; Nickerson and Nomaguchi, 1948; Medinets et al., 1948; Stone and

Loew, 1948; Üvnas, 1948; Coret, 1948). This blockade is specific for alpha receptors (Nickerson, 1949; Nickerson and Goodman, 1947; Nickerson and Nomaguchi, 1948; Furchgott, 1954; Allwood and Ginsberg, 1959). During the onset of the blockade an initial labile block is set up at the receptor which can be antagonised by catecholamines and sympathomimetic amines. After a latent period, the molecules undergo cyclization to form ethylinimonium ions which combine with the receptor by covalent bonding to cause an irreversible block (Nickerson and Gump, 1949; Harvey and Nickerson, 1953; Nickerson, 1957; Graham, 1957).

Beta-haloalkylamines, such as dibenamine, have been found to antagonize the uptake of catecholamines into the adrenergic store of postganglionic sympathetic nerves by an action at the hypothetical transfer site in the cell membrane (Furchgott, 1960; Thoenen et al., 1964). (Fig. 5; p.79).

Section 1.3226

Pronethalol (Nethalide; Alderline)

The first blocking agent specific for adrenergic beta receptors was dichlorisoprenaline, a derivative of the stimulating agent isoprenaline (Powell and Slater, 1958;

Moran and Perkins, 1958). Furchgott (1959) and Dresel (1960) demonstrated that blockade of beta receptors by this agent was preceded by stimulation. Pronethalol (Nethalide, Alderline) was developed as a competitive beta adrenergic receptor blocking agent which did not exhibit such initial stimulation (Black and Stephenson, 1962). This drug antagonizes the effects of adrenaline on mammalian heart receptors which are predominately of the beta variety (Fig. 5; p.79).

Section 1.323

Blocking agents acting in the adrenergic neurone: hypotensive drugs

Since Nickerson (1949) described adrenergic blocking agents as those chemicals which antagonize catecholamines at the adrenergic receptor, a number of drugs have been introduced which modify the uptake, storage and release of catecholamines in chromaffin cells and nerves. These drugs are not able to antagonize the effects of circulating catecholamines.

Section 1.3231

Reserpine

Reserpine, an alkaloid of Rauwolfia serpentina is a potent agent which is able to deplete tissues of catecholamines and serotonin and to increase central sympathetic activity. The central actions of reserpine lead to lowering of the catecholamine content of the hypothalamus and of the adrenal medulla. Interruption of the spinal cord and splanchnic nerves or the use of ganglionic blocking drugs prevents the adrenal depletion in cats and rabbits but not rats (Brodie et al., 1957a, b; Holzbauer and Vogt, 1956; Kroneberg and Schümann, 1958; Stjärne and Schapiro, 1958, 1959; Callingham and Mann, 1958a, b; 1962; Mirkin, 1961a, b).

The peripheral effects of reserpine lead to depletion of the stores of 5-hydroxytryptamine and catecholamines in platelets, heart, adrenal tissues and nerves (Brodie et al., 1957a, b; Pletscher et al., 1955, 1956; Shore et al., 1957; Holzbauer and Vogt, 1956; Bertler et al., 1956, 1961; Carlsson and Hillarp, 1956; Carlsson et al., 1957). The nature of this peripheral action is complex. Depletion of catecholamines may be caused by prevention of synthesis, prevention of active uptake of amines into the

adrenergic stores or by direct release of preformed amines from the stores (Paasonen and Krayer, 1958; Burn and Rand, 1957, 1958b; Muscholl and Vogt, 1958; Bertler et al., 1961; Dengler et al., 1961a, b, 1962). The uptake of C^{14}-noradrenaline into the available adrenergic stores (Axelrod et al., 1961; Herrting et al., 1961c) is not blocked by reserpine, but transfer of the catecholamine from this store to the more permanent "bound store" is inhibited (Weiner and Trendelenburg, 1962). Reserpine given after a noradrenaline infusion accelerates release of the amine from the store into the tissue fluids (Axelrod et al., 1961). The decreased ability of reserpinised chromaffin tissues to synthesise noradrenaline appears related to the prevention of dopamine from entering the stores (Kirshner, 1962; Kirshner et al., 1963; Kuntzman et al., 1962). Euler and Lishajko (1961a, b) proposed that the depletion of catecholamines from the adrenergic store by reserpine is due to inhibition of transport of catecholamines across subcellular membranes.

As a result of the depletion of adrenergic stores, impulses passing along postganglionic sympathetic fibres are ineffective. The indirect actions of sympathomimetic amines are reduced or abolished (Burn and Rand, 1958a; Paasonen and Krayer, 1958; Carlsson et al., 1957). The

direct actions of catecholamines and sympathomimetic amines are potentiated, but only after an interval of several days. Within 24 hours of a reserpine injection, the actions of tyramine are abolished at a time when the adrenergic stores are empty. At this time no potentiation of the direct action of catecholamines occurs (Trendelenburg 1963). Immediately after a reserpine injection, as the stored catecholamines are released into tissue fluids, a potentiation of the indirect actions of tyramine and amphetamine may be observed (Naysmith, 1962; Harrison et al., 1963).

Section 1.3232

Guanethidine

Maxwell et al. (1959, 1960a, b) demonstrated the ability of guanethidine to block the effect of sympathetic nerve stimulation without affecting the response of the end organs to circulating catecholamines. An initial sympathomimetic action was described together with a brief ganglionic and vagal blockade. The sympathomimetic stimulation is due either to a release of catecholamines from tissue stores or to a direct stimulation of the effector cell (Gaffney et al., 1961; Gillis and Nash, 1961; Herrting et al., 1962b; Kadzielawa, 1962; Krayer et al.,

1962; Abercrombie and Davies, 1963). Within 6-18 hours of treatment depletion of catecholamines in the sympathetic nerve stores is greatest but brain and adrenal stores are unaffected (Sheppard and Zimmerman, 1959; Bein, 1960; Cass et al., 1960; Butterfield and Richardson, 1961; Cass and Spriggs, 1961; Gillis and Nash, 1961; Krayer et al., 1962; Sanan and Vogt, 1962). The blockade of adrenergic nerve transmission resembles that following treatment with bretylium (see below), as impulses passing along the nerves fail to release noradrenaline (Gaffney et al., 1962; Herrting et al., 1962b). After an injection of guanethidine it is found that blockade and antagonism of injected tyramine precede depletion of the adrenergic stores (Gaffney et al., 1962; Kroneberg and Schümann, 1962). McCubbin et al. (1961) suggested that the initial blockade of nerve terminals following treatment with guanethidine depends on a bretylium-like action at the terminal axon twiglets but that blockade during prolonged treatment with guanethidine resembles that brought about by reserpinisation (Fig. 5, p. 79).

It has been shown that the responses of the end organs to circulating catecholamines and sympathomimetic amines resemble those found after reserpinisation. Potentiation of directly acting amines and antagonism and suppression of

indirectly acting amines occurs (Maxwell et al., 1960a; Page and Dunstan, 1959; Kroneberg and Schümann, 1962).

Section 1.3233

Bretylium and related compounds

Choline 2,6-Xylyl Ether Bromide (TM10) was found to have a wide variety of peripheral autonomic actions (Hey and Brown, 1952, 1956). The compound stimulated and fleetingly blocked autonomic ganglia, led to a brief decamethonium-like blockade at the neuromuscular junction and exhibited muscarinic stimulatory actions. It also antagonised the effects of histamine and adrenaline and inhibited monoamine oxidase. The most striking action of TM10 was its ability to block responses to sympathetic stimulation in doses which did not antagonise circulating noradrenaline. Exley (1956, 1957) and Exley and Fleming (1960) showed that this blockade was due to a failure of the sympathetic fibre to release noradrenaline. The liberation of catecholamines from the adrenal medulla was not affected. The doses used to cause blockade did not prevent the conduction of postsynaptic impulses along the adrenergic fibres. Prolonged treatment with TM10 led to a depletion of adrenergic stores (Coupland and Exley, 1957; Trendelenburg and Weiner, 1962).

Bretylium was shown to have many properties similar to those described above for TM10. It exerted a post-ganglionic blockade on the sympathetic system without antagonising the response of the end organs to circulating catecholamines and without preventing conduction of impulses along the sympathetic nerve fibre (Boura and Green, 1959; Exley and Fleming, 1960) (Fig. 5; p.79). Boura and Green (1959) showed that blocked sympathetic nerves were able to release only small amounts of transmitter when stimulated whereas cholinergic nerves were unaffected and Boura et al. (1960) showed that bretylium accumulated specifically in adrenergic nerves. The latter workers and also Exley and Fleming (1960) found that bretylium, like TM10, has a local anaesthetic effect on nerve tracts but showed that the doses used to cause axonal block were higher than those required to prevent release of catecholamines from the nerve endings. Green (1960) suggested that the site of action was at highly sensitive terminals of the adrenergic neurone near the neuro-effector junction. Bretylium has been shown to prevent both the uptake and release of radioactive noradrenaline from mammalian tissues but does cause a transient release immediately after its application (Herrting et al., 1962b). Prolonged treatment with the

drug can lead to a limited depletion of catecholamines in nerve stores (Cass and Spriggs, 1961) and to potentiation of the effects of catecholamines (Boura et al., 1959; Gokhale and Gulati, 1961).

The sympathomimetic effects which immediately follow bretylium injections, which are related to the release of noradrenaline described above, are prevented by pretreatment with reserpine, cocaine or amphetamine but are restored by small infusions of noradrenaline which refill the "available" (tyramine-sensitive) store (Day, 1962; Gokhale et al., 1963).

Section 1.324
The Storage of Catecholamines in Nerves and Chromaffin Cells

Mammalian adrenergic postganglionic sympathetic fibres contain considerable amounts of noradrenaline, dopamine and adrenaline (von Euler, 1948; Peart, 1949). These amines disappear from organs following degeneration of the sympathetic nerve supply (Cannon and Lissak, 1939). Blaschko and Welch 1953, Blaschko et al., 1955 and Hillarp et al. (1953, 1954) isolated catecholamine-containing granules from the cells of the adrenal medulla and Schümann (1958a, b) and von Euler (1958a, b) demonstrated the presence of similar granules in sympathetic fibres.

Other stores of catecholamines are found, according to Bertler et al. (1960b), in paraganglia, the organs of Zuckerkandl and in isolated dermal cells.

Burn and Rand (1958a) postulated that the noradrenaline released during sympathetic nerve activity comes from a store at or near the terminal branches of the adrenergic neurone. The disappearance of the catecholamines as the nerves degenerate and the presence of the granules in the cells suggests that the adrenergic store consists of these axoplasmic granules.

The adrenergic stores in the neurones can store catecholamines synthesised within the same cell or absorbed from blood and tissue fluids (Cervoni et al., 1960; Whitby et al., 1960; 1961; Herrting and Axelrod, 1961; Herrting et al., 1961a, b; Strömblad and Nickerson, 1961; Wolfe et al., 1962; Kirpekar et al., 1962). These latter amines are then available for release during nerve activity or under the influence of indirectly acting chemical agents (Herrting and Axelrod, 1961; Axelrod et al., 1962; Chidsey et al., 1963). Denervated organs, which have presumably also lost their adrenergic nerve store, are unable to take up circulating noradrenaline (Herrting et al., 1961).

Injection of tyramine into animals which have previously

received an acute dose of reserpine shows that the action of the amine is abolished at a time when the catecholamine content of the store is low. However the response to tyramine can be restored by treatment of the tissue with low concentrations of noradrenaline although little measurable increase in tissue noradrenaline can be detected. These observations have led many workers to suggest that the adrenergic store consists of two compartments:- a large "bound" store of noradrenaline and a smaller "available" store (Trendelenburg, 1961; Stjärne, 1961; Chidsey et al., 1962; Crout et al., 1962; Stone et al., 1962; Weiner et al., 1962a, b; Harrison et al., 1963). Further studies were made on the store using H^3-noradrenaline and the results indicated that soon after uptake into the tissues much of the amine is metabolised by catechol-O-methyl transferase (Axelrod, 1959). A proportion of the absorbed amine is retained in the store (Whitby et al., 1961; Herrting et al., 1962b; Wolfe et al., 1962; Chidsey and Harrison, 1963). This portion disappears at a slower rate and is believed to be transferred to the second, larger store (Axelrod et al., 1961, 1962; Kopin and Gordon, 1962a, b; Chidsey and Harrison, 1963; Crout, 1963). The amines in this store are predominantly metabolised by monoamine oxidase (Kopin and Gordon, 1962a,b).

Amines liberated by tyramine from the adrenergic store are metabolised by catechol-O-methyl transferase. The larger store of catecholamines is undepleted at a time when tachyphylaxis to tyramine has developed and is able to release sympathetic mediator in response to nerve stimulation (Harrison et al., 1963).

The adrenergic store is envisaged as granular stores of catecholamines within the nerve endings consisting of two compartments (Fig. 5; p.79). The first, "available" compartment may be emptied by nerve impulses, reserpine and sympathomimetic amines whilst the second may be depleted by nerve impulses, reserpine and guanethidine. Active transfer sites are also envisaged in the cell membrane through which catecholamines and sympathomimetic amines are absorbed. These transfer sites may be blocked by cocaine and by adrenergic blocking agents such as dibenamine and bretylium (Kirpekar and Cervoni, 1963; Potter and Axelrod, 1963; Thoenen et al., 1964).

Section 1.325
The Adrenergic Receptors

Lewandowsky (1899) showed that sympathetically denervated organs were still able to react to circulating adrenaline. Langley (1906) postulated the presence of

receptor sites interposed between the nerve endings and the effector cell with which adrenaline could combine. Ariëns (1960) proposed that in order to decide whether or not a receptor site was present in a tissue it was necessary to show that molecular specificity was required in any drug molecule to elicit a response from the end organ. Transmitter substances have highly specific structural relations with their receptor sites and changes in that molecular structure usually lead to decreased affinity for that receptor or even to antagonism with the natural transmitter.

In 1948 Ahlquist proposed that adrenergic receptors could be classified as "alpha" or "beta" according to their affinity for different classes of catecholamines (Fig. 5; p.79). Such a classification was based on the reactions of various organs to graded doses of noradrenaline, adrenaline and isoprenaline. Subsequent workers have modified the original proposals (Ahlquist and Levy, 1959; Furchgott, 1959). Alpha receptors are by definition those which are most easily stimulated by noradrenaline, less easily by adrenaline and least by isoprenaline. They are blocked by drugs such as dibenamine, phentolamine, piperoxane and ergotamine. Beta receptors are more easily stimulated by secondary amines such as isoprenaline and

adrenaline. This activity of adrenaline is exhibited following alpha receptor blockade. Frequently, for example in blood vessles, the effects of alpha and beta receptor stimulation are opposite. Thus, after alpha blocking agents such as ergotamine, the unmasked beta activity of adrenaline has been called "adrenaline reversal" (Dale 1913). Beta receptors may be blocked competitively by dichlorisoprenaline or pronethalol.

Section 1.326

Changes in sensitivity of sympathetically innervated organs

When organs are denervated (i.e. the last neurone in the motor supply is sectioned and the ending allowed to degenerate) a demonstrable increase in sensitivity to their normal neurohumours and related chemicals can be demonstrated (Cannon, 1934; Cannon and Rosenblueth, 1949). Budge (1855) described pupil dilatation in the denervated iris and subsequent workers were able to relate this and other phenomena to increased sensitivity of sympathetically denervated organs to circulating adrenaline (Lewandowsky, 1899; Anderson, 1903, 1905; Meltzer, 1904; Elliot, 1905). Tainter and Chang (1927) found that, after treatment with cocaine, the actions of adrenaline were similarly potentiated but that tyramine was antagonised. Tainter (1929) described

a similar subsensitivity to ephedrine after cocaine and Burn and Tainter (1931) and Bulbring and Burn (1938) found that the actions of tyramine and ephedrine were also antagonised by denervation. Burn and Hutcheon (1949) showed that the potentiation of catecholamines after denervation was greater than that caused by cocaine. Bulbring and Burn (1938) and Fleckenstein and Burn (1953) classified sympathomimetic amines according to their actions after denervation and cocaine (see Section 1.321). The classification of amines as "direct", "mixed" or "indirect" (Fleckenstein and Bass, 1955; Fleckenstein and Stöckle, 1957) has been modified by other workers. Thus if a beta-OH group is present together with a meta-phenolic OH in the amine molecule, potentiation can follow denervation or cocaine. Similar molecules with a para-OH group are not potentiated (Innes and Kosterlitz, 1954; Holtz et al., 1960). Carlsson et al., (1957) showed that the actions of tyramine could be prevented by reserpine and this led Burn and Rand (1958) to show that sympathomimetic amines could be divided into the same three classes according to their activity after reserpine as after cocaine or denervation. Innes and Krayer (1958) and Liebman (1961) carried out more precise observations on the effects of reserpine on indirect and mixed sympathomimetic amines. They plotted

dose/response curves for the amines before and after reserpinisation and concluded that the direct actions of mixed amines could be unmasked by the alkaloid whereas the indirect actions of both mixed and indirect sympathomimetic amines were always suppressed by reserpine.

Fleming and Trendelenburg (1961) showed that supersensitivity to directly acting amines was NOT invariably accompanied by subsensitivity to indirectly acting amines. They showed that a large single dose of reserpine which emptied the adrenergic store and abolished the actions of tyramine and nerve stimulation was not accompanied by supersensitivity to noradrenaline. Supersensitivity after denervation or reserpinisation develops after a time interval of several days (Krayer et al., 1962; Marley, 1962).

Trendelenburg et al. (1962a) and Schmidt and Fleming, (1963) studied the effect of short term pretreatment with reserpine on a variety of sympathomimetic amines. They concluded that the amines should not be divided into three separate groups but into a series, exhibiting wholly direct to wholly indirect actions. The majority of amines exhibit both types of action but the relative importance of each varies.

Section 1.3261

Cocaine

Cocaine sensitises sympathetically innervated organs to directly acting sympathomimetic amines (Fig. 6; p.79). It has been mentioned above that most sympathomimetic amines have both direct and indirect actions and that the latter are abolished by short term pretreatment with reserpine. Trendelenburg et al.(1962b) studied the potentiation of the direct actions of a variety of sympathomimetic amines by cocaine after the indirect actions were abolished by reserpine. They found that the direct actions of the amines were not all equally potentiated.

The indirect actions of sympathomimetic amines are competitively antagonised by cocaine (Tainter and Chang, 1927; Fleckenstein and Stöckle, 1957; Trendelenburg, 1961).

In an otherwise normal animal therefore, the effect of cocaine on any sympathomimetic amine will be the resultant activity of the amine after its indirect activity has been antagonised (constant for all amines) and its direct actions potentiated (this varies for each amine).

Section 1.3262

Hypotensive Drugs

Fleming and Trendelenburg (1961) showed that 3 mg/kg reserpine 24 hours previously abolished the effects of tyramine on the cat nictitating membrane by depleting the adrenergic store without causing supersensitivity to noradrenaline. However, long term pretreatment with smaller doses of reserpine, 0.1 mg/kg/day for many days, led to supersensitivity of the cat nictitating membrane to catecholamines after a week. Maximum sensitivity occurred after 14 days. In this respect reserpine differs from cocaine in that a time factor is involved in the development of supersensitivity to directly acting amines (Trendelenburg, 1959). In the cat cardiovascular system supersensitivity to circulating noradrenaline develops within three days. The supersensitivity to catecholamines is independent of the depletion of the adrenergic store as daily injections of 0.03 mg/kg/day into cats did lead to supersensitivity of the nictitating membrane without completely emptying the nerve store (Fleming and Trendelenburg, 1961). Trendelenburg (1963) pointed out that, unlike cocaine which only potentiates catecholamines at sympathetic neuroeffector junctions, reserpine can also

potentiate other substances which stimulate the effector cell.

Supersensitivity of organs to catecholamines has also been described after treatment with bretylium or guanethidine (Emmelin and Engstrom, 1961; Gokhale and Gulati, 1961).

Section 1.3263

Decentralization

Chronic preganglionic denervation (= decentralization) leads to a supersensitivity of the effector organ to a variety of agents (Fig. 6; p.79). In accordance with Cannon's Law of Denervation (Cannon, 1939) this sensitivity is not so great as that following postganglionic denervation. Hampel (1935) stated that a time of two weeks was required for decentralization sensitivity to become maximal. Trendelenburg and Weiner (1962) found that decentralization supersensitivity of the cat nictitating membrane also took time to develop and was unrelated to the content of the adrenergic store.

In decentralized animals which have become sensitized, it is found that sympathomimetic amines with mainly indirect actions are not antagonized. In fact, as the adrenergic

store is not affected (Rehn, 1958) and the effect of catecholamines displaced from that store is enhanced, an overall potentiation of agents such as tyramine can occur. Prolonged treatment with ganglion glocking drugs or with hypotensive drugs leads to a similar generalised supersensitivity (Trendelenburg, 1963).

Section 1.3264

Denervation

The supersensitivity which develops after postganglionic denervation resembles that following cocaine but several days must pass before it is maximal (Hampel, 1935). Burn and Hutcheon (1949) showed that the supersensitivity to catecholamines after denervation is greater than that elicited by cocaine. The degeneration of the nerve fibre leads to loss of the adrenergic store and the indirect actions of sympathomimetic amines are abolished. This supersensitivity, like that of cocaine, is specific in that amines with a beta- and meta-OH group are potentiated but those with a beta- and para-OH are not (Trendelenburg et al., 1962b). Other chemicals which can excite the effector cell, but which are unrelated to the sympathetic mediator, are potentiated to a degree similar to that following reserpine or decentralization (Trendelenburg, 1962).

Fig. 5 Diagram to show the arrangement of the pre- and post-ganglionic nerve fibres in the mammalian sympathetic and the site of action of various autonomic drugs.

o - acetylcholine store,

▮ - nicotinic acetylcholine receptor,

B - "bound" adrenergic store,

A - "available" adrenergic store,

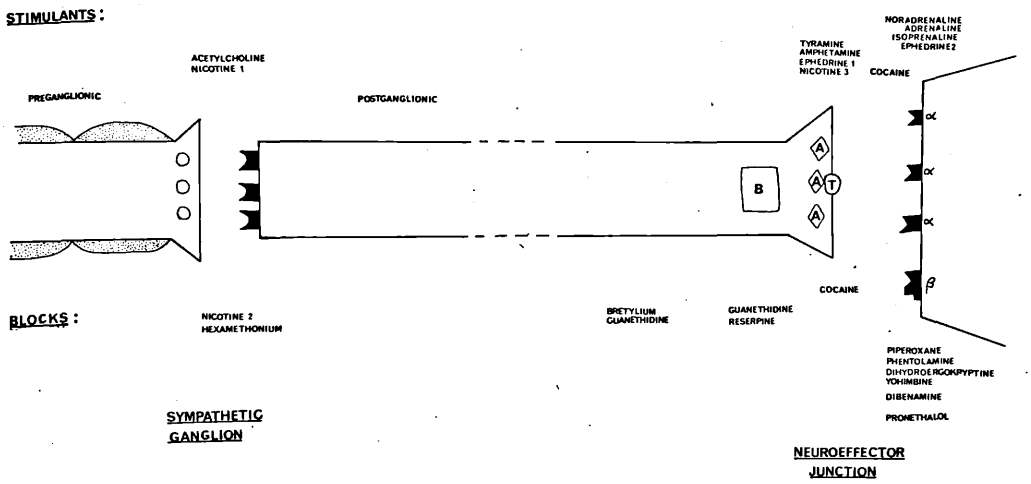
T - "transfer site" in cell membrane of post-ganglionic fibre,

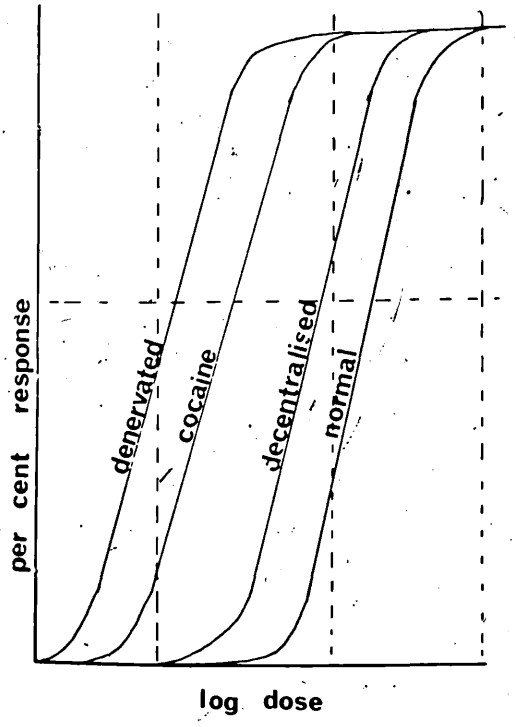
α - the alpha adrenergic receptor,

β - the beta adrenergic receptor.

Fig. 6 Diagram to show the change in position of the dose response curves of a sympathetically innervated organ to noradrenaline and adrenaline after various agents and procedures (after Trendelenburg, 1963).

MAMMAL


STIMULANTS :
ACETYLCHOLINE
NICOTINE 1
PREGANGLIONIC
POSTGANGLIONIC
TYRAMINE
AMPHETAMINE
EPHEDRINE 1
NICOTINE 3
NORADRENALINE
ADRENALINE
ISOPRENALINE
EPHEDRINE2
COCAINE
α
α
α
β
B
A
A
T
A
COCAINE
BLOCKS :
NICOTINE 2
HEXAMETHONIUM
BRETYLIUM
GUANETHIDINE
GUANETHIDINE
RESERPINE
PIPEROXANE
PHENTOLAMINE
DIHYDROERGOKRYPTINE
YOHIMBINE
DIBENAMINE
PRONETHALOL
SYMPATHETIC
GANGLION
NEUROEFFECTOR
JUNCTION


per cent response
denervated
cocaine
decentralised
normal
log dose

It seems that denervation sensitivity combines a generalised "decentralization" sensitivity component together with a specific "cocaine-like" component (Fig. 6; p.79) (Trendelenburg, 1963).

Section 1.3265

The mechanism which leads to supersensitivity

Cocaine has long been known to antagonize the action of monoamine oxidase (Philpot, 1940) and it was believed that this enzyme ended the active life of catecholamines. However, other agents which are more potent monoamine oxidase inhibitors do not cause supersensitivity and also amines which are known to be metabolised only by catechol O-methyl transferase are potentiated by cocaine (Kamijo et al., 1956; Wylie et al., 1960).

The cause of subsensitivity to tyramine and other amines after cocaine may be due to an action of the drug on active transfer sites through which the amines have to pass (see Section 1.324). Trendelenburg (1963) proposed that the mechanisms which cause subsensitivity and supersensitivity after cocaine are separate actions.

Denervation, decentralization, prolonged reserpinization and prolonged ganglion blockade all lead to blockade

of tonic impulses passing along the fibres and also to cessation of miniature end plate potentials which are presumed to be due to release of small amounts of noradrenaline (Burnstock and Holman, 1963). The receptors would presumably be less saturated with transmitter molecules under these conditions and may be able to offer more active sites to molecules of injected amines. Veldstra (1956) proposed that a further source of increased activity of injected molecules at the adrenergic receptors might lie in the loss of sites which normally prevented amines reaching the receptor. The adrenergic store itself disappears after denervation and cannot take up amines after reserpinisation or cocaine. This inability would lead to a relative increase of the amine concentration at the receptor. Trendelenburg (1963) pointed out that these concepts of supersensitivity do not explain the time factor in the development of supersensitivity nor do they explain the differing potentiation of para- and meta-OH analogues after cocaine and denervation. It has been proposed that structural changes in the receptor itself might be involved.

Section 1.4

The effect of autonomic drugs on fish chromatophores

The certainty of a sympathetic (adrenergic) pigment-aggregating system for the control of innervated chromatophores in teleost fishes, and the possibility of a cholinergic (parasympathetic) pigment-dispersing system has led many workers to investigate the effects of mammalian autonomic drugs on teleost colour changes.

The effects of the drugs tested are summarised in Table 2. In most of the species investigated, the effector cells studied were melanophores. In fish such as Phoxinus, for example, rapid changes in the state of the melanophores are controlled by the nervous system and slow changes by the pituitary gland hormones. On the other hand, in Labrus the erythrophores (Scheline, 1963) and in Fundulus the xanthophores as well as the melanophores (Fries, 1942a) are innervated.

It can be seen from Table 2 that innervated chromatophores are almost invariably aggregated by catecholamines and sympathomimetic amines. They are dispersed by adrenergic blocking agents. Parasympathomimetic agents have frequently been found to cause dispersion. Further details of the observations are included in relevant parts

of the text (Section 3). Recent workers in this field have been able to conclude that the aggregating neurohumour is most probably a catecholamine. Fujii (1961) and Watanabe et al. (1962b) concluded that the agent was adrenaline whilst Scott (1965) proposed that it might be adrenaline, noradrenaline or dopamine. The latter worker found that pyrogallol potentiated the effects of injected catecholamines and concluded that the metabolism of endogenous catecholamines involved the participation of the enzyme Catechol O-methyl transferase.

The work which is described in the experimental section of this thesis was carried out to investigate the actions of autonomic drugs, which have been of use in experimental studies on the autonomic nervous system of mammals, on the colour change mechanism of the minnow. The peripheral organisation of the autonomic chromatic fibres has been studied previously by several workers (Section 1.23 - 1.25).

Table 2

The response of innervated teleost chromatophores to treatment with autonomic drugs

EFFECT	ANIMALS	AUTHORS
Catecholamines		
Adrenaline		
Aggregation	Abudefduf	Rasquin,1958.
	Aequidens	Turner and Carl,1955.
	Ameiurus	Bray,1918; Bacq,1933; Parker,1941b,1942; Wykes,1938; Rasquin, 1958.
	Anguilla	Waring and Landgrebe, 1941.
	Apogonichthys	Rasquin,1958.
	Bathygobius	Rasquin,1958.
	Carapus	Rasquin,1958.
	Carassius	Fukui,1927; Beauvallet, 1938; Iwata et al., 1959a,b.
	Chasmichthys	Fujii,1958 et seq.
	Chrosomus	Saphir, 1935.

EFFECT	ANIMAL	AUTHORS
Adrenaline (cont.)		
	Crenilabrus	Beauvallet,1939.
	Cyprinodon	Ciabatti.,1929; Rasquin,1958.
	Cyprinus	Beauvallet,1939; Veil,1936.
	Erotelis	Rasquin,1958.
	Fundulus	Spaeth,1916,1918; Spaeth and Barbour, 1917; Wyman,1924; Smith,1931b,1939; Parker,1934; Abramowitz, 1936; Bogdanovitch, 1937; Foster,1937; Wykes,1938; Pierce, 1941.
	Gambusia	Ueda,1955.
	Gadus	Fänge,1962.
	Gobius	Meyer,1931.
	Labrus	Scheline,1963.
	Lebistes	Fänge,1962.
	Limanda	Hewer,1927.
	Lophopsetta	Osborne,1939.

EFFECT	ANIMAL	AUTHORS
Adrenaline (cont.)		
	Macropodus	Reidinger and Umrath, 1952.
	Mollienesia	Pierce,1941.
	Mugil	Rasquin,1958.
	Opsanus	Rasquin,1958.
	Oryzias	Ueda,1955; Ando,1960.
	Paralichthys	Osborne,1939.
	Peusorasbora	Ueda,1955.
	Phoxinus	Abolin,1925a; Hewer, 1926; Reidinger,1952; Fortune,1960; Pye, 1964b.
	Pleuronectes	Meyer,1931.
	Pseudopleuronectes	Osborne,1939.
	Rhodeus	Umrath,1957.
	Salmo	Gianferari,1922; Robertson,1951; Fortune,1960.
	Scardinius	Vialli,1927.
	Scophthalamus	Scott et al.1962a,b; Scott,1965.
	Strongylura	Rasquin,1958.

EFFECT	ANIMAL	AUTHORS
Adrenaline (cont.)		
	Thalassoma	Takaoka,1927.
	Tautoga	Smith,1941.
Aggregation then Dispersion	Oryzias	Watanabe et al.1962a.
Dispersion only	Parasilurus	Enami,1940.
Noradrenaline		
Aggregation	Carassius	Mori and Lerner (in Fujii,1961)
	Chasmichthys	Fujii,1961.
	Fundulus	Mori and Lerner (in Fujii,1961)
	Gadus	Fänge,1962.
	Labrus	Scheline,1963.
	Lebistes	Fänge,1962.
	Phoxinus	Pye,1964.
	Rhodeus	Umrath,1957.
	Scophthalamus	Scott et al.1962a,b; Scott,1965.
Dopamine		
Aggregation	Scophthalamus	Scott,1965.

EFFECT	ANIMAL	AUTHORS
Isoprenaline		
Aggregatinn	Scophthalamus	Scott et al.1962a,b; Scott,1965.
Sympathomimetic amines		
Ephedrine		
Aggregation	Aequidens	Turner and Carl,1955.
	Corydoras	Turner and Carl,1955.
	Cyprinodon	Ciabatti,1929.
	Gambusia	Ciabatti,1929.
	Salmo	Robertson,1951.
Ephetonin		
Aggregation	Cyprinodon	Ciabatti,1929.
	Gambusia	Ciabatti,1929.
Methamphetamine		
Aggregation	Scophthalamus	Scott,1965.
Neosynephrine		
Aggregation	Salmo	Robertson,1951.
Phenylethylamine		
Aggregation	Fundulus	Barbour and Spaeth, 1917.

EFFECT	ANIMAL	AUTHORS
p-oxyphenylethylamine		
Aggregation	Fundulus	Barbour and Spaeth, 1917.
Sympatol		
Aggregation	Cyprinodon	Ciabatti,1929.
	Gambusia	Ciabatti,1929.
Tyramine		
Aggregation	Fundulus	Barbour and Spaeth, 1917.
	Gasterosteus	Osterhage,1932.
	Labrus	Scheline,1963.
Other amines		
Indoethylamine		
Aggregation	Fundulus	Barbour and Spaeth, 1917.
Serotonin		
Aggregation	Labrus	Scheline,1963.
	Scophthalamus	Scott et al.1962a,b; Scott,1965.
No effect	Chasmichthys	Fujii,1961.

EFFECT	ANIMAL	AUTHORS
Adrenergic blocking agents		
Phentolamine		
Dispersion	Phoxinus	Pye,1964,b.
Ergotamine		
Dispersion	Scophthalamus	Scott,1965.
Aggregation	Phoxinus	Pye,1964b.
	Fundulus	Wyman,1924a; Smith, 1931b.
Dispersion followed by aggregation	Phoxinus	Giersberg,1930; Von Gelei,1942.
Dibenamine		
Dispersion	Chasmichthys	Fujii,1961.
	Oryzias	Watanabe et al.1962b.
	Scophthalamus	Scott et al.1962a,b; Scott,1965.
Dibenzylene		
Dispersion	Labrus	Scheline,1963.
	Scophthalamus	Scott et al.1962a,b.
Dichloroisoprenaline		
Dispersion	Scophthalamus	Scott et al.1962a,b.

EFFECT	ANIMAL	AUTHORS
Anaesthetics		
Cocaine		
Dispersion	Fundulus	Wyman,1924; Gilson, 1926.
	Phoxinus	Abolin,1925b.
	Salvelinus	Lowe,1917.
Aggregation	Fundulus	Smith,1931b; Gilson, 1926.
	Phoxinus	von Frisch,1911.
	Thalassoma	Takaoka,1927.
	Aequidens	Turner and Carl,1955.
Dibucaine		
Dispersion	Scophthalamus	Scott,1965.
Procaine		
Dispersion	Oryzias	Watanabe et al.1962b.
Hypotensive agents		
Reserpine		
Dispersion	Betta	Turner and Carl,1955.
	Brachydanio	Turner and Carl,1955.
	Corydoras	Turner and Carl,1955.
	Labrus	Scheline,1963.
	Macropodus	Turner and Carl,1955.

EFFECT	ANIMAL	AUTHORS
Reserpine (cont.)		
	Trichogaster	Turner and Carl,1955.
Cholinesters		
Acetylcholine		
Dispersion	Fundulus	Parker,1934d.
	Hoplias	Mendes,1942.
	Macropodus	Chin,1939; Reidinger and Umrath,1952.
	Oryzias	Ando,1960.
	Ophiocephalus	Chang et al.1939.
	Phoxinus	Giersberg,1930.
	Rhodeus	Umrath,1957.
	Salmo	Robertson,1951.
No effect	Fundulus	Barbour and Spaeth, 1917; Parker,1931.
	Rhodeus	Wunder,1931.
	Scorpaena	Smith and Smith,1934.
	Tautoga	Smith,1941.
Aggregation	Fundulus	Bogdanovitch,1937; Parker,1931.
	Carassius	Beauvallet,1938.

EFFECT	ANIMAL	AUTHORS
Acetyl-betamethylcholine		
Dispersion	Fundulus	Parker,1934d.
Anticholinesterases		
Physostigmine (eserine)		
Dispersion	Fundulus	Barbour and Spaeth, 1917; Gilson,1926; Bogdanovitch,1938.
	Hoplias	Mendes,1942.
	Ophiocephalus	Chang et al.1939.
	Oryzias	Watanabe et al.1962b.
	Phoxinus	Abolin,1926.
	Salmo	Robertson,1951.
	Scorpaena	Smith and Smith,1934.
Aggregation	Thalassoma	Takaoka,1927.
Parasympathomimetics		
Pilocarpine		
Dispersion	Fundulus	Barbour and Spaeth, 1917; Smith,1931b; Bogdanovitch,1938.
	Oryzias	Watanabe et al.1962b.
	Phoxinus	Abolin,1925b; Giersberg,1930.

EFFECT	ANIMAL	AUTHORS
Pilocarpine (cont.)		
	Rhodeus	Umrath,1957.
	Salmo	Robertson,1951.
	Scorpaena	Smith and Smith,1934.
Aggregation	Carassius	Beauvallet,1934.
	Thalassoma	Takaoka,1927.
Muscarinic blockers		
Atropine		
Dispersion	Carassius	Watanabe,1960.
	Chasmichthys	Fujii,1960.
	Crenilabrus	Beauvallet,1938.
	Cyprinus	Beauvallet,1938.
	Fundulus	Barbour and Spaeth, 1917; Spaeth,1916; Wyman,1924; Gilson, 1926; Bogdanovitch, 1937,1938; Smith, 1931b.
	Gasterosteus	Osterhage,1932.
	Macropodus	Reidinger and Umrath, 1952.
	Oryzias	Watanabe et al.1962b.

EFFECT	ANIMAL	AUTHORS
Atropine (cont.)		
Dispersion	Phoxinus	Abolin,1925b; Smith, 1931a; Pye,1964b.
	Rhodeus	Osterhage,1932; Umrath,1957.
	Salvelinus	Lowe,1917.
	Scorpaena	Smith and Smith,1934.
Antagonism of parasympatho-mimetic agents	Fundulus	Smith,1931b.
	Rhodeus	Wunder,1931; Umrath,1957.
	Thalassoma	Takaoka,1927.
Nicotinic blockers		
Nicotine		
Dispersion	Carassius	Verne and Vilter,1935a.
	Fundulus	Wyman,1924.
	Phoxinus	Abolin,1925b.
	Scorpaena	Smith and Smith,1934.
	Oryzias	Watanabe et al.1962b.
Aggregation	Salvelinus	Lowe,1917.
Hexamethonium		
Dispersion	Fundulus	Wilber,1960.

EFFECT	ANIMAL	AUTHORS
Effect of general anaesthesia on shade of fish		
Dispersion	Fundulus	Wyman,1924; Gilson, 1926.
	Oryzias	Ando,1960.
	Phoxinus	Healey,1948.

Section 2.0
Materials and Methods

The animals used in the experimental section were European minnows, Phoxinus phoxinus (L.), obtained from the River Lea in Hertfordshire. They were kept in the laboratory in porcelain sinks and were supplied with running tap water and compressed air. The fish were fed three times a week on finely minced ox heart from which the fat and connective tissue were removed. Bemax was supplied once a fortnight and the fish remained in good condition for long periods. The sinks in which the fish were kept were either painted black or left white so that fish completely adapted to either shade were available for study at any time.

Section 2.1
Drug injections

Injections were made into fish using an Everett 1 ml. hypodermic syringe with a "Star" No.22 needle. The fish to be injected was gently scooped from the water and held, belly uppermost, in a fine net. The needle was introduced through the net into the coelomic cavity from behind the

pelvic fin and to one side of the midline. Care was taken to hold the needle almost parallel to the body surface to avoid damage to the viscera. Only 0.1 ml. of fluid was injected in order to minimise subsequent leakage of fluid from the injection site as a result of increased pressure in the body cavity.

The vehicle for practically all injections was fresh-water teleost Ringer made up to the formula of Young (1933b). Adrenaline, dibenamine and yohimbine were made soluble in saline by adding a few drops of dilute HCl. Reserpine was dissolved in very dilute acetic acid and diluted to the required volume with distilled water to prevent reprecipitation of the alkaloid.

The dose of each drug injected into minnows was calculated in mg/kg. Only the weight of active agent was expressed in the dosage, allowance being made for the inactive radicles. The average weights of the minnows, which were usually between 6.5 and 9 cm. long, varied after complete recovery from operations (Sections 2.2 and 2.3) and were as follows:-

Mean weight of normal fish ..... 3.72 gm (100 animals)

Mean weight of spinal nerve sectioned fish (6 weeks after operation) ..... 3.20 gm (30 animals)

Mean weight of spinal sectioned fish (8 weeks after operation ... 2.90 gm (30 animals)

Section 2.2

Spinal section

Minnows were anaesthetised in 0.5% urethane in tap water and anaesthesia was maintained during the operation by a continuous flow of 0.25% urethane in tapwater over the gills (von Frisch and Stetter, 1932). The fish were then placed dorsal side up in a wax trough and supported by rolls of filter paper. A 0.5 cm. cut was made to one side of the midline with a fine knife and extended down through the body muscles to expose the vertebral column. The wound was held apart by thin hooks and bathed in a jet of Young's freshwater teleost Ringer. A section of the spinal cord was exposed and removed using a dentist's drill with a fine burr. More than fifty fish which had been operated on in this way, and which had been used in subsequent experiments, were preserved in formalin and transected to determine the site of the operation. It was found that all the operated animals had received a spinal section between vertebrae 7 and 12 and over 90% were cut between vertebrae 8 and 10. Section at this level interrupted the spinal chromatic tract without preventing the animal maintaining its normal equilibrium after recovery in tap-water. Immediately after the operation the wound was

stitched with fine nylon thread or with "Ethicon" braided silk thread using a fine suture needle. The wound was kept clean during healing by daily immersion of the fish into 3% NaCl solution. It was found that application of "Savlon" (I.C.I.) antiseptic cream to the fresh wound protected the tissues during the early stages of healing.

Section 2.3

Spinal nerve section

Minnows were prepared for the operation as described above for spinal section. A longer incision, 1 - 2 cm. long, was made and the vertebral column exposed. Four or five spinal nerves of one side were exposed and the rami lateralis and dorsalis of each were cut (Fig. 7; p.102). Immediately after a successful operation on a pale fish a dark patch appeared in the area deprived of chromatic innervation. Initially, such a band failed to appear in many fish but it was later found that sectioning the tracts further from the spinal cord was successful in the majority of individuals. As it was not possible to observe the level at which rami communicantes joined the spinal nerve, the appearance of a dark stripe was taken as a criterion for disruption of the chromatic tract to that

region. Section of the ramus ventralis alone did not lead to the appearance of a dark band in the dorsolateral region of the trunk which was the site of investigation of denervated melanophores. Completion of the operation and postoperative care were as described for spinal sectioned fish.

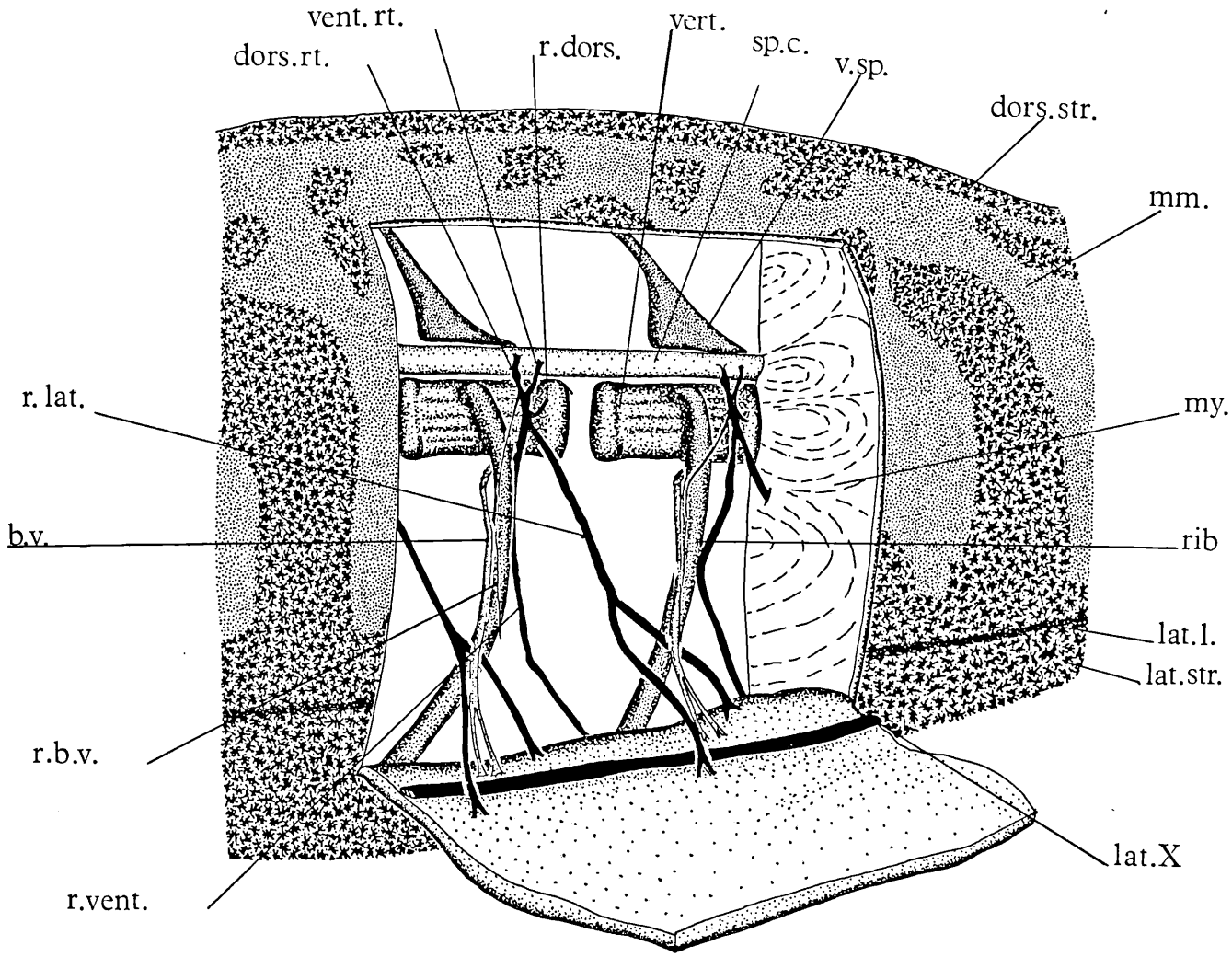
Section 2.4

The system for estimating melanophore activity

Healey (1948) described a method for estimating the overall shade of the skin of the minnow using shades of grey which approximated to standards of reflectivity laid down by Ostwald. In the minnow, in which background adaptation is predominantly brought about by melanophore activity, changes in colour are minimal and adaptation is seen as a change in shade of grey of the fish. The use of macroscopic observations is far quicker than use of the Hogben melanophore index (Section 1.1) and allows the effect of injected drugs to be followed by eye. Accordingly a series of standard grey papers was prepared with which the shade of a fish at any time could be compared.

Fig. 7 Diagram to show the arrangement of the spinal nerves of the trunk region of the minnow (R.H. side) in relation to the spinal cord and vertebral column. Spinal sections were made by removing several millimetres of the cord in the region of vertebrae 8 - 9 with a fine dentist's drill. Spinal nerve sections were performed by cutting the rami dorsalis and lateralis of a series of spinal nerves approx. 3 - 5 millimetres away from their separation from the ramus ventralis.

b.v., segmental artery; dors.rt., dorsal root of spinal nerve; dors.str., dorsal stripe of deeper pigmentation; lat.l., lateral line; lat.str., lateral stripe of deeper pigmentation; lat.X, lateralis branch of vagus; mm., background of micromelanophores; my., myotomes; r.bv., spinal nerve ramus accompanying segmental artery; r.dors., cut dorsal branch of spinal nerve; r.lat., lateral branch of spinal nerve; r.vent., ventral branch of spinal nerve; rib, rib; sp.c., spinal cord; vent., ventral root of spinal nerve; vert., centrum of 8th vertebra; v.sp., vertebral spine.


vent. rt.
dors. rt.
r. dors.
vert.
sp. c.
v. sp.
dors. str.
mm.
r. lat.
my.
b.v.
rib
lat. l.
lat. str.
r.b.v.
lat. X
r.vent.

Section 2.41

Preparation of the standard papers.

Fresh barium sulphate was precipitated from a mixture of barium chloride and sodium sulphate, was centrifuged down at 1,500 r.p.m. and removed as a thick moist paste from the tubes. A little Winton picture varnish was added to increase cohesion and the paste was applied to coarse glass paper. After drying, the surface was a smooth matte finish, sufficiently robust to be used in a disappearing-spot photometer. The white barium sulphate was taken as a standard reflectance of 100% for comparison with bristol board and a good black paper. During photometric observations, each surface was illuminated separately by its own lamp and the surfaces were reversed to counteract assymetric characteristics in the apparatus. Calculation of the reflectivity of the bristol board and black paper were made using the formula:-

$$\frac{\text{Reflectance A}}{\text{Reflectance B}} = \frac{(\text{Distance A from photometer})^2}{(\text{Distance B from photometer})^2}$$

$$= \frac{d_A^{\,2}}{d_B^{\,2}}$$

The ratio of reflectances of two pieces of bristol board were compared to measure the efficiency of the photometer.

$\frac{d_A}{d_B^*}$ = $\frac{104.6 \text{ cm}}{103.4 \text{ cm}}$; $\frac{90.1 \text{ cm}}{89.9 \text{ cm}}$; $\frac{83.3 \text{ cm}}{83.7 \text{ cm}}$.

Hence, R_A; R_B = 1.007

Comparison between barium sulphate and bristol board (bb)

$\frac{d_{BaSO_4}}{d_{bb}^*}$ = $\frac{85.1 \text{ cm}}{81.9 \text{ cm}}$; $\frac{91.7 \text{ cm}}{88.3 \text{ cm}}$; $\frac{106.1 \text{ cm}}{101.9 \text{ cm}}$.

Hence the reflectivity of bristol board = 92.5%

Comparison of black paper (black) with bristol board (bb)

d_{black}^*	32.6 cm	28.2 cm	39.7 cm	31.1 cm	21.0cm
d_{bb}^*	218.0 cm	208.7 cm	221.6 cm	242.4 cm	188.3cm

Hence the black paper has reflectivity = 2.1%

Ostwald standard greys were obtained by constructing discs of bristol board and sticking sectors of black paper to them. Rotation of the discs in good light "mixed" the sectors to produce the grey. The angles of the black and white sectors were calculated as follows:-

The reflection from the white segment of the rotating disc is proportional to the angle of the sector and the reflectivity of the white material. It is given by,

---

* Each figure in this row is the mean of several readings with the comparator fixed.

$$R_w = \frac{\theta}{360} \times \frac{a}{100}$$

where θ is the angle of the segment, and a is the percentage reflectance of the white material.

Similarly, the reflection from the black segment is given by:-

$$R_b = \frac{(360 - \theta)}{360} \times \frac{b}{100}$$

where b is the percentage reflectance of incident light from the black material.

The overall reflectance of the spinning disc is given by:-

$$\frac{n}{100} = \frac{\theta}{360} \times \frac{a}{100} + \frac{360 - \theta}{360} \times \frac{b}{100}$$

$$= \frac{\theta a}{360} + \frac{(360 - \theta)b}{360}$$

$$= \frac{\theta a - \theta b + 360b}{360}$$

$$= \frac{\theta (a - b) + 360b}{360}$$

$$\text{Hence } \theta = \frac{360 (n-b)}{a - b}$$

By substituting the desired reflectivity from the Ostwald scale for n, the standard grey discs were constructed (Table 3).

TABLE 3

Construction of discs to obtain
a series of Ostwald Grey Standards

Reflectivity	θ_w°	θ_b°	No. on derived Ostwald Scale.
71%	274.4	85.6	0
45%	170.8	189.2	1
28%	103.1	256.9	2
18%	63.3	296.7	3
11%	35.5	324.5	4
7.1%	1919	340.1	5
4.5%	9.6	350.4	6
2.8%	2.8	357.2	7
2.1%	0.0	360.0	8

It was necessary to adapt the last unit, 2.1% from the Ostwald scale, 1.8%, as no paper with sufficiently low albedo was obtainable.

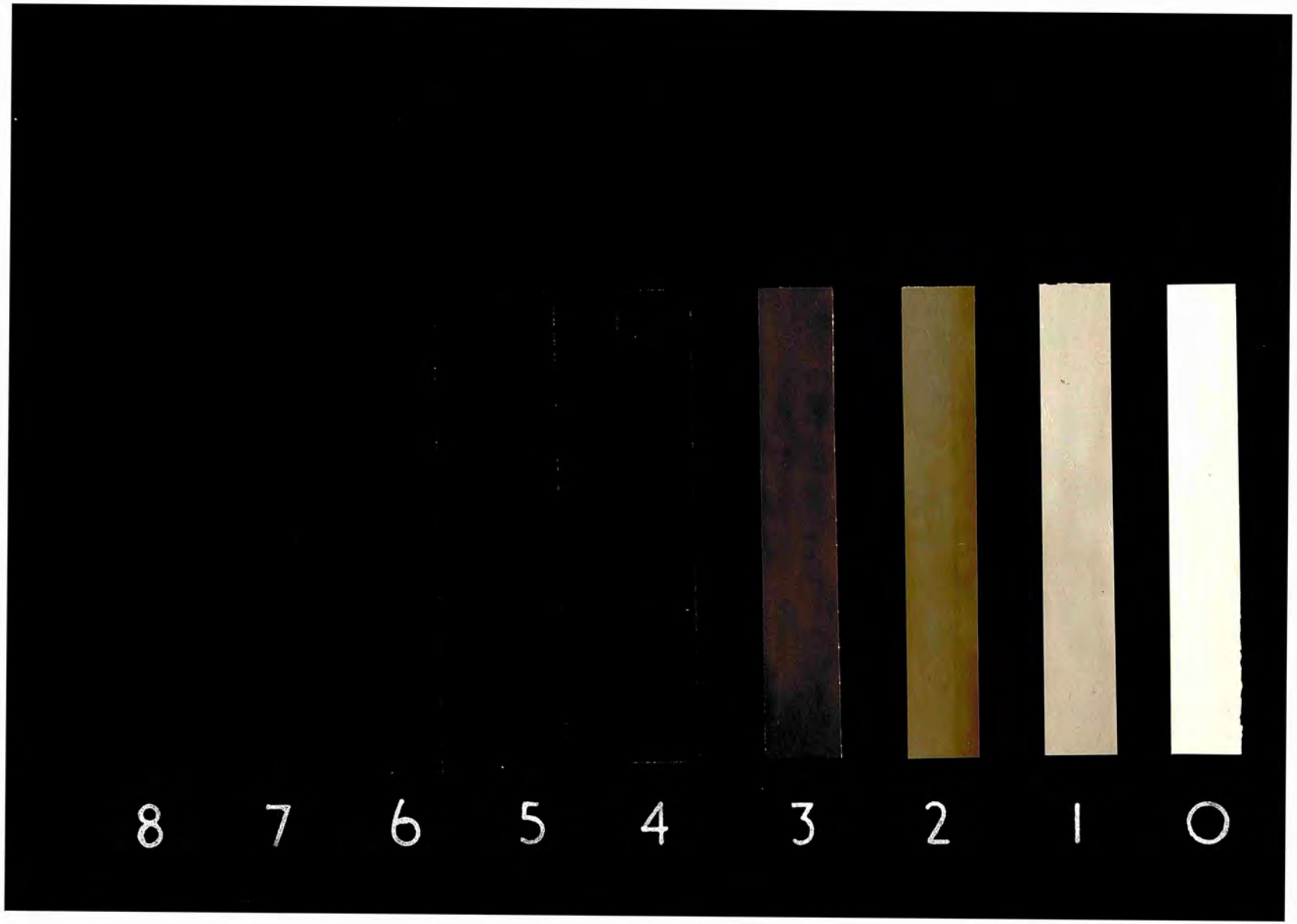
Permanent standard papers were prepared for use in the laboratory, as the spinning discs were too cumbersome for comparisons. Strips of grey photographic paper were prepared in the following manner.

Test strips of Ilford B4-1P photographic paper were progressively exposed at 5 sec intervals to a 5-8 watt bulb, working at 240-250 volts at 50 cps, 180 cms above the bench. The strips were developed for 90 secs in Ilford

Id20 developer, fixed with acid hypo for 1½ hours and subsequently compared with the spinning discs in direct sunlight. Those areas which were closest in shade to the spinning discs were used as guides toward exposing large sheets of photographic paper to the same darkroom conditions. Eventually thirty or more sheets were obtained from which nine sheets were selected as being indistinguishable from the standard discs and are shown in Figure 8; p.*108*. These were used in the experimental studies.

It was found that cutting strips of each sheet to a size similar to that of the minnow facilitated estimation of the shade of the fish. The fish were observed on black, white and grey backgrounds during the course of the experiments, and subjective errors in estimating the shade of the fish were overcome by mounting a complete series of the strips on boards painted to each of the background shades used. A small colour contribution to the shade of minnows is made by xanthophores in the minnow skin, seen especially following pituitary injections (Section 3.5). Accordingly the colour standards were prepared using a very slight coloration with dilute Orange G stain. Finally, it was observed that variation in the light source and the depth of water through which the fish was observed led to

Fig. 8 Portions of the standard grey papers used in the estimation of melanophore activity. The units of the derived Ostwald scale represented here (0 - 8) served as axes for the graphs in Section 2.


8
7
6
5
4
3
2
1
0

subjective errors in observing the shade of the fish. These errors were not large, but care was taken to ensure that the depth of water in the flasks in which the fish were observed was constant and that the light source, an "Anglepoise" lamp, was kept two feet vertically above the top of the flask.

In the text of this work it has been found convenient to describe colour changes of the minnow in a shorthand way. The chromatophores in fish possess branched processes along which the pigment granules can be moved (Fig. 3; p.19). Matthews (1931) showed that during such pigment migrations the outline shape of the cell with its processes remains unchanged. For brevity, chromatophores may be described as "aggregated" or "dispersed" when, in fact, it is the pigment within the chromatophores which has taken up either a central position or a state of dispersion throughout the cell and its processes. In addition, when referring to the shade of fish when compared with the series of Ostwald grey standards, the shade is described as Derived Ostwald Scale (D.O.S.) 4.0, 5.0 etc.

Section 3.0

Results

Section 3.1

The colour changes of normal minnows when subjected to background reversal

The Ostwald series of greys, which were described in the introduction, were used to follow the colour change of normal minnows which were transferred from a white background to a black background and vice versa. A variety of groups of fish were taken which had spent different lengths of time on black or white backgrounds. Observations were made at different times of year when water temperatures were markedly different.

Results were taken in the following way. Fish which had been adapted to a white background (for example) in the stock aquaria for a given length of time were transferred to 1 litre glass beakers placed in a wide, deep tray. This tray was also painted white and was filled with water to cut down light reflection at the beaker surfaces. Only one or two fish were placed in each beaker which contained fresh tap water to two thirds of its depth. During the course of the experiment the temperature of the water was recorded by means of a thermometer and

adjusted by siphoning off the water and adding fresh. In this way the fouled water was gradually removed during the experiment with minimum disturbance of the fish. The beakers were lit by means of a 60 watt Anglepoise lamp approximately 60 cm. above the tray.

The beakers containing the fish were then transferred to a similar, black tray which also contained water to cut down total internal reflection. The shade of the fish, which is related to the degree of pigment aggregation or dispersion in the skin melanophores, was recorded for each fish at the start of, and at subsequent intervals during, adaptation to the new background. Similar observations were made on black-adapted fish transferred to a white background.

When the observed shades of the adapting fish were plotted against time (Fig. 9, a - d; p.114) it was seen that the adaptation was at first fast but subsequently slower. This shape of the colour change curve has been described for Anguilla (Neill, 1940), Gasterosteus (Hogben and Landgrebe, 1940) and Phoxinus (Healey, 1948; 1951) using the melanophore index of Hogben and macroscopic shade of grey (Healey, 1948). During this shade change it was seen that melanophores in different regions reacted at different rates. Those in the barred region of the

skin (Fig. 7; p.102) aggregated more slowly than elsewhere in the dorsal surface and dispersed more rapidly. Accordingly the overall shade of the fish appeared "blotchy" between stages 2 to 6 of the Derived Ostwald Scale. It was necessary to estimate the shade of the fish from a distance of approximately two feet in order to obtain a comparison with the standard greys.

The effect of the previous history of the fish and of temperature on the rate of adaptation to the new background is illustrated in the figures. Fish at 20 - 22°C subjected to the change of background after one year on black reacted a little more slowly in the first few minutes than did fish at the same temperature which had spent only three months on black. After a half hour the shades of these two groups of fish were noticeably different, that of the first group being darker. A third group of fish, brought into the laboratory later in the summer, which had also spent three months on a black background was subjected to background reversal at 15 - 16°C. The initial rapid shade change was slower than fish with a similar history changing shade at 20 - 22°C but the shade reached after 30 minutes was paler than that of "one-year" fish at the same time. A final group of fish, also black-adapted for three months, was

subjected to the same background reversal at 10°C. In this group some fish paled at a rate similar to that of fish at 15 - 16°C but others had impaired paling ability.

A second series of observations was made on fish which had been adapted to a white background for three months or more and which had been placed on a black background for 30 or 60 minutes so that they darkened (Fig. 9b; p.114). They were then returned to a white background and allowed to pale. Because the fish were observed at different times of the year they adapted at different temperatures. Fish which had been on a black background for only one hour and which changed shade at 20 - 22°C paled much faster than fish in the first experiment. Other groups of fish kept on black for only thirty minutes were allowed to pale at 20 - 22°C, 15 - 16°C and 10°C. In this group it was seen that the rate of paling was fast but that the rate became slower with decrease in temperature.

Other observations were made on groups of fish adapting to a black background after adaptation to white backgrounds (Fig. 9c; p.114). One group of fish was kept on a white background in the aquarium for three years and allowed to adapt to a black background at 20 - 22°C. These fish changed shade at a slower rate than other fish in this group and could not achieve complete darkening.

* Fig. 9

a. The adaptation of minnows, which had spent a prolonged period on a black background, to a white background at different temperatures.

○ fish after one year on black (20-22°C)(12 animals)
◎ fish after three months on black (20-22°C)(10 animals)
▼ fish after three months on black (15-16°C)(10 animals)
□ fish after three months on black (10°C)(15 animals)

b. The adaptation of minnows, which had spent only a short period on a black background, to a white background at different temperatures.

○ fish after one hour on black (20-22°C)(10 animals)
◎ fish after half hour on black (20-22°C)(11 animals)
▼ fish after half hour on black (15-16°C)(18 animals)
□ fish after half hour on black (10°C)(5 animals)

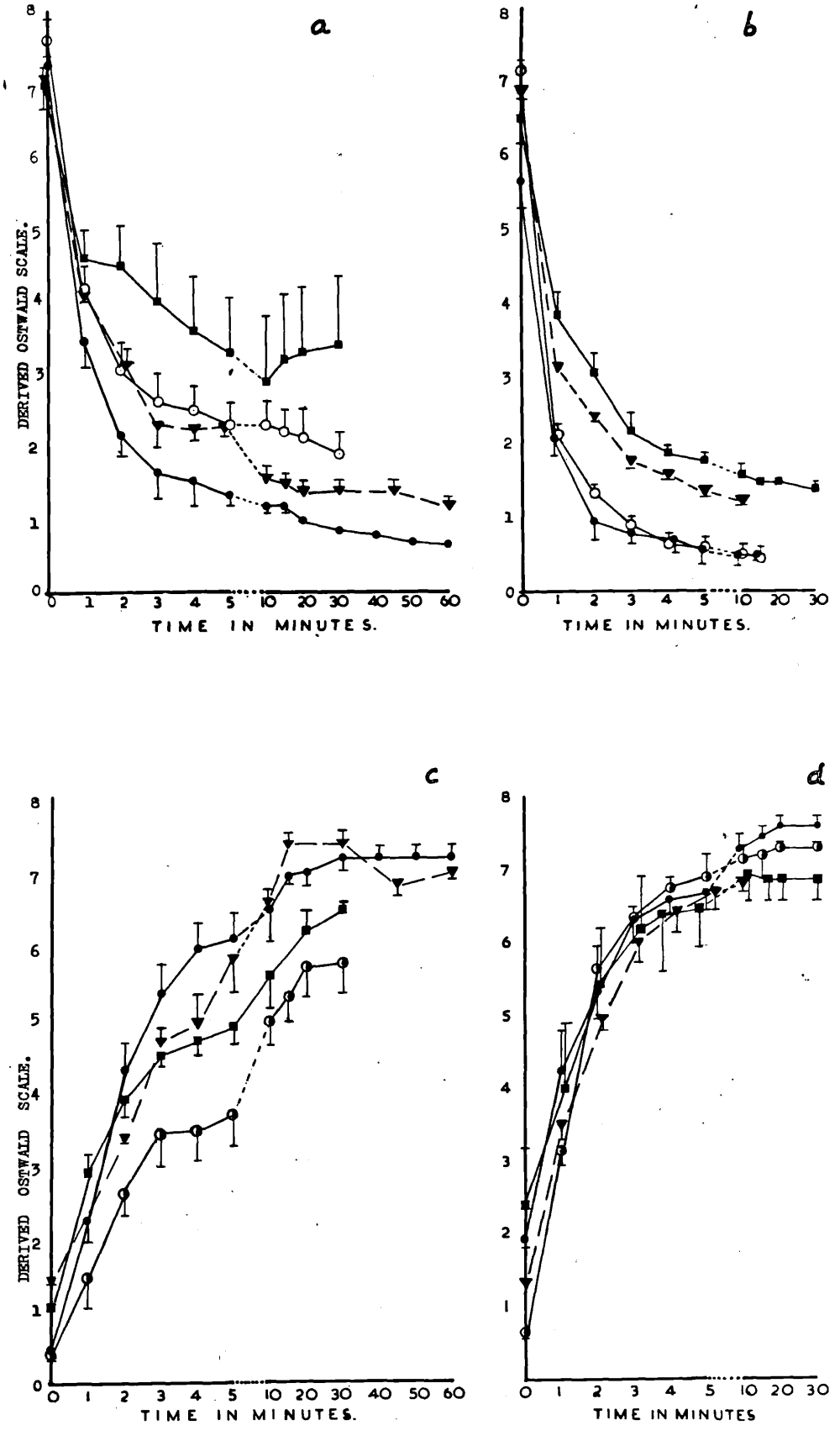
c. The adaptation of minnows, which had spent a prolonged period on a white background, to a black background at different temperatures.

◑ fish after two to three years on white (20-22°C) (11 animals)
◎ fish after three months on white (20-22°C)(9 animals)
▼ fish after three months on white (15-16°C)(25 animals)
□ fish after three months on white (10°C)(5 animals)

d. The adaptation of minnows after a brief stay on a white background, to a black background at different temperatures.

◑ after one hour on white (20-22°C)(10 animals)
◎ after half hour on white (20-22°C)(12 animals)
▼ after one and a quarter hours on white (15-16°C) (7 animals)
□ after half hour on white (10°C)(4 animals)

* In this figure, and in all subsequent graphical representations of colour changes in this text, the plotted points show the mean and size of the standard error for the number of fish in the group.


a
b
c
d
DERIVED OSTWALD SCALE.
TIME IN MINUTES.
0 1 2 3 4 5 10 20 30 40 50 60
0 1 2 3 4 5 10 20 30

Other fish were kept on a white background for three months and were subjected to background reversal at 20 - 22°C, 15 - 16°C or 10°C. In these groups it was again seen that the rate of colour change decreased progressively with temperature.

Finally, several groups of fish which had been black adapted for more than three months were allowed to adapt to a white background for thirty minutes or one hour and were then returned to a black background. It can be seen from Fig. 9d; p.114 that the rate of adaptation to black after a brief stay on white is noticeably faster than fish which have spent a long time on a white background. Unlike the shade changes seen when fish adapted to black for only a brief period are returned to white, these fish did not seem to be greatly affected by decreases in temperature.

These observations on minnow colour changes have been limited to a period of only half an hour. Healey (1951) observed the melanophore index of minnows adapting to different backgrounds and concluded that colour changes were not complete until two or more hours had passed. Hogben (Hogben and Landgrebe, 1940) believed that the very slow terminal parts of the curves he obtained for Gasterosteus were mediated by pituitary hormones (Section1.24).

The shape of the curves obtained in the present experiments using the Ostwald scale agrees with those obtained by the previous workers. This shape may be due to the rate at which melanophores can redistribute their pigment granules under the influence of endogenous nervous stimuli. It can be shown (Figs. 14 and 15, Section 3.22) that a similar shaped shade-change curve follows injections with catecholamines.

Healey (1951, 1954) and Pye (1964) have previously described groups of melanophores in the minnow skin which have different thresholds to stimuli which cause colour changes. Waring and Landgrebe (1941) record similar threshold differences among groups of perfused Anguilla melanophores when treated with melanophore dispersing hormone. Preliminary investigations on another teleost fish, Acanthocottus bubalis, have shown that melanophores with different thresholds to paling stimuli are found in alternate annuli along the body (Fig. 10; p.*119*). The differential response of groups of chromatophores during colour changes may allow fish to approximate their shade in nature to the shade of the background whilst also producing patterns which break up the body outline.

Figure 11; p.121 (Table 4) was constructed to relate the macroscopic shade of minnows, as judged using the derived Ostwald scale, to the values of the melanophore index (M.I.) obtained for the same species by Healey (1951). Healey's fish were kept at 12°C and the M.I. recorded for a small region of macromelanophores near the tail. Values of D.O.S. obtained for the same species at 10°C and 15 - 16°C were plotted against the M.I. achieved by Healey's fish at the same time intervals after the start of the colour change. The figure indicates the way in which changes in distribution of melanophore pigment (M.I.) affects the overall shade of the fish (D.O.S.).

It can be seen from Figure 9; p.114 that fish which have been longest on a particular background change shade slowest. In addition, these fish cannot reach the extremes of adaptation to the new background, within the time limits of the experiments, which are reached by previously less long adapted fish. It is well known that continued exposure to a white or black background leads to decrease or increase in the amount of pigment and in the number of melanophores in the skin (Kuntz, 1917; Murisier, 1920 - 21; Parker, 1948). It is therefore important to know the history of individual fish which are compared in colour change experiments. The changes in

rate of paling may in part be due to the morphological changes described above (e.g. fish in Fig. 9a and 9d with different histories but changing shade at the same temperature). It is also possible that changes in the speed of reaction of melanophores or of central coordinating chromatic centres may result from sustained exposure to one background. Parker and Brower (1937) found little difference in the rate of colour change of Fundulus when subjected to repetitive background reversal. On the other hand, Umrath and Walcher (1951) claimed that Phoxinus was able to speed up the rate of colour changes with practice. Their observations on the latter species indicated that their specimens of the minnow could adapt in only one or two minutes, a much faster rate of adaptation than was found in the present work. It seems likely that the faster changes which are found with continued background reversals in the minnow are associated with the titre of circulating chromatic hormone(s) released from the pituitary after exposure to one background for at least a number of hours. The results represented in Figs. 9b and 9d; p.114 suggest that in the short time that minnows were adapted to a new background and then returned to the original colour only small changes in chromatic hormones occured in the blood. Umrath and Walcher (1951) also

Fig. 10 Black-adapted Acanthocottus(=Cottus) bubalis after injections of 6 mg/kg noradrenaline (20°C). The regions of the body with a lower threshold to the amine are clearly visible, but are difficult to distinguish in completely black-adapted fish.

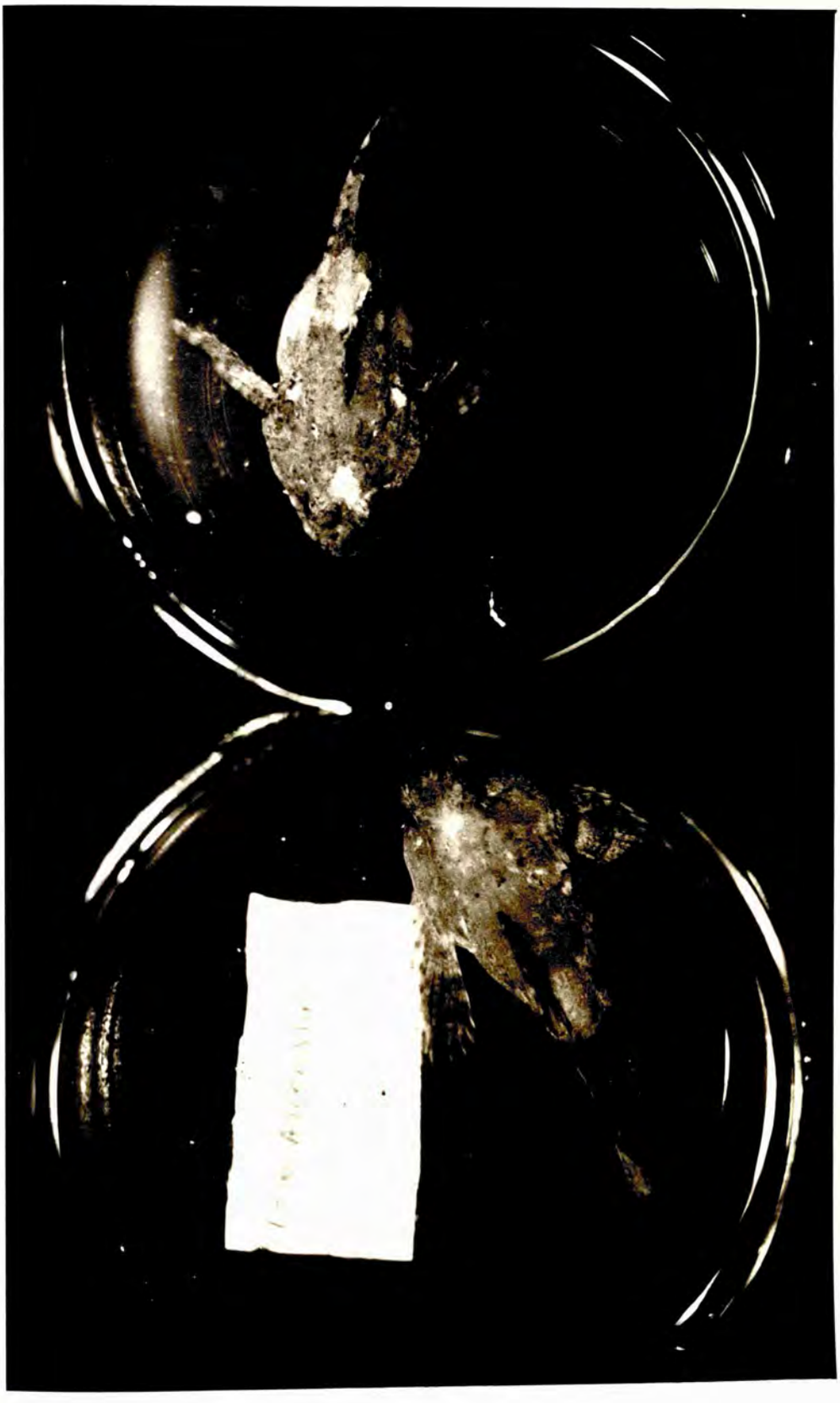


Table 4

Relationship between overall shade and melanophore index in the minnow

Y = M.I. (Healey)	X = Derived Ostwald Scale
1.0	0.0
1.2	1.1
1.3	1.1
1.3	1.46
1.4	1.2
1.4	1.4
1.7	1.4
2.0	2.9
2.0	3.75
2.1	3.75
2.2	3.92
2.4	3.83
2.5	4.33
2.6	2.3
2.9	4.5
2.9	4.7
3.1	2.3
3.4	4.9
3.4	5.82
3.9	5.6
3.9	6.6
3.6	5.25
4.0	5.7
4.0	7.4
4.2	4.1
4.2	4.6
4.3	6.5
4.3	7.4
4.5	6.85
4.5	7.1
4.8	7.0
4.8	7.1
5.0	8.0

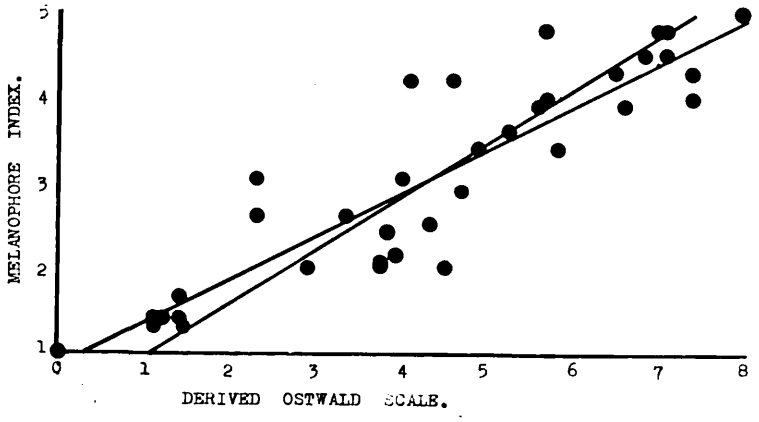
Correlation Coefficient, $r = 0.91$

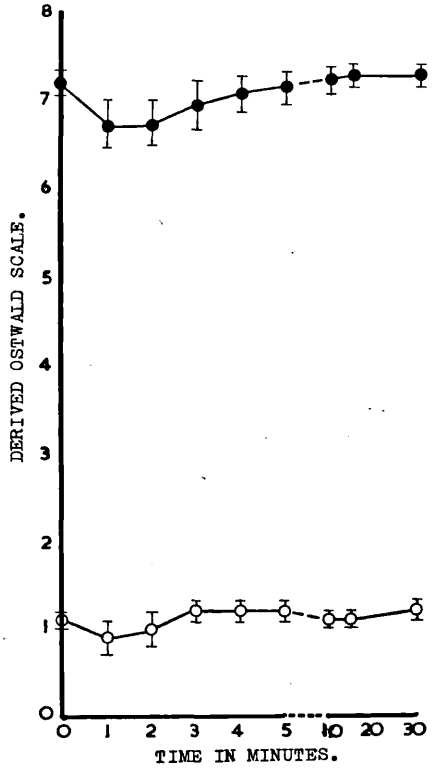
$Y = .51 + .54X$

$X = 1.8Y - 1.14$

Fig. 11 The relation between overall shade, as measured by the D.O.S. scale, and the melanophore index (Healey, 1951) in the minnow.

Fig. 12 The effects of control injections of Young's freshwater teleost Ringer on the shade of 10 black- and 7 white-adapted minnows (16°C).


MELANOPHORE INDEX.
1
2
3
4
5
0
1
2
3
4
5
6
7
8
DERIVED OSTWALD SCALE.


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
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30
TIME IN MINUTES.

suggest that during a long stay on one background, neurohumours associated with the activity of one set of chromatic fibres may pile up in the skin and be stored there temporarily. To this end they quote the work of Parker (1933, 1934b) that acetylcholine and adrenaline may be stored in lipoids in the skin.

The observed differences in colour change between fish of dissimilar histories may therefore be attributed to long term morphological changes and more short-lived changes of physiological agents acting locally on melanophores. It has also been shown (p.114) that fish of the same history change colour at decreased speed at lower temperature. This phenomenon may be a measure of the decreased metabolic rate in poikilothermic animals at lower temperatures. Colour changes in both directions are affected. Previous workers have found that local temperature changes affect chromatic tracts differentially. Low temperatures should enhance paling. In the present experiments paling was more affected by lower temperatures than was darkening. Some fish at 10°C failed to adapt at all to a white background. Zaimis (1960) found that conduction in the adrenergic fibres of mammals was impaired by hypothermia and compared the treatment with adrenergic blackade. It is possible to suggest that general cooling

of a fish may have such a specific effect on the aggregating sympathetic fibres whereas local cooling exerts its effects in a different way.

Section 3.2
Injections of adrenergic drugs into intact fish

Section 3.21
Control injections of Young's freshwater teleost Ringer

Von Frisch (1911b) and subsequent workers described the fright pallor or "Shreckreaktion" which often follows disturbance of black-adapted fish. It was important to determine the extent of this pallor before interpreting the effect of drug injections on the chromatic system of fish. Animals which had become accustomed to handling and to movements in their vicinity were chosen for these injections as subsequent experiments were to be performed on similar groups of fish. The majority of injections in later experiments consisted of drugs dissolved in Young's freshwater teleost Ringer. Fig. 12 (p.121) shows the responses of several fish injected with 0.1 ml. Ringer solution whilst adapted to black or white backgrounds. No significant colour change could be detected in the

white-adapted fish but the black-adapted fish showed a transient pallor lasting a few minutes. Most fish had returned to normal well within 10 minutes and rarely paled below 6 on the Ostwald scale.

Section 3.22

Catecholamines

Section 3.221

Noradrenaline

In Table 2 there appears a list of workers who have found that noradrenaline causes the aggregation of teleost chromatophores. Interest in this amine as a melanophore aggregating agent is comparatively recent. It had long been known that adrenaline was a potent aggregating agent but most workers considered that its role in fish colour change was hormonal. Von Euler (1948 et seq.) demonstrated the presence of large amounts of noradrenaline in sympathetic postganglionic nerves of mammals and proposed that it acted as the neurotransmitter. It has been shown that teleost aggregating chromatic fibres are sympathetic in nature and this has led to tests with the primary amine.

In the present study, groups of black-adapted fish were injected with noradrenaline dissolved in Young's Ringer (Fig. 13; p.127). Doses of the catecholamine in excess of 2 mg/kg produced a pallor of D.O.S. 1.0 to 1.5 within 30 to 45 minutes. During this pallor patches of melanophores were seen to react at a slower rate than those in other regions of the skin. These patches of melanophores with a "high threshold" to paling stimuli were described earlier during normal colour changes (Section 3.1). As the effect of the drug wore off, these patches were the first to reappear against the pale adjacent regions of skin. Smaller doses of noradrenaline, between 0.5 and 1.5 mg/kg, produced imcomplete pallor as the refractory melanophores failed to aggregate completely. Doses below 0.2 mg/kg produced only slight pallor which was indistinguishable from that produced in control fish by Young's Ringer alone.

Section 3.222

Adrenaline

The aggregating influence of adrenaline on teleost chromatophores has been described earlier (Table 2). Black adapted minnows were injected with doses of the amine

(Fig. 14; p.127) and the resultant pallor was similar to that already described for noradrenaline. Doses exceeding 2.5 mg/kg produced complete pallor of the fish within 15 to 30 minutes, together with a "blotchy" appearance of the body during the intermediate stages of aggregation and dispersion. Fish injected with doses of the drug below 0.3 mg/kg were indistinguishable from control fish injected with Ringer whilst the small group of fish injected with 1.35 mg/kg paled incompletely.

Section 3.223

Isopropylnoradrenaline

Isopropylnoradrenaline (isoprenaline) has been found to react more readily with mammalian beta adrenergic receptors (Section 1.3214). The potency of this amine for alpha receptors is low in mammals. Injections of this amine were made into black or white adapted minnows (Fig. 15; p.130) to discover whether it resembled noradrenaline in causing pallor or whether it acted to cause darkening by stimulating beta-receptors preferentially.

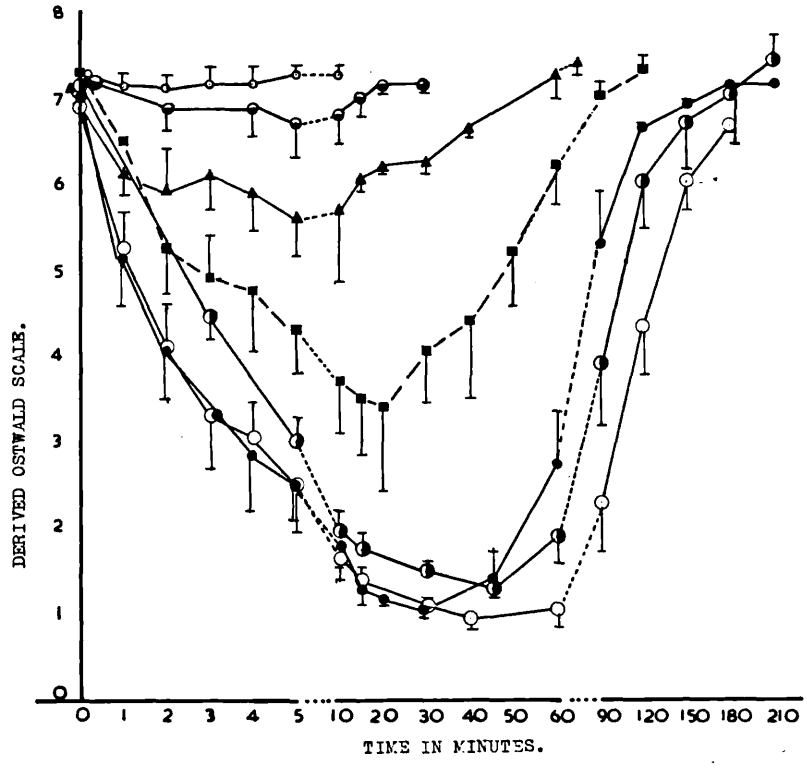
White-adapted fish injected with 11.4 mg/kg (15 - 16°C) showed no obvious change in shade but the same dose in black-adapted fish produced a considerable pallor

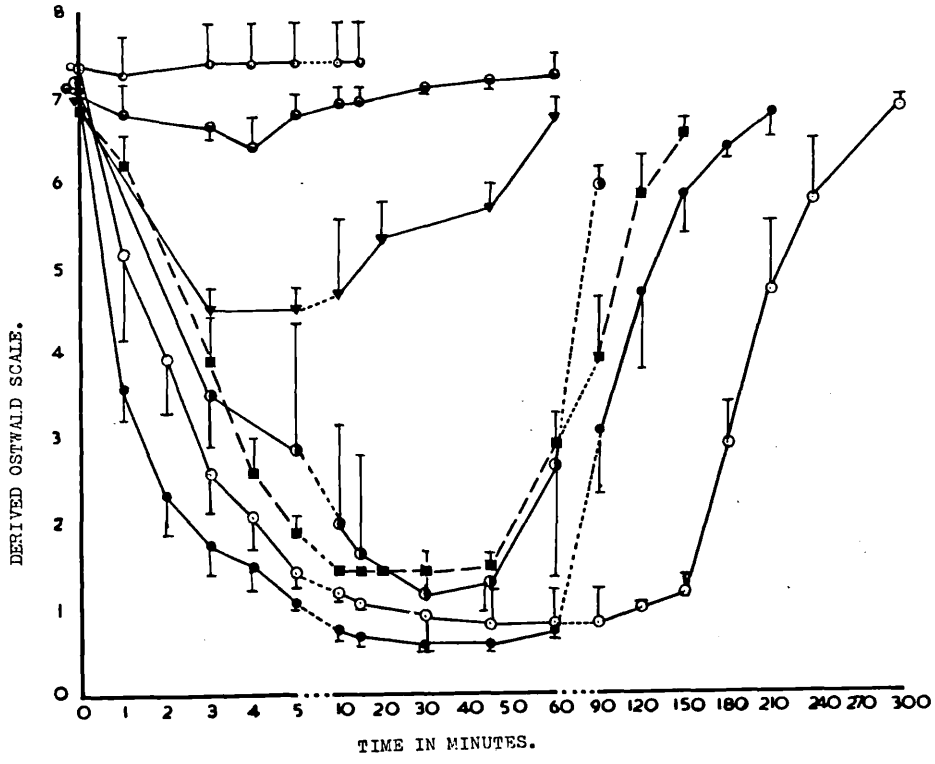
Fig. 13 The effect of noradrenaline injections on the shade of black-adapted minnows

○ 6.5 mg/kg; 22°C. (10 animals)
● 2.75 mg/kg; 22°C. (7 animals)
◑ 2.1 mg/kg; 22°C. (8 animals)
□ 1.4 mg/kg; 20°C. (10 animals)
▲ 0.7 mg/kg; 20°C. (7 animals)
◒ 0.15 mg/kg; 20°C. (7 animals)
○ 0.015 mg/kg; 20°C. (8 animals)

Fig. 14 The effect of adrenaline injections on the shade of black-adapted minnows

○ 13.2 mg/kg; 19°C. (6 animals)
● 6.5 mg/kg; 19°C. (6 animals)
◑ 4.0 mg/kg; 19°C. (3 animals)
□ 2.7 mg/kg; 20°C. (5 animals)
▲ 1.35 mg/kg; 19°C. (3 animals)
◒ 0.27 mg/kg; 20°C. (7 animals)
○ 0.027 mg/kg; 20°C. (4 animals)


DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210
TIME IN MINUTES.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TIME IN MINUTES.

lasting approximately 30 minutes. During this pallor a "blotchy" appearance of the skin, due to a differential response of melanophores in the barred regions of the skin was seen. A subsequent injection of 5.7 mg/kg in another group of fish at 22°C. produced a more complete pallor of black adapted fish. In no fish was there indication of a dispersing action of isoprenaline on the melanophores.

The three amines tested in these experiments have been shown to combine directly with the adrenergic receptors in the postsynaptic region of the sympathetic neuro-effector junction of mammals. In view of the demonstration of the presence of both noradrenaline and adrenaline in teleost nerves and organs (von Euler 1952, 1961; von Euler and Fänge, 1961) it is reasonable to suggest that either noradrenaline or adrenaline, might be the sympathetic neurotransmitter in teleost aggregating fibres. The aggregating influence of these agents and of isoprenaline has a differential effect on the skin melanophores similar to that produced by the melanophore-aggregating during background adaptation of untreated fish. The paling action of isoprenaline may be interpreted either as an action on alpha receptors or as stimulation of beta receptors which act in the same manner as the alpha variety. Such a duplication of sympathetic receptors has been

described in the mammalian gut (Ariens et al., 1964).

Dopamine, the catecholamine precursor of noradrenaline, has been suggested as a sympathetic neurotransmitter in ruminant viscera (Schümann, 1959). Healey (personal communication) and Scott (1965) described a potent melanophore-aggregating property of this catecholamine as well. It is not possible at this stage to identify teleost colour change neurohumours by injection techniques.

Section 3.23
Sympathomimetic amines

Section 3.231
Ephedrine

In the introduction (Section 1.321) ephedrine was described as an amine with "mixed" actions. It can stimulate mammalian adrenergic receptors directly and also displace sympathin from the adrenergic nerve terminals. In the few fish examined previously, this drug brought about melanophore aggregation (Table 2). The largest dose injected in the present study, 11.3 mg/kg, led to a pronounced pallor of black-adapted fish which was maximal after forty-five minutes (Fig. 16; p.130). Thereafter the

Fig. 15 The effect of isoprenaline injections on the shade of black- and white-adapted minnows

● 11.4 mg/kg; 15-16°C. (5 animals on Black; 5 animals on White)

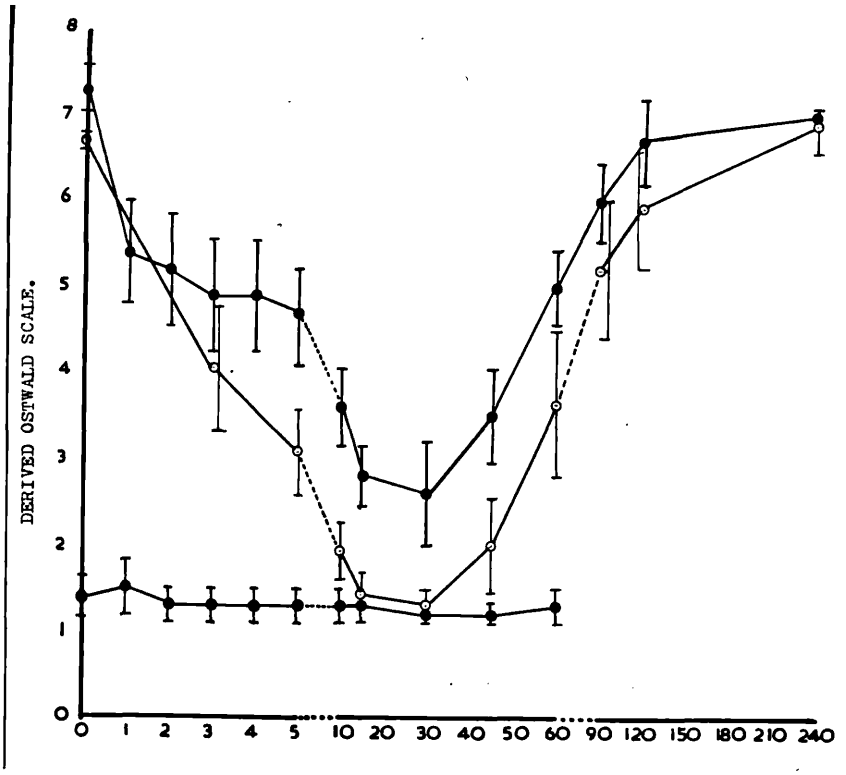
○ 5.4 mg/kg; 20-22°C. (7 animals on Black)

Fig. 16 The effect of injections of ephedrine on the shade of black-adapted minnows

● 11.3 mg/kg; 20°C. (10 animals)

◐ 6.8 mg/kg; 18°C. (5 animals)

○ 3.4 mg/kg; 18°C. (4 animals)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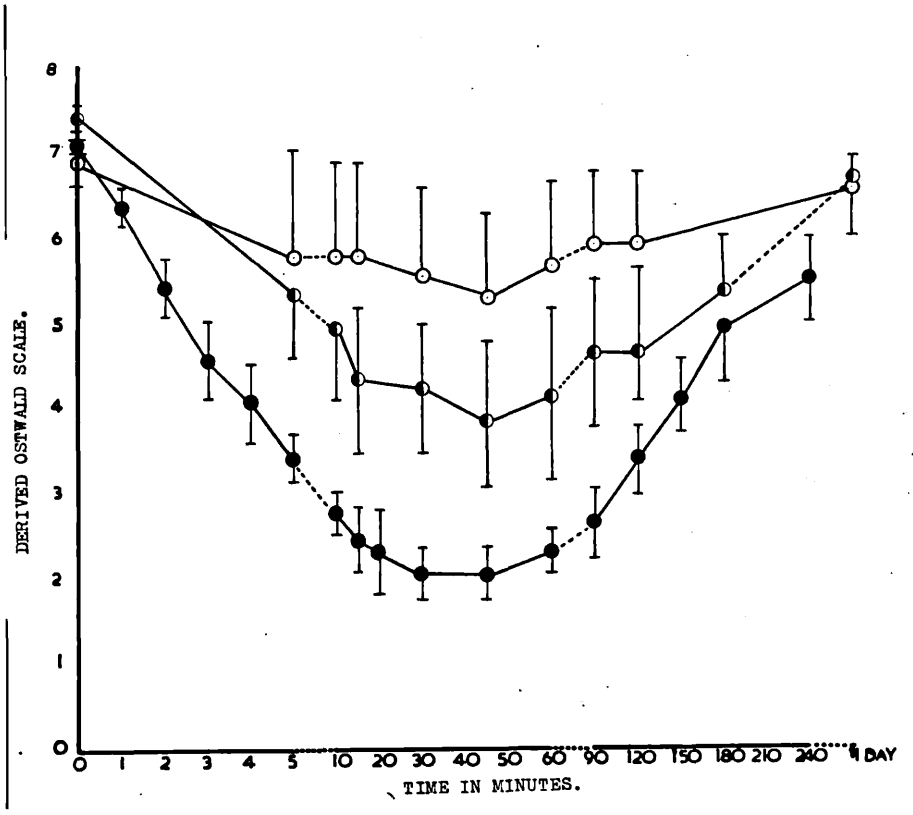
DERIVED OSTWALD SCALE.
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TIME IN MINUTES.

pallor slowly subsided but did not disappaar until the next day. The lower doses which were used, 6.8 and 3.4 mg/kg, were less effective, on a weight for weight bases, than adrenaline or noradrenaline. Their actions were prolonged however, and throughout the paling which followed ephedrine injections it was possible to distinguish melanophores reacting differentially to produce a "blotchy" pattern.

Section 3.232

Tyramine

Tyramine has also received brief study for its chromatic actions in teleosts (Table 2). In mammals it has been shown to exert its action by displacing noradrenaline from the adrenergic nerve store (Section 1.321). In the present study, injections of as much as 21 mg/kg tyramine produced a short-lived, incomplete pallor (Fig. 18; p.133). During this response the barred regions of the skin reacted only slightly and most of the paling apparently took place in the adjacent areas which have a lower threshold to paling stimuli.

Section 3.233

Amphetamine

Amphetamine, like tyramine, exerts its effects in mammals mainly by an indirect action on the adrenergic stores of sympathetic neurones (Section 1.321). When injected into black adapted minnows however, the degree and duration of the resultant pallor more nearly resembled that following treatment with ephedrine (Fig. 17; p.133). Once again an overall "blotchy" appearance of the fish during the onset and subsidence of pallor was seen. Doses of amphetamine greater than 12.2 mg/kg were found to be toxic.

Section 3.24

Adrenergic blocking agents

In recent years several groups of workers have discovered that agents which compete with adrenaline at the adrenergic receptor or which specifically block sympathetic neurones in mammals are less specific in the vertebrates. Thus adrenergic blocking agents may show acetylcholine-like activity, atropine activity (Ross, 1936; Kosterlitz and Lees, 1961), anticholinesterase activity (Boyd et al., 1960, 1963) or even cholinergic blockade

Fig. 17 The effect of amphetamine injections on the shade of black-adapted minnows

● 12.2 mg/kg; 20°C. (5 animals)

◐ 6.2 mg/kg; 20°C. (8 animals)

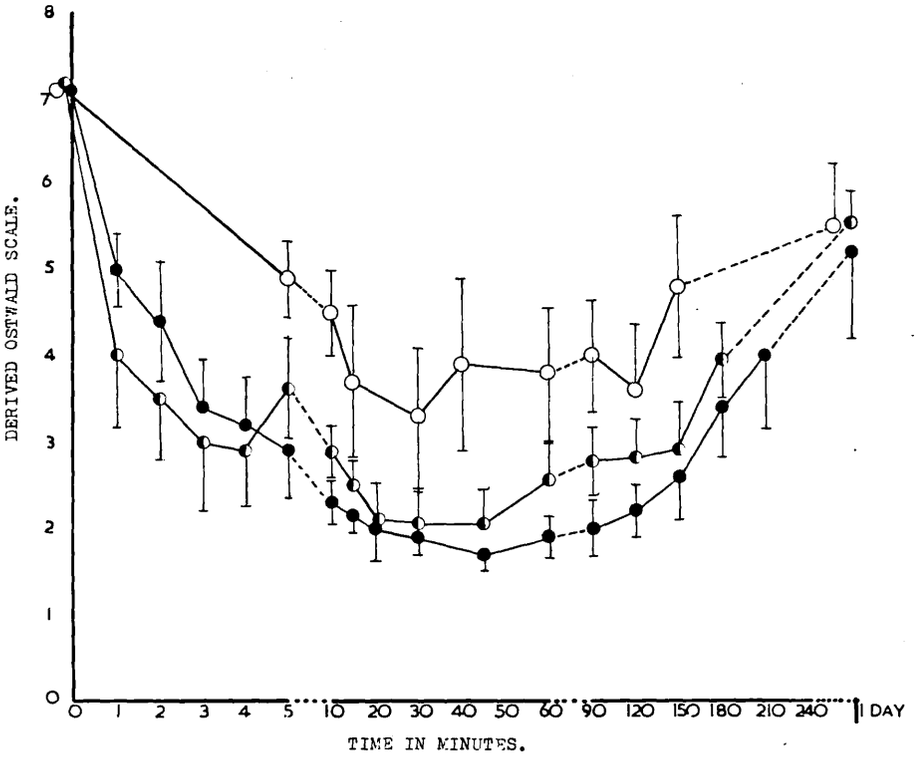
○ 3.5 mg/kg; 18°C. (5 animals)

Fig. 18 The effect of injections of tyramine on the shade of black-adapted minnows

● 21.0 mg/kg; 15°C. (5 animals)

◐ 11.5 mg/kg; 20°C. (18 animals)

○ 5.75 mg/kg; 15°C. (4 animals)


DERIVED OSTWALD SCALE.
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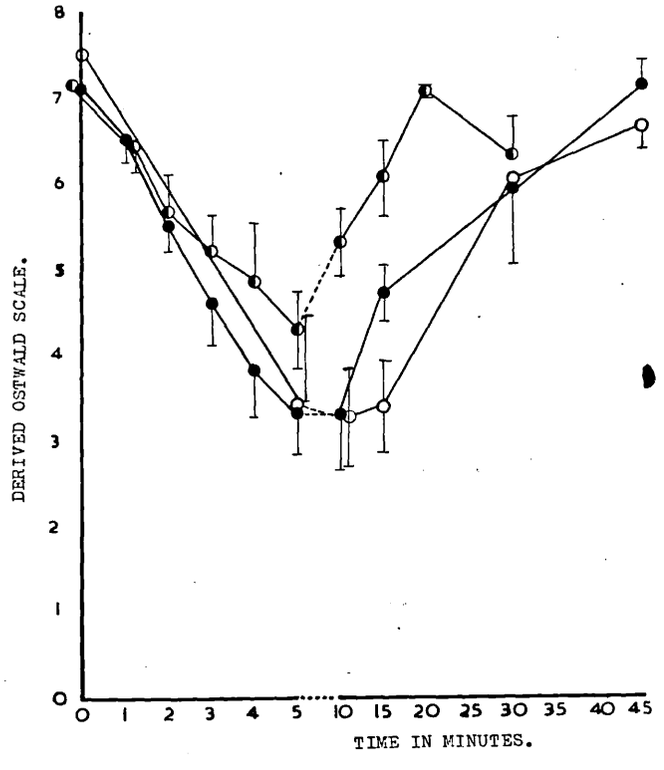
8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240 1 DAY
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
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0 1 2 3 4 5 10 15 20 25 30 35 40 45
TIME IN MINUTES.

(Boyd et al., 1963). Accordingly it is not possible to determine the nature of a neurotransmitter in a lower vertebrate neuroeffector mechanism from a study of adrenergic blocking agents alone. In the present study a variety of blocking agents have been chosen for use in investigation the minnow chromatic nerves but discussion of their effects will be postponed to Sections 3.254, 3.35, 3.44, 3.5 and 4.14.

Section 3.241

Piperoxane

Piperoxane (933F) is described in mammals as a competitor with catecholamines at the alpha adrenergic receptor (Section 1.322). It has been shown to have pronounced anti-Ach effects (Boyd et al., 1963) in doses lower than were required to block adrenergic nerves. In addition these workers described a direct stimulatory action of the drug on smooth muscle whilst Boyd et al. (1960) showed that the drug also possessed considerable anti-cholinesterase activity.

In the present study, injections of piperoxane were made into black- and white-adapted fish (Fig. 19a, b; p.137). In white-adapted fish, doses of 5 mg/kg and above led to an

almost complete dispersion of the melanophores, the responses differing only in the duration of the dispersion which was dose-dependent. Lower doese of 2.1 and 1.0 mg/kg caused only incomplete darkening. In every case the first melanophores to disperse lay in those areas previously described as having a higher threshold for paling stimuli. The lower doses were not able to bring about dispersion of melanophores outside the barred regions of the skin but the higher doses were able to cause these to disperse almost completely. Black-adapted minnows injected with 10.5 mg/kg piperoxane were seen to darken a little more during the time when similarly treated white-adapted fish were darkening (Fig. 19b; p.137). Simultaneous injection of 7.1 mg/kg noradrenaline into both groups led to a rapid pallor within 15 minutes. The black-adapted fish did not recover from this noradrenaline injection until a further hour had elapsed.

Section 3.242

Phentolamine

Boyd et al. (1963) demonstrated that phentolamine possesses anti-Ach and anticholinesterase properties but to a lesser extent than piperoxane. Pye (1964b) found

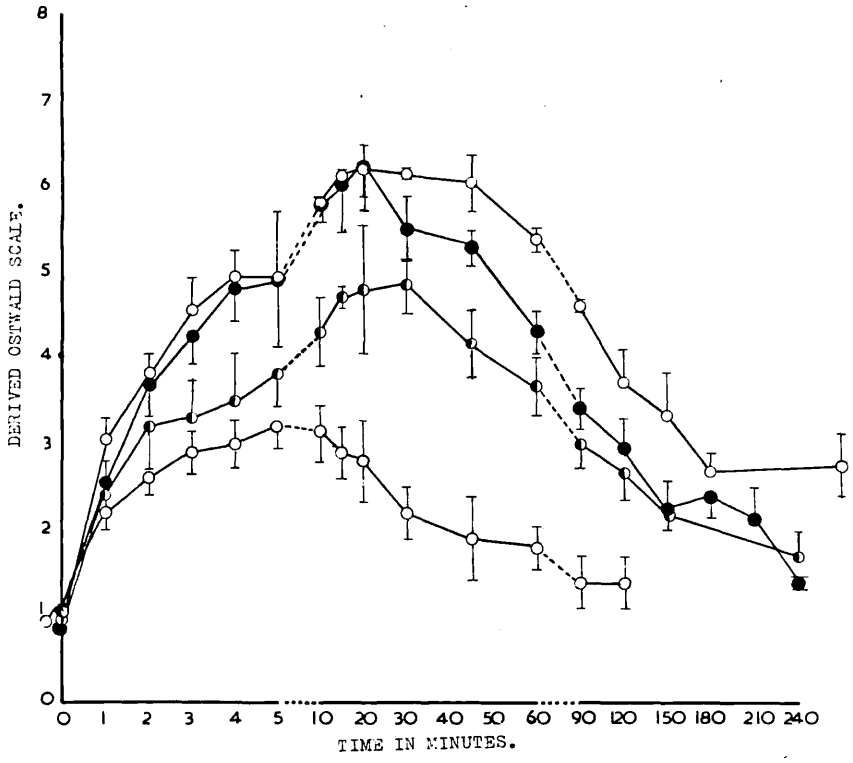
that, whereas ergotamine reversed the effect of electrical chromatic nerve stimulation, phentolamine prevented both aggregation and dispersion of melanophore pigment in the minnow.

Injections of 12 mg/kg phentolamine into white-adapted minnows led to incomplete darkening of the fish to approximately 4.0 D.O.S. (Fig. 20; p.138). A similar injection, at 20 - 22°C instead of 15 - 16°C, led to similar effects. A lower dose of 6 mg/kg at 20°C led to diminished darkening in a third group of fish. In most cases the melanophores which dispersed were localised in the barred regions of the skin. A fourth group of black-adapted fish were injected with 12 mg/kg phentolamine and then placed on a white background. After an initial rapid paling these fish reached the same shade as fish which had previously been white-adapted. Black-adapted fish injected with 12 mg/kg phentolamine paled a little in the first few minutes but this pallor was indistinguishable from that following injections of Ringer into controls. These fish then resumed their original dark shade and showed no other change. Finally, a group of white-adapted minnows were injected with 12 mg/kg phentolamine and after 35 minutes, when dark, were injected with 7.1 mg/kg noradrenaline. Within 5 minutes these fish had paled again to the normal white-adapted state.

Fig. 19 a. The effect of piperoxane injections on the shade of white-adapted minnows

○ 10.5 mg/kg; 20°C. (12 animals)
● 5.25 mg/kg; 20°C. (13 animals)
◐ 2.1 mg/kg; 20°C. (10 animals)
○ 1.05 mg/kg; 20°C. (5 animals)

b. The effect of piperoxane injections on the shade of five black- and five white-adapted minnows and the effect of noradrenaline on the response to piperoxane. Fish were injected at the start with 10.5 mg/kg piperoxane and then at A with 7.1 mg/kg noradrenaline (19°C.)


DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240
TIME IN MINUTES.

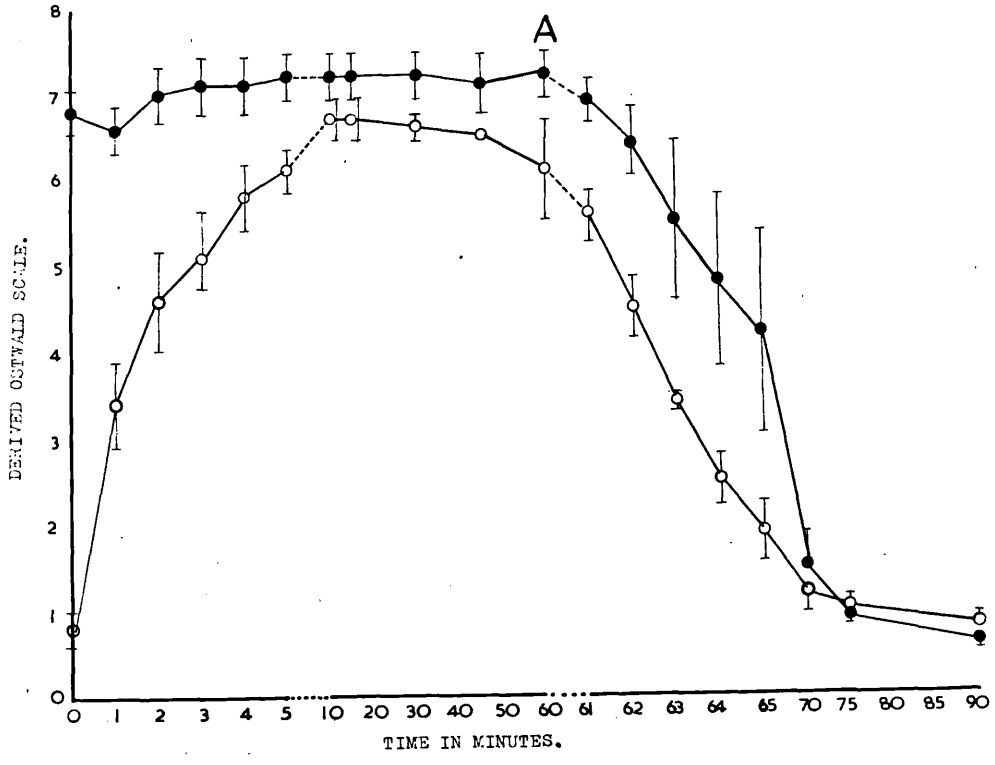

A
DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 40 50 60 61 62 63 64 65 70 75 80 85 90
TIME IN MINUTES.

Fig. 20 a. The effect of phentolamine (rogitine) injections on the shade of white-adapted or white-adapting minnows

◒ 12 mg/kg; 20-22°C. (Black-adapted: put on white)(5 animals)

● 12 mg/kg; 20-22°C. (White-adapted)(5 animals)

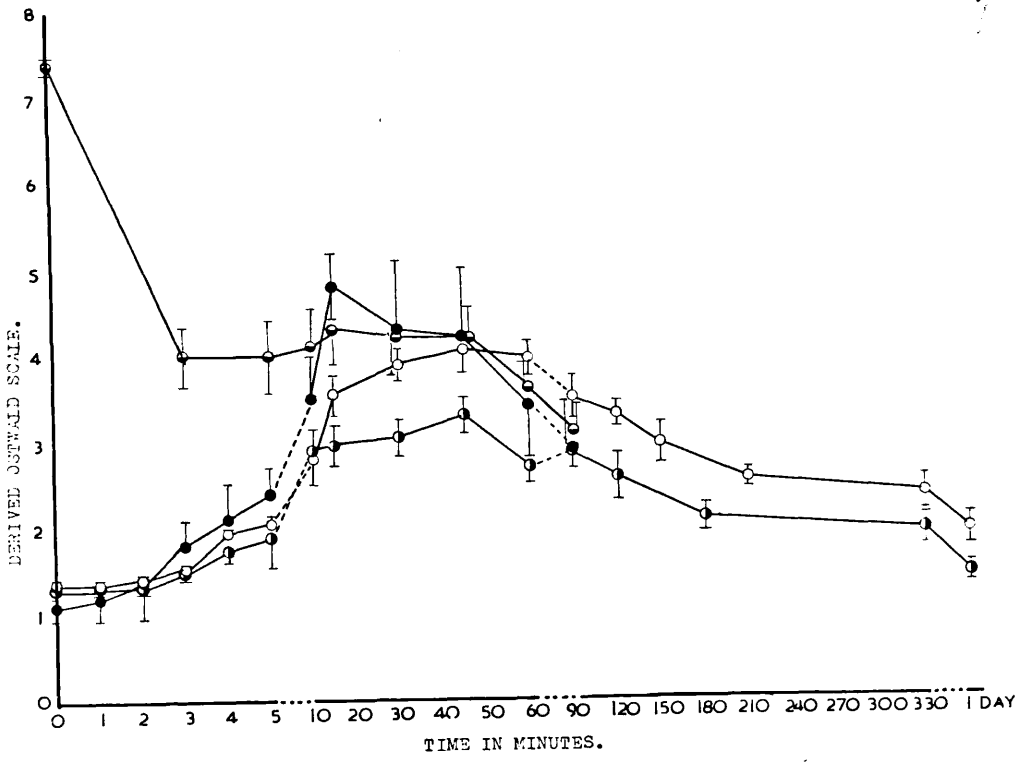
○ 12 mg/kg; 15-16°C. (White-adapted)(13 animals)

◑ 6 mg/kg; 20-22°C. (White-adapted)(19 animals)

b. The effect of phentolamine injections on the shade of black-adapted minnows and the effect of noradrenaline on the darkening action of phentolamine on white-adapted minnows

● 12 mg/kg phentolamine in black-adapted fish; 20°C. (5 animals)

○ 12 mg/kg phentolamine in white-adapted fish, followed by 7.1 mg/kg noradrenaline at A; 20°C. (5 animals)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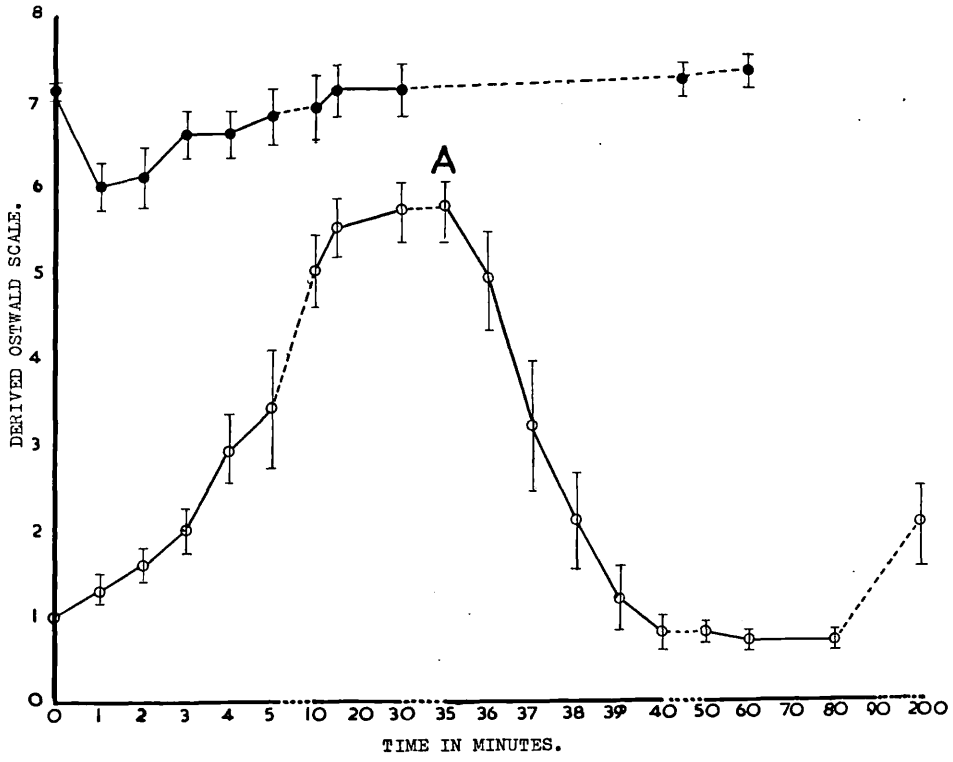
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240 270 300 330 1 DAY
TIME IN MINUTES.


A
DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 35 36 37 38 39 40 50 60 70 80 90 200
TIME IN MINUTES.

Section 3.243

Dihydroergokryptine

In the introduction (Section 1.3221) it was pointed out that the dihydro-derivatives of the ergot alkaloids possessed less pronounced side effects than the natural alkaloids. Dihydroergokryptine is a member of the synthetic derivatives which has less direct stimulating activity at the mammalian alpha adrenergic receptor. In the past, workers using the natural alkaloids have found a considerable paling activity associated with ergotamine- and ergotoxine-blocking activity which has enabled reversal of injected adrenaline (Spaeth and Barbour, 1917) or of nerve stimulation (Giersberg, 1930; Pye, 1964) to be seen.

Doses of dihydroergokryptine (DHEK) injected into white-adapted minnows led to a strong dispersion of the melanophores. This dispersion did not develop so rapidly as that following injection with the previous blocking agents (Fig. 21; p.141). Doses of 17.7 and 5.9 mg/kg injected at 15 - 16°C. were less active than a dose of 11.8 mg/kg injected at 19°C. However all three injections caused considerable darkening, after a latent period of 5 to 10 minutes, and this did not disappear for several days. During the onset and subsidence of the change

"blotchy" patterns appeared as a result of differential melanophore reactions in the skin of the fish.

Section 3.244

Yohimbine

According to Boyd et al. (1962) yohimbine is more selective in its blocking action for adrenergic nerves than are most other adrenergic blocking agents. However Boyd et al. (1963) demonstrated that this drug could block Ach and cholinergic nerves and also exert anti-cholinesterase effects. In fish, yohimbine has been found to disperse teleost chromatophores. ~~(Table 2, p. 117).~~

Minnows proved to be more sensitive to injections of yohimbine than they were to the previous blocking agents. Only rarely did injections greater than 6 mg/kg fail to cause death. However doses of 6.0, 2.4 or 1.2 mg/kg led to almost complete darkening of white-adapted fish, recovery occurring only after several days (Fig. 22; p.141). As with other blocking agents it was seen that the barred regions of the skin "escaped" from paling stimuli more readily than adjacent areas.

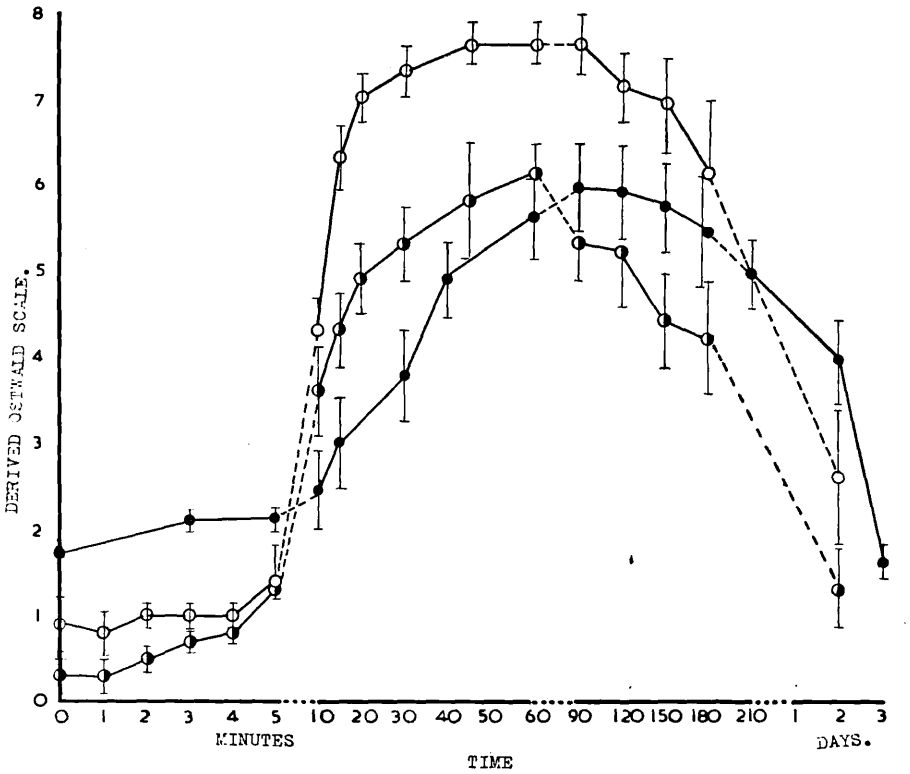
Another group of white-adapted minnows injected with 6 mg/kg yohimbine darkened to 6.0 on the Ostwald scale.

Fig. 21 The effect of dihydroergokryptine injections on the shade of white-adapted minnows

11.8 mg/kg; 19°C. (5 animals)
17.7 mg/kg; 15°C. (9 animals)
5.9 mg/kg; 15°C. (5 animals)

Fig. 22 The effect of yohimbine injections on the shade of white-adapted minnows

6 mg/kg; 12.5°C. (7 animals)
2.4 mg/kg; 12.5°C. (4 animals)
1.2 mg/kg; 12.5°C. (4 animals)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0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 1 2 3
MINUTES
DAYS.
TIME

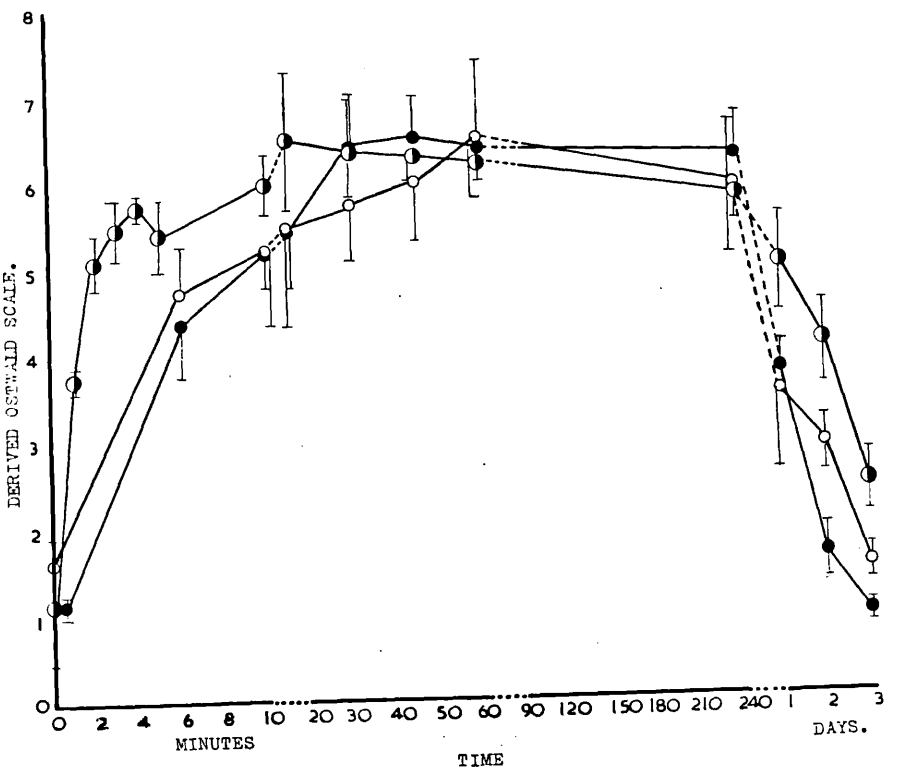
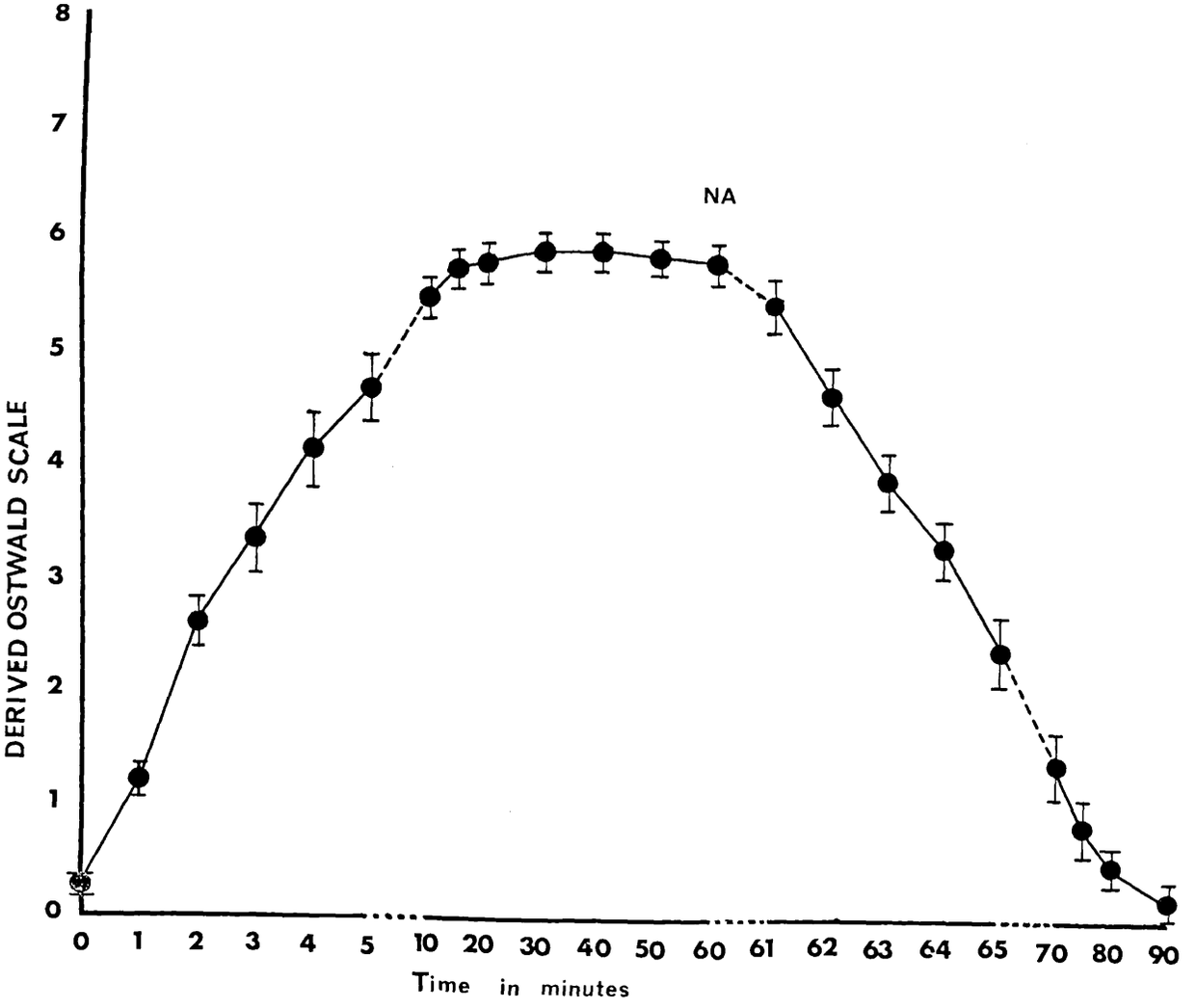

DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 2 4 6 8 10 20 30 40 50 60 90 120 150 180 210 240 1 2 3
MINUTES
DAYS.
TIME

Fig. 23 The effect of yohimbine injections on the shade of white-adapted minnows and the effect of a subsequent injection of noradrenaline. Fish were injected with 6 mg/kg yohimbine at the start and with 6.5 mg/kg noradrenaline at NA. (10 fish, 15°C.)


NA
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Time in minutes

After 1 hour, these fish were injected with 7.1 mg/kg noradrenaline (Fig. 23; p.142) and were seen to pale completely within 30 minutes.

Section 3.245

Dibenamine

Boyd et al. (1963) studied dibenzyline (phenoxybenzamine) which, like dibenamine, exerts a powerful, noncompetitive blockade at the mammalian alpha-adrenergic receptor. The former drug was shown to have very strong cholinergic blocking activity. Both dibenamine and dibenzylene have been shown to cause dispersion of teleost chromatophores (Table 2; p.90).

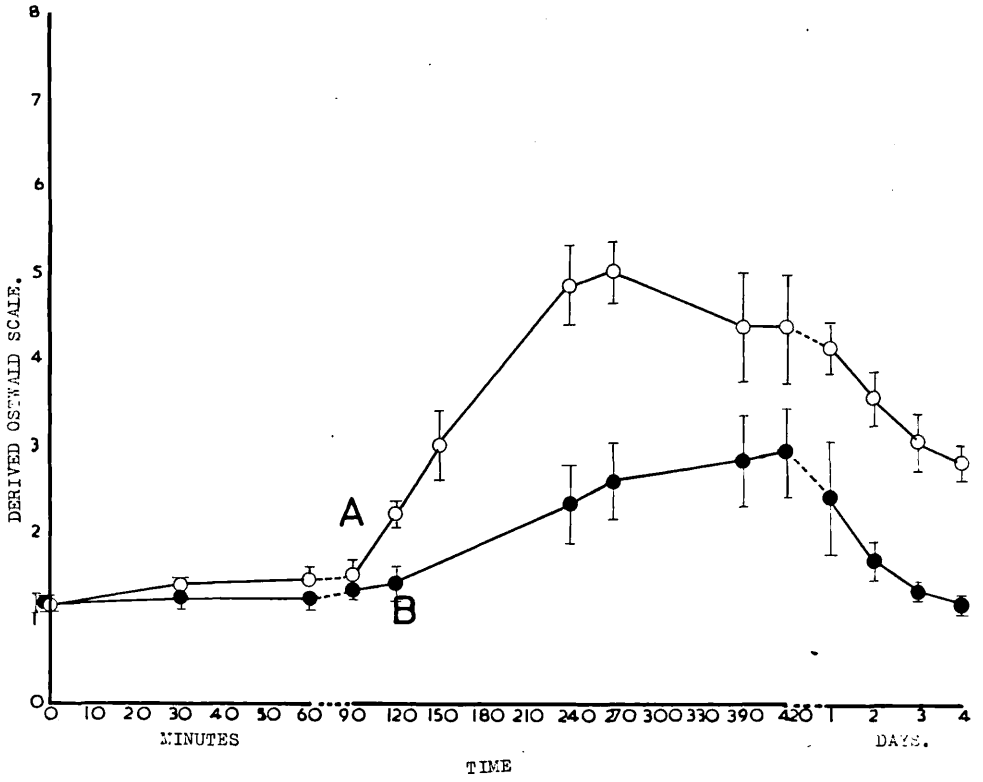
Single injections of dibenamine into white-adapted minnows led to only a partial dispersion of the melanophores (Fig. 24; p.144). The first group of white-adapted fish injected with 47 mg/kg dibenamine showed little sign of adrenergic blockade with 1½ hours. A second injection of 47 mg/kg in the same fish, at A in Fig. 24, led to considerable darkening which was mainly due to the melanophores in the barred skin regions. The maximum dispersion was reached 4 hours after the beginning of the experiment. Recovery to the pale state took between one

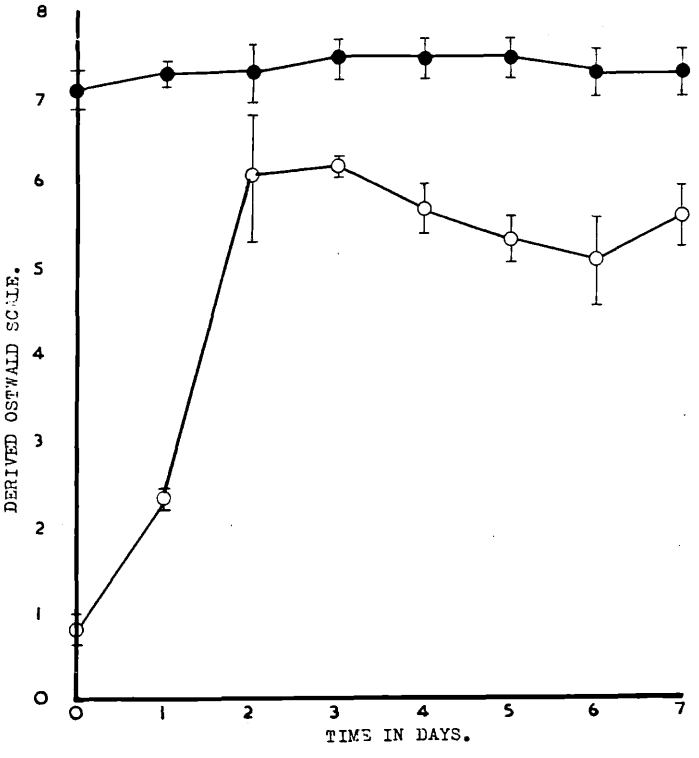
Fig. 24 a. The effect of acute injections of dibenamine on the shade of white-adapted minnows

○ 47 mg/kg at start and again at A; (15°C.) (12 animals)

● 11.75 mg/kg at start and again at B; (12°C.) (6 animals)

b. The effect of chronic (daily) injections of dibenamine (11.75 mg/kg) on the shade of six black- and six white-adapted minnows (8 - 12°C.)


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
A
B
0 10 20 30 40 50 60 90 120 150 180 210 240 270 300 330 390 420 1 2 3 4
MINUTES
DAYS.
TIME


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 6 7
TIME IN DAYS.

and two weeks. A second group of fish received doses of 11.75 mg/kg at the same time intervals but the extent and duration of the dispersion was not so great.

In a second experiment, two groups of fish were kept one on a white, the other on a black background. Each fish received one injection of 11.75 mg/kg dibenamine daily over a period of several weeks. This chronic treatment led to a gradual deepening of the shade of the white-adapted fish to about D.O.S. 5 or 6 but complete dispersion was not achieved (Fig. 24b; p.144). The black-adapted fish remained dark throughout this treatment.

In mammals (Section 1.3225) it is known that dibenamine and its congeners are first changed to ethyliniminium ions prior to the development of non-competitive adrenergic blackade. Injection of catecholamines during this transformation leads to failure of dibenamine blockade presumably because the early stages of the blockade involve competitive attachment to the adrenergic receptor. In the present experiment, the failure of single injections of dibenamine to darken white-adapted fish may be due to a CONTINUED release of catecholamines from sympathetic paling fibres or chromaffin cells elsewhere in the body (c.f. Section 3.34). The ability of competitive adrenergic blocking agents to darken fish which have been on a white

background for several months may represent a more efficient competitive blockade of such continuously released catecholamine.

Injections of noradrenaline and adrenaline were made into white-adapted fish in which dibenamine blockade was developing or which had been subjected to chronic treatment with the blocking agent for more than a week (Fig 25a; p.147). In the first group, injections of 2.75 mg/kg noradrenaline and 5.35 mg/kg adrenaline produced some pallor but this was not so great as that produced in fish of a similar shade adapted to a grey background and not injected with dibenamine (Section 3.255). The second group of fish, which were black-adapted, paled to a greater extent after 0.15 mg/kg noradrenaline or 0.27 mg/kg adrenaline than did normal black-adapted fish injected with the same doses. This latter response may represent the reaction of unblocked adrenergic receptors, possibly of a "beta" variety. The potentiation of these small doses might be brought about by an action of dibenamine preventing the uptake of the amines into adjacent adrenergic stores (Section 1.324).

Other groups of black-adapted fish which had been chronically pretreated with dibenamine were injected with 11.5 mg/kg tyramine, 11.3 mg/kg ephedrine or 8.8 mg/kg amphetamine (Fig. 25b; p.147). The failure of these agents

Fig. 25 a. The effect of catecholamines in minnows pretreated with dibenamine.

1. White-adapted fish darkening after acute injections of 47 mg/kg dibenamine.

○ 2.75 mg/kg noradrenaline; 14°C. (6 animals)

● 5.35 mg/kg adrenaline; 14°C. (6 animals)

2. Black-adapted fish after several weeks daily injections of 11.75 mg/kg dibenamine.

● 0.15 mg/kg noradrenaline; 14°C. (4 animals)

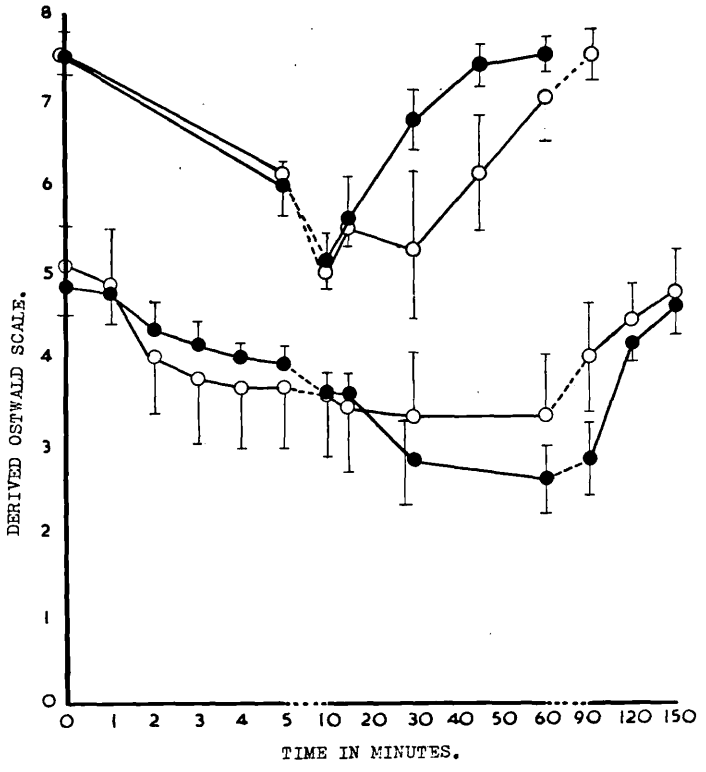
○ 0.27 mg/kg adrenaline; 14°C. (4 animals)

b. The effect of sympathomimetic amines in minnows which had been given daily injections of 11.75 mg/kg dibenamine for several weeks.

○ 11.5 mg/kg tyramine; 14°C. (6 animals)

● 11.3 mg/kg ephedrine; 14°C. (4 animals)

◑ 8.8 mg/kg amphetamine; 14°C. (4 animals)


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150
TIME IN MINUTES.

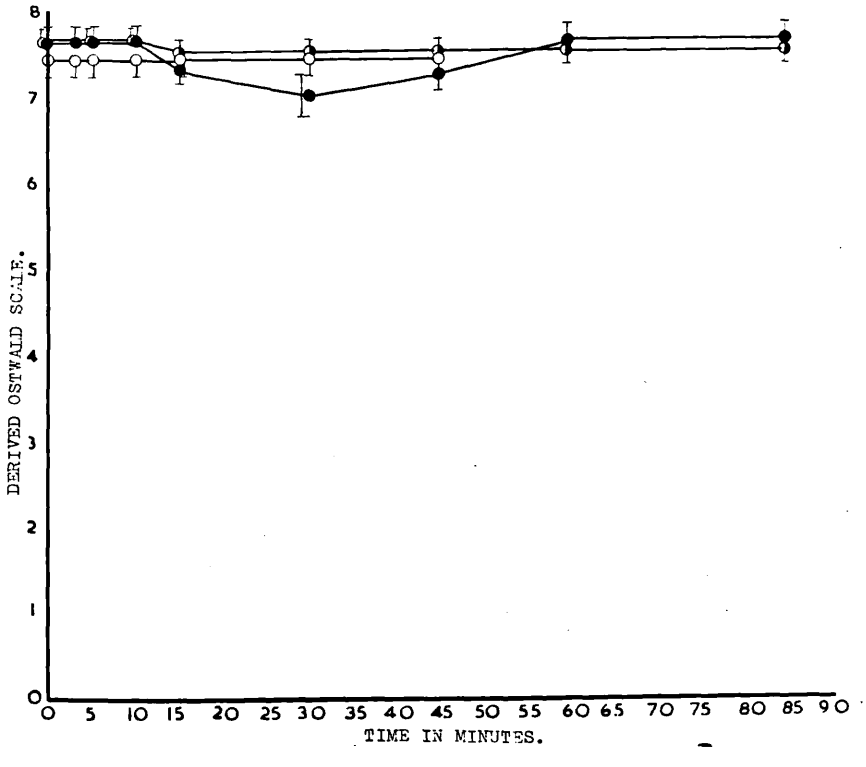
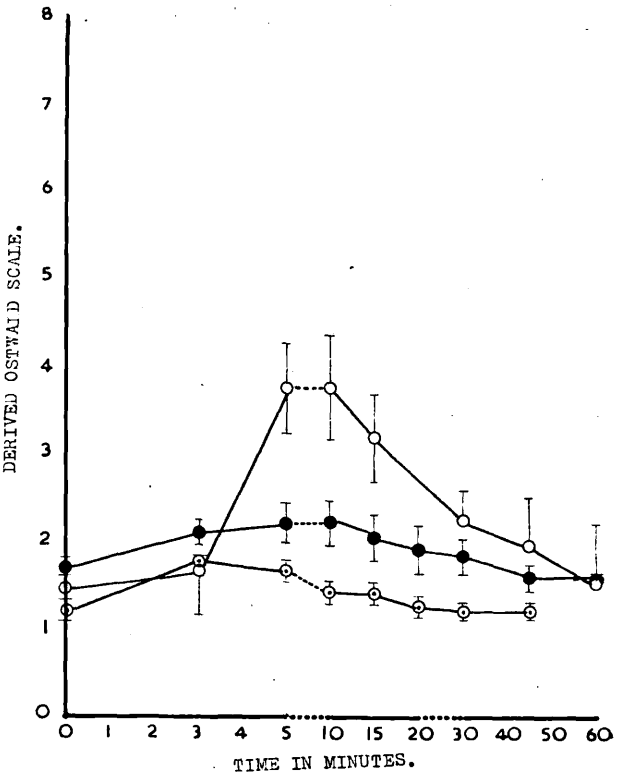

DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
TIME IN MINUTES.

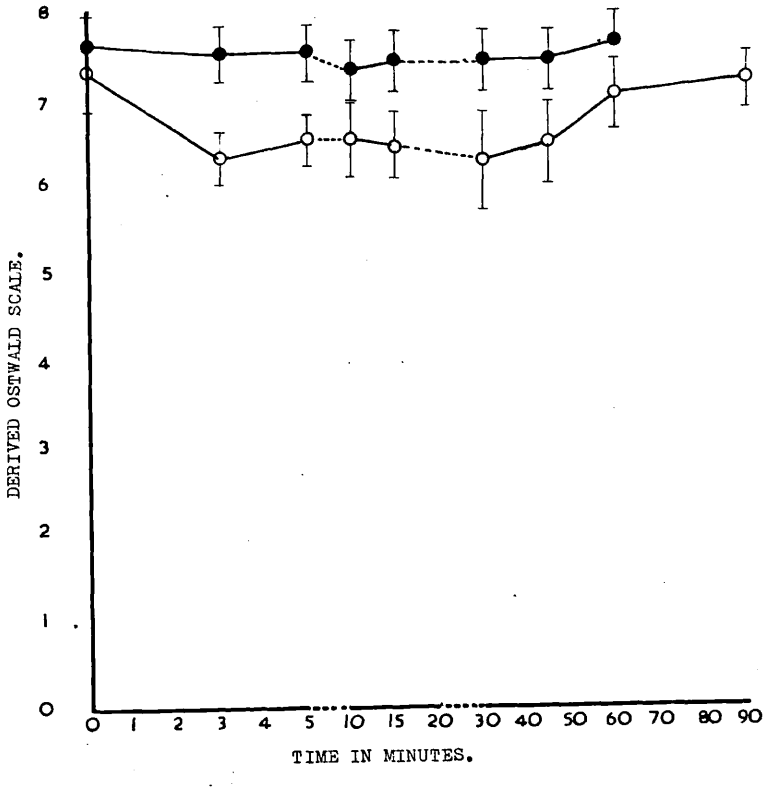
Fig. 26 a. The effect of alderline (pronethalol; nethalide) on the shade of white-adapted minnows.

● 50 mg/kg; 13°C. (19 animals)
⊙ 25 mg/kg; 13°C. (10 animals)
○ 13 mg/kg; 13°C. (9 animals)

b. The effect of alderline (pronethalol; nethalide) on the action of isoprenaline in black-adapted minnows.

● 5.7 mg/kg isoprenaline + 13.4mg/kg alderline 15°C. (5 animals)
○ 11.4 mg/kg isoprenaline + 13.4mg/kg alderline 15°C. (10 animals)


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 10 15 20 30 40 50 60
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 10 15 20 30 40 50 60 70 80 90
TIME IN MINUTES.

to produce paling may also represent blockade of the transfer site to the adrenergic store as well as receptor blockade.

Section 3.246

Pronethalol (methelide; alderline)

This drug is know to block specifically the beta receptors of mammals (Section 1.3225). Injections into white-adapted minnows rarely had profound darkening effects (Fig. 26a; p.148). Slight darkening after 50 and 25 mg/kg was seen and on one occasion 13 mg/kg darkened a group of fish. This effect could not be demonstrated in subsequent experiments. Injections of isoprenaline were antagonised by pronethalol (Fig. 26b; p.148).

Section 3.247

Mixtures of alpha and beta blocking agents

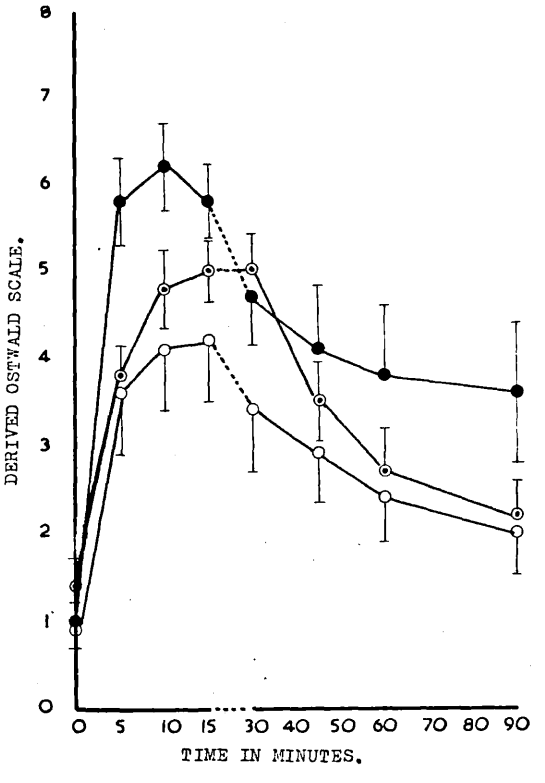
White-adapted fish were injected with 5.25 mg/kg piperoxane, 6 mg/kg phentolamine or 3.6 mg/kg yohimbine together with 13.4 mg/kg pronethalol (Fig. 27; p.150). In all cases the degree of darkening did not differ greatly from that produced in white-adapted fish by the alpha

Fig. 27 The effect of injections of combined alpha and beta adrenergic blocking agents on the shade of white-adapted minnows

● 3.6 mg/kg yohimbine + 13.4 mg/kg alderline (5 animals) 21°C.

○ 6.0 mg/kg phentolamine + 13.4mg/kg alderline (5 animals) 21°C.

⊙ 5.2 mg/kg piperoxane + 13.4 mg/kg alderline (5 animals) 21°C.


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 5 10 15 30 40 50 60 70 80 90
TIME IN MINUTES.

blocking agent alone. It was noticed however that the duration of the response to yohimbine was decreased in the presence of pronethalol.

Section 3.25

Hypotensive drugs

In the last decade several drugs have been widely studied which cause sympatholysis by way of actions on or in the adrenergic neurone. These drugs (Section 1.323) do not antagonize the effects of injected catecholamines but do modify the responses to the indirect actions of sympathomimetic amines. The action of bretylium tosylate has been studied by Boyd et al. (1962, 1963) in lower vertebrates. They concluded that it was more specific for adrenergic nerves than were many other mammalian adrenergic blocking agents but that both anti-acetycholine and antiacetylcholinesterase properties were present. Guanethidine exerted similar cholinergic properties to a greater extent.

Section 3.251

Bretylium

Section 3.2511

Acute treatment

Black- or white-adapted minnows were injected with a single dose of 8.2 mg/kg bretylium (Fig. 28; p.155). In the first few minutes following the injection the dark fish paled somewhat but this pallor was only slightly greater than that caused in control fish by injections of Ringer. As this pallor subsided the white-adapted fish began to darken and then both groups of fish began to pale simultaneously. After one hour the two groups of fish were seen to be unable to adapt completely to their respective backgrounds, the white-adapted fish becoming 3.0 - 3.5 and the black-adapted fish 6.0 - 6.5 on the D.O.S. scale. In mammals bretylium has been shown to exert both transient ganglionic blockade and to have sympathomimetic actions prior to its main sympatholytic effect (Section 1.3233). The darkening of fish after 15 minutes and the subsequent pallor may represent these actions on the aggregating nervous system of the minnow.

On the next day and successive days, the fish which had been injected with bretylium were subjected to background reversal for periods of five minutes and then returned to their original background. Colour changes were recorded at one minute intervals and comparison of the responses with those of normal fish (Fig. 9; p.114) shows that the white-adapted fish show greatly impaired colour changes after bretylium. Black-adapted fish however were able to achieve a shade of D.O.S. 4.5 on the white background fairly rapidly but thereafter were unable to pale. If, as Parker 1931 et seq. has suggested, the chromatic nervous system consists of antagonistic adrenergic paling fibres and cholinergic dispersing fibres, it would have been expected that paling of dark fish would have been inhibited but not the darkening of pale fish. The rapid darkening of fish from the test white background when returned to black may depend on the levels of circulating pituitary chromatic hormones present in the blood of these fish. During the five days following the injection a gradual reappearance of the fast colour change occurred and recovery was complete in three weeks.

Section 3.2512

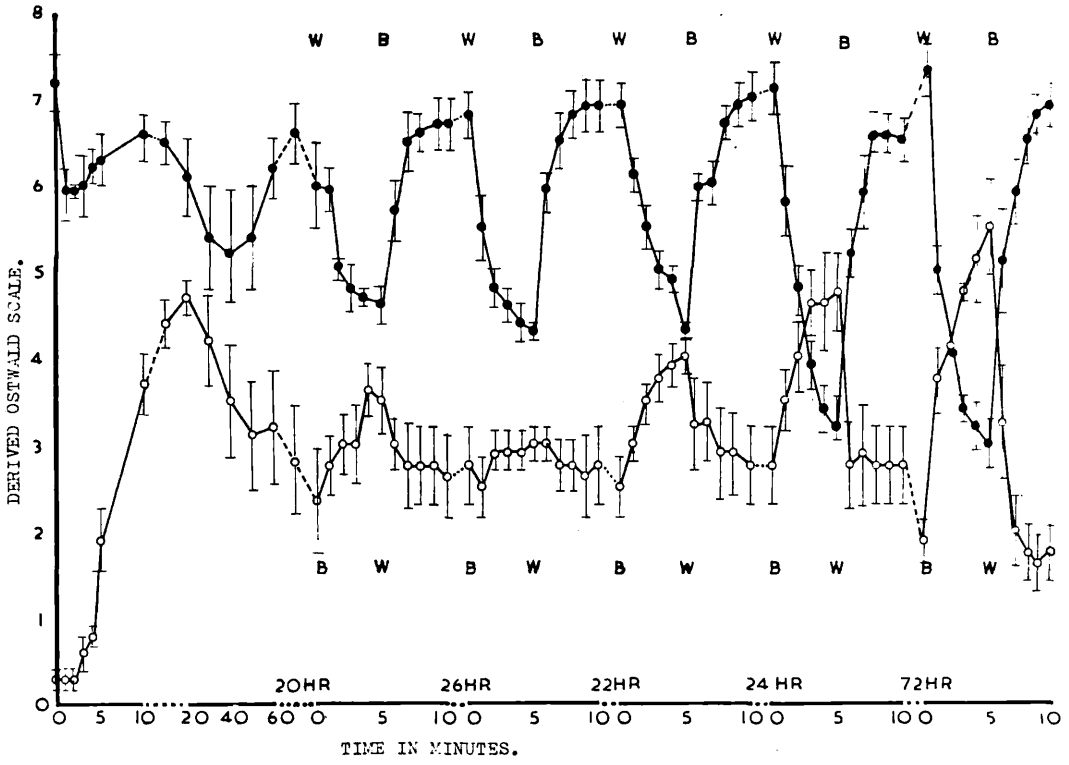
Chronic treatment

Daily injections of 4.1 mg/kg bretylium were made into black and white minnows for several weeks (Fig. 28b; p.155). Readings of the shade of these fish were taken both before and fifteen minutes after each injection. The black-adapted fish showed rather greater pallor than described above for the first few days but subsequently returned to their normal black-adapted shade. The white-adapted fish however darkened considerably at first to D.O.S. 5.0 - 6.0 but eventually reached a steady state between D.O.S. 3.0 and 4.0. The appearance of these fish with intermediate colouration was not so "blotchy" as that described previously during normal colour changes. Each daily injection caused a transitory pallor in all fish.

In the section on acute treatment with bretylium, it was shown that fast colour changes in both directions were impaired by bretylium. (Fig. 29; p.156) shows that white-adapted fish were only able to adapt to a black background very slowly. Black-adapted fish (recordings not made) were seen to be unable to adapt quickly to a white background. Under these conditions it seems

Fig. 28 a. The effect of acute injections of 8.2 mg/kg bretylium on the shade of four black- and five white-adapted minnows and on the ability of these fish to adapt to background reversal. Black or white backgrounds were presented to the fish at B or W respectively (16-20°C.)

b. The effect of chronic (daily) injections of 4.1 mg/kg bretylium on the shade of six black- and six white-adapted minnows (8-12°C.)


DERIVED OSTWALD SCALE.
W B W B W B W B W B
B W B W B W B W B W
20HR 26HR 22HR 24 HR 72HR
0 5 10 20 40 60 0 5 10 0 5 10 0 5 10 0 5 10 0 5 10
TIME IN MINUTES.

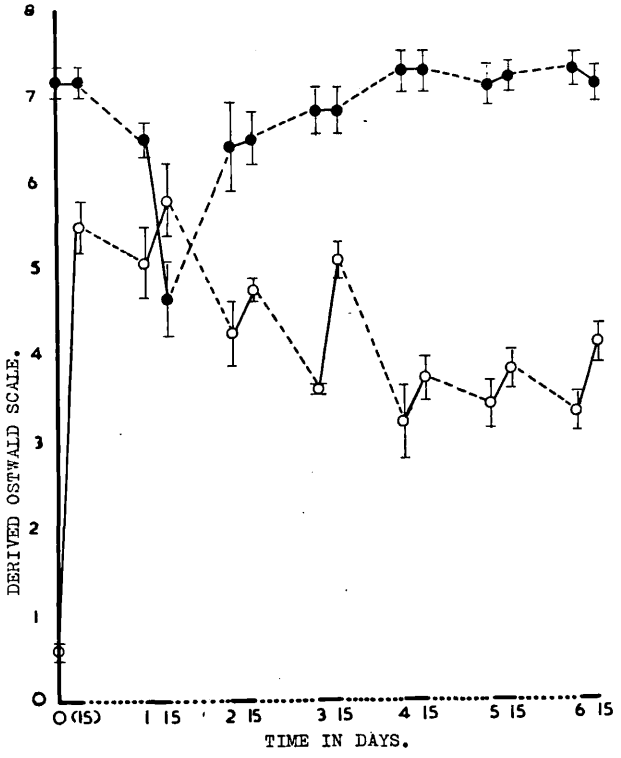
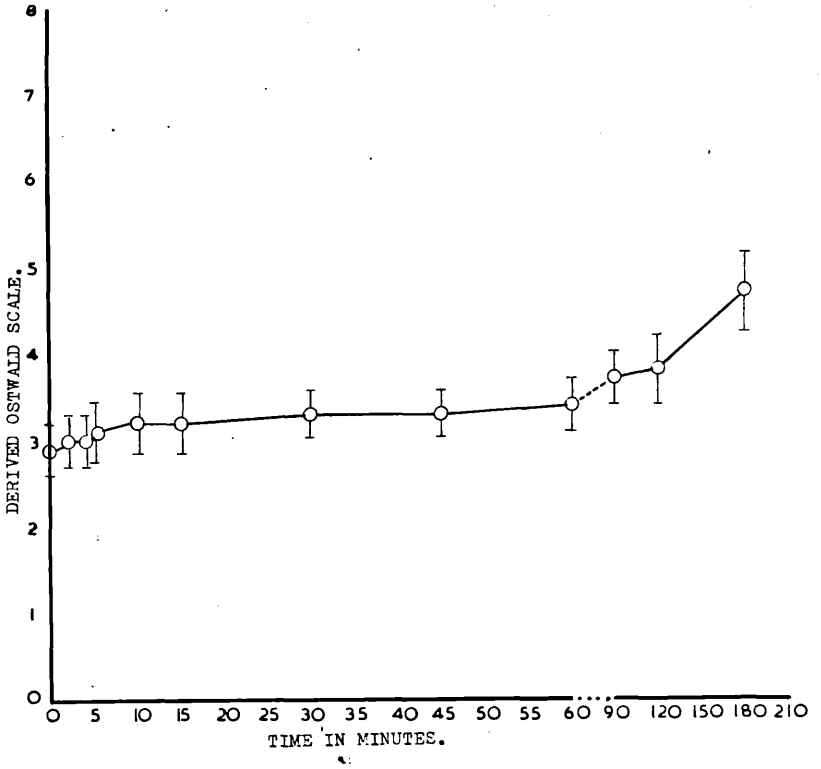

DERIVED OSTWALD SCALE.
0 (15) 1 15 2 15 3 15 4 15 5 15 6 15
TIME IN DAYS.

Fig. 29 The response of white-adapted minnows, which had been chronically pretreated with 4.1 mg/kg bretylium for several weeks, to background reversal (20°C.)(5 animals)

Fig. 30 The effect of a single injection of 12 mg/kg reserpine on the shade of seven black- and eight white-adapted minnows (22°C.)


DERIVED OSTWALD SCALE.
0
1
2
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4
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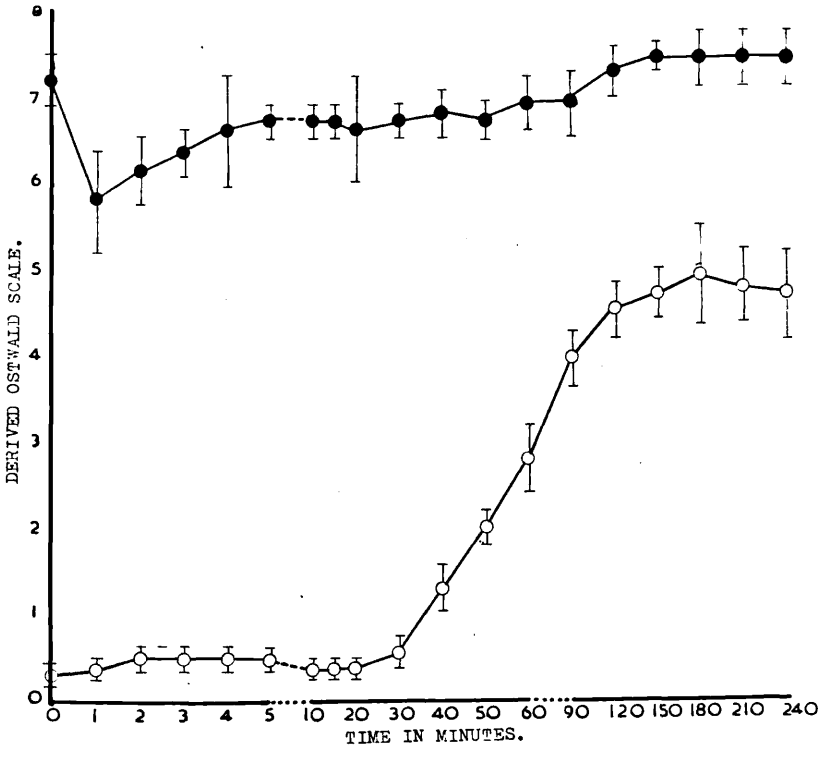
6
7
8
0 5 10 15 20 25 30 35 40 45 50 55 60 90 120 150 180 210
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240
TIME IN MINUTES.

probable that the lack of fast changes may be associated with failure of chromatic nerve activity and that the resultant shade on white or black depends on pituitary hormones. Healey (1951) described the slow colour changes of Phoxinus after spinal section which are abolished by hypophysectomy (Healey, 1948). The slow colour changes found after continued treatment with bretylium have time relations of the same order as these humorally controlled colour changes.

It is suggested (Section 4.22) that the darkening following the injection of adrenergic blocking agents into normal, white-adapted fish may represent the unmasking of a darkening agency, possibly the postulated darkening fibres of Parker, which is antagonized by impulses in the paling fibres and by pituitary paling hormone. The intermediate shade of white-adapted bretylium-treated fish may represent the action of unsupported pituitary hormones due to the suppression of the antagonistic nervous agencies.

Section 3.252

Reserpine

Reserpine has been shown to deplete adrenergic nerve terminals and other adrenergic stores of their

catecholamines (Section 1.3231). Injection of the amine into fish has always led to dispersion of chromatophores (Turner and Carl, 1955; Scheline, 1963).

Section 3.2521

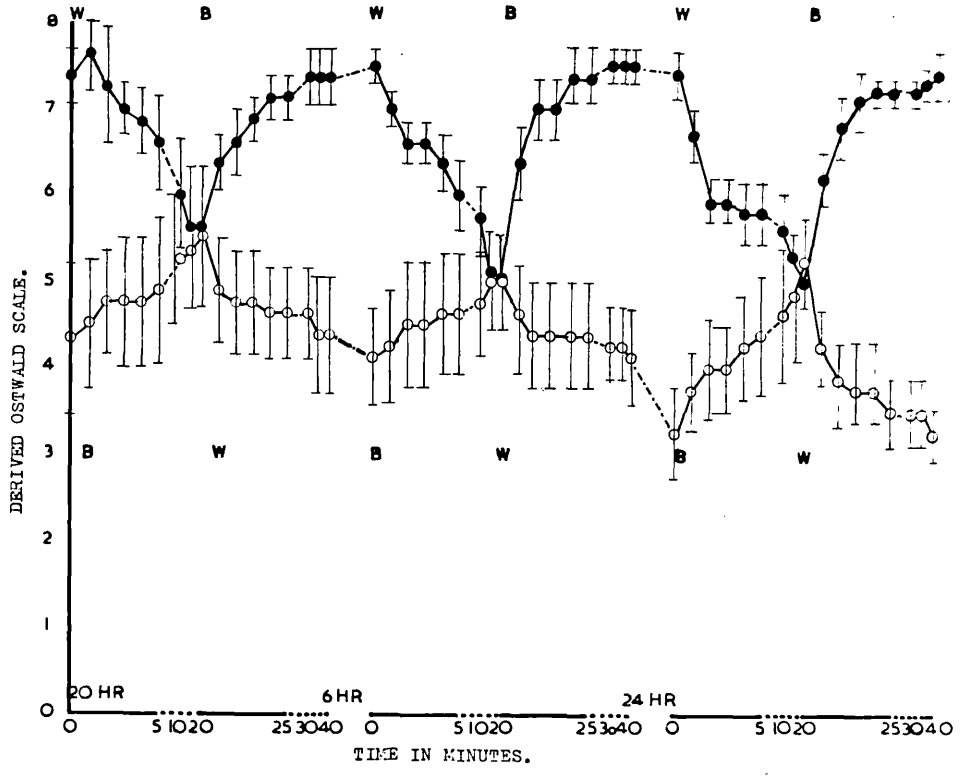
Acute treatment

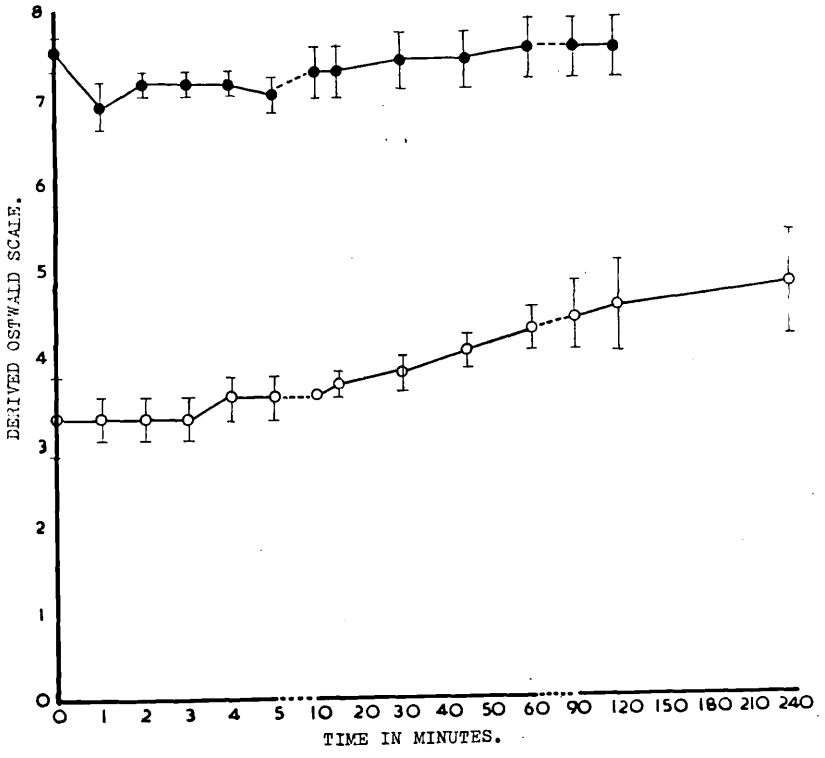
Black- and white-adapted fish were injected with 12 mg/kg reserpine (Fig. 30; p.156) as a single injection. Soon after the injection the black-adapted fish developed a slight but long-lasting pallor. As this pallor subsided, the white-adapted fish slowly darkened to between D.O.S. 4.0 and 5.0 after three hours. As was described following bretylium injections, these fish were not "blotchy" at this intermediate shade and observations on fin and body melanophores showed all to be partially dispersed. White-adapted reserpine-treated fish were always darker than fish treated with bretylium.

The two groups of fish were subjected to background reversal for twenty minutes every day following the injection of reserpine (Fig. 31; p.159). The presence of the hypotensive agent considerably impaired the colour changes of these fish but, as the effect of the drug wore off in succeeding days, recovery of the fast change

Fig. 31 The effect of acute treatment with 12 mg/kg reserpine on the ability of four black- and four white-adapted minnows to adapt to background reversal (20 - 23°C.)

Fig. 32 The effect of a single injection of 12 mg/kg reserpine on the shade of four black- and four white-adapted minnows which had received a similar injection four days previously (20°C.)


W
B
W
B
W
B
B
W
B
W
B
W
DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
20 HR
6 HR
24 HR
0 5 10 20 25 30 40 0 5 10 20 25 30 40 0 5 10 20 25 30 40
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
0
1
2
3
4
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7
8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240
TIME IN MINUTES.

occurred. This recovery took two weeks.

Two groups of fish, one black- the other white-adapted, which had been injected with 12 mg/kg reserpine four days earlier were given a second injection of the same dose of the drug. The black-adapted fish showed a considerably reduced pallor after the injection, possibly due to the prior depletion of the catecholamine stores in the body. The white-adapted fish, which had equilibrated at D.O.S. 3.0 - 4.0 slowly darkened further to between D.O.S. 4.0 and 5.0 (Fig. 32; p.159).

Section 3.2522

Chronic treatment

Black- and white-adapted fish were injected daily with 2.7 mg/kg reserpine over a period of several weeks (Fig. 33; p.161). The black fish at first showed slight pallor but then became very dark and remained in this condition. The white-adapted fish equilibrated to about D.O.S. 4.0. Some of these fish were tested for their ability to react to background reversal (Fig. 34; p.161). In no fish was there any sifnificant ability to adapt to the new background within thirty minutes. Other groups of black- and white-adapted, chronically-reserpinized fish

Fig. 33 The effect of chronic (daily) injections of 2.7 mg/kg reserpine on the shade of six black- and twenty-one white-adapted minnows (8 - 12°C.)

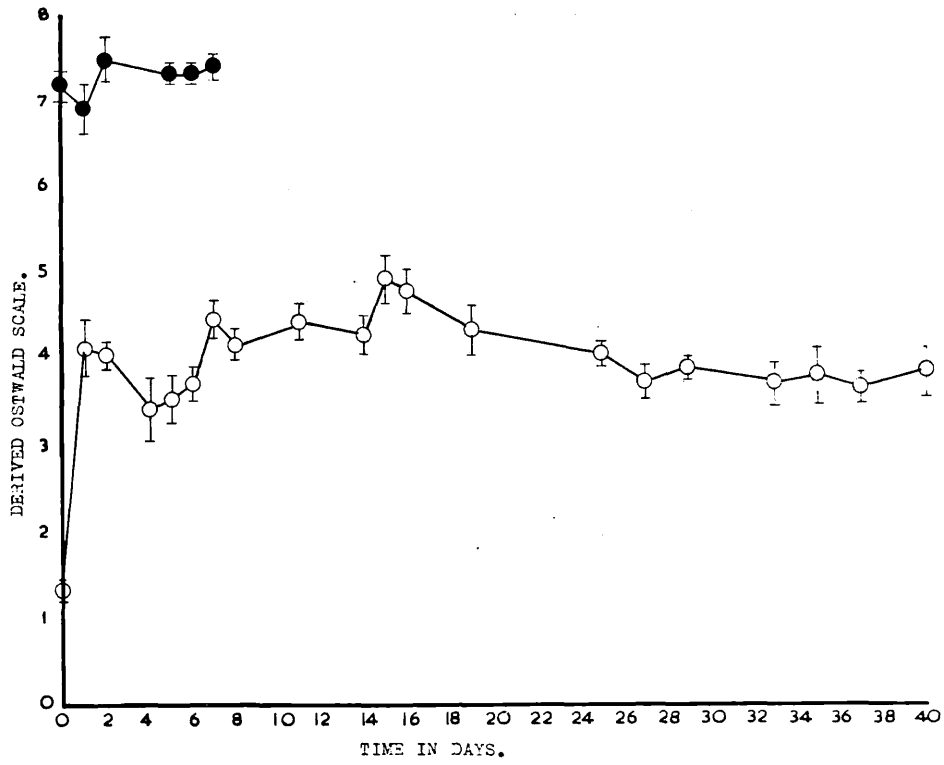
Fig. 34 The effect of reserpine pretreatment on the ability of 34 black- and white-adapted minnows to adapt to change of background

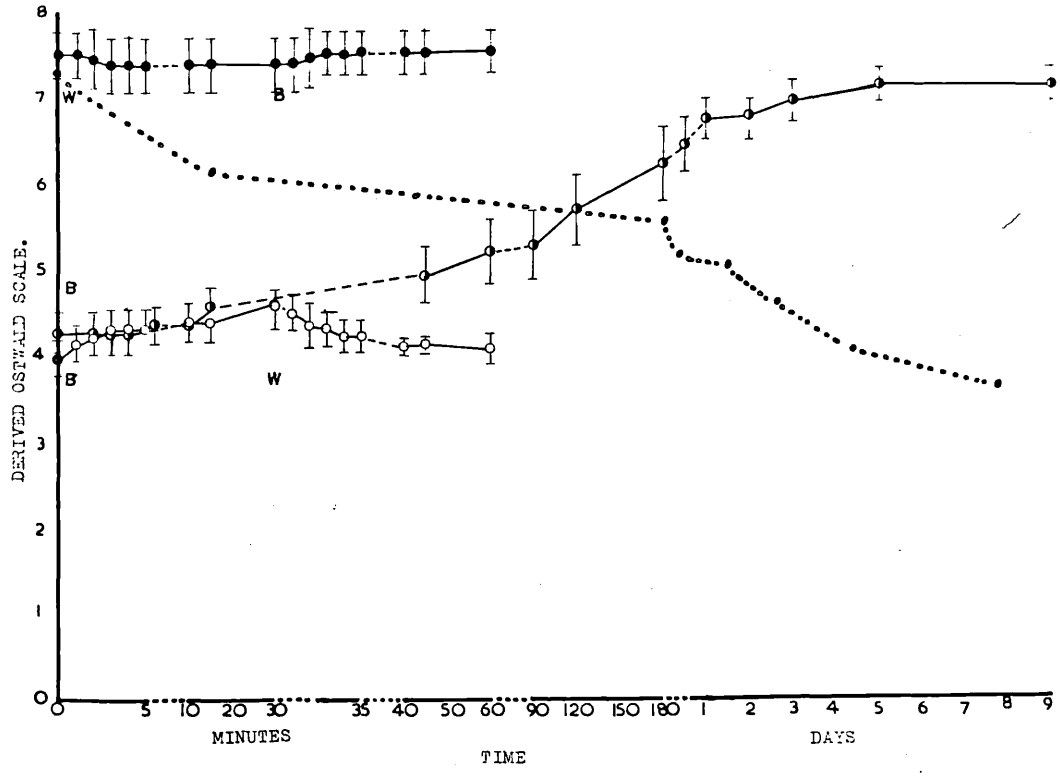
◑ white adapted fish put on black after several weeks reserpinization (8°C.)(6 animals)

◐ black adapted fish put on white after several weeks reserpinization (20°C.)(5 animals)

○ white adapted fish transferred to black (B) and then to white(W) after 16 days reserpinization (20°C.) (20 animals)

● black adapted fish transferred to white (W) and then to black (B) after 10 days reserpinization (8°C.) (8 animals)


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
TIME IN DAYS.


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
W
B
B
B
W
0 5 10 20 30 35 40 50 60 90 120 150 180 1 2 3 4 5 6 7 8 9
MINUTES
DAYS
TIME

were subjected to background reversal and the time required to adapt to the new background recorded. The time from white to black was 120 hours and from black to white 210 hours. The latter change is slower than that described by Healey (1951) for spinal fish adapting from black to white backgrounds.

In general, the effects following treatment with reserpine resemble the effects of bretylium in that rapid colour changes in both directions are abolished and that white-adapted fish assume an intermediate shade of grey.

Section 3.253

Guanethidine

Section 3.2531

Acute treatment

Minnows adapted to black- and white backgrounds were injected with 10.75 mg/kg guanethidine (Fig. 35; p.164). White-adapted fish darkened to D.O.S. 3.0 after 5 minutes while the black-adapted fish began to pale. After this time all fish paled so that one hour after the injection the black-adapted fish were approximately D.O.S. 2.5 and the white fish 1.5. After a further hour both groups of

fish began to darken again. By the next day the fish on a black background were almost completely black whilst those on a white background assumed an equilibrium shade of D.O.S. 3.0. Some of the white-adapted fish were given a second dose of 10.75 mg/kg guanethidine. These fish, unlike white-adapted fish given a first injection with guanethidine, not only failed to darken but also paled for two or three hours before regaining their intermediate shade. Guanethidine is known to have sympathomimetic actions in mammals and presumably the pallor which develops in grey- or black-adapted fish is a result of this activity. In addition, like bretylium, this agent is known to exert a fleeting blockade on sympathetic ganglion transmission. The darkening following its injection into white-adapted, normal fish may represent failure of transmission in autonomic ganglia of the aggregating fibre system leading to the manifestation of a darkening agency which can overcome the effect of circulating pituitary paling hormones.

Fish which had been injected only once with guanethidine on either a black or a white background were subjected to background reversal (Fig. 37; p.166). A slow colour change persisted in these fish. White-adapted fish were able to darken from D.O.S. 3.0 to 5.5 in half an hour

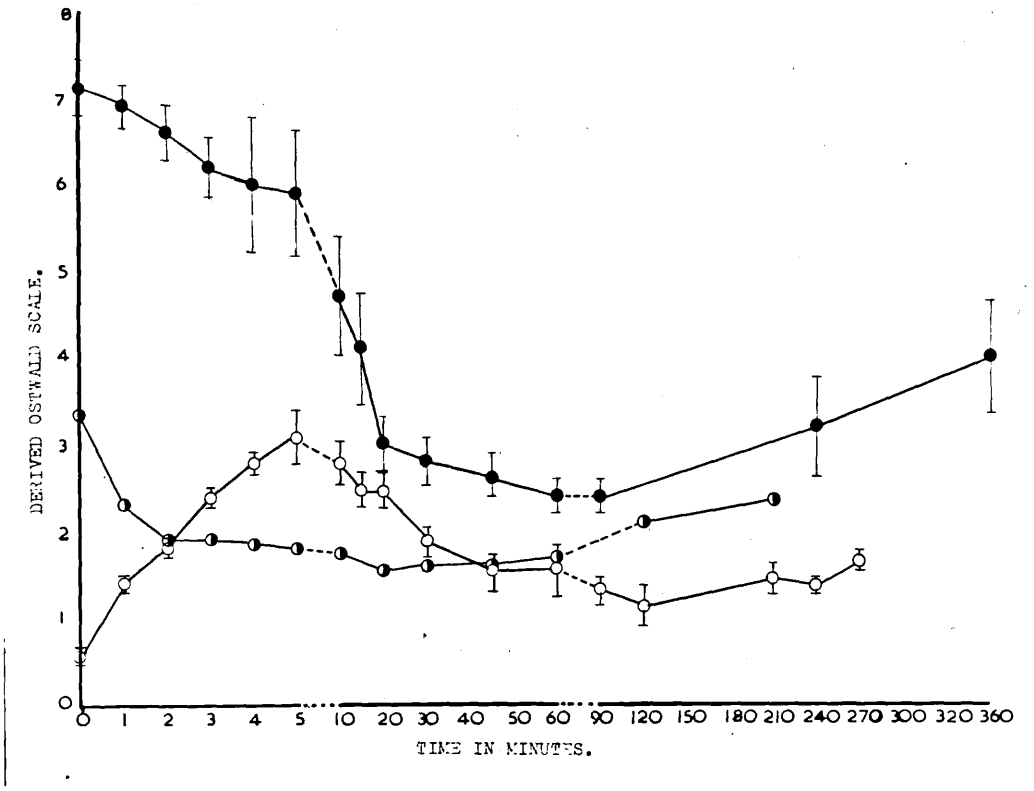
Fig. 35 The effect of injections of guanethidine on the shade of black- and white-adapted minnows

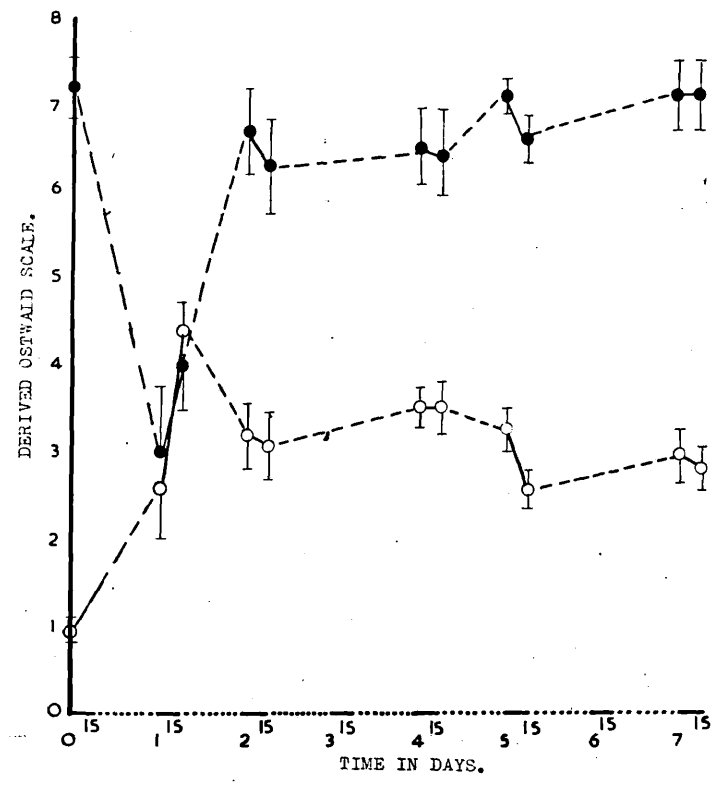
● 10.75 mg/kg in black-adapted fish 20°C. (5 animals)

○ 10.75 mg/kg in white-adapted fish 20°C. (20 animals)

◑ 5.4 mg/kg in white-adapted fish which had received 10.75 mg/kg one day previously 20°C. (10 animals)

Fig. 36 The effect of chronic (daily) injections of 4.3 mg/kg guanethidine on the shade of five black- and eight white-adapted minnows


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240 270 300 320 360
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
0
1
2
3
4
5
6
7
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0 15 1 15 2 15 3 15 4 15 5 15 6 15 7 15
TIME IN DAYS.

on a black background but required over an hour to return to 3.0 when returned to white. Similarly, another group of white-adapted fish was able to adapt completely to a black background after two hours and on return to the original white background required a much longer time to recover. In this respect guanethidine in acute doses seemed more effective against rapid paling fibres whereas acute treatment with bretylium or reserpine antagonized darkening more readily (Sections 3.2511 and 3.2521). Black-adapted fish which had received a single dose of 10.75 mg/kg guanethidine required four hours to adapt completely to the white background (D.O.S. 3.0)

Section 3.2532

Chronic treatment with guanethidine

Black- and white-adapted fish were given daily injections of 4.3 mg/kg guanethidine (Fig. 36; p.164) and readings of the shade of the fish were taken before and fifteen minutes after each injection. Black-adapted fish paled during the first day of treatment but subsequently returned to their normal dark colour on this background. White-adapted fish darkened at first but eventually reached a steady state between D.O.S. 2.5 and 3.0. Each daily

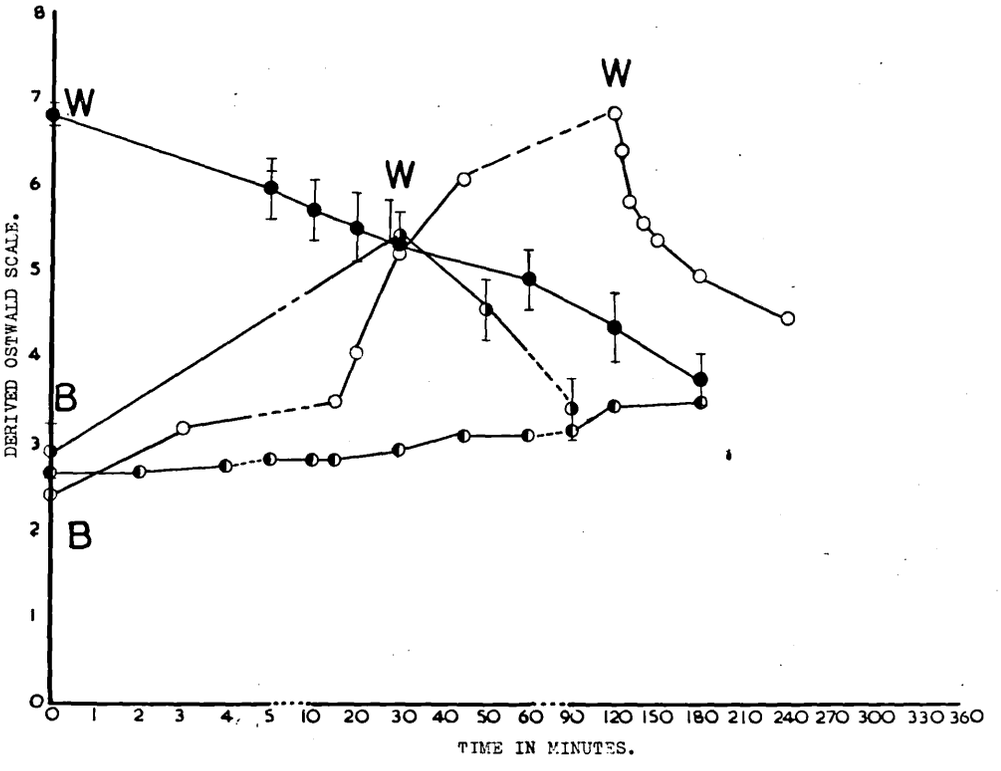
Fig. 37 The effect of guanethidine pretreatment on the ability of minnows to adapt to a new background

◑ white-adapted minnows previously injected with a single dose of 10.75 mg/kg guanethidine, placed on a black background (B) and then returned to white (W). (10 animals) (20°C.)

○ white-adapted minnows previously injected with 10.75 mg/kg and 5.4 mg/kg guanethidine (four days apart) placed on a black (B) and then a white (W) background. (10 animals) (20°C.)

● black-adapted minnows which were previously injected with 10.75 mg/kg guanethidine and then placed on a white (W) background (10 animals) (20°C.)

◐ white-adapted minnows which had been chronically pretreated with 4.3 mg/kg/day guanethidine for several weeks and then placed on a black (B) background (7 animals) (16°C.)


DERIVED OSTWALD SCALE.
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0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240 270 300 330 360
TIME IN MINUTES.

injection was followed by a brief but intense pallor lasting less than 15 minutes. A group of white adapted fish subjected to background reversal was found to have lost the ability to adapt within a few hours to the new background (Fig. 37; p.166).

All three drugs tested in this section (bretylium, reserpine and guanethidine) antagonize rapid colour changes in both directions and permit white adapted fish to assume only an intermediate shade. This latter shade is not of the "blotchy" variety described for fish which have achieved an intermediate shade in response to grey backgrounds or in response to adrenergic blocking agents which were used (Section 3.24).

Further discussion of these results obtained with hypotensive drugs is postponed until a more complete discussion of the effect of adrenergic drugs on the minnow chromatic system can be presented (Sections 3.35 and 4.14).

Section 3.254

The response of minnows pretreated with hypotensive drugs to catecholamines and sympathomimetic amines

In the introduction to this work (Section 1.3262) it was pointed out that hypotensive drugs not only prevent

functional activity in adrenergic neurones but also lead to changes in sensitivity of end organs to sympathomimetic amines and catecholamines. The following series of experiments describes the reactions of fish pretreated with hypotensive agents for varying periods of time to some of these amines.

Since white-adapted, chronically pretreated minnows assume an intermediate shade of grey (Section 3.251 - 3.253) it was necessary to make injections of sympathomimetic and catechol amines into fish adapted to an intermediate shade of grey corresponding to D.O.S. 4 (Figs. 38a, b; 39a, b; pp.170,171). The effects of amines in experimental fish could then be compared over the same range of melanophore pigment migration. Black-adapted hypotensive pretreated fish may be compared directly with normal black-adapted fish injected with the same amines, as the hypotensive fish are able to adapt to the black background relatively well.

Section 3.2541

Noradrenaline

The responses of fish chronically pretreated with reserpine, bretylium or guanethidine to injections of noradrenaline are represented in Figs. 40, 45, 46 (pp.174,177).

Table 5 (p.276) and Fig. ~~102~~ 101 (p.278) summarise the responses of the experimental fish to noradrenaline and relate the degree of potentiation of the amine to the length of time of chronic treatment with the hypotensive drugs. The injections of noradrenaline used were those expected to cause approximately 50% response or less in normal fish (cf. Fig. 100; p.277). It was found that not only did all three hypotensive agents cause pronounced supersensitivity to noradrenaline but also that the degree of supersensitivity caused by reserpine increased with the duration of chronic treatment.

Section 3.2542

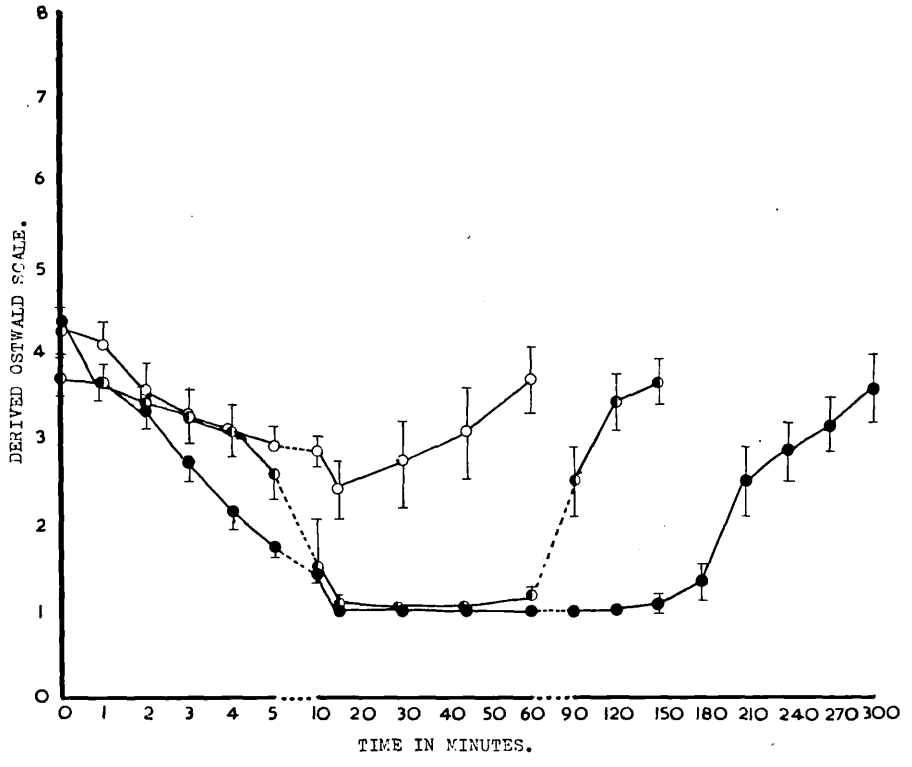
Adrenaline

The responses of experimental fish to injections of adrenaline are represented in Figs. 40, 45 and 47 (pp.174, 177 & 178). It was found that doses of adrenaline which in normal fish produced 50% paling or less (Fig. 100; p.277) were strongly potentiated by prolonged treatment with hypotensive agents. Like noradrenaline, the supersensitivity to adrenaline caused by reserpine increased with the length of chronic treatment.

Fig. 38 The effect of catechol- and sympathomimetic amines on the shade of minnows adapted to a grey background

a) ● 10.8 mg/kg adrenaline (12°C.)(6 animals)
◐ 5.4 mg/kg adrenaline (12°C.)(6 animals)
○ 2.7 mg/kg adrenaline (12°C.)(6 animals)

b) ● 5.5 mg/kg noradrenaline (12°C.) (6 animals)
◐ 2.75 mg/kg noradrenaline (12°C.) (6 animals)
○ 1.4 mg/kg noradrenaline (12°C.) (5 animals)


DERIVED OSTWALD SCALE.
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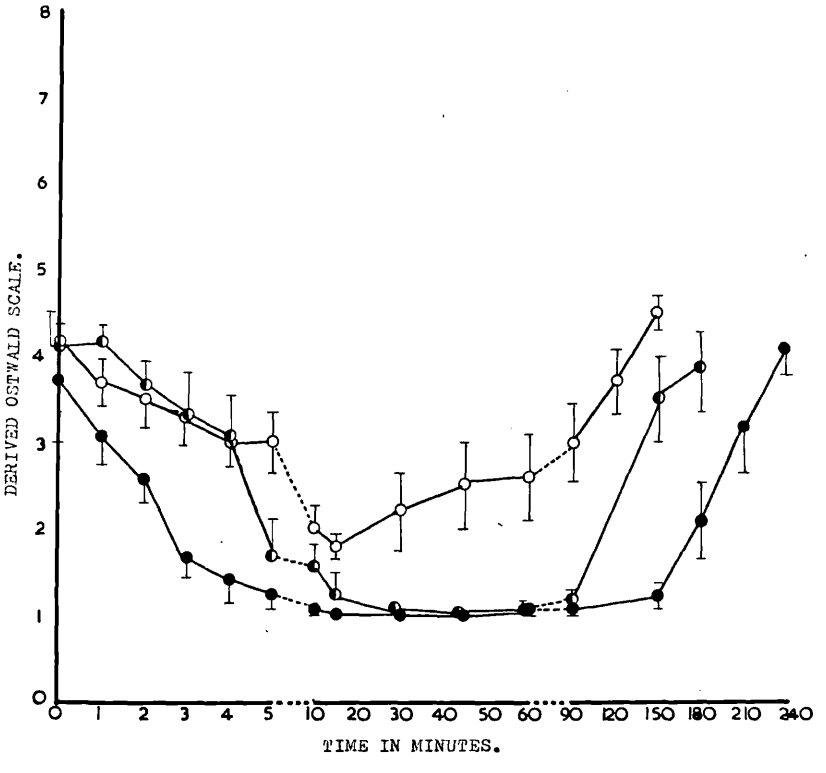
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
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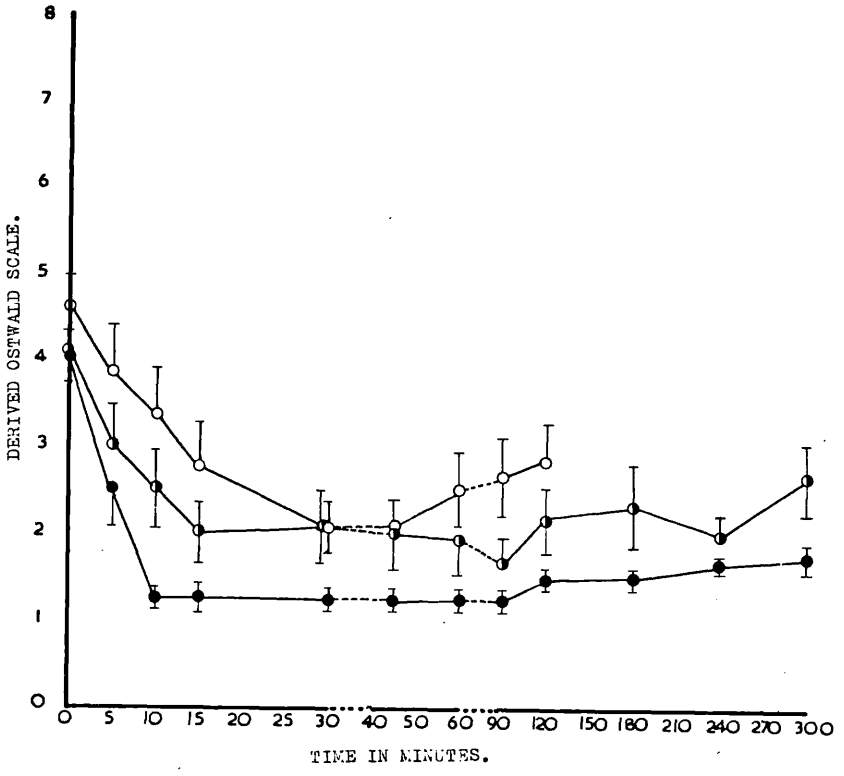
TIME IN MINUTES.

Fig. 39 The effect of injections of sympathomimetic amines on the shade of minnows adapted to a grey background.

a) ● 11.3 mg/kg ephedrine (13°C.)(6 animals)
◑ 5.7 mg/kg ephedrine (13°C.)(6 animals)
○ 2.5 mg/kg ephedrine (13°C.)(6 animals)

b) ● 21.0 mg/kg tyramine (11.5°C.)(6 animals)
◑ 11.5 mg/kg tyramine (11.5°C.)(6 animals)
○ 4.2 mg/kg tyramine (11.5°C.)(6 animals)


DERIVED OSTWALD SCALE.
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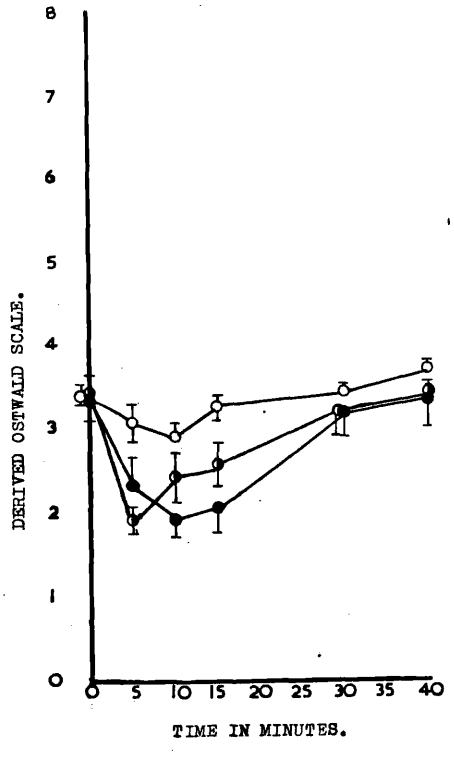
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
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TIME IN MINUTES.

Section 3.2543

Ephedrine

Injections of doses of ephedrine into fish chronically pretreated with hypotensive drugs (Figs. 43, 46 and 48; pp.175, 177 & 178) showed that both reserpine and bretylium were able to antagonize the influence of this amine after five weeks of treatment (Fig. 101; p.278). During the first week of reserpine treatment small doses of ephedrine were somewhat enhanced. Guanethidine did not depress the activity of ephedrine.

Section 3.2544

Tyramine

Injections of tyramine were made into minnows which had received a single dose (12 mg/kg) of reserpine 24 hours before (Fig. 42; p.175) and into other fish which had been subjected to chronic treatment with reserpine for various lengths of time. Acute treatment strongly antagonized the effects of tyramine. During the first ten days of chronic treatment, the response to injections of tyramine was re-established but continued reserpinization subsequently depressed these responses once more. The paling effects

of tyramine were never completely abolished by reserpine. After five weeks chronic treatment of black-adapted minnows with bretylium or guanethidine (Figs. 46 and 48; pp.177,178) the effects of tyramine were respectively unaffected and potentiated (Fig. 101; p.278).

Section 3.2545

Amphetamine

Injections of amphetamine were made into black-adapted fish which had been chronically treated for five weeks with reserpine, bretylium or guanethidine (Figs. 44, 46 and 47; pp.176,177 & 178). Accordingly, nothing is known about the time factor in the development of sensitivity changes to this amine. However, after five weeks, the actions of the agent were markedly antagonized by reserpine, little affected by bretylium and potentiated by guanethidine.

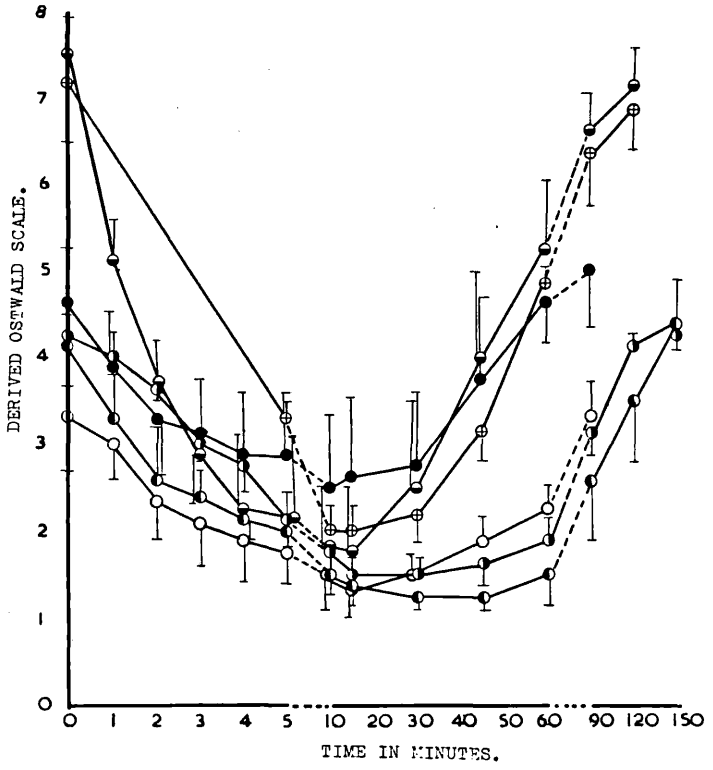
In this context it is necessary to interpret sensitivity changes as represented in Fig. 101 with caution. The degree of potentiation or antagonism of any amine can be misleading if it is used without a knowledge of the complete dose/response curve (Trendelenburg, 1963). Tentative, but incomplete, dose/response curves have been calculated for the amines used in this section for

Fig. 40 The effect of injections of noradrenaline on the shade of black- and white-adapted minnows which were previously injected with reserpine

- ● white-adapted fish after five days chronic treatment with 2.7 mg/kg reserpine, injected with 0.7 mg/kg noradrenaline (13.5°C.) (4 animals)
- ◒ black-adapted fish after seven days chronic reserpinization, injected with 1.4 mg/kg noradrenaline (21°C.) (4 animals)
- ◑ White-adapted fish, after ten days chronic reserpinization, injected with 0.7 mg/kg noradrenaline (9°C.) (4 animals)
- ◐ white-adapted fish, after ten days chronic reserpinization, injected with 2.1 mg/kg noradrenaline (10°C.) (4 animals)
- ○ white-adapted fish, after four weeks chronic reserpinization, injected with 0.35 mg/kg noradrenaline (12°C.) (5 animals)
- ⊕ black-adapted fish, after five weeks chronic reserpinization, injected with 0.15 mg/kg noradrenaline (14°C.) (3 aminals)

Fig. 41 The effect of injections of adrenaline on the shade of black- and white-adapted minnows which were previously treated with reserpine

- ○ white-adapted fish, after 10 days chronic reserpinization, injected with 4 mg/kg adremaline (10°C.) (4 animals)
- ◑ white-adapted fish, after ten days reserpinisation injected with 1.35 mg/kg adrenaline (10°C.) (5 animals)
- ◐ white-adapted fish, after four weeks chronic reserpinization, injected with 0.81 mg/kg adrenaline (12°C.) (6 animals)
- ● black-adapted fish, after five weeks chronic reserpinization, injected with 0.27 mg/kg adrenaline (14°C.) (3 animals)


DERIVED OSTWALD SCALE.
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0 1 2 3 4 5 10 20 30 40 50 60 90 120 150
TIME IN MINUTES.

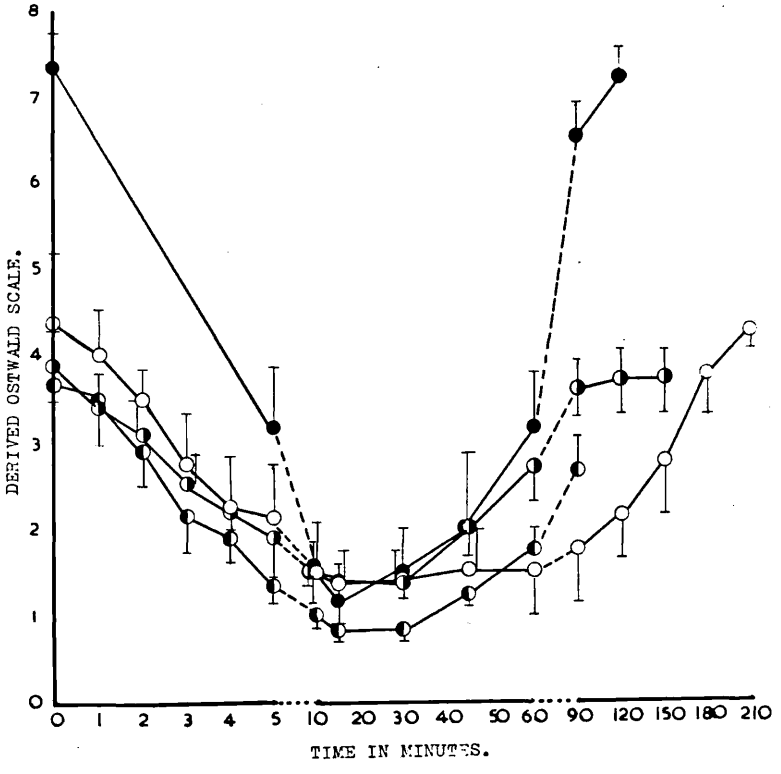

DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210
TIME IN MINUTES.

Fig. 42 The effect of injections of tyramine on the shade of minnows previously treated with reserpine

◑ white-adapted fish injected with 11.5 mg/kg tyramine after five days chronic reserpinization (13°C.) (4 animals)

⊕ white-adapted fish injected with 11.5 mg/kg tyramine after ten days chronic reserpinization (10°C.) (4 animals)

◐ white-adapted fish injected with 21 mg/kg tyramine after ten days chronic reserpinization (10°C.) (4 animals)

◕ white-adapted fish injected with 11.5 mg/kg tyramine after three weeks chronic reserpinization (10°C.) (4 animals)

○ white-adapted fish injected with 21 mg/kg tyramine after three weeks chronic reserpinization (10°C.) (4 animals)

● black-adapted fish injected with 11.5 mg/kg tyramine within 24 hours of a single injection of 12 mg/kg reserpine (20°C.) (10 animals)

◒ black-adapted fish injected with 11.5 mg/kg tyramine after five weeks chronic reserpinization (13°C.) (4 animals)

Fig. 43 The effect of injections of ephedrine on the shade of minnows previously treated with reserpine

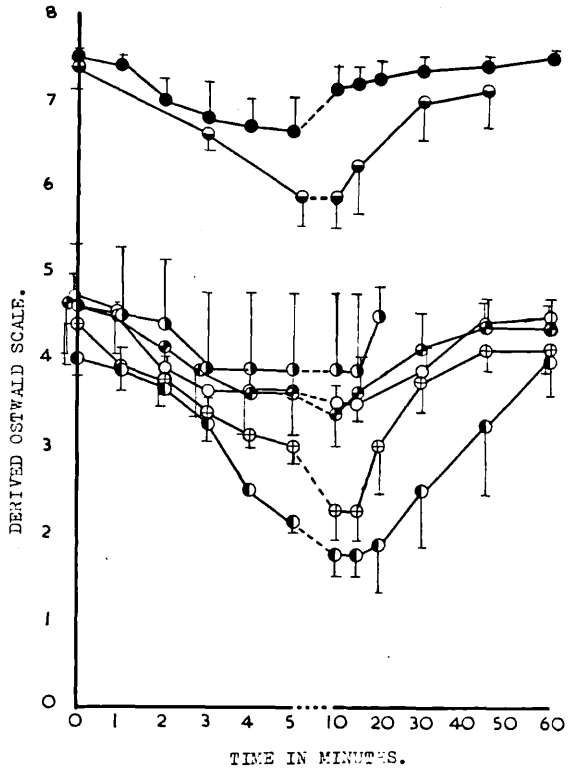
◑ white-adapted fish injected with 11.3 mg/kg ephedrine after five days chronic reserpinization (10°C.) (4 animals)

◒ white-adapted fish injected with 4.5 mg/kg ephedrine after five days chronic reserpinization (10°C.) (4 animals)

○ white-adapted fish injected with 4.5 mg/kg ephedrine after three weeks chronic reserpinization (10°C.) (3 animals)

◒ white-adapted fish injected with 2.25 mg/kg ephedrine after three weeks chronic reserpinization (10°C.) (4 animals)

● black-adapted fish injected with 11.3 mg/kg ephedrine after five weeks chronic reserpinization (14°C.) (3 animals)


DERIVED OSTWALD SCALE.
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0 1 2 3 4 5 10 20 30 40 50 60
TIME IN MINUTES.

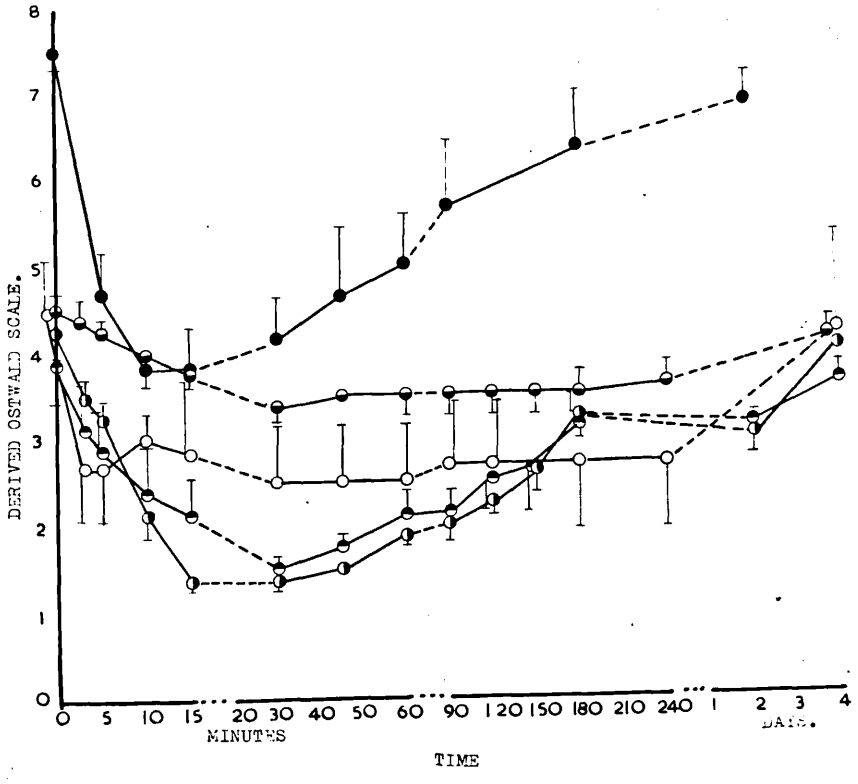
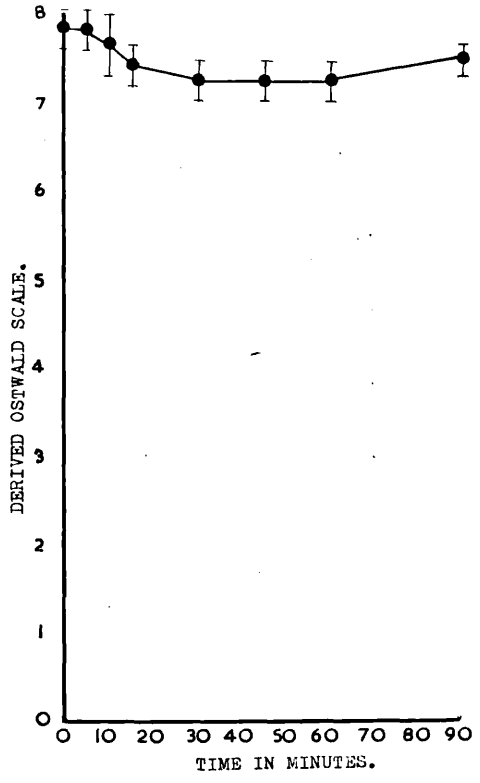

DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 5 10 15 20 30 40 50 60 90 120 150 180 210 240 1 2 3 4
MINUTES
TIME

Fig. 44 The effect of amphetamine (8.8 mg/kg) on the shade of black-adapted fish which had undergone chronic reserpinization for five weeks (6 fish, 14°C.).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DERIVED OSTWALD SCALE.
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TIME IN MINUTES.

Fig. 45 The effect of catecholamines in black-adapted minnows which had been chronically treated with bretylium for five weeks.

○ 0.15 mg/kg noradrenaline (14°C.) (4 animals)

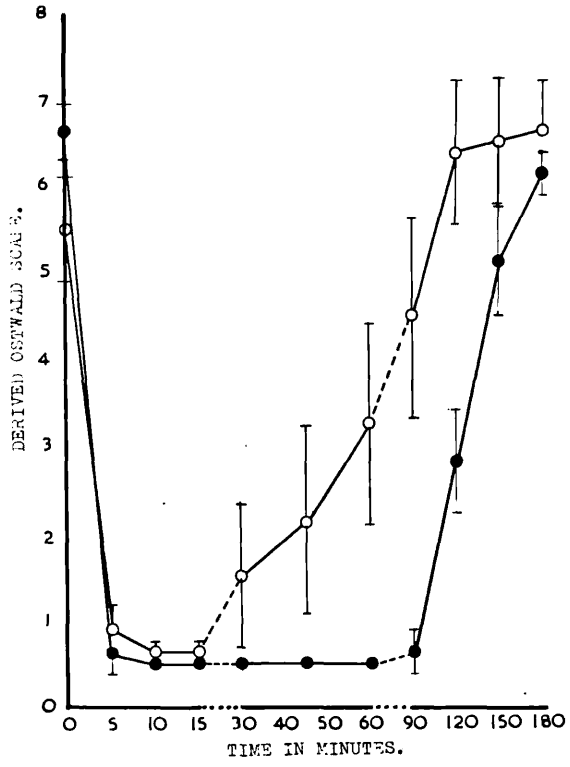
● 0.27 mg/kg adrenaline (14°C.) (4 animals)

Fig. 46 The effect of sympathomimetic amines on the shade of black-adapted minnows which had been chronically treated with bretylium for five weeks.

● 11.5 mg/kg tyramine (14°C.) (6 animals)

○ 11.3 mg/kg ephedrine (14°C.) (4 animals)

◑ 8.8 mg/kg amphetamine (14°C.) (4 animals)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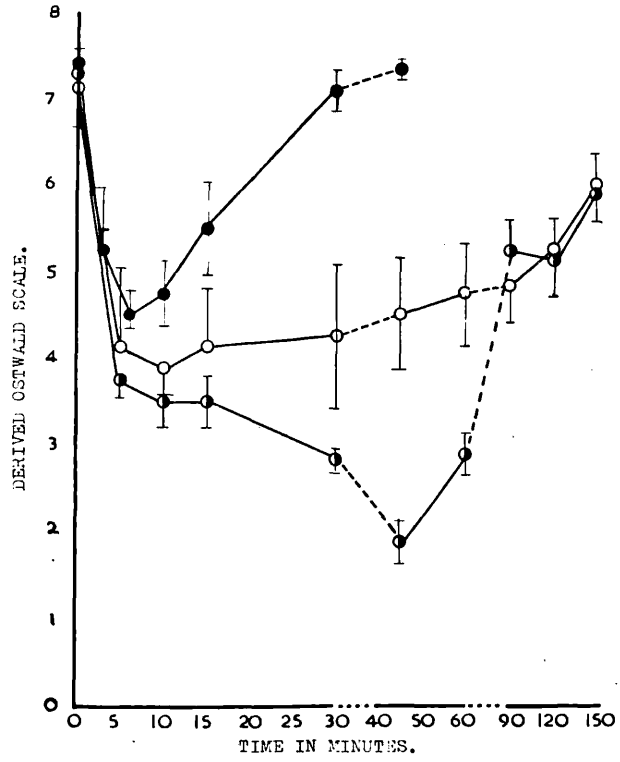
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
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TIME IN MINUTES.

Fig. 47 The effect of catecholamines on the shade of black-adapted minnows which had been chronically treated with guanethidine for five weeks.

○ 0.15 mg/kg noradrenaline (14°C.) (4 animals)

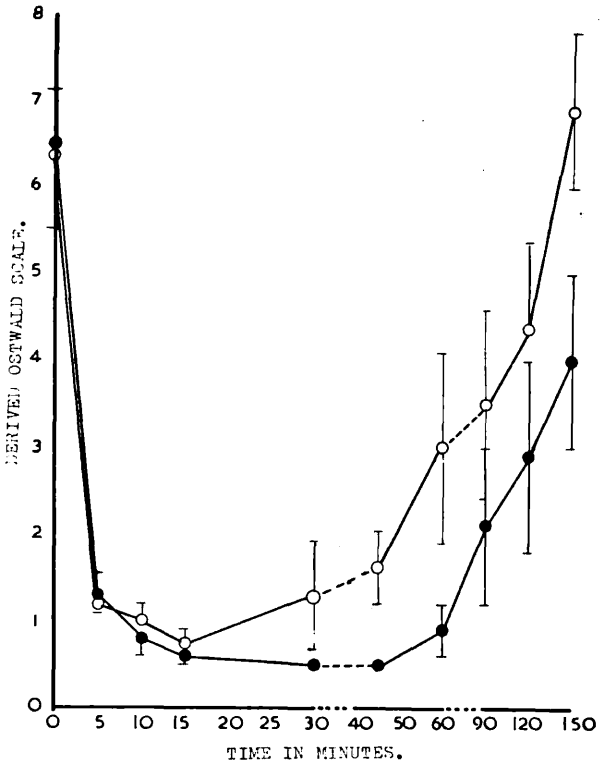
● 0.27 mg/kg adrenaline (14°C.) (5 animals)

Fig. 48 The effect of injections of sympathomimetic amines on the shade of black-adapted minnows which had been chronically treated with guanethidine for five weeks.

● 11.5 mg/kg tyramine (14°C.) (6 animals)

◑ 11.3 mg/kg ephedrine (14°C.) (4 animals)

○ 8.8 mg/kg amphetamine (14°C.) (4 animals)


DERIVED OSTWALD SCALE.
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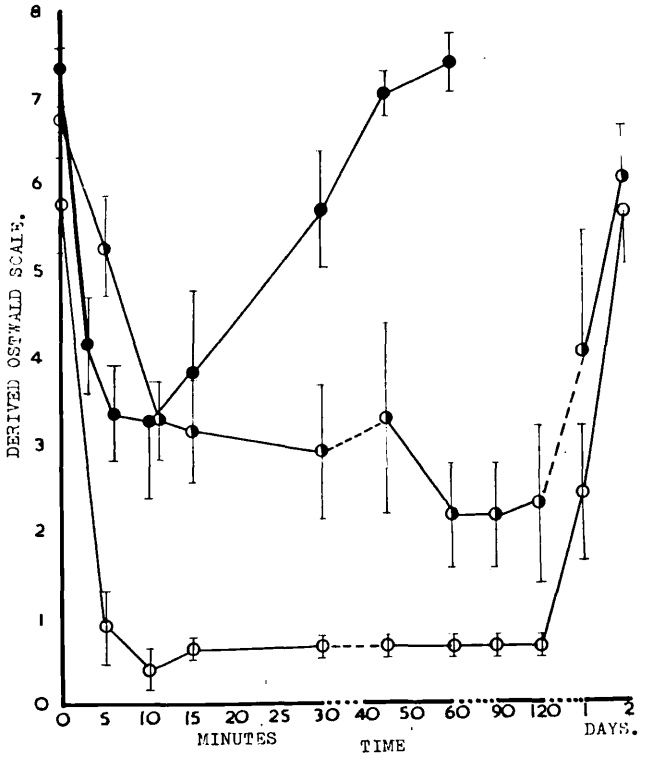
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
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MINUTES
TIME
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untreated minnows (Fig. 100; p.277 , Table 5; pp.272). Where possible doses have been chosen which would produce 50% response or less when potentiation was expected and 50% or more where antagonism was likely.

The significance of these changes in sensitivity to catecholamines and sympathomimetic amines is discussed in Section 4.13, together with the observations on spinal-sectioned and spinal nerve-sectioned fish and on cocaine-treated fish.

Section 3.255

The effect of agents and procedures likely to darken normal minnows on the shade of minnows subjected to chronic treatment with hypotensive agents

Section 3.2551

Acetylcholine

Parker (1931 et seq.) suggested that the postulated dispersing fibres in teleosts are cholinergic and antagonise the effects of sympathetic (adrenergic) aggregating fibres on the melanophores. The preliminary experiments on hypotensive drugs showed that these agents abolish the rapid colour changes of the minnow

(Section 3.251 - 3.253). Injections of acetylcholine into normal and operated minnows (Section 3.611) were not found to cause darkening, but the possibility remains that a darkening action of the injected cholinester was masked by an indirect action which led to the release of tissue catecholamines. Injections of acetylcholine were therefore made into minnows which had been subjected to chronic treatment with hypotensive agents whilst on a white background (Fig. 49; p.182).

White-adapted fish which had been given daily injections of reserpine (2.7 mg/kg) for 10 days were injected with 4 mg/kg acetylcholine. A considerable pallor follwed this injection. It has been shown earlier (Section 3.2544) that "indirect" amines such as tyramine are able to pale fish treated in this way even though nervously coordinated colour changes are abolished. An injection of 1.3 mg/kg acetylcholine in a similarly treated group of fish gave rise to a less prolonged pallor. A group of fish which had been treated with reserpine for four weeks failed to pale when injected with 8 mg/kg acetylcholine. At this time during chronic treatment with reserpine, indirectly acting amines are strongly antagonized whilst direct actions are potentiated (Fig. 101; p.278). Pretreatment of white-adapted minnows for four weeks with

bretylium or guanethidine also prevented any pallor following injection of 8 mg/kg acetylcholine. Guanethidine is not able to block indirect actions of sympathomimetic amines (Section 3.254).

It seems that the paling action of acetylcholine is related to the content of adrenergic stores within which sympathomimetic amines exert their indirect effects and perhaps also on the ability of terminal branches of sympathetic neurones to conduct action potentials (Section 1.323). Experiments on spinal sectioned and spinal nerve sectioned minnows indicates that the paling action of acetylcholine lies outside the central nervous system (Section 3.611). No indication of a dispersing action of acetylcholine was seen in hypotensive pre-treated fish.

Section 3.2552

Dihydroergokryptine (DHEK)

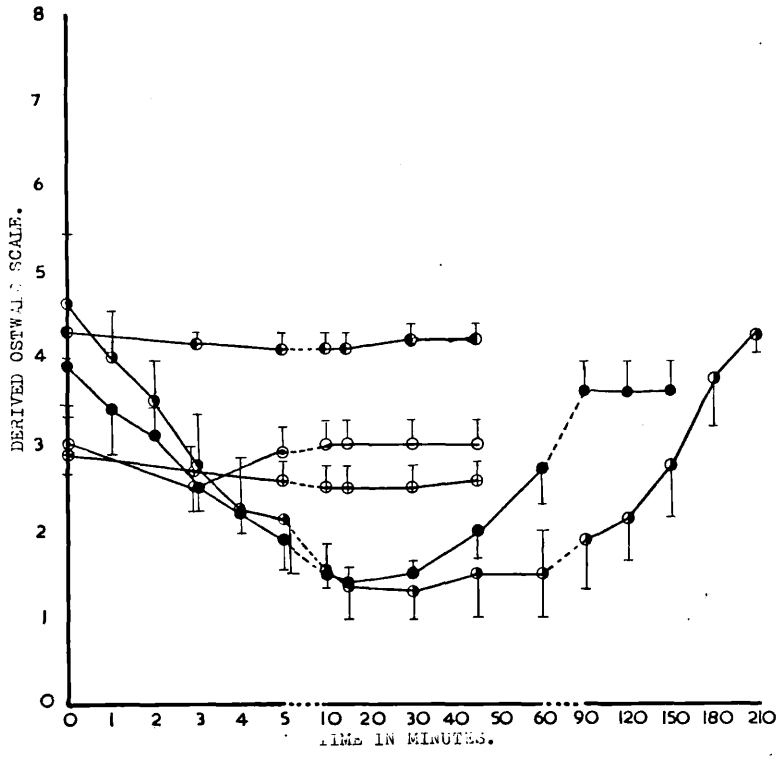
Injections of alpha adrenergic blocking agents into white-adapted minnows usually led to pronounced darkening (Section 3.24). Injections of dihydroergokryptine were made into white-adapted fish chronically pretreated with reserpine, bretylium or guanethidine (Fig. 50; p.182). Reserpinized fish, equilibrated at ca. D.O.S. 5.0 and

Fig. 49 The effect of injections of acetylcholine on the shade of white-adapted minnows which had been chronically pretreated with hypotensive drugs.

- ● 1.35 mg/kg acetylcholine in fish after ten days chronic reserpinization (10°C.) (5 animals)
- ◑ 4.05 mg/kg acetylcholine in fish after ten days chronic reserpinization (10°C.) (4 animals)
- ◐ 8.1 mg/kg acetylcholine in fish after four weeks chronic reserpinization (12°C.) (5 animals)
- ⊕ 8.1. mg/kg acetylcholine in fish after four weeks chronic treatment with guanethidine (12°C.) (8 animals)
- ○ 8.1 mg/kg acetylcholine in fish after four weeks chronic treatment with bretylium (12°C.) (5 animals)

Fig. 50 The effect of injections of dihydroergokryptine in white-adapted minnows which had been chronically treated with hypotensive drugs.

- ● 11.8 mg/kg DHEK in fish after five weeks reserpinization (12.5°C.) (5 animals)
- ◐ 11.8 mg/kg DHEK in fish after five weeks chronic treatment with guanethidine (12.5°C.) (7 animals)
- ○ 11.8 mg/kg DHEK in fish after five weeks chronic treatment with bretylium (12°C.) (5 animals)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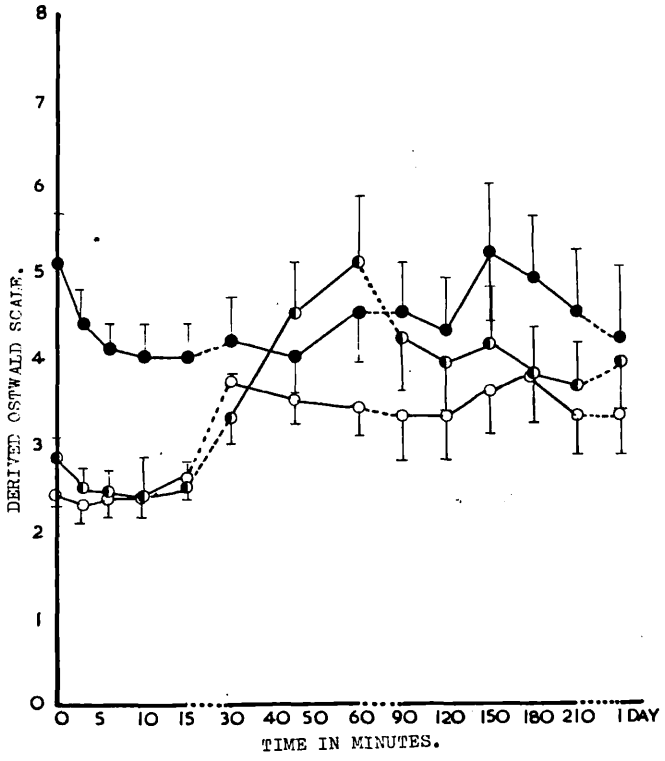
TIME IN MINUTES.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DERIVED OSTWALD SCALE.
0 5 10 15 30 40 50 60 90 120 150 180 210 1 DAY
TIME IN MINUTES.

injected with 11.8 mg/kg DHEK, were seen to show a small but prolonged pallor after the injection. No darkening comparable to that found in normal fish was seen. Bretylium pretreated fish darkened from less than D.O.S. 3.0 to less that 4.0 after the same treatment but guanethidine pretreated fish were seen to darken considerably from D.O.S. 2.5 to 5.0 in one hour. The latter drug seems less able to deplete adrenergic stores which are available for the indirect actions of sympathomimetic amines whilst it does potentiate the direct actions of catecholamines (Section 3.254). It has been observed that chronic treatment with guanethidine, while abolishing fast colour changes in the minnow, leads to a paler equilibrium shade on white than is obtained with bretylium and reserpine. It is possible that this paler state is maintained by small amounts of catecholamines which can be blocked by DHEK. It is also possible that DHEK exerts a direct stimulatory effect on the melanophores of the minnow to cause dispersion.

Section 3.2553

Spinal section between vertebrae 7 and 11

Section through the chromatic tract of the minnow leads to dispersion of melanophores thus deprived of nervous supply (Sections 1.23, 3.31, 3.41). White-adapted fish which had been chronically pretreated with one of the three hypotensive drugs were subjected to spinal section and their response recorded (Fig. 58; p.195). Reserpinized fish, which showed no signs of darkening during the urethane anaesthesis, darkened quite markedly from D.O.S. 4.0 to 6.5 in the hour following the operation. Recovery to the preoperated shade took a further two to three hours. Guanethidine pretreated fish darkened to a lesser extent following the operation whilst bretylium pretreated fish were little affected.

The shade of fish which have been chronically pretreated with the three hypotensive drugs and kept on a white background differs with the hypotensive drug. All such fish are an intermediate shade of grey but reserpine-pretreated fish are generally somewhat darker. The slow background adaptations which follow background reversal suggest that such intermediate coloured fish still retain a hormonal mechanism of colour change in the absence of a

functional nervous component. The failure of the white-adapted fish to achieve full pallor is difficult to explain. Injections of adrenergic blocking agents (such as dihydroergokryptine) darken the guanethidine- and bretylium-treated fish to some extent. This darkening might represent blockade of small amounts of circulating catecholamines which aggregate the melanophores. Guanethidine does not seem to be able to empty adrenergic stores in the minnow as indirectly acting amines are not antagonized by long term treatment. On the other hand it is possible that the intermediate shade of the white-adapted, chronically pretreated fish is achieved by failure of the nervous control of melanophores together with a decreased release of pituitary paling hormone. There seemed to be no peripheral antagonism of the effects of injected paling hormone into chronic hypotensive fish (Section 3.52). There is some evidence that the more conventional adrenergic blocking agents (piperoxane, dibenamine) antagonize the effect of paling hormone peripherally and might also cause decreased release of pituitary paling hormone (Section 3.53).

The failure of acetylcholine to produce melanophore dispersion under conditions which empty the adrenergic stores and prevent paling fibre activity is surprising in

the light of the reports of previous workers (Table 2). If acetylcholine were the dispersing neurohumour of the postulated darkening fibres it should theoretically be able to overcome the effects of circulating titres of paling hormone. Normal minnows which have been white-adapted for many months are still able to darken rapidly despite the presence of such paling hormone in the blood.

Section 3.26

The effect of cocaine on the responses of fish to catechol and sympathomimetic amines

In the introduction to this thesis (Section 1.3261) it was pointed out that cocaine has been found to antagonize competitively the indirect effects and to potentiate the direct effects of sympathomimetic amines in mammalian tissues. In teleosts, treatment with cocaine has been variously reported to disperse and to aggregate the melanophores of the same species. Thus, Wyman (1924a, b) and Abolin (1926) found that Fundulus and Phoxinus melanophores dispersed after cocaine but von Frisch (1911) and Smith (1931b) found the reverse. Von Frisch also found that lesions in the chromatic tract of fish made pale by cocaine were still able to darken the regions thus

deprived of nervous control (Quoted by Smith, 1931a).

Section 3.261

Preliminary injections of cocaine

Preliminary injections of cocaine were made into black-adapted minnows to investigate the degree of pallor which developed. The doses used were 13.5 and 6.75 mg/kg (Fig. 51; p.189). A considerable pallor developed in these fish which was much greater than that following injections of Ringer into control fish. It is not known whether this pallor is caused by a direct effect of cocaine on nerves or on melanophores or whether it represents a potentiation of small amounts of catecholamine released as a result of handling the fish. It was found that a second injection of cocaine into black fish which had darkened again after a cocaine injection produced much less pallor. This pallor was indistinguishable from control injections of Ringer. Accordingly, when the interaction between cocaine and a sympathomimetic amine was investigated, the fish were injected with 6.75 mg/kg cocaine two hours prior to the beginning of the experiment. The injection at the start of the experiment proper contained a similar dose of cocaine and also the amine

under consideration.

The effects of single doses of cocaine on the ability of white-adapted fish to adapt to background reversal were also studied (Fig. 51; p.189). White-adapted fish injected with either 13.5 or 6.75 mg/kg cocaine showed delayed darkening when placed on a black background, final adaptation taking up to two hours. After the first ten minutes on the new background these fish did not differ markedly from black-adapted fish injected with cocaine and kept on a black background.

Section 3.262

Catecholamines

Small doses of adrenaline or noradrenaline, together with cocaine, were injected into black-adapted fish (Figs. 52, 53; pp.189,191). A strong potentiation of the paling effects of these amines was seen when the degree of pallor produced by given doses was compared with that found in untreated fish. Fig. 100 (p.277) represents this potentiation as a shift of the dose/response curve to the left. During the pallor following injection of the mixture, a differential response of skin melanophores, as described earlier (Section 3.22) was seen.

Fig. 51 The effects of injections of cocaine on the shade of black- or white-adapted minnows and on the rate of adaptation to a new background.

◐ First injection of 6.75 mg/kg cocaine into black-adapted fish (16°C.) (7 animals)

⊖ First injection of 13.5 mg/kg cocaine into black-adapted fish (16°C.) (6 animals)

● Effect of 0.1 ml Young's F.W. teleost Ringer in black-adapted fish previously injected with 13.5 mg/kg cocaine (16°C.) (4 animals)

○ 13.5 mg/kg cocaine in black-adapted fish which had been injected with cocaine two hours earlier (16°C.) (4 animals)

◐ 13.5 mg/kg cocaine injected into white-adapted fish which were then placed on a black background (16°C.) (6 animals)

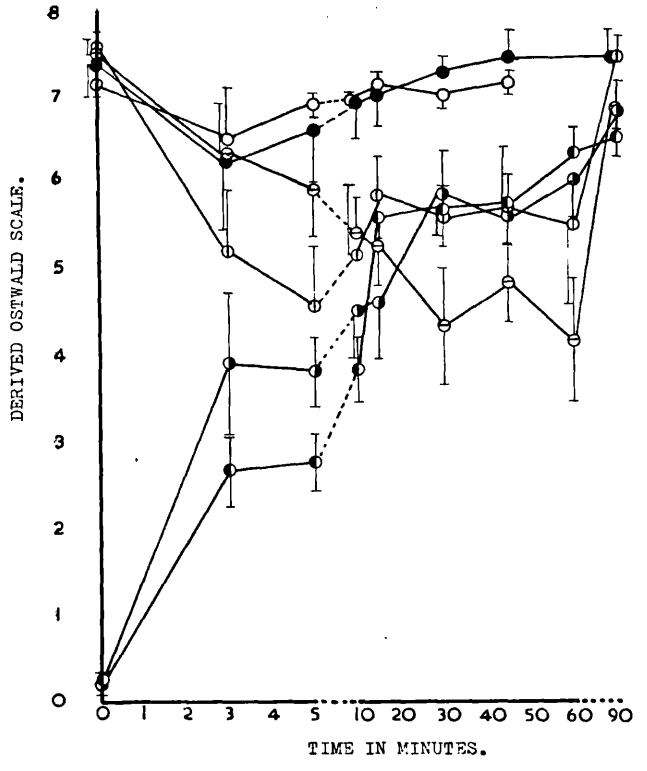
◑ 6.75 mg/kg cocaine injected into white-adapted fish which were then placed on black (16°C.) (5 animals)

Fig. 52 The effect of cocaine on the action of adrenaline in black-adapted minnows (16°C.)

● 13.5 mg/kg cocaine + 1.35 mg/kg adrenaline (4 animals)

⊙ 13.5 mg/kg cocaine + 0.27 mg/kg adrenaline (5 animals)

○ 13.5 mg/kg cocaine + 0.14 mg/kg adrenaline (4 animals)


DERIVED OSTWALD SCALE.
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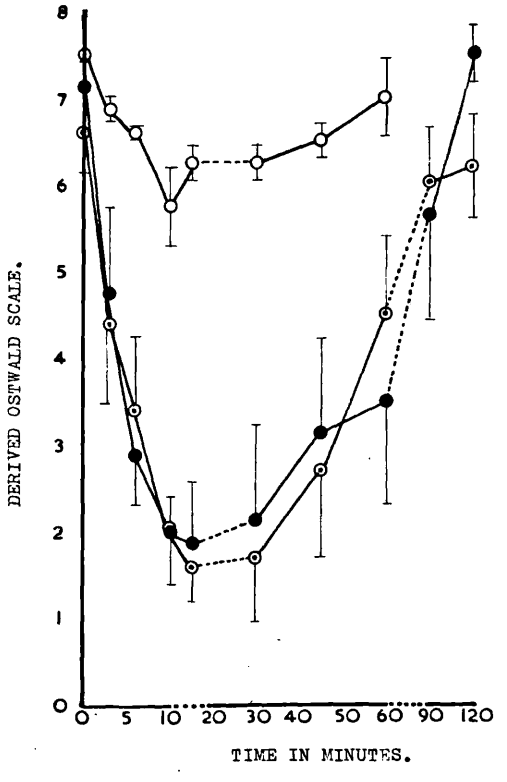
TIME IN MINUTES.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TIME IN MINUTES.

Section 3.263

Sympathomimetic amines

Injections of tyramine (21, 17.25 and 11.5 mg/kg), ephedrine (11.3, 6.7 and 2.7 mg/kg) and amphetamine (16, 8 and 4 mg/kg) were made into black-adapted, cocainized fish. Tyramine (Fig. 54; p.191) was now found to produce almost complete pallor, albeit short-lived, after the highest dose. Such pallor was never found in untreated fish injected with tyramine. It is possible that this dose is able to overcome the competitive blockade of cocaine and thus displace catecholamine from an adrenergic nerve store. The enhanced paling activity could then be explicable in terms of a potentiation of this displaced catecholamine such as has been described in the previous paragraph. The lowest dose used was less active than 5.75 mg/kg tyramine in normal black fish. In Fig. 101, p.278 and Table 5, p.272 these actions are represented as a change in the % response to a given dose before and after pretreatment with cocaine. All the doses tested here were in the upper region of the dose/response curve for tyramine (Fig. 100; p.277).

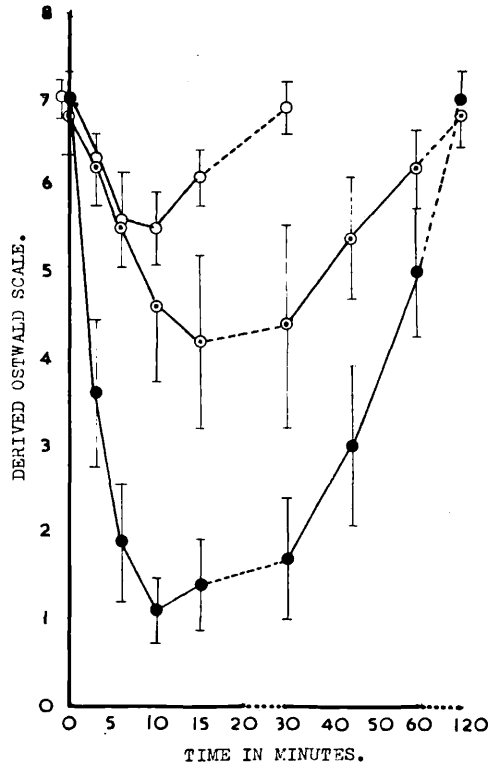
Similarly, injections of amphetamine (Fig. 56; p.193), which in normal fish produce 50% response or more, were

Fig. 53 The effect of cocaine on the action of noradrenaline in black-adapted minnows (16°C.)

- ● 13.5 mg/kg cocaine + 0.7 mg/kg noradrenaline (5 animals)
- ⊙ 13.5 mg/kg cocaine + 0.15 mg/kg noradrenaline (5 animals)
- ○ 13.5 mg/kg cocaine + 0.08 mg/kg noradrenaline (5 animals)

Fig. 54 The effect of cocaine on the action of tyramine in black-adapted minnows (17°C.)

- ● 13.5 mg/kg cocaine + 21.0 mg/kg tyramine (4 animals)
- ⊙ 13.5 mg/kg cocaine + 17.2 mg/kg tyramine (4 animals)
- ○ 13.5 mg/kg cocaine + 11.5 mg/kg tyramine (4 animals)


DERIVED OSTWALD SCALE.
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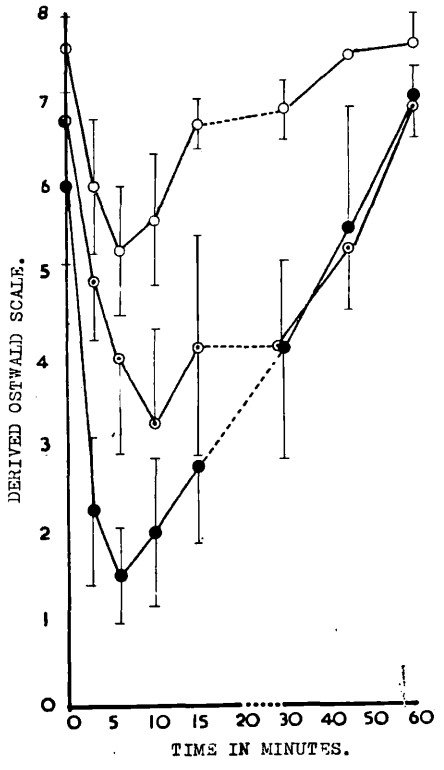
TIME IN MINUTES.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TIME IN MINUTES.

only antagonized in low doses. Once again the highest dose of the sympathomimetic amine was potentiated. Possibly this dose was able to overcome the competitive blocking action of cocaine at the transfer site to the adrenergic store. The responses of minnow to ephedrine and cocaine differed from those found for tyramine and amphetamine. Low doses of the amine were potentiated, possibly because the potentiation of direct actions was greater than the suppression of indirect actions. However the actions of larger doses were suppressed. Apparently treatment with cocaine changes the slope of the dose/response curve for ephedrine.

Section 3.3
Injections of adrenergic drugs into spinal sectioned minnows

Section 3.31
Shade changes following spinal section

The pioneer work of Pouchet and von Frisch (Section 1.23) enabled the pathways of chromatic fibres from the medulla oblongata to the spinal nerves to be traced. These and other workers described the chromatophore dispersion which follows section of the chromatic nerve

Fig. 55 The effect of cocaine on the action of ephedrine in black-adapted minnows (17°C.)

● 13.5 mg/kg cocaine + 11.3 mg/kg ephedrine (5 animals)

⊙ 13.5 mg/kg cocaine + 6.7 mg/kg ephedrine (5 animals)

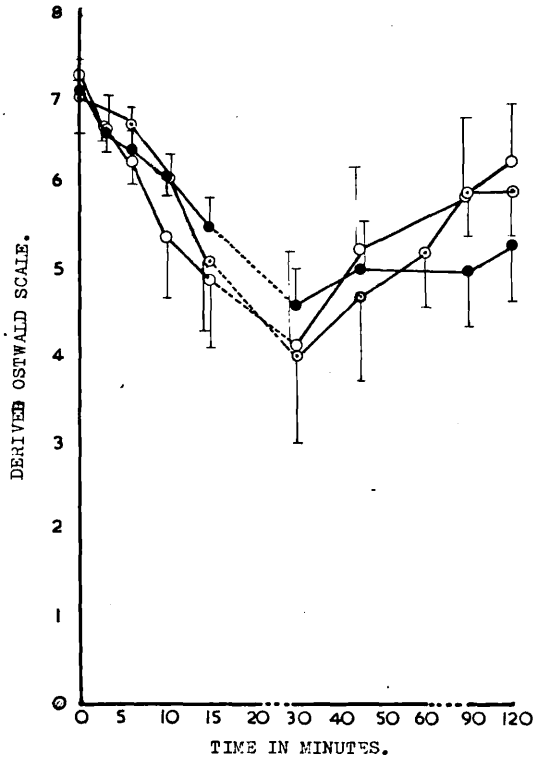
○ 13.5 mg/kg cocaine + 2.7 mg/kg ephedrine (4 animals)

Fig. 56 The effect of cocaine on the action of amphetamine in black-adapted minnows (17°C.)

● 13.5 mg/kg cocaine + 16 mg/kg amphetamine (4 animals)

⊙ 13.5 mg/kg cocaine + 8 mg/kg amphetamine (4 animals)

○ 13.5 mg/kg cocaine + 4 mg/kg amphetamine (4 animals)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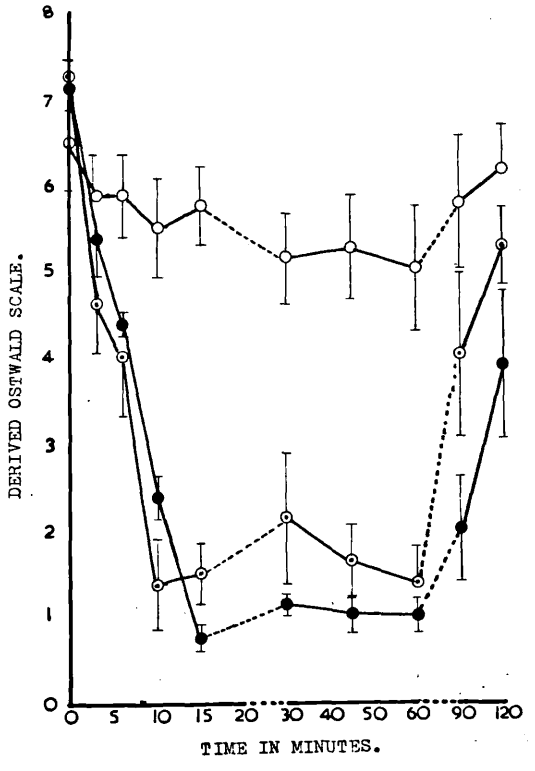
DERIVED OSTWALD SCALE.
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TIME IN MINUTES.

fibres in teleosts (Parker, 1933, et seq.; Abramowitz, 1935 et seq.; Healey, 1948; Gray, 1955, 1956a; Pye, 1964). Fig. 57, p. represents the darkening of white-adapted fish during the spinal operation. After complete anaesthesia in 0.5% urethane the fish darkened to D.O.S. 3.0 to 4.0. Cutting the spinal cord led to a more intense darkening which was maximal one hour after the operation, at which time the fish was quite conscious and was on a white background. During the next three days this dispersion lessened from 5.5 to about 4.0 on the Ostwald scale. For many days after this the shade of the operated fish remained between D.O.S. 2.5 and 4.5 but then a slow pallor set in leading to almost complete white background adaptation after two or three weeks. On a few occasions fish were found which did not darken after spinal section. These were discarded as being atypical.

Section 3.32

The responses of freshly spinal-sectioned fish to adrenaline and noradrenaline

White-adapted fish which had been subjected to spinal section 45 minutes previously were injected with doses of catechol amines as dispersion of the melanophores became

Fig. 57 The change in shade of white-adapted minnows before, during and after the operation of spinal section. (20°C.) (6 animals)

Anst; anaesthesia in urethane, 0.5%

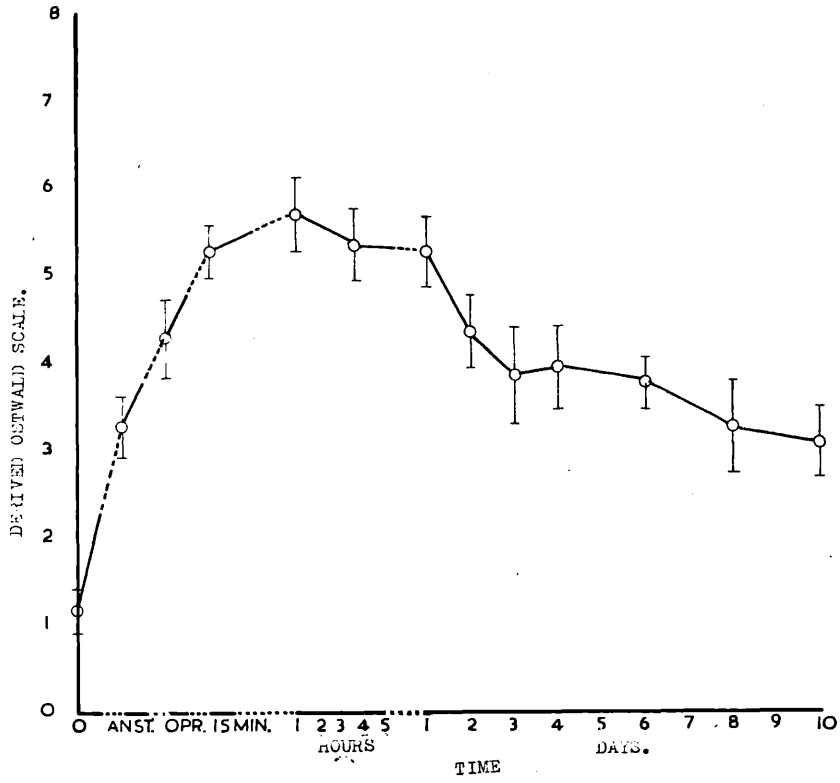
Opr; the duration of the operation.

Fig. 58 The change in shade of white-adapted minnows which had been chronically treated with hypotensive drugs, following spinal section. (12°C.)

● reserpinised fish (5animals)

◐ guanethidine-pretreated fish (7 animals)

○ bretylium pretreated fish (5 animals)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HOURS
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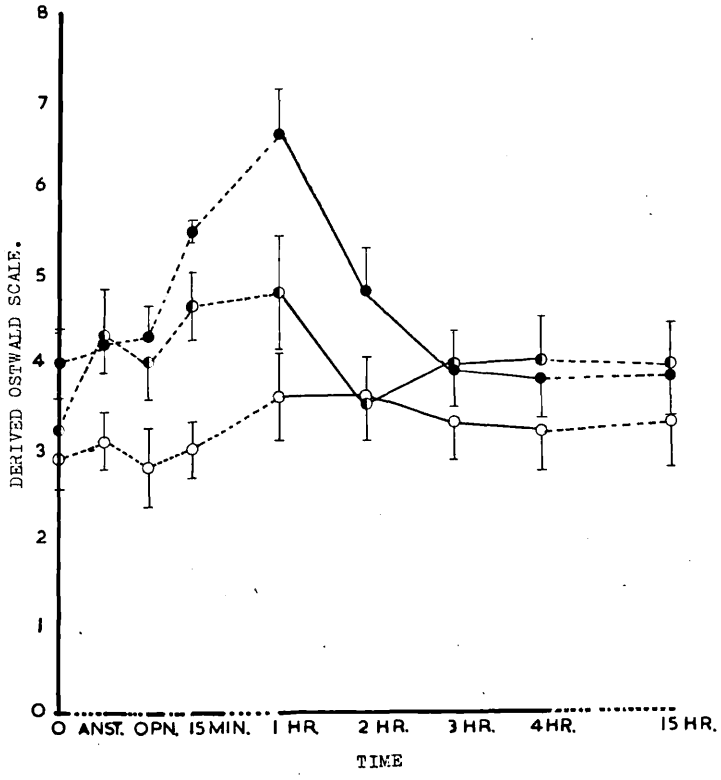
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TIME

maximal at about D.O.S. 5.0. The response of grey-adapted fish to similar injections, described in Section 3.255, were taken as controls as they, too, had partially dispersed melanophores. The doses of adrenaline and noradrenaline injected were chosen to produce 50% or more response in normal black-adapted fish (Figs. 59 and 60; pp. 198). A pronounced tendency for decreased pallor in the spinal-sectioned fish as compared with the grey fish followed injections of these amines and a more rapid recovery of the former occurred. This pattern of responses is not inconsistent with the hypothesis of Parker and his school (Section 1.23) that chromatic nerve section not only cuts off chromatic aggregating influences but also stinulates an antagonistic set of dispersing nerve fibres. Grey-adapted fish might be considered (Section 4.21, Fig. 102; p.294) as having a darkening system active at all times during stimulation by overhead light but this system may be counteracted to a greater or lesser extent by aggregating fibres controlled from the "W" retina. Lower albedo would cause decreased tonus in the aggregating fibres.

Section 3.33

The responses of black-adapted, spinal-sectioned minnows to injections of adrenaline and noradrenaline

Freshly spinal-sectioned minnows were kept on a white background until the operative darkening had worn off. Fish whose shade had fallen to between D.O.S. 1.0 and 2.5 were transferred to a black illuminated background and allowed to adapt for one or two weeks. Thus every black-adapted fish studied in this section had been spinal sectioned for at least four weeks. The fish were not used after two months as Healey (1962) found evidence for subsequent regeneration of chromatic tracts after a number of months.

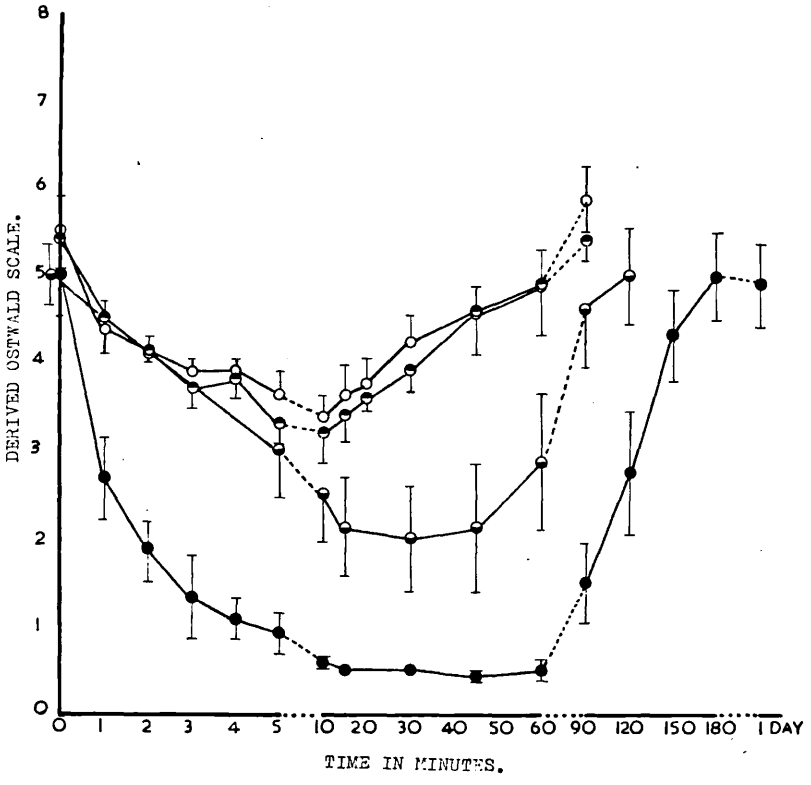
Injections of noradrenaline (Fig. 61; p.200) caused pallor in black-adapted spinal fish. The maximum pallor was less complete than that found in normal black-adapted fish. It seemed that isolated melanophores were not reacting to noradrenaline, perhaps as a result of physiological changes in the colour cells following prolonged separation from central influences. Similar observations were made using adrenaline (Fig. 62; p.200). The noradrenaline responses have been plotted on the dose response curve (Fig. 100; p.277) and it was found that some potentiation of the responses occurred following

Fig. 59 The effect of noradrenaline in minnows on a white background which had darkened immediately after spinal section.

● 8.2 mg/kg noradrenaline (17°C.) (6 animals)
◒ 4.3 mg/kg noradrenaline (19°C.) (4 animals)
◒ 2.75 mg/kg noradrenaline (17°C.) (5 animals)
○ 0.7 mg/kg noradrenaline (17°C.) (4 animals)

Fig. 60 The effect of adrenaline on the shade of white-adapted minnows which had darkened immediately after spinal section.

● 8 mg/kg adrenaline (20°C.) (6 animals)
⊙ 4 mg/kg adrenaline (20°C.) (4 animals)
○ 2.1 mg/kg adrenaline (22°C.) (4 animals)


DERIVED OSTWALD SCALE.
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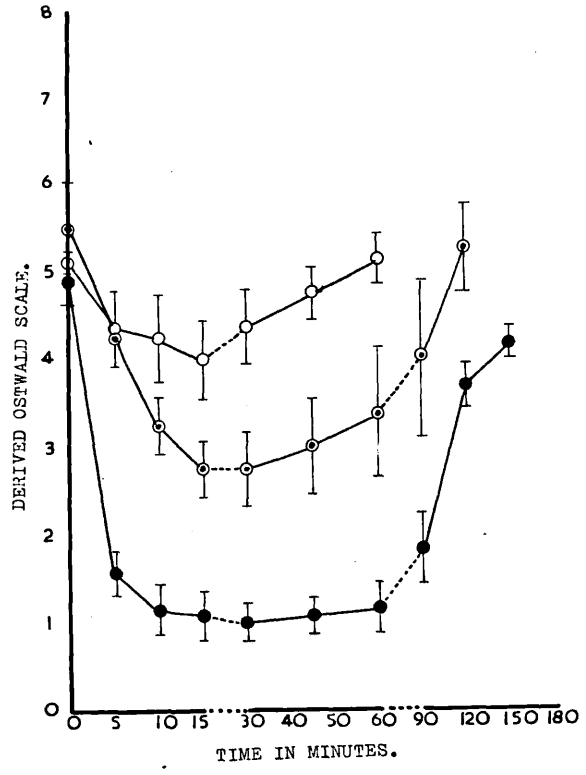
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
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spinal section. This enhancement was not so great as that following treatment of normal fish with cocaine. The significance of such changes in sensitivity after various agents and procedures is discussed in Section 4.13.

In this study it must be borne in mind that part of the enhancement of the aggregating influence of catecholamines such as noradrenaline after spinal section may be due to the cessation of impulses in postulated darkening fibres which might otherwise be continuously active in illuminated environments.

The responses of black-adapted spinal-sectioned fish to the sympathomimetic amines ephedrine, tyramine and amphetamine are represented in Fig. 63; p.202 . The actions of tyramine (21 and 11.5 mg/kg) were unaffected when compared with normal fish but the actions of both amphetamine (13.5 mg/kg) and ephedrine (11.3 mg/kg) were to produce a less intense but greatly prolonged pallor.

Section 3.34

Adrenergic blocking agents

Spinal-sectioned minnows were kept on a white background for several weeks until they paled. These fish were not always as pale as normal fish on a white background

Fig. 61 The effect of noradrenaline injections on the shade of black-adapted minnows two months after spinal section.

● 5.5 mg/kg noradrenaline (19°C.) (4 animals)

⊙ 2.75 mg/kg noradrenaline (17°C.) (6 animals)

○ 1.4 mg/kg noradrenaline (10°C.) (4 animals)

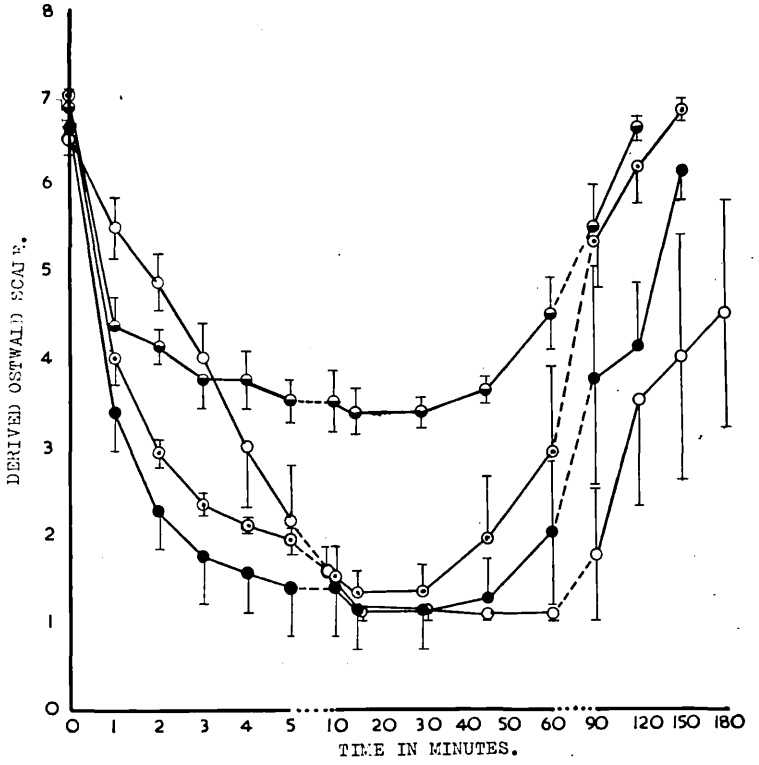
◒ 0.8 mg/kg noradrenaline (17°C.) (4 animals)

Fig. 62 The effect of adrenaline injections on the shade of black-adapted minnows two months after spinal section.

● 13.2 mg/kg adrenaline (19°C.) (8 animals)

⊙ 6.5 mg/kg adrenaline (19°C.) (4 animals)

○ 2.7 mg/kg adrenaline (11°C.) (5 animals)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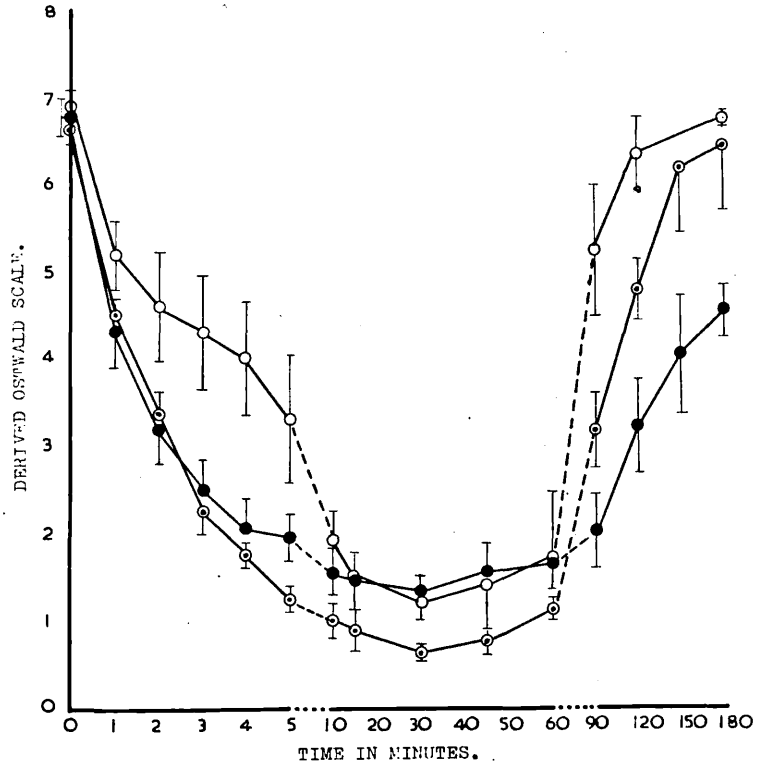
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TIME IN MINUTES.

and it was frequently noticed that dark flecks of dispersed melanophores were present in the skin. Injections of adrenergic blocking agents were made into these fish.

Section 3.341

Piperoxane (Fig. 64; p.202)

Injections of 14.8, 12.0 and 9.0 mg/kg piperoxane into white-adapted spinal fish led to a marked darkening of the skin. This darkening, however, was far less intense than that produced in normal white-adapted fish. Equivalent shades in the latter would have been caused by between 1 and 2 mg/kg piperoxane. In most fish a tendency for the "refractory" skin melanophores to disperse first was seen.

Section 3.342

Phentolamine (Fig. 65; p.205)

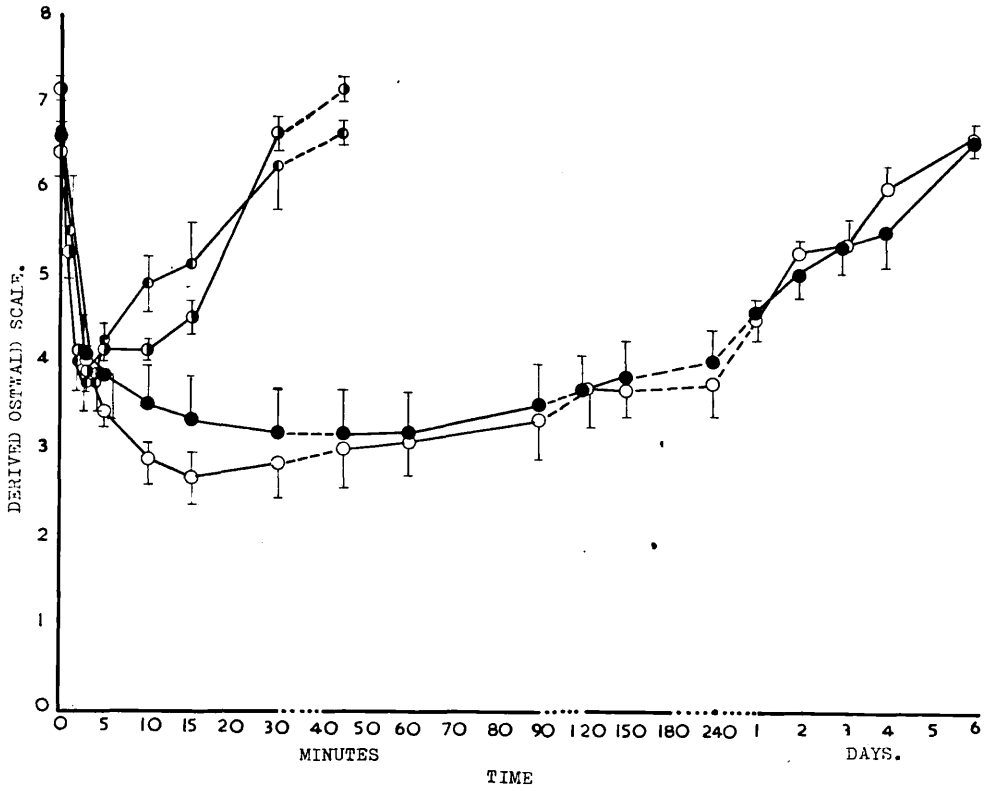
15.4 and 7.7 mg/kg phentolamine in the experimental fish led to an incomplete darkening similar to that found in normal fish and in the piperoxane injections described above.

Fig. 63 The effects of injections of sympathomimetic amines on the shade of black-adapted minnows two months after spinal section.

◐ 11.5 mg/kg tyramine (15°C.) (4 animals)
◑ 21.0 mg/kg tyramine (15°C.) (4 animals)
● 11.3 mg/kg ephedrine (15°C.) (6 animals)
○ 13.5 mg/kg amphetamine (15°C.) (6 animals)

Fig. 64 The effect of injections of piperoxane on the shade of white-adapted minnows two months after spinal section.

● 14.8 mg/kg (16°C.) (5 animals)
◑ 12.0 mg/kg (15°C.) (5 animals)
○ 9.0 mg/kg (10.5°C.) (5 animals)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MINUTES
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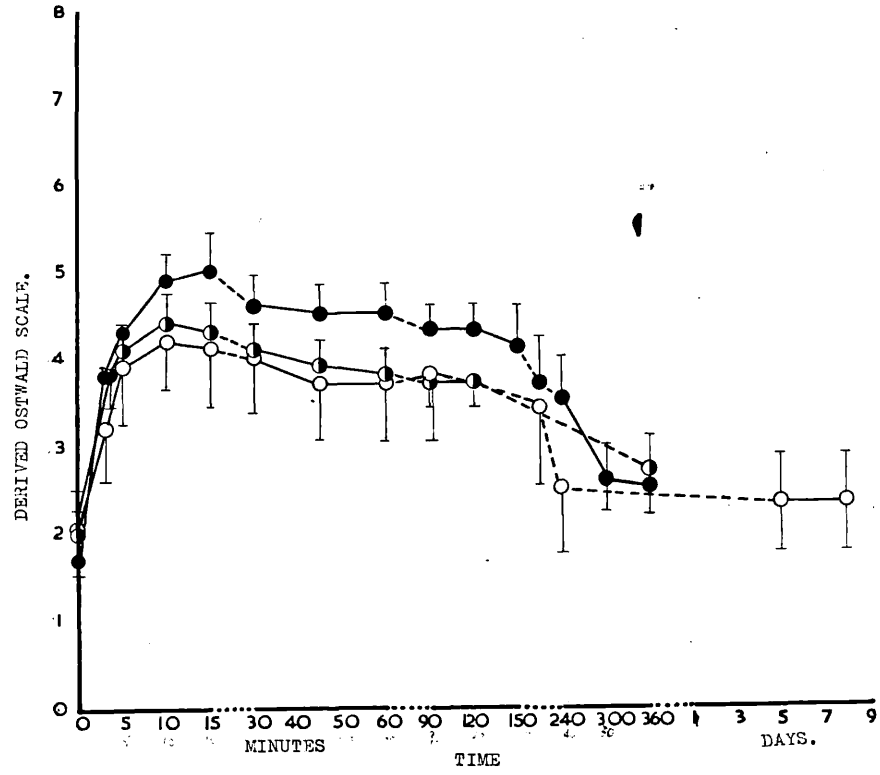
TIME


DERIVED OSTWALD SCALE.
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MINUTES
DAYS.
TIME

Section 3.343

Dihydroergokryptine (Fig. 66; p.205)

Injections of 15 and 7.5 mg/kg DHEK in white-adapted spinal fish also produced incomplete darkening. A similar shade would have followed the injection of normal white-adapted fish with less than 6 mg/kg DHEK.

All the above groups of fish resembled, after injection, those fish which had been spinal sectioned and maintained on a white background for one or two days or normal fish which had been chronically treated with hypotensive drugs.

Section 3.344

Yohimbine (Fig. 67; p.206)

White-adapted spinal fish proved less sensitive than normal fish to yohimbine. A large dose of 12.4 mg/kg yohimbine produced considerable darkening of some fish whereas others only darkened part way, as described above for other blocking agents. None of these fish appeared adversely affected by the drug; all survived.

Section 3.345

Dibenamine (Fig. 68; p.206)

A small dose of 12 mg/kg dibenamine into white-adapted, spinal fish led to a slow darkening to an intermediate shade. It has been shown earlier that normal white-adapted fish require a series of injections to darken completely after dibenamine.

It is possible to suggest that the decreased darkening effects of adrenergic blockers in spinal fish are related to interruption of chromatic darkening tracts (Section 1.23). Thus adrenergic blockade unmasks a tendency for the melanophores to disperse but the degree of dispersion is dependant on the activity of an antagonistic innervation. A further consideration of the paling mechanism at work in white-adapted spinal fish is necessary however. It has been shown that the melanophores of spinal sectioned fish become supersensitive to noradrenaline (Section 3.33). Trendelenburg (1963) has discussed the significance of the time delay required for this supersensitivity to develop in various mammalian tissues. It can be suggested that, when activity in a postulated darkening tract ceases after spinal section, the intermediate shade which persists for several days

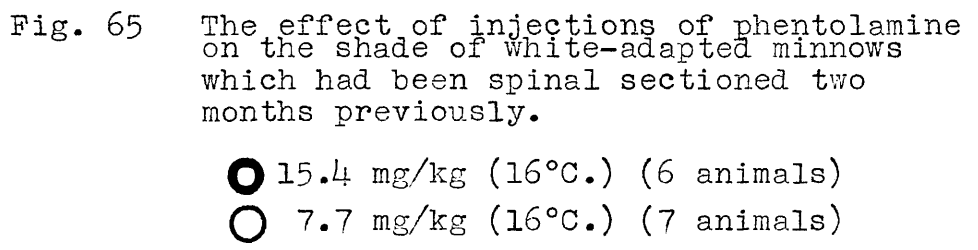


Fig. 65 The effect of injections of phentolamine on the shade of white-adapted minnows which had been spinal sectioned two months previously.

● 15.4 mg/kg (16°C.) (6 animals)
○ 7.7 mg/kg (16°C.) (7 animals)

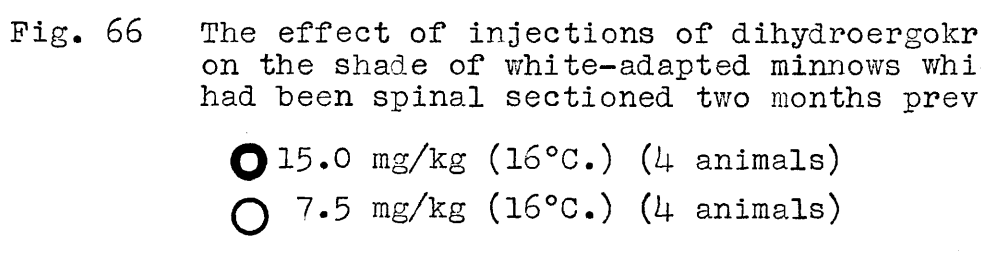
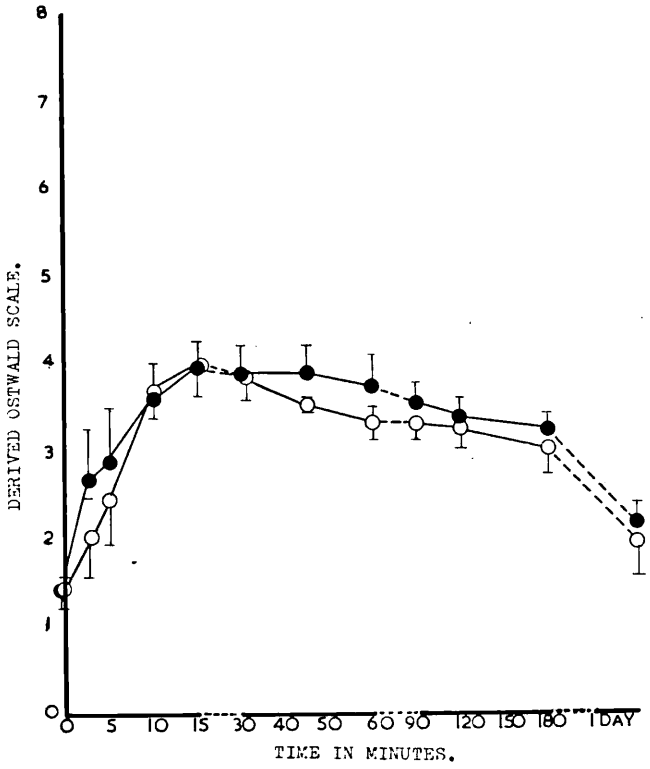


Fig. 66 The effect of injections of dihydroergokryptine on the shade of white-adapted minnows which had been spinal sectioned two months previously.

● 15.0 mg/kg (16°C.) (4 animals)
○ 7.5 mg/kg (16°C.) (4 animals)


DERIVED OSTWALD SCALE.
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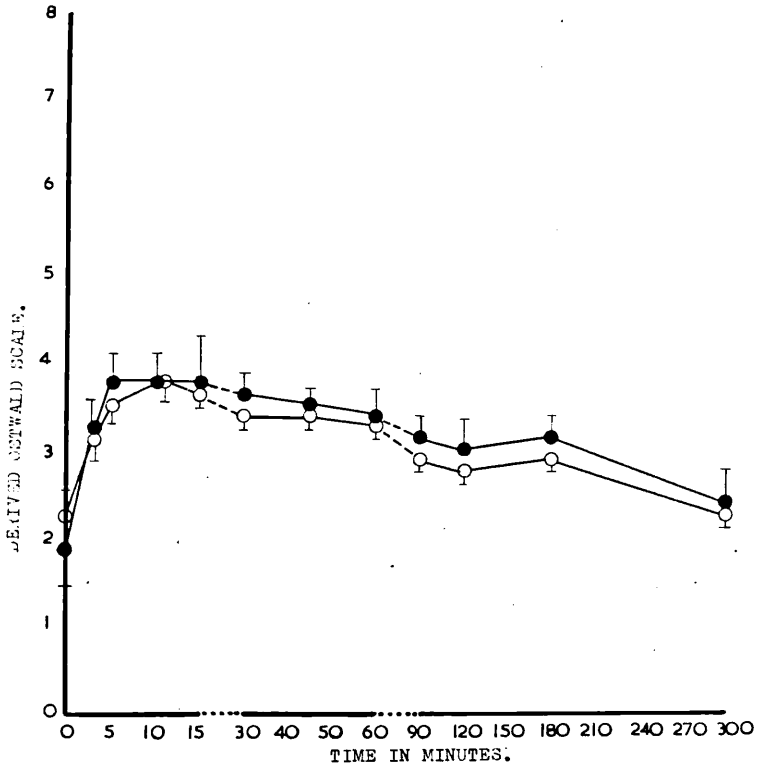
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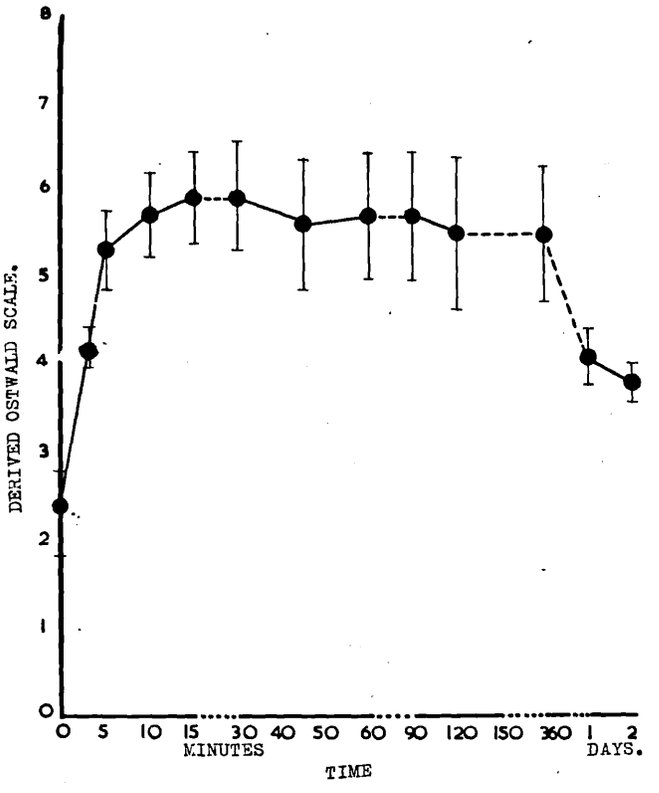
TIME IN MINUTES.

Fig. 67 The effect of yohimbine injections on the shade of white-adapted minnows which had been spinal sectioned two months previously.

12.5 mg/kg (15°C.) (5 animals)

Fig. 68 The effect of dibenamine injections on the shade of white-adapted minnows two months after spinal section.

12 mg/kg (12°C.) (4 animals)


DERIVED OSTWALD SCALE.
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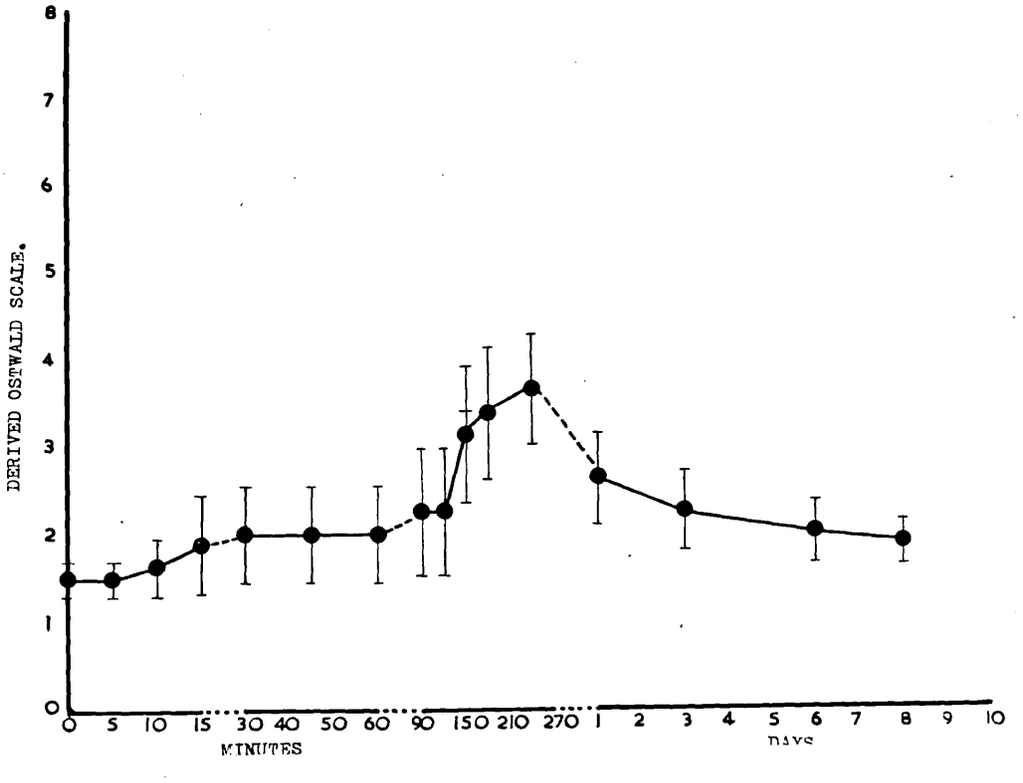
DERIVED OSTWALD SCALE.
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represents the unsupported activity of circulating pituitary paling hormone. The subsequent complete pallor of operated fish on a white background may represent additional stimulation by low doses of catecholamines released endogenously which initially were unable to affect the state of unsensitized melanophores. Umrath and Walcher (1951) proposed that when sympathetic ganglia were separated from the central nervous system by preganglionic lesions, these ganglia eventually initiate tonic impulses in the paling fibres which cause the darkened areas of the skin to blanch. Whatever the source of catecholamines involved in complete white background-adaptation in spinal fish it is necessary to propose either that they disappear, together with pituitary paling hormone, when the fish are exposed to a black background or that they are unable, in the absence of pituitary hormone, to antagonize the effects of a darkening agency, such as a second pituitary hormone, resembling intermedine, released when the fish is placed on a black background.

On the other hand, the darkening brought about by injections of adrenergic blocking agents into white-adapted spinal fish may be caused by other means. Decreased release of pituitary paling hormine may occur as a result of the central actions of the drugs or peripheral actions

of the hormone may be antagonized (Sections 3.2533, 3.52, 3.53 and 3.54). Finally, the drugs may themselves exert a direct effect on minnow melanophores to bring about pigment dispersion. Such an action has been described for dibenamine in denervated melanophores of Chasmichthys (Fujii, 1961) and Oryzias (Watanabe et al., 1962b). The less pronounced darkening of white-adapted fish could then be attributed to the antagonistic effect of small amounts of circulating catecholamines in white-adapted fish to which the melanophores have become sensitized.

Section 3.35
The responses of spinal sectioned minnows to hypotensive drugs

Section 3.351
Reserpine

Black- and white-adapted spinal fish were injected with 10 and 7 mg/kg reserpine respectively (Fig. 69; p.209). The pallor which developed in black-adapted fish was greater than that found in unoperated fish injected with 12 mg/kg reserpine. This alkaloid is known to release

Fig. 69 The effects of reserpine injections into black- and white-adapted minnows two months after spinal section.

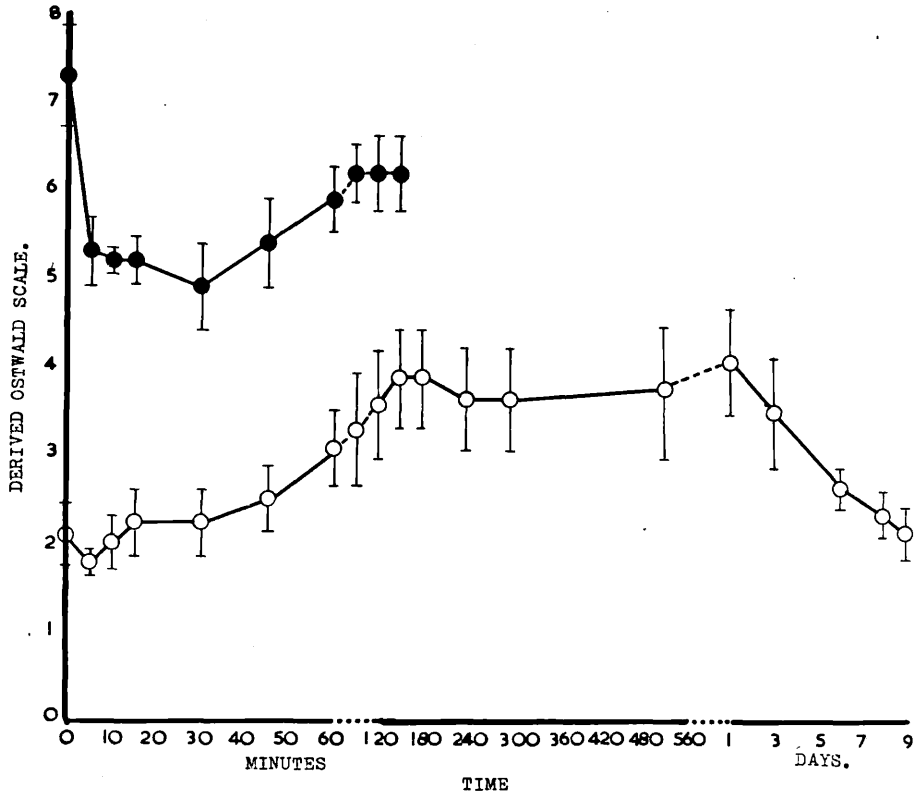
● black-adapted fish injected with 10 mg/kg (13°C.) (5 animals)

○ white-adapted fish injected with 7 mg/kg (12°C.) (7 animals)

Fig. 70 The effects of injections of bretylium or guanethidine on the shade of black- and white-adapted monnows two months after spinal section.

○ 10.5 mg/kg bretylium (12°C.) (5 black-, 5 white-adapted animals)

● 14.0 mg/kg guanethidine (12°C.) (4 black-, 4 white-adapted animals)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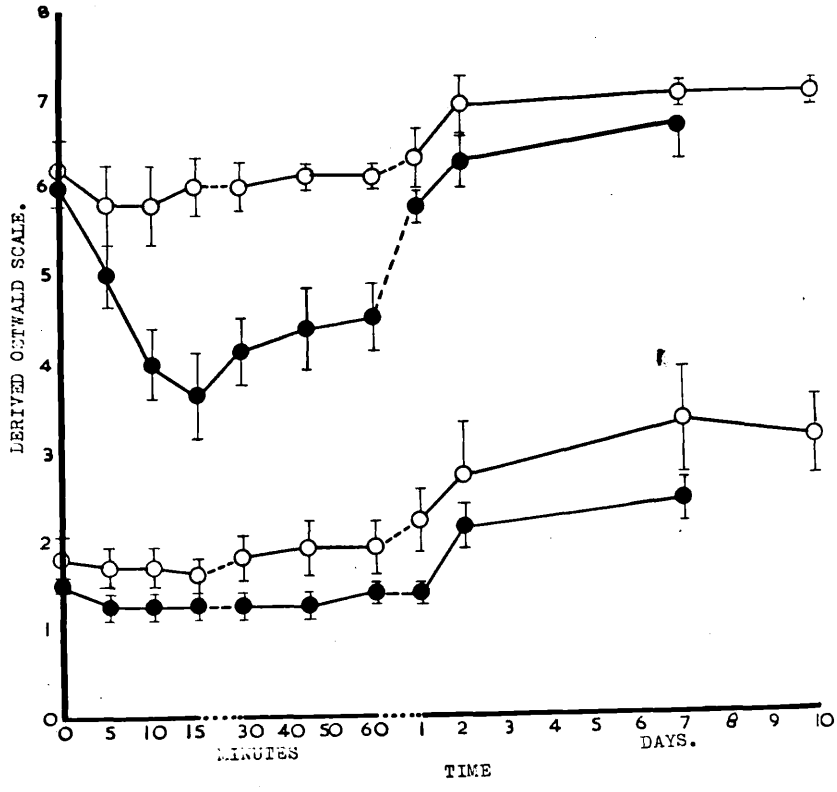
MINUTES
DAYS.
TIME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DAYS.
TIME

catecholamines from mammalian adrenergic stores and it has been shown (Section 3.33) that the melanophores of spinal sectioned fish are supersensitive to noradrenaline. It seems likely that the reserpine-induced pallor in these spinal black-adapted fish is related to this adrenergic depleting action. At the time when the pallor in these fish started to subside the white-adapted fish started to darken slowly. After two hours the latter fish assumed a shade similar to that produced in spinal sectioned fish after the initial dispersion had worn off. A similar shade has also been described in normal fish treated with hypotensive agents or with phentolamine. This intermediate shade lasted four or five days and disappeared after one week.

Section 3.352

Guanethidine

Black- and white-adapted spinal minnows were injected with 14 mg/kg guanethidine (Fig. 70; p.209). The black-adapted fish did not pale to the same extent as normal black-adapted fish injected with 10.75 mg/kg guanethidine. The pallor of the spinal fish lasted several days. It is possible that part of the intense pallor found in normal

fish after guanethidine depends on the integrity of the chromatic aggregating tract in the spinal cord. After the pallor subsided, the white-adapted fish darkened a little to D.O.S. 2.5. A similar coloration followed chronic injections of guanethidine into normal white fish.

Section 3.353

Bretylium

Black- and white-adapted spinal fish were injected with 10.5 mg/kg bretylium (Fig. 70; p.209). Only slight pallor occurred in the black-adapted fish during the first fifteen minutes but the white-adapted fish darkened slowly to D.O.S. 3.5. In this respect they resembled normal chronically-treated fish (Section 3.2512).

When normal white-adapted fish were injected with acute doses of bretylium or reserpine (Sections 3.2511 and 3.2521) a brief but intense darkening occurred. Guanethidine did not have this short-lived effect. Subsequent injections of bretylium or reserpine (chronic treatment) did not cause brief darkening but at this time no fast darkening could be elicited from the fish on background reversal. Spinal sectioned fish were not found to show this brief but intense darkening as described for

"acute" fish. It is possible that acute treatment with hypotensive drugs in normal fish unmasks an active darkening agency in white-adapted fish whose function depends on the integrity of the spinal cord. Subsequently this agency, as well as the paling fibres, is blocked by the drugs. The possible nature of this agency is discussed in Section 4.22.

Section 3.4
Injections of adrenergic drugs into spinal nerve-sectioned minnows

Section 3.41
Shade changes following spinal nerve section

White-adapted minnows were subjected to spinal nerve section (Section 2.3) so that five or more consecutive spinal nerves on one side of the body were cut without interference with the blood supply to that region. The responses of the area of skin thus affected, which appeared as a dark stripe on the body, are represented in Fig. 71 (p.215). During anaesthesia all the melanophores of the body dispersed to some extent but, as the normally innervated melanophores re-aggregated during recovery, the

denervated effectors dispersed further. After 15 minutes, during which time the darkening reached D.O.S. 6.0, the band began to pale and had disappeared after one day. The band did not pale as a homogeneous area. Some melanophores, notably those in the "barred" region of the body, were the last to aggregate fully.

Section 3.42

The response of freshly operated minnows to adrenaline and noradrenaline

Several groups of freshly operated fish were injected with adrenaline or noradrenaline soon after the operation had been performed. Some of these fish were white-adapted, so that normally innervated areas adjacent to the dark band might release neurohumours into the blood and tissue fluids. Other fish were black-adapted prior to the operation and during the injection (i.e. were not placed on a white background at any time).

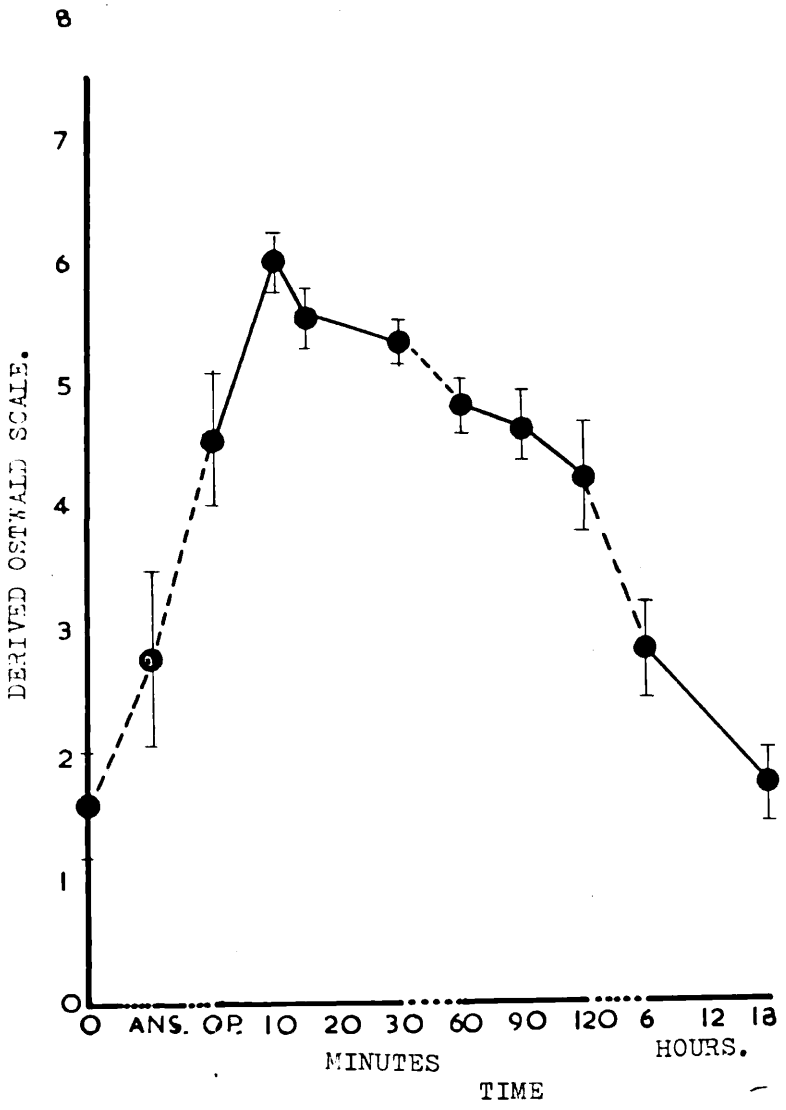
Adrenaline (Fig. 72; p.216) in doses of 8.0, 4.0 and 1.6 mg/kg was injected into white-adapted fish. The dark stripe of these fish paled a little more slowly than did the skin of grey adapted fish (Section 3.254). Moreover, some of the fish showed a tendency for the dark stripe to

reappear after the drug effect wore off. The main effect of the injections of adrenaline, however, was to hasten the disappearance of the stripe so that it was no longer ivsible after 10 hours. Similar injections were made into black-adapted, freshly operated fish in which adjacent areas were not receiving nervous paling stimuli. Injections of 4.0, 1.35 and 0.27 mg/kg adrenaline paled the stripe region rather more slowly than adjacent regions of normally innervated skin. The denervated area stood out against a paler background.

It must be remembered that section of the spinal nerves, whilst not cutting the segmental arteries, might cause vaso-motor disturbances in the peripheral blood vessels in the skin. Such changes could lead to a slower arrival of injected drug molecules to the denervated area. However, the redispersion of the stripes in white-adapted fish may well reflect the presence of an active darkening agency in the affected area.

Similar injections were made using noradrenaline, (Fig. 73; p.216). Injections of 7.1, 3.5 and 1.4 mg/kg noradrenaline into white-adapted fish paled the stripe less easily thandid similar doses pale the skin of grey-adapted fish. As the effects of the injection subsided the dark stripe reappeared again against the surrounding

Fig. 71 The change in shade of the denervated area of white-adapted minnows during and after the operation of spinal nerve section. (9 animals, 19°C.).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MINUTES
HOURS.
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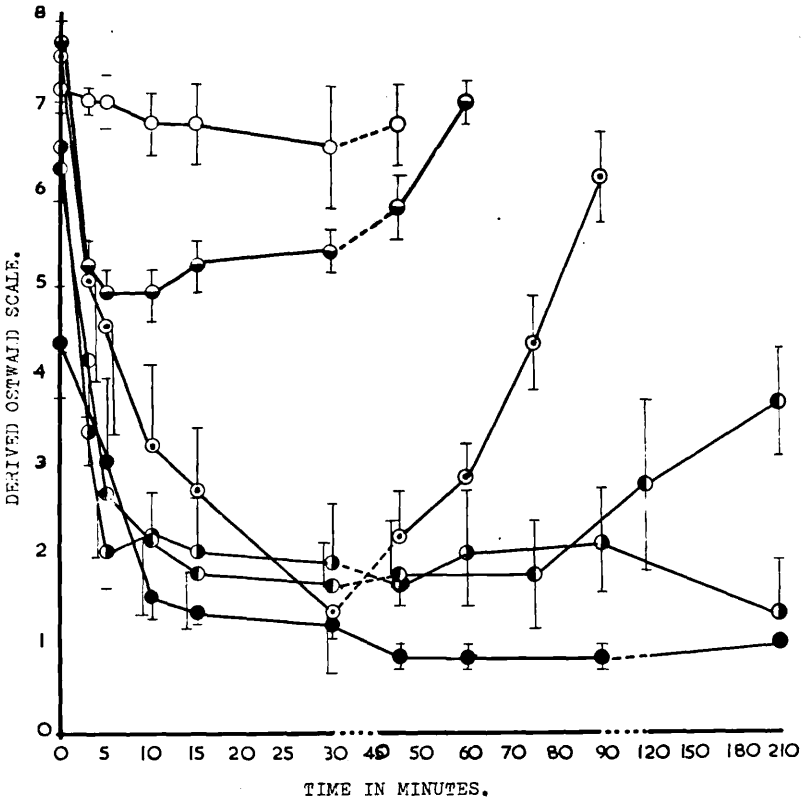
Fig. 72 The effect of adrenaline on the denervated area of black- and white-adapted minnows immediately after spinal nerve section (21°C.)

- ● 8 mg/kg adrenaline, white-adapted fish (3 animals)
- ◐ 4 mg/kg adrenaline, white-adapted fish (4 animals)
- ◑ 1.6 mg/kg adrenaline, white-adapted fish (3 animals)
- ⊙ 4.0 mg/kg adrenaline, black-adapted fish (3 animals)
- ◒ 1.35 mg/kg adrenaline, black-adapted fish (3 animals)
- ○ 0.27 mg/kg adrenaline, black-adapted fish (4 animals)

Fig. 73 The effect of noradrenaline injections on the denervated area of black- and white-adapted minnows immediately after spinal nerve section.

- ● 7.1 mg/kg in white-adapted fish (18°C.) (4 animals)
- ◑ 3.5 mg/kg in white-adapted fish (18°C.) (3 animals)
- ◐ 1.4 mg/kg in white-adapted fish (18°C.) (3 animals)
- ◒ 4.1 mg/kg in black-adapted fish (23°C.) (4 animals)
- ⊙ 2.1 mg/kg in black-adapted fish (23°C.) (3 animals)
- ○ 0.7 mg/kg in black-adapted fish (23°C.) (3 animals)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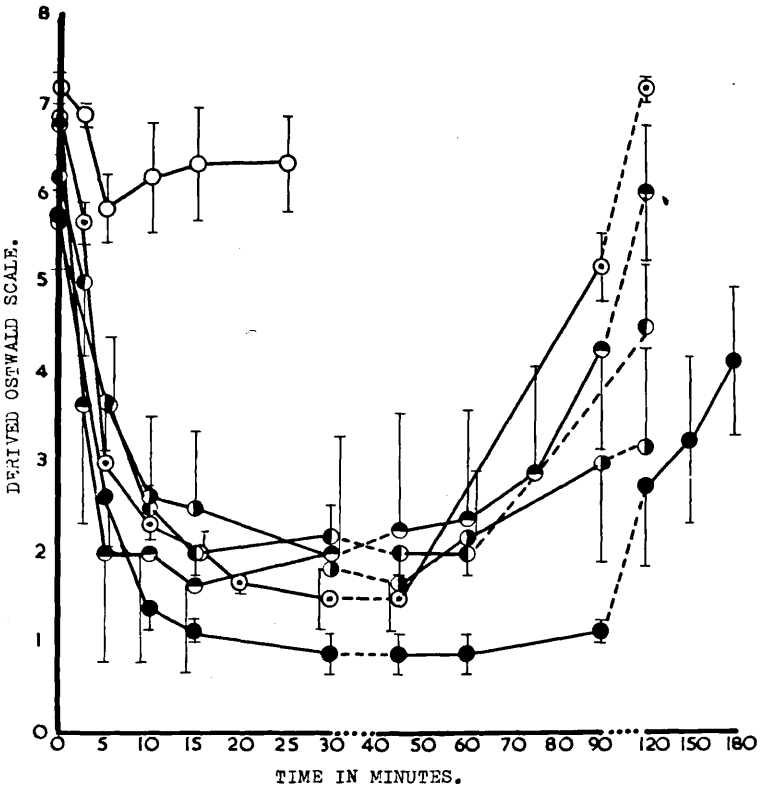
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TIME IN MINUTES.

pale regions. This tendency was more readily seen than was the case using adrenaline. Freshly operated black-adapted fish were injected with 4.1, 2.1 and 0.7 mg/kg noradrenaline. In these fish the operated area was less readily paled by the drug and always stood out as a darker, but nevertheless paling, area.

It is difficult to explain the redispersion of melanophores aggregated by the catecholamines (occasionally after adrenaline and in most cases after noradrenaline) when adjacent areas are aggregated by tonic nerve impulses and when the fish have spent sufficient time on a white background to build up a titre of blood-borne pituitary paling hormone. Gray (1955, 1956) described the influence of adjacent innervated areas on freshly denervated caudal bands of Phoxinus which presumably depend on diffusing neurohumours. The melanophores of the band were caused to aggregate first at the margins and subsequently all were recruited. The present observations were made on much larger areas than were Gray's, and may require greater times for the diffusion of active aggregating agents into the whole area. It is also possible that the melanophores have an inherent tendency to disperse when deprived of tonic sympathetic stimulation. Subsequent aggregation of melanophores in a denervated band depends on the

acquisition of supersensitivity to circulating agents released from adjacent paling fibres. Such sensitivity changes in mammals take time to develop (Section 1.326). If this is the case, the dispersion of denervated melanophores after lesions in the chromatic tract may be solely due to an inherent tendency of melanophores to disperse in the absence of direct nervous stimuli unless acted upon by circulating aggregating substances in concentrations to which they are sensitive. Gray (1956a) suggested that the final aggregation of denervated melanophores of minnows on a white background might be caused by sensitivity changes whilst Umrath and Walcher (1951) proposed that the entry of water into the body at the site of section stimulated the cut nerves to become rhythmically excited. Both groups of workers attributed fleeting antidromic and orthodromic dispersion to mechanical stimulation of dispersing fibres.

Section 3.43

The response of black-adapted, spinal nerve-sectioned fish to catecholamines and sympathomimetic amines

The fish used in the following experiments had been subjected to spinal nerve-section and then allowed to adapt to white and black backgrounds for two to five weeks. During this time several fish were subjected to background reversal in an attempt to observe different response rates of the normal melanophores and those deprived of their nerve supply. Only occasionally was it possible to see that the denervated area changed shade more slowly than adjacent regions of skin and in general no difference in rate of shade change was observable (cf. Gray, 1956a). As it was not possible to determine whether regeneration of the nerve tract to the melanophores had occurred by the simple criterion of adaptation to reversed backgrounds, fish were only used which had been operated on not less than two weeks and not more than five weeks previously. At this time it is believed that the chromatic fibres sectioned in the operation have degenerated, the initial darkening following this section has disappeared, super-sensitivity to injected catecholamines will have developed and regeneration of the chromatic tract to the

melanophores will not have occurred.

Injections of small amounts of noradrenaline into such black-adapted fish were made (Fig. 74p.221). Doses of 0.15 mg/kg noradrenaline and above caused greater pallor in the denervated region than in other parts of the skin. Comparison of the time course of this pallor with that of normally innervated melanophores showed that the duration of the pallor, as well as the intensity, following a given dose was increased. The response to lower doses, 0.015 mg/kg noradrenaline, was indistinguishable from that of Ringer in control fishes.

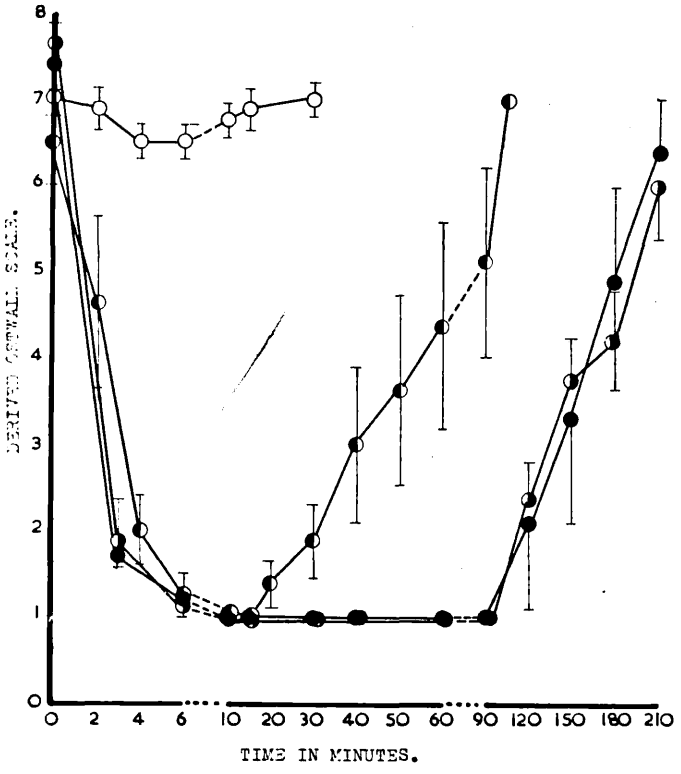
Comparison of the dose/response curves of noradrenaline before and after the operation shows that not only is the drug potentiated to a greater degree than is found after spinal section but that the potentiation is also greater than that which follows cocaine. Following the arguments of Cannon (1935) and Trendelenberg (1963) it is possible to suggest that at least one synapse lies in the chromatic tract between the sites of operation in the spinal cord and in the spinal nerve. It has been pointed out (Section 1.3264) that the potentiation of catecholamines following denervation of the nictitating membrane of the cat was approximately equal to the sum of the potentiations brought about by cocaine and by

Fig. 74 The effect of noradrenaline injections on the denervated area of black-adapted minnows several weeks after spinal nerve section

● 2.3 mg/kg (16°C.) (5 animals)
◑ 1.2 mg/kg (16°C.) (4 animals)
◐ 0.15 mg/kg (16°C.) (4 animals)
○ 0.015 mg/kg (16°C.) (4 animals)

Fig. 75 The effect of adrenaline injections on the shade of the denervated area of black-adapted minnows which had been spinal nerve sectioned several weeks earlier.

● 2.9 mg/kg (18°C.) (4 animals)
⊙ 0.29 mg/kg (17°C.) (4 animals)
○ 0.029 mg/kg (17°C.) (4 animals)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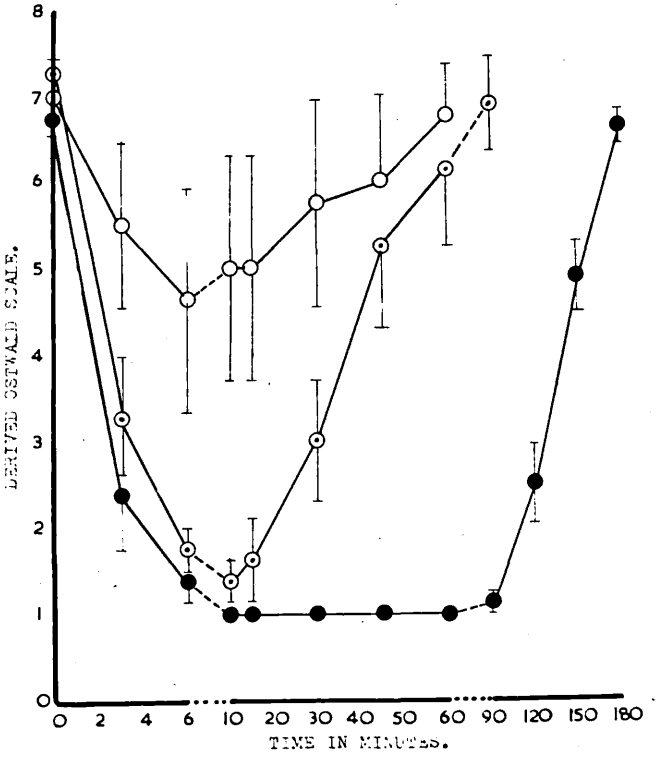
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decentralisation. It is possible to postulate that a synapse occurs in the chromatic tract of the minnow in the region of the sympathetic chain, and another in the skin (the nerve/melanophore junction).

Similar injections of 2.9, 0.29 and 0.029 mg/kg adrenaline were made into operated fish (Fig. 75; p.221). Again a strongly potentiated paling action of the amine occurred in the denervated stripe and the shift of the dose/response curve for adrenaline after the operation was greater than that following cocaine (Fig. 100; p.277). A potentiation of the effect of adrenaline has been described in denervated chromatophores of Ameiurus (Parker, 1941b),1942; Chasmichthys (Fujii, 1958); Lophopsetta, (Osborne, 1939) and Tautoga (Smith, 1941).

The actions of sympathomimetic amines were also investigated in black-adapted, operated fish. The denervated areas however are not separated from the circulatory system and hence are not isolated from adjacent regions whose adrenergic stores are unaffected by the operation. In mammalian studies it has been possible to use perfused, isolated tissues whose adrenergic stores degenerate after denervation thus abolishing the indirect actions of sympathomimetic amines.

Black-adapted fish injected with 11.5 and 5.75 mg/kg tyramine were seen to pale to a much greater extent in the denervated region than in the other, normal areas (Fig. 76; p.225). Similarly, the actions of amphetamine (Fig. 76; p.225) in doses of 6.0 and 3.0 mg/kg were potentiated. If these amines are purely indirectly acting in fish, as has been established in mammals, the pronounced pallor in the denervated region may be caused by the potentiated response of the melanophores to catecholamines displaced from nearby adrenergic stores. The responses of denervated regions to 7.5 and 3.8 mg/kg ephedrine were also enhanced but it was not possible to say whether this potentiation was due to that of displaced catecholamines or to potentiation of the direct actions of ephedrine.

Comparison of the "skeleton" dose/response curves of the sympathomimetic amines with those for catecholamines (Fig. 100; p.277) shows that the former are less potent aggregating agents. The ability of dibenamine, which exerts a blockade at the alpha adrenergic receptor and at the hypothetical transfer site in the adrenergic store of mammals, to abolish their actions may be due to the indirect component of activity of the amines as well as to their lower potency. After pretreatment with cocaine, the changes in response to amphetamine and tyramine may be

interpreted as a competitive antagonism of the indirect actions of these amines at the adrenergic store. Similarly the responses to amphetamine and tyramine after treatment with bretylium and reserpine suggest that indirect actions are antagonized as increased sensitivity to catecholamines is developing (Fig. 101; p.278). However, the pattern of activity of ephedrine is not clear. After cocaine, low doses of ephedrine were potentiated whereas higher doses were inhibited. A similar result was found after 7 days reserpinization. It can only be concluded that further experiments should be performed using reserpine and reserpine + cocaine to investigate the dose/response curves for direct and indirect actions and to see if the direct actions can be potentiated. Such experiments have not been performed in this work.

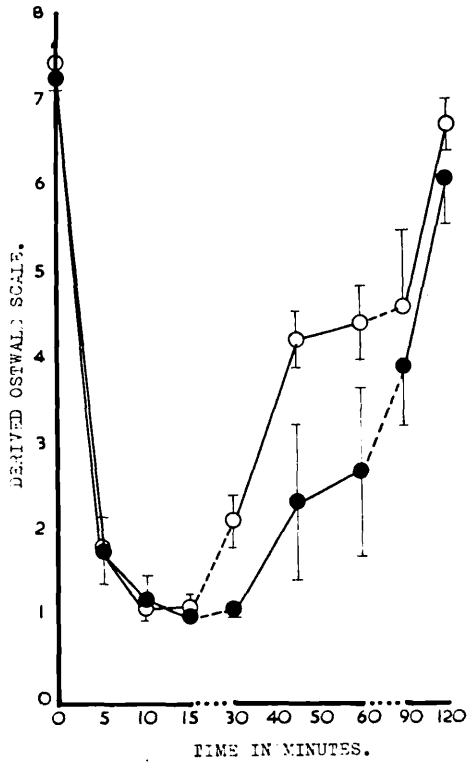
The observed subsensitivity (Fig. 101; p.278) of decentralized fish to the sympathomimetic amines is probably only apparent. The doses used to cause pallor were such as would produce almost maximum pallor in normal fish. It has already been mentioned (Section 3.33) that decentralised melanophores appear to undergo some physiological change which prevents complete aggregation of their pigment and the degree of pallor following sympathomimetic amines in these fish was similarly reduced.

Fig. 76 The effect of tyramine injections on the shade of the denervated area of black-adapted minnows several weeks after spinal nerve section.

● 11.5 mg/kg tyramine (14°C.) (6 animals)
○ 5.75 mg/kg tyramine (14°C.) (5 animals)

Fig. 77 The effects of injections of ephedrine and amphetamine on the shade of the denervated area of black-adapted minnows several weeks after spinal nerve section.

● 7.5 mg/kg ephedrine (15°C.) (5 animals)
○ 3.8 mg/kg ephedrine (15°C.) (5 animals)
◐ 3.0 mg/kg amphetamine (15°C.) (4 animals)
◑ 6.0 mg/kg amphetamine (15°C.) (4 animals)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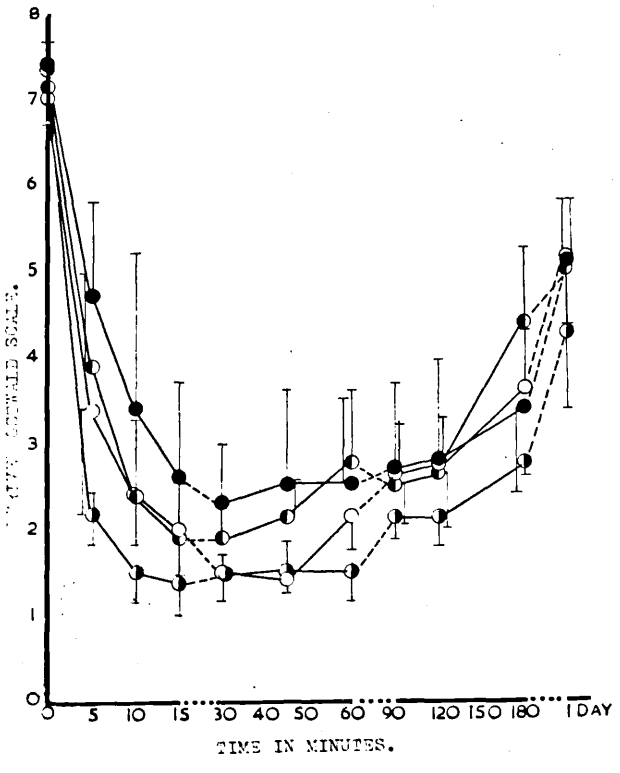
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TIME IN MINUTES.

However, the duration of the pallor following injection of amphetamine or ephedrine was prolonged compared with normal fish. It does appear, therefore, that some potentiation of the actions of these amines has occurred.

Section 3.44
Adrenergic blocking agents

Section 3.441
Phentolamine

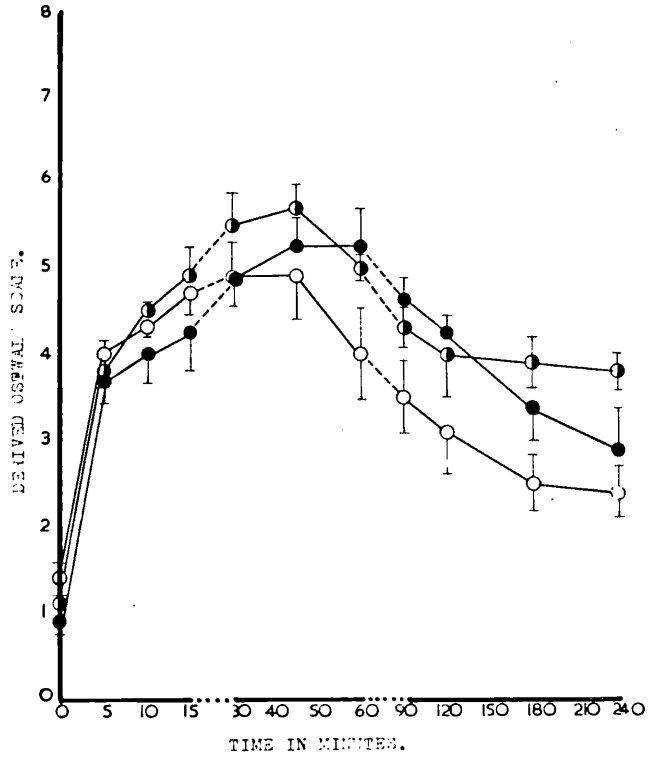
White-adapted fish which had been subjected to spinal nerve section several weeks previously were injected with 13 or 6.5 mg/kg phentolamine (Fig. 79; p.227). Those fish which received the higher dose were found to show similar darkening of both the normal and denervated areas. However, on recovery it was noticed that some groups of melanophores in the denervated area were less able to aggregate than others and were still visible 24 hours later. The lower dose of phentolamine darkened the denervated area more readily than adjacent, normal regions. Once again, some melanophores which were more resistant to paling stimuli delayed the final recovery to the white background.

Fig. 78 The effects of piperoxane injections on the shade of the denervated areas of white-adapted minnows several weeks after spinal nerve section.

● 12.5 mg/kg (12°C.) (4 animals)
◑ 6.25 mg/kg (12°C.) (5 animals)
○ 2.5 mg/kg (12°C.) (5 animals)

Fig. 79 The effects of phentolamine injections on the shade of the denervated areas of white-adapted minnows several weeks after spinal nerve section.

● 13 mg/kg (12°C.) (13 animals)
○ 6.5 mg/kg (12°C.) (11 animals)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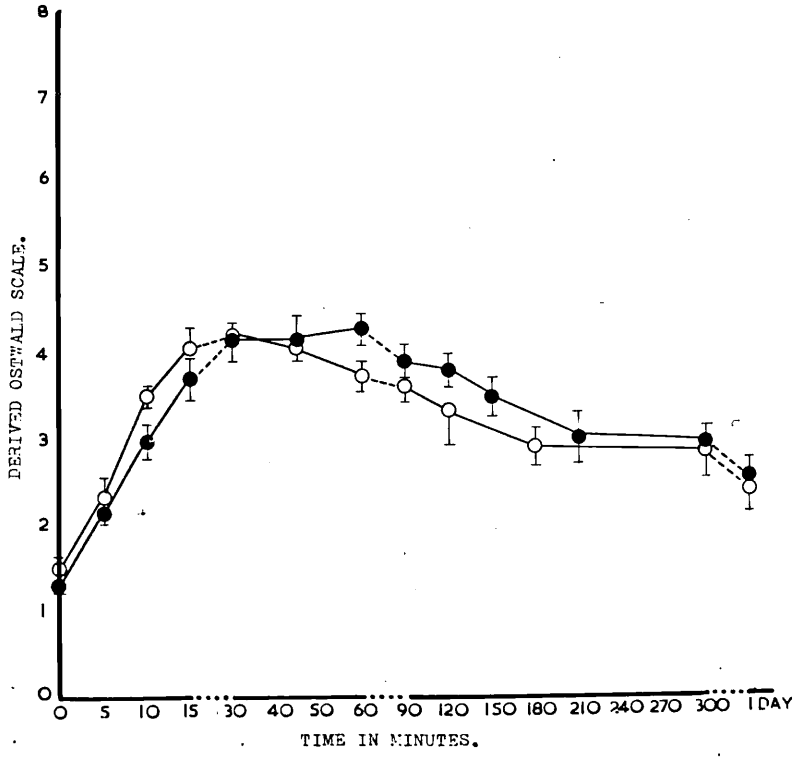
DERIVED OSTWALD SCALE.
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TIME IN MINUTES.

Section 3.442

Piperoxane

White-adapted fish were injected with 12.5, 6.25 or 2.5 mg/kg piperoxane (Fig. 78; p.227). The largest dose led to a slower dispersion in the denervated stripe than in other regions of the skin and subsequent paling in the stripe as the drug effects wore off was delayed. Similarly, following the injection of 6.25 mg/kg piperoxane, the denervated area stood out as a pale area during the darkening of the fish and with this dose and the last, 2.5 mg/kg, the denervated area was the last to pale.

Section 3.443

Yohimbine

Injections of 6.6, 2.7 or 1.35 mg/kg yohimbine into white-adapted spinal nerve-sectioned fish (Fig. 80; p.231) caused darkening of the whole body but a delayed dispersion in the operated region was observed. During recovery of the original pallor, some parts of the denervated region stood out as dark patches.

The demonstration by Boyd et al. (1960, 1962, 1963) and Burnstock et al. (1963) that adrenergic blocking agents may have anti-acetylcholine, anticholinesterase and other pharmacological actions in lower vertebrates underlines the necessity for caution in interpreting the responses to such agents in the minnow. The darkening which follows their injection into white-adapted fish is usually both consistent and pronounced and melanophores which seem less sensitive to catecholamines and sympathomimetic amines disperse their pigment first. Of the several drugs tested in normal minnows only phentolamine failed to cause complete darkening. Pye (1964a) showed that large doses of this agent could bring about intense darkening. He also showed that, whereas ergotamine blocked paling of the skin following electrical stimulation and also unmasked the ability of such stimulation to actively darken the innervated area, phentolamine prevented both responses to electrical stimulation. When adrenergic drugs are injected into animals which have the spinal chromatic tract cut, most of the adrenergic blocking agents resemble phentolamine (Section 3.34). If spinal section, as Pye (1964c) has suggested, also sections the postulated darkening tract (see Section 4.22) the decreased melanophore dispersion in these fish may be due to a failure in the

dispersing system.

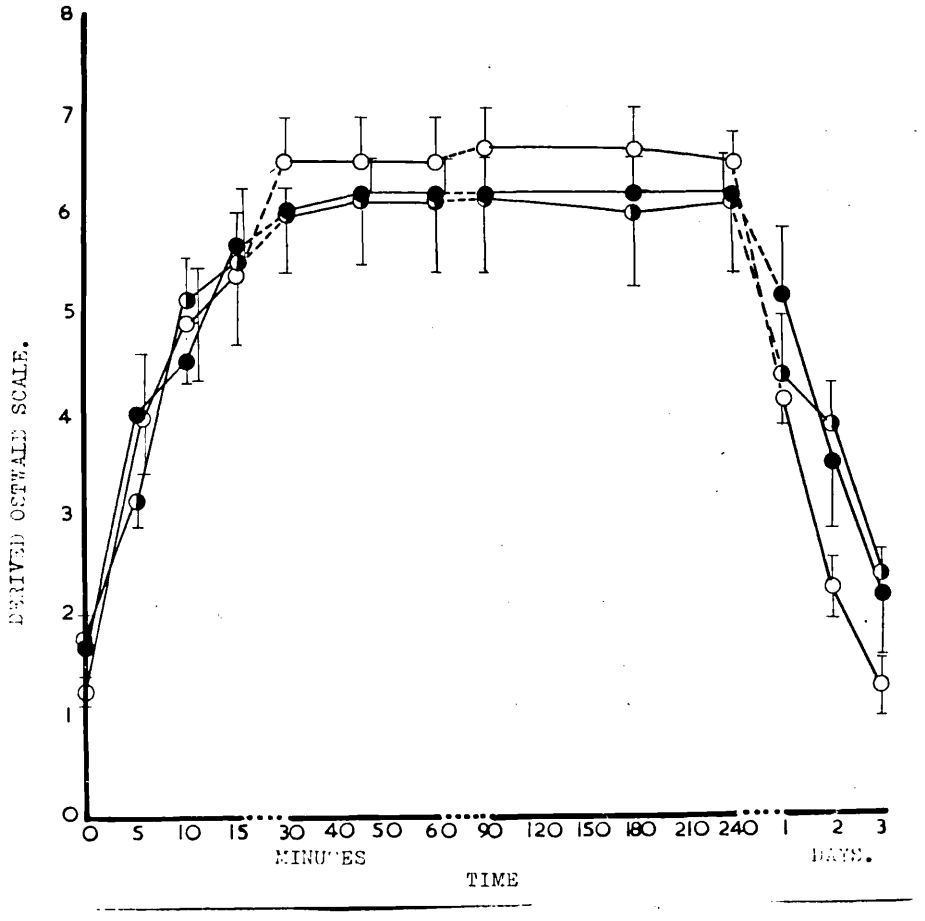
Alternative actions for the "adrenergic blocking agents" in fish may be proposed if they are not so specific as mammalian studies suggest. The agents amy exert a direct stimulation on minnow melanophores to disperse their pigment (Section 3.34). This action, which could be independent of any adrenergic blocking activity, would be less effective in operated fish if the supersensitive melanophores were acted upon by circulating catecholamines which might normally oppose the dispersing action of the blocking agents. If, in the absence of nervous or other chromatic influences, the adrenergic blocking agents have a direct dispersing action on the melanophores, their use as pharmacological tools will be open to serious criticism.

At present the action of these drugs in the minnow may still be tentatively interpreted as causing darkening by competition with catecholamines at the adrenergic receptors. This action unmasks an active dispersing agency, which may be the postulated darkening fibres.

Sawyer *et al.* (1947, 1949) and Everett (1964) have shown that the release of follicle stimulating hormone (FSH) in the rabbit may be under the control of an adrenergic system. Injections of dibenamine prevented ovulation in

Fig. 80 The effects of yohimbine injections on the shade of the denervated areas of white-adapted minnows several weeks after spinal nerve section.

● 6.6 mg/kg (20.C.) (3 animals)
◑ 2.7 mg/kg (13°C.) (4 animals)
○ 1.35 mg/kg (13°C.) (4 animals)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DERIVED OSTWALD SCALE.
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MINUTES
DAYS.
TIME

female rabbits following coitus. It is not known whether this blockade is hypothalamic. The darkening of white-adapted fish after adrenergic blocking agents might conceivably involve blockade of release of the pituitary paling hormone which, like FSH, is formed in the adenohypophysis, or an antagonism of its effects peripherally. The latter hypothesis is discussed in Section 3.5.

Section 3.5
The response of intact minnows to pituitary extracts.

Minnows which have been adapted to a white background for some time are believed to secrete pituitary hormone into the blood which reinforces the action of the aggregating fibres. Regions of the skin separated from central nervous control still retain a slow shade change when subjected to background reversal and this is believed to be mediated by the pituitary. Healey (1948) found that hypophysectomised minnows which were otherwise intact were unable to maintain their background adaptation and showed fluctuations in shade. The slow, final stages of background adaptation are attributed to reinforcement of nervous activity by changes in the level of pituitary hormones in the blood (Section 1.24).

However, it has been shown that spinal fish assume an intermediate shade after the operative darkening has subsided and then pale slowly. Fish pretreated with agents which deplete the adrenergic store, whether spinal sectioned or not, also assume this intermediate coloration as do white-adapted spinal fish injected with phentolamine, piperoxane or dihydroergokryptine. It is possible that adrenergic blocking agents darken white-adapted fish either by interfering with catecholamines which maintain that pallor or by affecting the hormonal mechanism. The intense darkening which follows adrenergic blocking agent injections into normal, but not spinal-sectioned, fish suggests that maintenance of the pale state requires a continued activity of the chromatic fibres if the hormonal mechanism is unaffected.

Preliminary experiments have been performed to investigate the action of various adrenergic drugs on the paling activity of injected pituitary extracts.

Section 3.51

The effects of injections of pituitary extracts alone on the shade of black-adapted minnows

Pituitary glands were removed from plaice heads and placed in acetone. After several changes of acetone at

one day intervals the glands were dried, powdered and stored in a desiccating chamber. Injections of weighed amounts of powder were made by grinding the powder with a small volume of Young's Ringer and injecting 0.1ml into the fish. The responses of black-adapted minnows to such injections are represented in Fig. 81; p.235. All the doses used, between 10.8 and 35 mg/kg powder, aggregated the melanophores of black-adapted fish and maximally dispersed the erythrophores. Patches of red appeared at the base of the fins, the mouth and opercula of the fish as the latter chromatophores reacted. No erythrophore dispersing action was found using any of the amines which paled fish in the previous sections. The pallor of fish injected with pituitary hormone was maximal only after 1-1½ hours and recovery took 4 to 20 hours depending on the dose.

Four small minnows, approximately 4 cm. long, were each injected with the extract of three minnow pituitaries. These fish paled more rapidly and more intensely than did minnows injected with the plaice pituitary previously described. Strong erythrophore dispersion occurred.

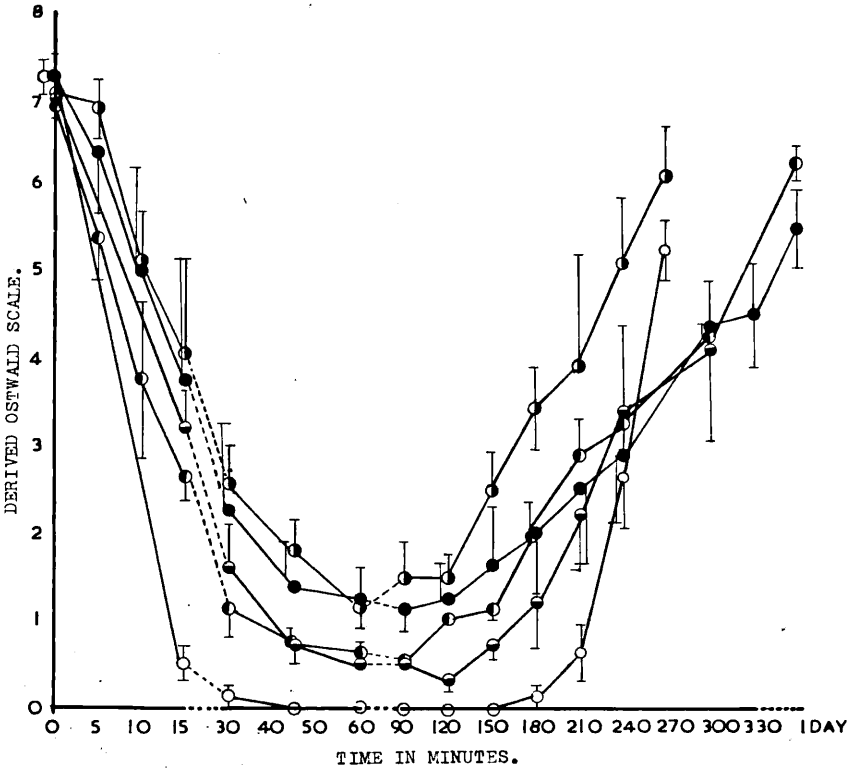
In all these fish, whether injected with large or small amounts of pituitary extract, the pattern of the skin during paling differed from that brought about by injections of catecholamines or sympathomimetic amines.

Fig. 81 The effect of pituitary extracts of the plaice or the minnow on the shade of black-adapted minnows.

- ● 35mg/kg plaice pituitary powder (13°C.) (4 animals)
- ◒ 27mg/kg plaice pituitary powder (19°C.) (5 animals)
- ◐ 17.5mg/kg plaice pituitary powder (13°C.) (4 animals)
- ◑ 10.8mg/kg plaice pituitary powder (19°C.) (13 animals)
- ○ 3 minnow pituitaries/fish (19°C.) (4 animals)

Fig. 82 The effect of pituitary extracts of the plaice on the shade of black- or white-adapted minnows which were pretreated for several weeks with hypotensive drugs or with dibenamine (12°C.)

- ⊕ 12mg/kg plaice pit. in white-adapted reserpinised fish (6 animals)
- ⊖ 12mg/kg plaice pit. in black-adapted reserpinised fish (6 animals)
- ◐ 12mg/kg plaice pit. in white-adapted guanethidine treated fish (8 animals)
- ⊗ 12mg/kg plaice pit. in black-adapted guanethidine treated fish (5 animals)
- ⊙ 12mg/kg plaice pit. in white-adapted bretylium treated fish (7 animals)
- ⊙ 17.5mg/kg plaice pit. in white-adapted bretylium treated fish (4 animals)
- ○ 12mg/kg plaice pit. in black-adapted bretylium treated fish (5 animals)
- ● 35mg/kg plaice pit. in white-adapted dibenamine treated fish (5 animals)
- ◐ 12mg/kg plaice pit. in white-adapted dibenamine treated fish (5 animals)
- ◑ 35mg/kg plaice pit. in black-adapted dibenamine treated fish (4 animals)
- ◒ 12mg/kg plaice pit. in black-adapted dibenamine treated fish (5 animals)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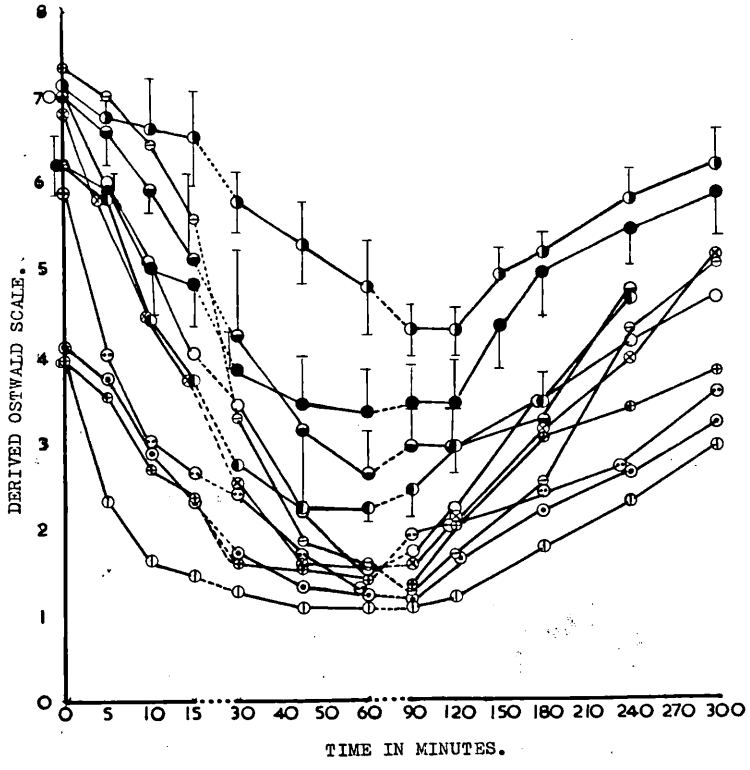
0 5 10 15 30 40 50 60 90 120 150 180 210 240 270 300 330 1 DAY
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
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0 5 10 15 30 40 50 60 90 120 150 180 210 240 270 300
TIME IN MINUTES.

The "resistant" melanophores in the bars and stripes of the skin did not stand out as transiently darker patches. In addition, the delayed onset of paling following the injections distinguished the two types of active agent.

Section 3.52

The effects of injections of pituitary extracts on the shade of minnows pretreated with hypotensive drugs

White- and black-adapted fish which had been chronically pretreated with reserpine for three weeks (2.7 mg/kg/day) were injected with 12 mg/kg powdered plaice pituitary. Both groups of fish paled completely within 1-1½ hours but recovered a little more slowly than did black-adapted fish described in Section 3.51 (Fig. 82;p.235) Reserpinization did not prevent erythrophore dispersion.

Guanethidine pretreated fish paled rather more quickly when injected with a similar dose of pituitary extract, reaching maximum pallor within 45 min. Once again a strong red coloration showed that erythrophore dispersion occurred. Recovery to the original shade was delayed when compared with normal fish.

Similarly, fish chronically treated with bretylium showed melanophore aggregation and erythrophore dispersion after plaice pituitary injection, but required over five

hours to recover. It may be concluded that the intermediate colouration which develops in normal white-adapted fish after prolonged treatment with hypotensive agents is not due to a peripheral antagonism of paling hormone. It is possible that a central action might lead to decreased release of hormone in the white-adapted, chronically pretreated fish. However, the intermediate shade found in spinal fish soon after operation might suggest that the amount of normone released naturally by the minnow is, by itself, insufficient to maintain pallor in the absence of nervous activity. Similarly, Healey's experiments suggest that prolonged nervous activity is not maintained in hypophysectomised fish. It is suggested that the two agencies summate to cause normal, long-term white background adaptation.

Section 3.53

The effects of pituitary extracts combined with adrenergic blocking agents

Black- and white-adapted fish were given daily injections of 11.75 mg/kg dibenamine for 10 days and were then injected with either 12 or 35 mg/kg plaice pituitary powder extract (Fig. 82; p.235). In these fish,

melanophore aggregation was antagonized but erythrophore dispersion was maximal. Other black-adapted fish were injected with a mixture of plaice pituitary extract and piperoxane (Fig. 83;p.241). One group received 35 mg/kg plaice pituitary and 6.3 mg/kg piperoxane and after a strong pallor recovered more quickly than did fish given the pituitary extract alone. The second group was given 17.5 mg/kg plaice pituitary powder extract with the same dose (6.3 mg/kg) piperoxane. This group also recovered more quickly than did fish given pituitary extract alone. In both groups of fish strong erythrophore dispersion occurred.

Further injections of pituitary extracts were made into black-adapted fish together with combined alpha and beta adrenergic blocking agents (Fig. 84;p.241). One group of fish received 27 mg/kg pituitary extract, 10.5 mg/kg piperoxane and 13.5 mg/kg pronethalol. A very rapid and intense pallor followed this injection which lasted three hours, but recovery took more than 24 hours. Erythrophore dispersion was marked. The second group of fish, injected with the same doses of adrenergic blocking agents and only 17.5 mg/kg plaice pituitary, showed very much reduced paling. Erythrophore dispersion occurred as before.

With the doses of pituitary extract used, there is some indication that the paling potency of the injection is reduced by the presence of blocking agents. This reduction in potency may be due to a peripheral interaction at the melanophore between the hormone and the drug or to competition between the drug and catecholamine contamination in the crude extract. The action of the erythrophore dispersing hormone of the plaice pituitary is unaffected by the presence of adrenergic blocking drugs.

Section 3.54

Pituitary extracts and cocaine

In view of the possibility that plaice pituitary extracts might contain, in addition to paling hormone, catecholamine contaminants which also cause pallor, fish were pretreated with cocaine and then injected with pituitary extract and cocaine. Black-adapted fish injected with 27 mg/kg plaice pituitary and 13.5 mg/kg cocaine (Fig. 83; p.241) paled considerably. The time course of this pallor resembled that found in normal fish injected with pituitary extract alone. A smaller dose of pituitary extract, 10.8 mg/kg, together with 13.5 mg/kg cocaine again caused a similar pallor to that found in control fish.

A similar lack of effect of cocaine was found when it was used in fish injected with the extract of three minnow pituitaries.

The decreased potency of injected pituitary extracts in minnows injected with adrenergic blocking agents implies that some antagonism between these agents occurs peripherally. Dibenamine has been found to disperse melanophores by an apparent direct stimulation (Fujii, 1961; Watanabe et al. 1962b). The intermediate shade which develops in white-adapted spinal fish when adrenergic blocking agents are injected might be due to such antagonism and also to a decreased release of the hormone from the pituitary gland (cf. Section 3.44). The nature of the peripheral interaction is not known. The hormone and drugs may act at different sites to cause pigment aggregation and dispersion by direct actions. On the other hand, it is conceivable that the pituitary hormone might bring about melanophore aggregation by releasing an amine locally in the skin. Davey (1960) concluded that the dispersion caused by amphibian chromatic pituitary hormones depends on the local release of indolalkylamines. The failure of reserpine to prevent the action of injected paling hormone in fish with apparently depleted adrenergic stores, may be used as an argument against an indirect action of the hormone in the minnow.

Fig. 83 The effect of plaice or minnow pituitary extract on the shade of black-adapted minnows simultaneously injected with piperoxane or cocaine.

● 35 mg/kg plaice pit. + 6.3 mg/kg piperoxane (13°C.) (4 animals)

○ 17.5 mg/kg plaice pit. + 6.3 mg/kg piperoxane (13°C.) (4 animals)

◑ 27 mg/kg plaice pit. + 13.5 mg/kg cocaine (19°C.) (5 animals)

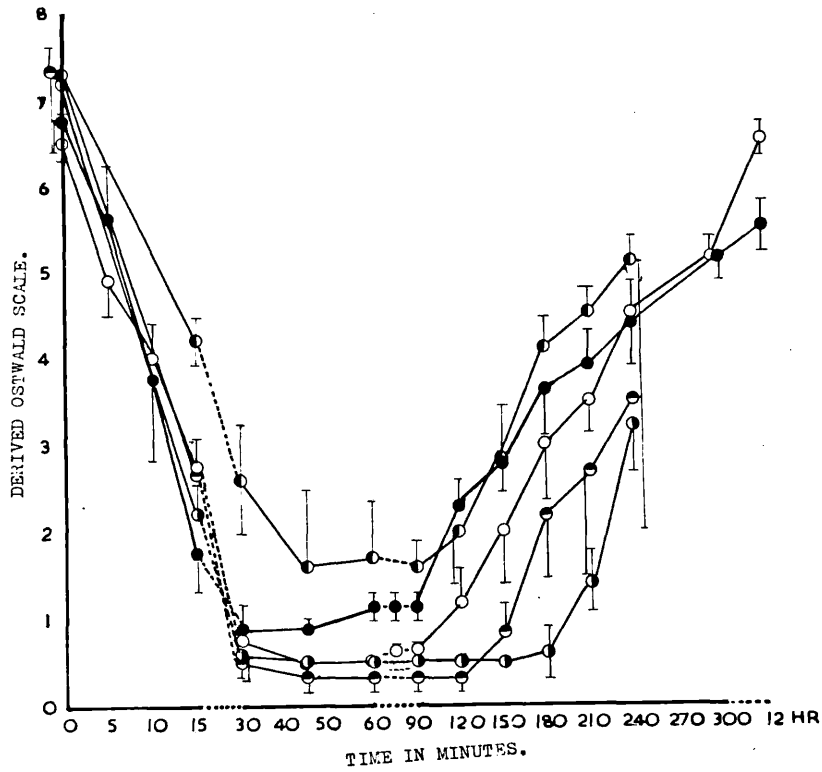
◐ 10.8 mg/kg plaice pit. + 13.5 mg/kg cocaine (19°C.) (5 animals)

◓ 3 minnow pituitaries + 13.5 mg/kg cocaine (19°C.) (3 animals)

Fig. 84 The effect of plaice pituitary hormone on the shade of black-adapted minnows when injected simultaneously with alpha and beta adrenergic blocking agents.

○ 27 mg/kg plaice pit. + 10.5 mg/kg piperoxane + 13.5 mg/kg alderline (19°C.) (4 animals)

● 10.8 mg/kg plaice pit. + 10.5 mg/kg piperoxane + 13.5 mg/kg alderline (19°C.) (5 animals)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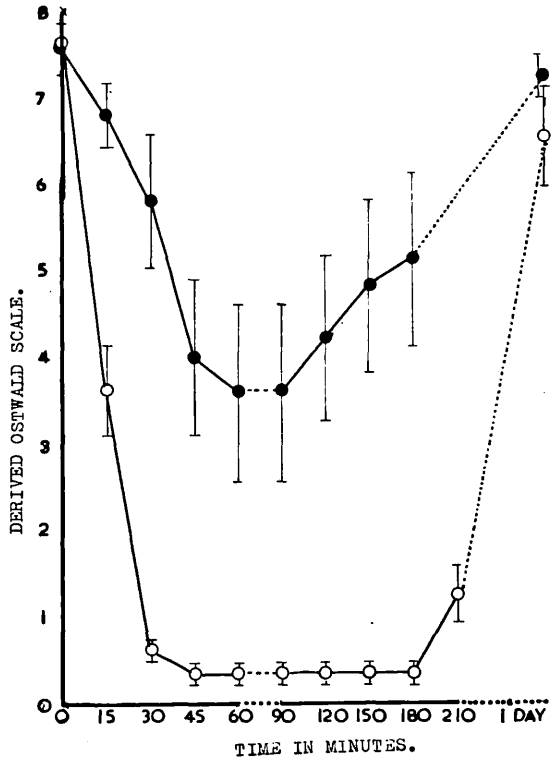
TIME IN MINUTES.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TIME IN MINUTES.

Kent (1961) has shown that the erythrophore-dispersing hormone of the teleost pituitary can be separated from the minnow-aggregating/frog-dispersing melanophore-stimulating hormone. None of the drugs used above affected the activity of pituitary extracts on erythrophores of the minnow skin.

Section 3.6
Cholinergic agents

It has been suggested (Sections 3.33, 3.345, 3.353, 3.42, 3.443) that an active darkening agency, the postulated melanophore-dispersing fibres, antagonizes the action of the sympathetic paling fibres in the minnow. Previous workers proposed that such dispersing fibres are cholinergic and resemble the parasympathetic system of mammals (Section 1.23). The following experiments were carried out to investigate the reactions of normal and operated minnows to various mammalian cholinergic drugs.

Section 3.61
Cholinesters

Section 3.611
Acetylcholine

Normal black- and white-adapted minnows were injected with acetylcholine (Fig. 85; p.244). The largest dose which did not prove lethal, 6.7 mg/kg, did not darken white-adapted fish when injected with eserine. In these fish respiratory stress was observed, seen as gulping movements of the mouth and opercula, together with tremor of the fins and emesis. These characteristics were less prevalent when lower doses of acetylcholine, with or without eserine, were injected. No darkening of the fish, such as was found with adrenergic blocking agents, was observed. Black-adapted fish, which also showed some signs of stress when injected, were seen to pale to some extent when injected with 2.7 mg/kg acetylcholine together with 5.4 mg/kg eserine. This pallor was maintained for a much longer time than that which followed control injections of Young's Ringer (Section 3.21). Lower doses of acetylcholine, 0.27 and 0.027 mg/kg were injected into black-adapted fish but no significant pallor developed.

Fig. 85 The effect of acetylcholine injections on the shade of black- and white-adapted minnows

● 6.7 mg/kg acetylcholine + 6.7 mg/kg eserine (13°C.) (8 animals)

◑ 2.7 mg/kg acetylcholine + 5.4 mg/kg eserine (12°C.) (5 animals on each background)

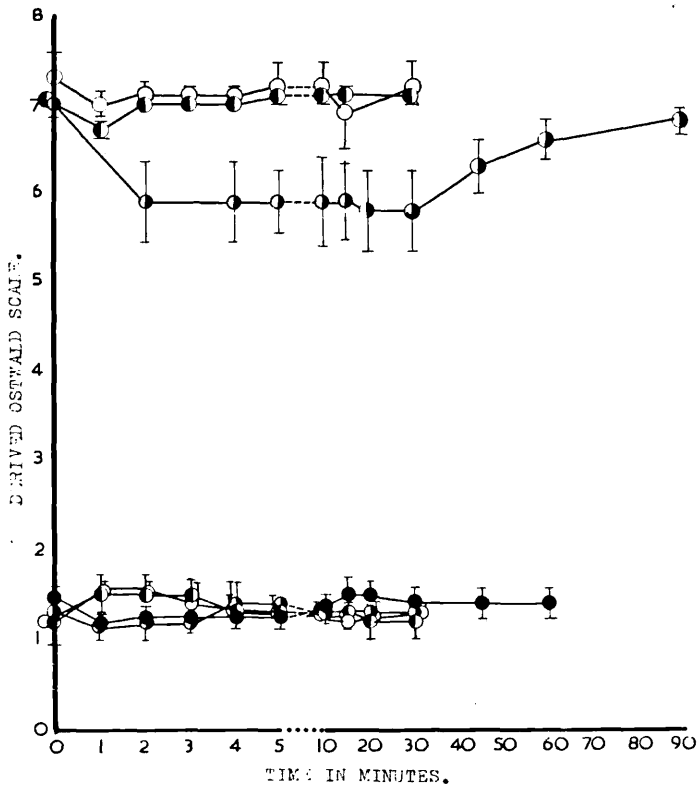
◐ 0.27 mg/kg acetylcholine (19°C.) (5 animals on each background)

○ 0.027 mg/kg acetylcholine (19°C.) (5 animals on each background)

Fig. 86 The effect of acetylcholine injections on the shade of black- and white-adapted minnows two months after spinal section (12°C.)

● 3.5 mg/kg acetylcholine + 7 mg/kg eserine (white) (8 animals)

○ 3.5 mg/kg acetylcholine + 7 mg/kg eserine (black) (5 animals)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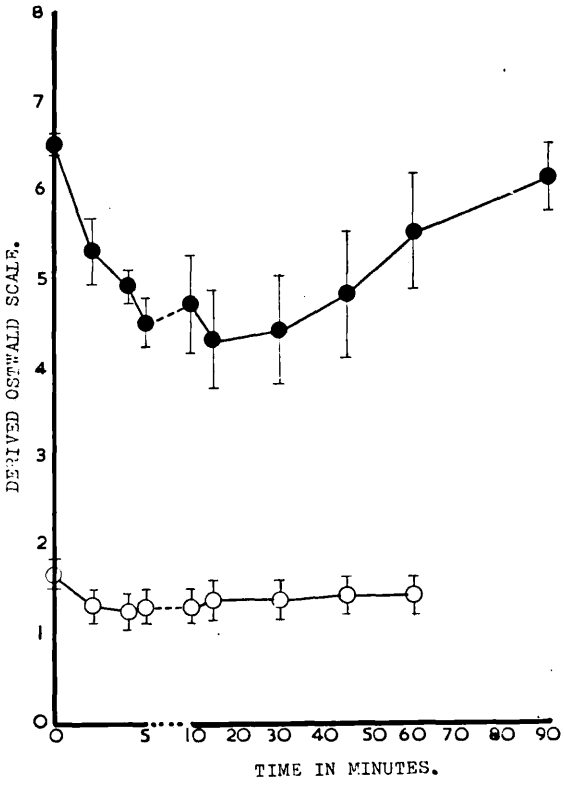
TIME IN MINUTES.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If acetylcholine were an antagonistic neurohumour to the sympathetic paling system, injections of the ester into white-adapted spinal fish could possibly lead to melanophore dispersion in the absence of activity in the paling fibres. Injections of acetylcholine (3.5 mg/kg) together with eserine (7 mg/kg) (Fig. 86; p.244) did not lead to darkening. In fact, a similar dose in black-adapted fish led to a pronounced pallor which lasted approximately 3/4 hours.

Injections of acetylcholine into black- and white-adapted minnows which had denervated stripes on one side of their body (Fig. 87; p.249) led to no darkening of the denervated area. The dose used was 3.0 mg/kg acetylcholine with 6 mg/kg eserine. The same dose in black-adapted fish caused a strong pallor in the denervated area which was as intense as, but less prolonged than, that found in spinal fish. Parker (1942) found that denervated chromatophores of Ameiurus showed potentiated responses to adrenaline (cf. Section 3.43) but not to acetylcholine.

The mechanism of the paling action of acetylcholine is not clear. The ester has been found to disperse the melanophores of many teleosts (Table 2, p.85). The continued activity of the ester in spinal-sectioned fish implies that it does not act on the medullary paling centre.

It is possible that the action may involve a "nicotinic" stimulation of sympathetic ganglia from which impulses reach the skin by way of the spinal nerves. The enhanced responses of spinal-sectioned fish could then be attributed to the potentiated action of neurohumours on the melanophores. However, if this were the case, denervated melanophores would be expected to react even more strongly to injections of acetylcholine. Adjacent areas of skin, with unaffected nerve terminals, would be expected to release transmitter which on reaching the denervated melanophores would produce maximal responses. Such an action has been previously suggested (Section 3.43) for the indirectly acting sympathomimetic amines tyramine and amphetamine. Were acetylcholine to cause pallor by displacing catecholamines from adrenergic stores as suggested in mammals (Burn 1961) a similar difference in effect between decentralised and denervated melanophores would be expected. According to Trendelenburg (1963), only "specific" exciting agents which combine with the receptor sites of the end organ are further potentiated by denervation. He maintained that "non-specific" agents, which stimulated the smooth muscles of the cat nictitating membrane at non-adrenergic receptor sites, are potentiated to the same extent by both denervation and decentralisation. It seems possible

therefore that acetylcholine, which is a "non-specific" stimulant of the cat nictitating membrane, exerts such an action on minnow melanophores. The failure of acetylcholine-induced pallor after chronic reserpinisation (Section 3.255) does however suggest that the pallor involves an action on the adrenergic system.

Section 3.612

Carbachol

Preliminary injections of the acetylcholinesterase-resistant ester carbachol into minnows showed that it was much more toxic than acetylcholine. Doses of less than 0.5 mg/kg were not lethal but, like acetylcholine, induced fin tremors, emesis and respiratory difficulty. Lower doses of carbachol, 0.07 mg/kg, were without effect on white-adapted fish but caused slight pallor in black-adapted fish similar to that caused by Young's Ringer alone (Fig. 88a; p.249).

White-adapted spinal fish injected with 0.07, 0.03 and 0.015 mg/kg carbachol were in general unaffected by the injection. Some fish were however seen to darken a little after the intermediate dose (Fig. 88b; p.249). No injections were made into spinal nerve-sectioned fish.

Section 3.613

Methachol and bethanechol (Fig. 89; p.250)

Only one series of injections was possible with these drugs as difficulty was found in obtaining samples. White-adapted fish injected with 2.1 mg/kg methachol were seen to darken to some extent ten minutes after the injection. Injections of 2.0 and 1.0 mg/kg bethanechol also brought about limited darkening.

Carbachol, unlike methachol and bethanechol, is known to have strong nicotinic activity in mammals. However, its high toxicity in the minnow appears to limit its experimental use. The slight darkening action of the mainly "muscarinic" esters is suggestive. Any tendency for "muscarinic" acetylcholine to darken minnows may be masked in the present study by a strong "nicotinic" activity on sympathetic paling ganglia or on paling fibre nerve terminals. It has not been possible to confirm that cholinesters are potent darkening agents in the present study (cf. Parker, 1940c; Mendes, 1942). Paling actions of acetylcholine have been described in Fundulus and Carassius (Table 2; p.85).

Fig. 87 The effect of acetylcholine injections on the shade of the denervated area of black- and white-adapted minnows several weeks after spinal nerve section (12°C.)

● 3.0 mg/kg acetylcholine + 6 mg/kg eserine (white) (5 animals)

○ 3.0 mg/kg acetylcholine + 6 mg/kg eserine (black) (5 animals)

Fig. 88 a. The effect of carbachol on the shade of black- and white-adapted minnows (19°C.)

○ 0.07 mg/kg in white adapted fish (4 animals)

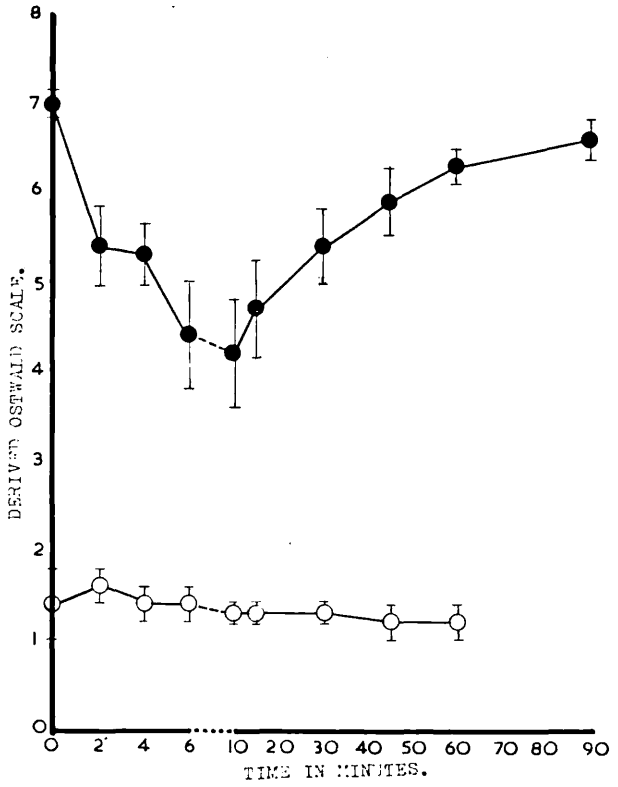
● 0.07 mg/kg in black adapted fish (4 animals)

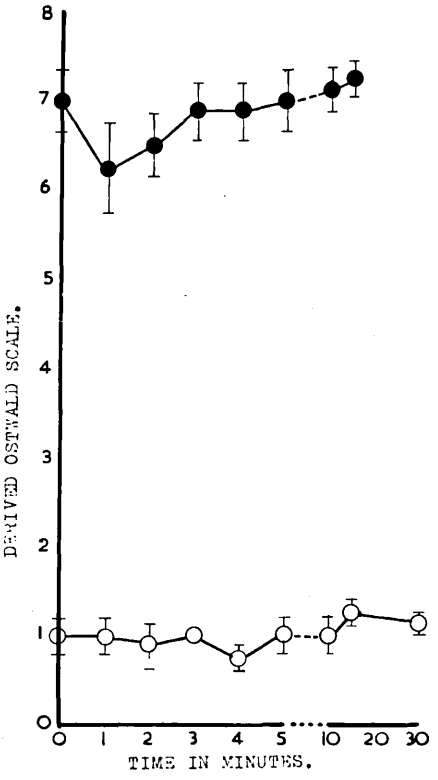
b. The effect of carbachol injections on the shade of white-adapted minnows two months after spinal section.

◑ 0.07 mg/kg (18°C.) (6 animals)

● 0.03 mg/kg (18°C.) (6 animals)

○ 0.015 mg/kg (19°C.) (4 animals)


DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 2 4 6 10 20 30 40 50 60 70 80 90
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30
TIME IN MINUTES.

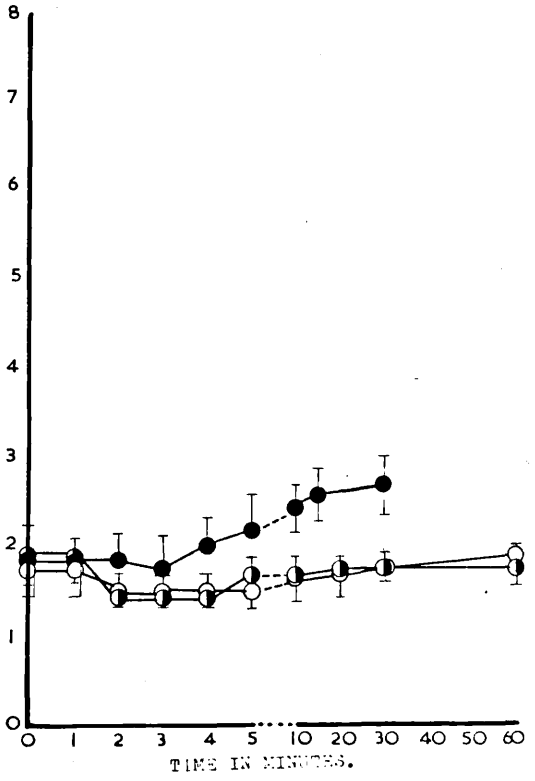
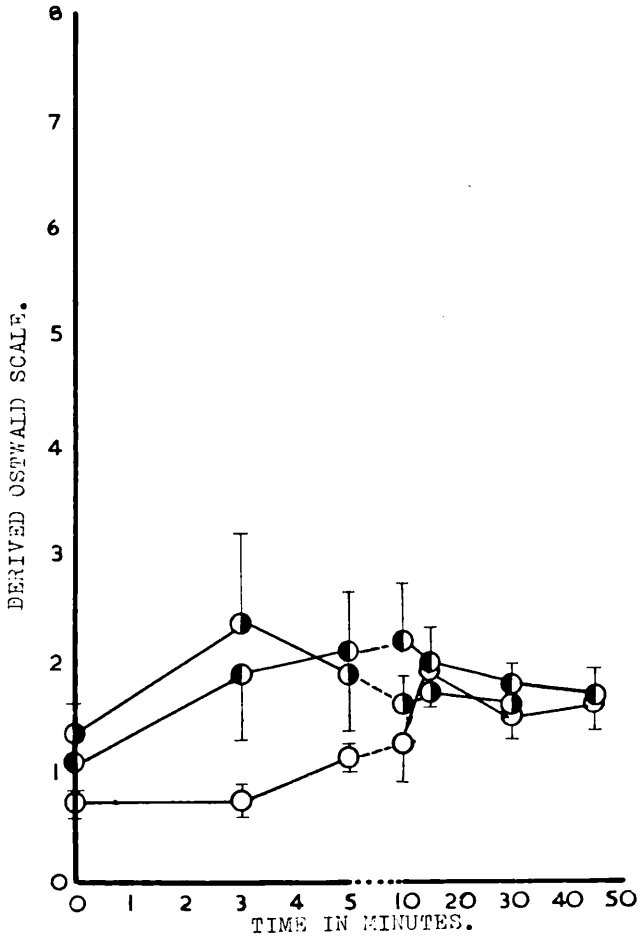

0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 10 20 30 40 50 60
TIME IN MINUTES.

Fig. 89 The effects of injections of methachol or bethanechol on the shade of white-adapted minnows (21°C.).

◐ 2.0 mg/kg bethanechol (5 animals)
◑ 1.0 mg/kg bethanechol (4 animals)
○ 2.1 mg/kg ~~bethanechol~~ methachol (4 animals)


DERIVED OSTWALD SCALE.
0
7
6
5
4
3
2
1
0
0 1 2 3 4 5 10 20 30 40 50
TIME IN MINUTES.

Section 3.62

Pilocarpine

Pilocarpine was injected into white-adapted minnows in doses of 21, 15.8, 10.5, 7.4 and 4.25 mg/kg (Fig. 90; p. 252). Fluctuations in the shade of the fish occurred, especially after higher doses, but no darkening similar to that following adrenergic blocking agents was seen. Black-adapted fish injected with 10.5 mg/kg pilocarpine were unaffected.

Black- and white-adapted spinal minnows were also tested (Fig. 91; p. 252). The largest dose used, 10.5 mg/kg, did darken one white-adapted fish considerably but other fish were unaffected by this and lower doses. Black-adapted fish on the other hand darkened to some extent after 10 mg/kg but paled slightly after 6 and 3 mg/kg.

Finally white-adapted fish which had been spinal nerve-sectioned several weeks previously were injected with 7.5 or 5.0 mg/kg pilocarpine. The denervated melanophores were not significantly affected by the drug.

The weak and varied effects of injected pilocarpine are inconcludive. If a cholinergic dispersing system were present in the minnow a stronger darkening would be expected to follow injection of this muscarinic stimulant.

Fig. 90 The effects of pilocarpine on the shade of black- and white-adapted minnows.

◐ 21 mg/kg (12°C.) (3 animals)

◑ 15.8 mg/kg (12°C.) (3 animals)

● 10.5 mg/kg (12°C.) (15 animals on white: 6 animals on black)

⊙ 7.4 mg/kg (14°C.) (6 animals)

○ 4.25 mg/kg (14°C.) (5 animals)

Fig. 91 The effects of pilocarpine on black- or white-adapted minnows which have previously been subjected to spinal section or spinal nerve section.

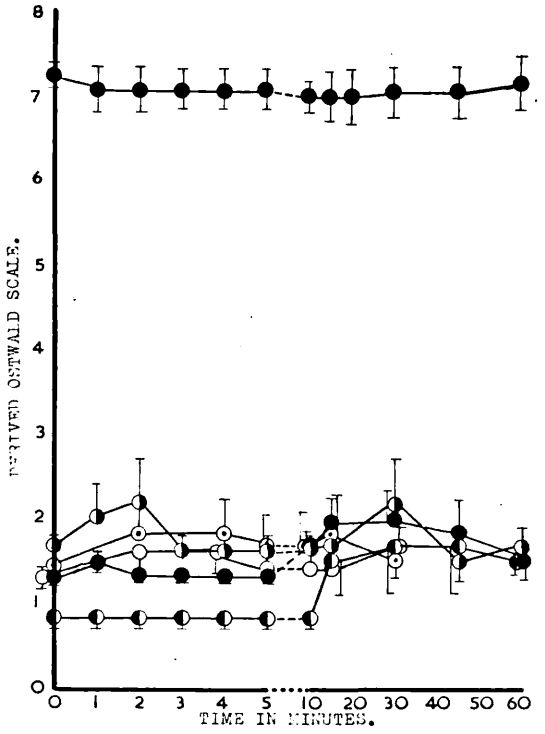
● 10mg/kg in spinal fish (16°C.) (3 animals on white: 3 animals on black)

◑ 6.0mg/kg in spinal fish (18°C.) (3 animals on white: 3 animals on black)

○ 3.0mg/kg in spinal fish (18°C.) (3 animals on white: 3 animals on black)

···● 7.5mg/kg in denervated areas (14°C.) (4 animals on white)

···◑ 5.0mg/kg in denervated areas (14°C.) (5 animals on white)


8
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6
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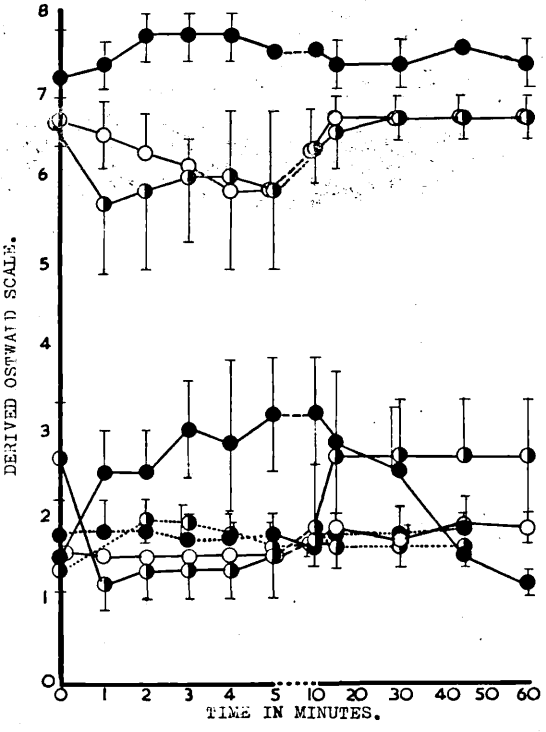
4
3
2
1
0
0 1 2 3 4 5 10 20 30 40 50 60
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
8
7
6
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4
3
2
1
0
0 1 2 3 4 5 10 20 30 40 50 60
TIME IN MINUTES.

Section 3.63

Muscarinic blocking agents

Section 3.631

Atropine

Atropine in mammals is known to antagonise the cholinergic parasympathetic system by a muscarinic blockade of acetylcholine and thus unmask the actions of the antagonistic sympathetic system. In fish, it would be expected to enhance the activity of the sympathetic paling fibres and perhaps prevent or antagonize adaptation to a black background. Previous workers however (Table 2;p.85) have found that this agent darkens pale fish and does not affect black background adaptation.

Injections of 13.5 and 8.0 mg/kg atropine into white-adapted minnows led to pronounced darkening (Fig. 92;p.255). Black-adapted fish remained dark after 13.5 mg/kg atropine. When these fish were subjected to background reversal it was found that the rate of colour change of all fish was rapid but that fish adapting to a white background could only assume an intermediate shade. A similar effect of atropine on colour change has been described in Fundulus by Smith (1931b).

Fujii (1960) and Watanabe (1960) proposed that the action of atropine involved a direct stimulation of melanophores to disperse their pigment. Denervated melanophores of Chasmichthys were still able to disperse when treated with atropine and were more sensitive to this agent. It is also possible that part of the darkening activity of atropine is caused by adrenergic blockade; such activity has been found in mammals (Section 1.3141).

Injections of atropine were also made into black- and white-adapted spinal minnows (Fig. 93; p.235). Both groups of fish received 14 mg/kg atropine. The white-adapted fish were found to darken considerably but, unlike unoperated fish, only the regions of skin immediately overlying the body cavity were affected. There was no darkening of the head and tail. The values of D.O.S plotted in Fig. 93, p. apply only to the middle part of the fish body. Black-adapted spinal fish were seen to darken a little more after the injection.

The localised effect of atropine in white-adapted spinal fish is surprising. In the area affected, the sympathetic chain is in close communication with the body cavity. Atropine has been found to exert weak nicotinic blocking actions in mammalian ganglia but in spinal fish, in which white-adaptation does not depend on activity in

Fig. 92 The effects of atropine injections on the shade of black- and white-adapted minnows and on their ability to adapt to a new background. Fish were transferred to a white (W) or black (B) background as indicated in the figure.

● 13.5 mg/kg atropine (black adapted fish) (14°C.) (5 animals)

○ 13.5 mg/kg atropine (white adapted fish) (14°C.) (5 animals)

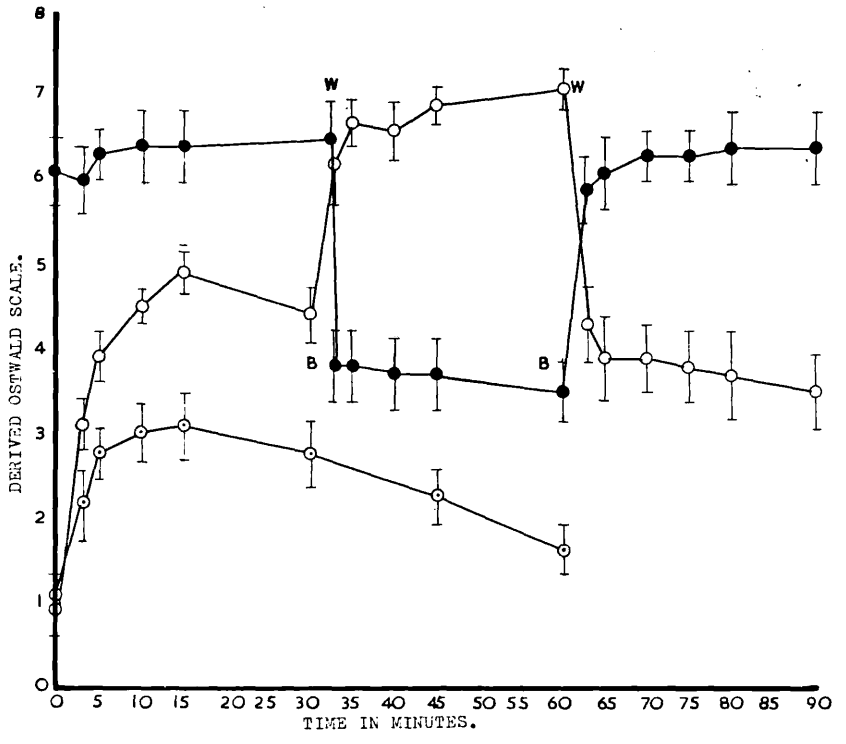
⊙ 8.0 mg/kg atropine (white adapted fish) (21°C.) (6 animals)

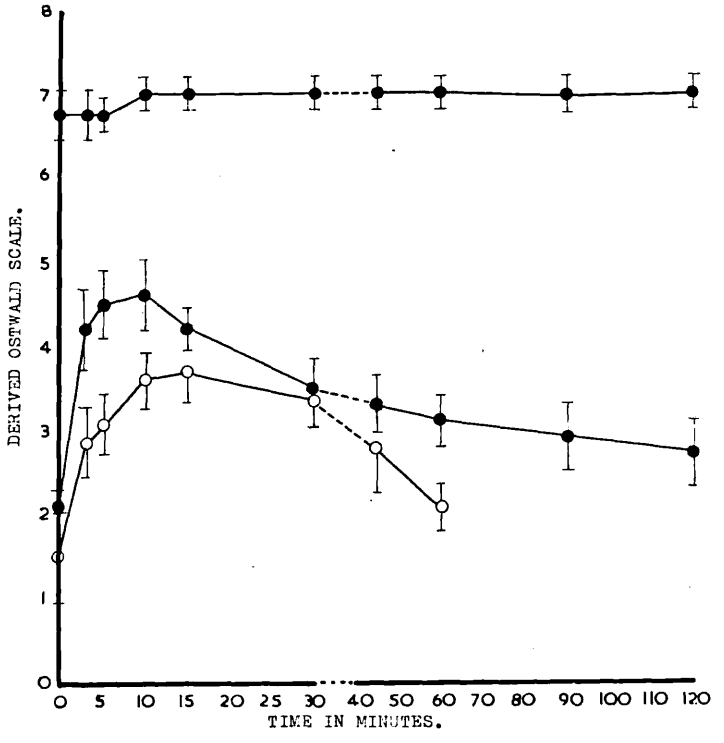
Fig. 93 The effects of atropine injections on the shade of black- or white-adapted minnows which had been previously subjected to spinal section or spinal nerve section.

● 14.mg/kg atropine (black adapted spinal fish) (18°C.) (4 animals)

● 14 mg/kg atropine (white adapted spinal fish) (18°C.) (5 animals)

○ 8 mg/kg atropine (white adapted spinal nerve sectioned fish) (21°C.) (6 animals)


DERIVED OSTWALD SCALE.
TIME IN MINUTES.
W
W
B
B


DERIVED OSTWALD SCALE.
TIME IN MINUTES.

the paling fibres, ganglionic blockade would not be expected to cause darkening. It is possible that the uptake of atropine from the body cavity is impaired after spinal section, perhaps due to vasomotor disturbances, and the injected alkaloid is only able to affect nearby melanophores.

Spinal nerve-sectioned, white-adapted fish were injected with 9 mg/kg atropine (Fig. 93; p.255). Darkening of the general body surface occurred, including the head and tail, but the denervated area darkened to a greater extent despite the increased sensitivity of melanophores in this region to circulating catecholamines (Section 3.43). Fujii (1960) found that Chasmichthys denervated melanophores were supersensitive to atropine and it seems probable that such a mechanism occurs in the minnow. It remains to be seen whether the potentiation of atropine depends on the failure locally of impulses in the chromatic paling fibres or on a change in the receptors of the melanophores.

Section 3.632
Homatropine

The actions of a related alkaloid, homatropine, were investigated to see if this agent exerts an effect on minnow melanophores similar to that of atropine. Black-

and white-adapted fish were injected with 11.5 mg/kg homatropine (Fig. 94; p.258). Black-adapted fish were unaffected by the injection but white-adapted fish darkened a little. When subjected to background reversal both groups of fish changed shade at a slower rate than uninjected fish (Section 3.1). Similar injections into spinal fish were without effect on black-adapted fish but did darken white-adapted fish to some extent.

Homatropine in mammals is a weaker muscarinic blocking agent than atropine. In the minnow it appears to have some ability to mimic the darkening activity of atropine but this effect is weak.

Section 3.64
Nicotinic blocking agents

Section 3.641
Hexamethonium (C_6)

White- and black-adapted minnows were injected with 9.0 or 4.5 mg/kg of the ganglion blocking agent hexamethonium (C_6) (Fig. 95; p.260). These doses led to a darkening of white-adapted fish to between D.O.S. 4.5 and 5.5 which lasted one to two hours. The larger dose also paled the black-adapted fish to about 5.5 on the scale

Fig. 94 The effects of homatropine injections on the shade of intact and spinal-sectioned minnows (14°C.).

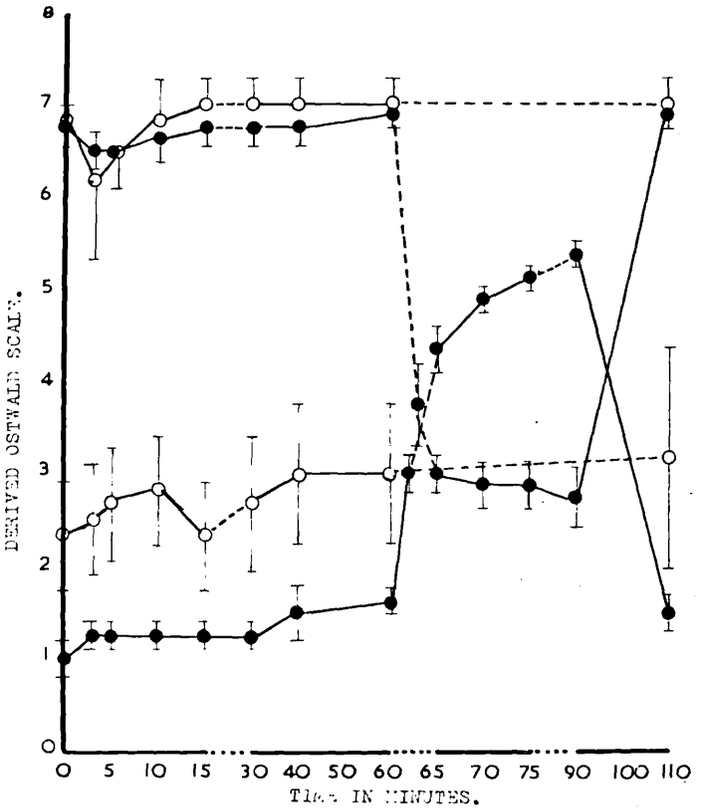
● 11.5 mg/kg in black-adapted fish* (4 animals)

● 11.5 mg/kg in white-adapted fish* (4 animals)

○ 11.5 mg/kg in black-adapted spinal fish (3 animals)

○ 11.5 mg/kg in white-adapted spinal fish (3 animals)

* Intact fish were subjected to background reversal at sixty and ninety minutes.


DERIVED OSTWALD SCALE.
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0 5 10 15 30 40 50 60 65 70 75 90 100 110
TIME IN MINUTES.

after 20 minutes. This pallor was much greater than caused in normal fish by an injection of Young's Ringer. In a second series of injections 9 mg/kg C_6 were injected into black- and white-adapted fish which were subjected to background reversal. The fish transferred from black to white at first paled rapidly to between D.O.S. 2.0 and 2.5 in the first five minutes but then darkened to between D.O.S. 3.5 and 4.5 as the drug began to exert its effect. This darkening subsided after 2-2½ hours. Similarly the white adapted fish placed on black began to darken rapidly at first, but as the drug began to exert its effect this darkening slowed and some fish even began to pale. After 1-1½ hours the fish completed their adaptation.

Two other groups of fish, one black- the other white-adapted, were injected with a combination of 2.7 mg/kg Ach, 2.7 mg/kg eserine and 4.5 mg/kg C_6 (Fig. 96;p.260). The strong pallor previously found in black-adapted fish was no longer seen, only a small pallor similar to that following saline injections occurred. The white-adapted fish showed complex shade changes however. The head and tail of each fish darkened considerably but the region of the body overlying the body cavity darkened much more slowly and to a lesser degree. Recovery of the fish

Fig. 95 The effects of hexamethonium injections on the shade of black- or white-adapted minnows and on their ability to adapt to a new background (19°C.)

● 9.0 mg/kg hexamethonium
(5 white-adapted fish: 6 black-adapted fish)

◑ 9.0 mg/kg hexamethonium
(Subjected to background reversal)
(6 animals on white: 6 animals on black)

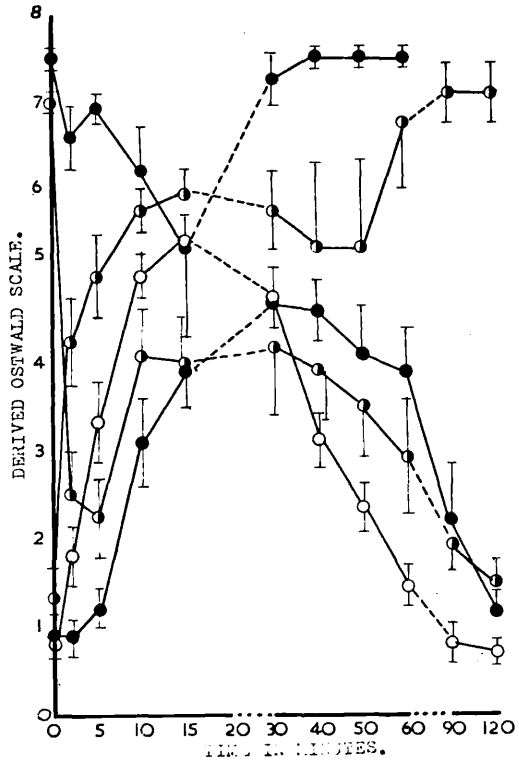
○ 4.5 mg/kg hexamethonium
(13 animals) (White adapted)

Fig. 96 The effects of an injection of 2.7 mg/kg acetylcholine, 2.7 mg/kg eserine and 4.5 mg/kg hexamethonium on black- or white-adapted minnows (19°C.)

● black-adapted fish (5 animals)

◑ head and tail regions of white-adapted fish (5 animals)

○ trunk region of white-adapted fish (5 animals)


DERIVED OSTWALD SCALE.
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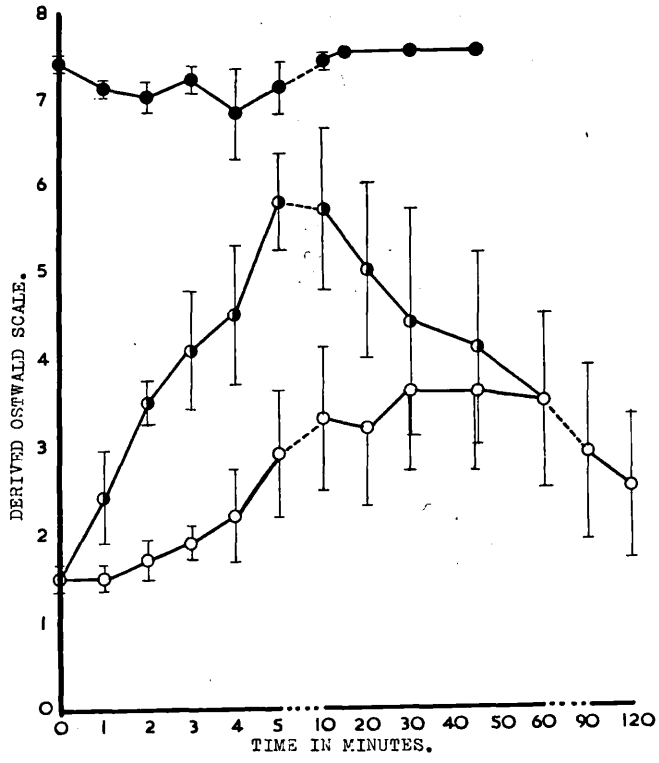
DERIVED OSTWALD SCALE.
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0 1 2 3 4 5 10 20 30 40 50 60 90 120
TIME IN MINUTES.

occurred after 2-2½ hours.

These actions of hexamethonium may be explained in terms of ganglionic blockade. The inability of fish to adapt completely to a new background and to maintain their shade on a background to which they were adapted already implies that C_6 is preventing synaptic transmission in nervous tracts responsible for aggregation or dispersion of melanophore pigment. It is suggested (Section 4.13) that the sensitivity changes of melanophores to catecholamines after spinal-section and spinal nerve-section imply that synapses occur in the chromatic tract between the operative sites. It is reasonable to suppose that these synapses lie in the sympathetic ganglia. The darkening of both white-adapted and white-adapting fish may represent failure of nervous transmission at this site. If an antagonistic nervous system, which is responsible for rapid melanophore dispersion, is also present in the minnow it appears to involve a synaptic transmission involving nictoinic acetylcholine which is susceptible to C_6 blockade. To test this, the effect of C_6 was investigated in white-adapted spinal fish which had been spinal-sectioned fifteen minutes earlier. The intense darkening which follows the operation has been described earlier (Section 3.31) and has been attributed by earlier workers to

stimulation of chromatic darkening fibres. The fish, which had already darkened to 5.0 on the scale (Fig. 97; p.263) would normally have continued to darken to D.O.S. 6.0 in the next 1-2 hours. In this experiment however, the fish began to pale after the injection of 9 mg/kg C_6 and only darkened to the normal shade after a further 45 minutes. It seems, therefore, that C_6 can antagonize the system which leads to melanophore dispersion in much the same way as it antagonizes the sympathetic paling fibres.

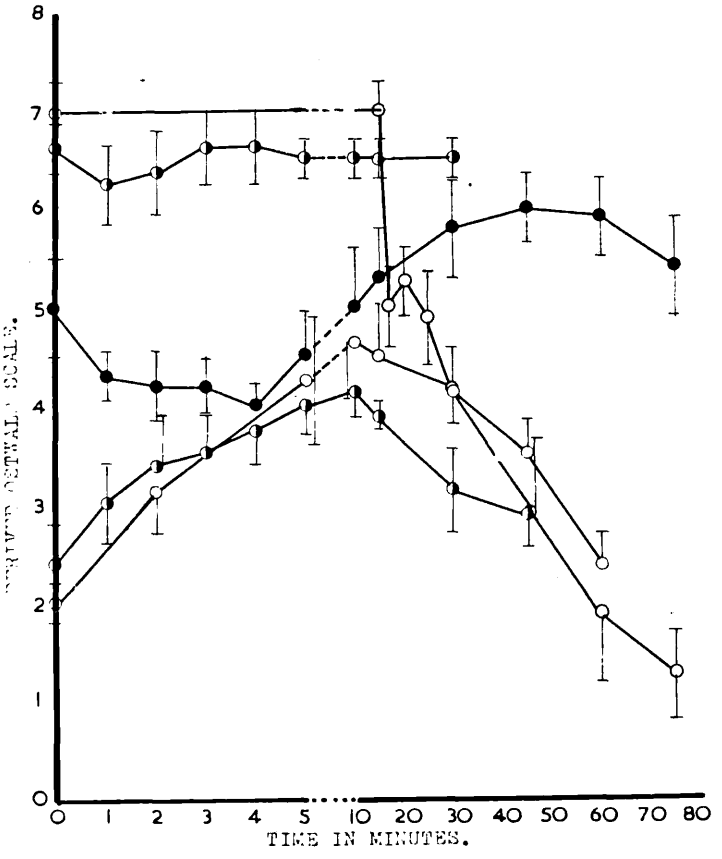
Injections of C_6 were also made into black- and white-adapted spinal fish (Fig. 97;p.263). The black-adapted fish were unaffected by 5.8 mg/kg C_6 but the white-adapted fish were found to darken from 2.5 to 4.0 on the scale. The mechanism of this darkening is not known but it may represent a direct stimulation on melanophores leading to pigment dispersion. If such a direct action of the drug exists interpretation of the effects on unoperated fish described above becomes difficult. There is no reason to suppose that hexamethonium has pronounced anti-adrenergic activity which might account for the dispersion of decentralised melanophores. If the proposal of Umrath and Walcher (1951) is correct, that decentralised sympathetic ganglia develop tonic impulses in the paling

Fig. 97 The effect of hexamethonium on the shade of operated minnows.

● 9 mg/kg in freshly spinal-sectioned minnows (5 animals) (20°C.)

◑ 5.8 mg/kg in black- and white-adapted spinal fish two months after spinal section (4 fish on white: 4 fish on black) (16°C.)

○ 5 mg/kg in five black- and four white-adapted fish which had been subjected to spinal nerve section several weeks previously (22°C.).
After 15 min. the black-adapted fish were transferred to a white background and the shade of the denervated area recorded.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0 1 2 3 4 5 10 20 30 40 50 60 70 80
TIME IN MINUTES.

fibres, it is possible that C_6 is able to depress this action and so cause the shade of the fish to return to the intermediate state seen in the week following the operation of spinal section.

Black- and white-adapted fish with denervated lateral "stripes" were injected with 5.0 mg/kg C_6 (Fig. 97;p.263). It is suggested (Section 4.21) that the melanophores of denervated stripes are affected by neurohumours released from adjacent, normally innervated areas. This control is probably reinforced by circulating chromatic hormones of pituitary origin. The denervated areas of the white-adapted fish did not darken to the same extent as other regions and so stood out as paler areas. This difference may have been due to the increased sensitivity of the denervated melanophores to paling neurohumours present in the tissue fluids and to the added presence of paling hormone in the blood. Adjacent melanophores were presumably less able to react to undestroyed neurotransmitter and their innate capacity to disperse caused the skin to darken. Black-adapted fish, on the other hand, paled to some extent after the injection but the denervated areas did not. Fifteen minutes after the injection these fish were transferred to a white background. Neither the innervated nor the denervated melanophores were able to aggregate

rapidly. The state of the denervated melanophores closely followed that of the melanophores which were unaffected by the section of spinal nerves.

Section 3.642

Nicotine

Nicotine, which is also a potent ganglionic blocking agent in mammals, was injected into black- and white-adapted minnows in doses of 3.6 and 1.5 mg/kg (Fig. 98; p.267). Doses larger than 4 mg/kg were toxic. Neither injection caused dispersion of melanophores of white-adapted fish which might have followed ganglionic blockade of the aggregating fibres. The larger dose caused almost complete pallor in black-adapted fish whilst the lower dose was less effective. 3.6 mg/kg nicotine were injected into other black- and white-adapted fish which were immediately subjected to background reversal. The pale fish, when put on black, at first started to darken but then paled again. Twenty minutes after the injection these fish began to darken slowly but did not become fully dark for another $1\frac{1}{2}$-2 hours. Black-adapted fish, placed on white after the injection, paled rapidly for the first few minutes but were unable to pale completely. Some fish

in fact darkened as the effect of the injected drug became maximal and final adaptation did not occur until one hour after the injection.

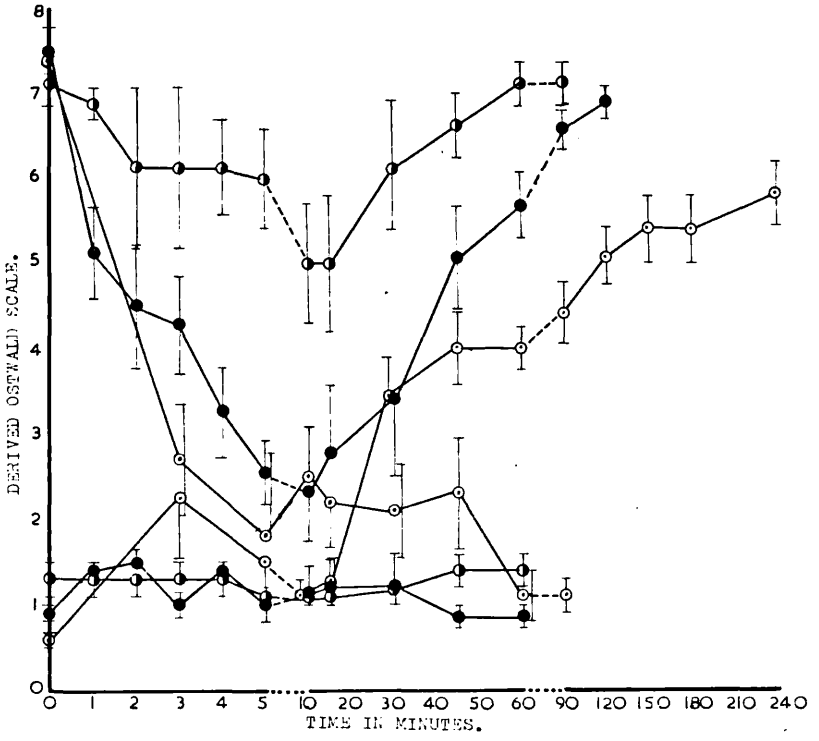
It appears that nicotine has a strong paling action as well as an ability to prevent rapid shade change in both directions. The paling action may represent nicotinic stimulation of sympathetic ganglia, which is only transitory in mammals, or may represent a release of catecholamines from adrenergic nerve stores or chromaffin cells as described by Burn (1961). The inability of black fish to adapt quickly to a white background after nicotine may represent blockade of aggregating nerve stimuli which unmasks a titre of pituitary chromatic hormone in the blood.

A final injection of 7 mg/kg nicotine, 3.5 mg/kg Ach and 3.5 mg/kg eserine was made into white-adapted spinal fish to see if a muscarinic dispersing action of Ach could be detected (Fig. 99; p.267). No such action was seen; in fact the pale fish paled further, reaching a maximal pallor after 20 minutes.

Fig. 98 The effect of injections of nicotine on the shade of black- and white-adapted or adapting minnows.

- ● 3.6 mg/kg (remain on black or white) (21°C.) (11 animals on white: 9 animals on black)
- ⊙ 3.6 mg/kg (subjected to background reversal) (22°C.) (5 in each group)
- ◑ 1.5 mg/kg (remain on black or white) (19°C.) (4 animals on black: 5 animals on white)

Fig. 99 The effect of an injection of 7 mg/kg nicotine, 3.5 mg/kg acetylcholine and 3.5 mg/kg eserine on the shade of white-adapted minnows two months after spinal section. (13°C.) (4 animals)


DERIVED OSTWALD SCALE.
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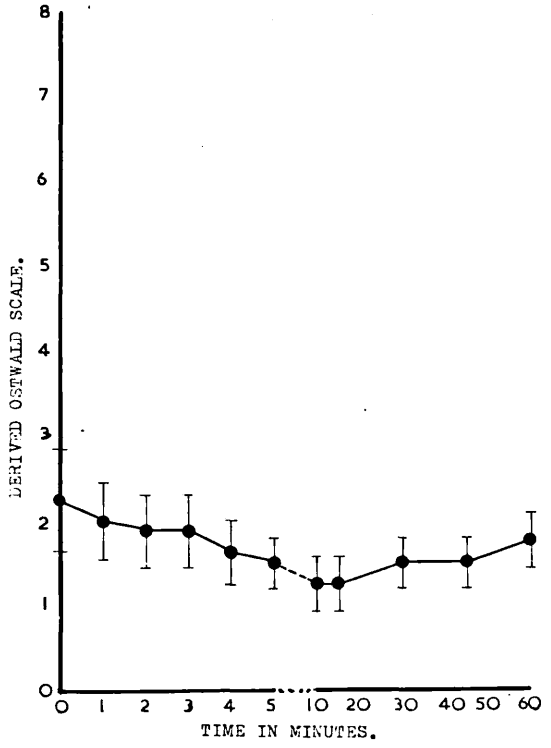
8
0 1 2 3 4 5 10 20 30 40 50 60 90 120 150 180 210 240
TIME IN MINUTES.


DERIVED OSTWALD SCALE.
0
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4
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6
7
8
0 1 2 3 4 5 10 20 30 40 50 60
TIME IN MINUTES.

Section 4.0 DISCUSSION

Section 4.1
The melanophores

Section 4.11
The value of D.O.S. in following colour changes in the minnow

The use of a macroscopic index to follow melanophore pigment changes has proved useful in the minnow. In fish with other colours and with more variegated patterns a macroscopic index would be much more difficult to use. In the latter case it would be necessary to resort to the more laborious Melanophore Index of Hogben or to photo-electric measurements on localised areas of skin. In the present study, which can only be considered a preliminary investigation of the pharmacology of the pigmento-motor system of the minnow, the use of the Derived Ostwald Scale has allowed a simple, graphical reconstruction of shade changes with minimum distrubance of the test animals.

Section 4.12

The varied response of melanophores in the minnow

The groups of melanophores in the lateral stripes and bars of the minnow skin (Fig. 7; p.102) which were less susceptible to aggregating stimuli from the sympathetic nervous system or from injected amines have been described previously by Healey (1951, 1954) and Pye (1964). In addition, Healey (personal communication) observed that a series of sections through the spinal cord of pale minnows, passing progressively forward through the point of exit of the chromatic tract, at first caused dispersion of the "resistant" melanophores and only subsequent dispersion of other effector cells. This difference in reactivity may be caused by different numbers of chromatic fibres to individual melanophores or by differences in the reactivity of the cells themselves. Differential reactions of the annuli of Acanthocottus bubalis also followed injections of noradrenaline and piperoxane. Waring (quoted in Waring 1964) found that the melanophores of the eel also showed varying sensitivities when perfused with melanophore dispersing hormone (MDH). The value of the differential response may be to produce camouflage patterns which break up the outline of the animal and so render it less easily seen.

Section 4.13

The changes in sensitivity of minnow melanophores after various agents and procedures

The normal reactions of minnows to catecholamines and sympathomimetic amines (Sections 3.22 and 3.33) have been shown to change after treatment with cocaine (Section 3.26), hypotensive drugs (Section 3.254) and after operations (Sections 3.33 and 3.43). "Skeleton" dose/response curves for the amines are shown in Fig. 100, p.277 (Table 5, p.272) and sensitivity changes after various agents and procedures are also indicated in Fig. 101, p.278 .

Spinal section led to a gradual development of moderate supersensitivity to noradrenaline. Such supersensitivity has been described in decentralised mammals (Section 1.3263). This enhancement of the action of noradrenaline might be caused by physiological changes at the neuro-effector junction of the chromatic system or may be due to the elimination of an active darkening process which follows spinal section (Section 4.22). Treatment with cocaine enhanced the actions of both noradrenaline and adrenaline to a greater degree than did spinal section, but spinal nerve section produced the most pronounced potentiation of these amines. Trendelenburg (1963)

maintained that denervation sensitization was greater than the sensitivity which followed cocainization (Section 1.326). It may be concluded therefore that at least one synapse lies between the site of spinal section (vertebrae 8-10) and the region where cuts were made in the dorsal and lateral rami of the spinal nerves (Fig. 7;p.102). Between this last operative site and the melanophore lies a single synapse, the neuroeffector junction. As the aggregating chromatic tract passes from the spinal cord into the autonomic chain and thence to the spinal nerves, it is reasonable to suppose that synaptic connections between pre- and post-ganglionic fibres occur in the sympathetic ganglia. Young (1931a) has shown that teleost ganglia are anatomically comparable with those of higher vertebrates.

The injections of amphetamine, tyramine and ephedrine which were made into normal fish proved less potent than did injections of adrenaline and noradrenaline. The resistant melanophores in the patterned areas of the minnow skin were rarely completely aggregated by these agents. Increased doses of amphetamine and ephedrine proved toxic and did not produce complete pallor. The incomplete pallor which has been described may be related to the amount of neurotransmitter present in available adrenergic stores

TABLE 5

The Dose/Response Relationship of Catecholamines and sympathomimetic Amines in the Minnow

NORADRENALINE		ADRENALINE	
Log_{10} Dose	% Response	Log_{10} Dose	% Response
Normal fish			
0.81	86	1.12	88.5
0.44	85	0.81	92
0.32	81.5	0.60	84
0.15	44.5	0.43	80
1.85	20.5	0.13	35.5
1.18	6.2	1.43	10.2
2.18	1.7	2.48	0
0.74	76	1.03	76
0.44	76	0.73	72
0.15	56	0.43	43
Reserpinised fish			
	After one week chronic treatment (white)		
1.85	45.5		
	After one week chronic treatment (black)		
0.15	76		
	After two weeks chronic treatment (white)		
1.85	64	0.60	68
0.32	69	0.13	64

Table 5 Cont.

NORADRENALINE		ADRENALINE	
Log_{10} Dose	% Response	Log_{10} Dose	% Response
	After four weeks chronic treatment (white)		
1.54	59	1.91	78
	After five weeks chronic treatment (black)		
1.18	72	1.43	83
Guanethidine			
	After five weeks chronic treatment (black)		
1.18	88	1.43	92
Bretylium			
	After five weeks chronic treatment (black)		
1.18	88	1.43	92
Cocaine (black)			
1.85	84	0.13	73
1.18	38	1.43	76
2.90	21.5	1.15	16.8
Fresh spinal section (white)			
0.94	90	0.90	79
0.62	60	0.60	50
0.44	41	0.32	22
1.85	38.5		
Decentralised (black)			
0.74	81	1.12	81
0.44	81	0.81	91
0.15	82.5	0.43	82.5
1.85	51		

Table 5 Cont.

NORADRENALINE		ADRENALINE	
Log_{10} Dose	% Response	Log_{10} Dose	% Response
Freshly denervated fish			
black			
0.61	76	0.60	82
0.32	78	0.13	35
1.85	19	1.43	5.3
white			
0.85	85	0.90	81
0.54	70	0.60	72
0.15	67	0.20	69
Denervated (black)			
0.36	86.5	0.46	85
0.08	85.5	1.46	80
1.18	84	2.46	37
2.18	7.2		

Sympathomimetic amines

TYRAMINE		EPHEDRINE		AMPHETAMINE	
Log_{10} Dose	% Response	Log_{10} Dose	% Response	Log_{10} Dose	% Response
Normal fish					
1.32	53	1.05	71	1.09	76
1.06	40	0.83	48.5	0.79	72
0.76	52	0.53	23.5	0.54	53
1.32	42	1.05	69		
1.06	43.5	0.82	59		
0.62	15.1	0.35	54		

Table 5 Cont. Sympathomimetic amines

TYRAMINE		EPHEDRINE		AMPHETAMINE	
Log_{10}Dose	% Response	Log_{10}Dose	% Response	Log_{10}Dose	% Response
Reserpinised fish					
		Acute (white)			
1.06	11.2				
		One week chronic treatment (white)			
1.06	15.8	1.05	67.5		
		0.65	61		
		Two weeks chronic treatment (white)			
1.06	49				
1.32	56				
		Three weeks chronic treatment (white)			
1.06	26.8	0.65	44.5		
1.32	24.5	0.35	22		
		Five weeks chronic treatment (black)			
1.06	20	1.05	46.5	0.95	7.4
Bretylium					
		Five weeks chronic treatment on black			
1.06	39	1.05	45.5	0.95	74
Guanethidine					
		Five weeks chronic treatment on black			
1.06	55	1.05	68	0.95	89
Cocaine (black)					
1.32	75	1.05	35.5	1.20	89
1.24	52	0.83	43	0.90	79
1.06	31	0.43	43	0.60	21

Table 5 Cont. Sympathomimetic amines

TYRAMINE		EPHEDRINE		AMPHETAMINE	
Log_{10}Dose	% Response	Log_{10}Dose	% Response	Log_{10}Dose	% Response
Decentralised (black)					
1.32	45	1.05	51	1.30	59
1.06	43				
Denervated (black)					
1.06	86	0.88	69	0.78	81
0.76	84	0.58	80	0.48	74

Fig. 100 "Skeleton" dose response curves for catechol and sympathomimetic amines. The potentiation of the effects of catechol amines by cocaine, spinal section (=decentralization) and spinal nerve section (=denervation) is represented by a shift of the dose response curves to the left. Dose/response curves for freshly spinal-sectioned minnows show that the sensitivity to catecholamines is lowered.

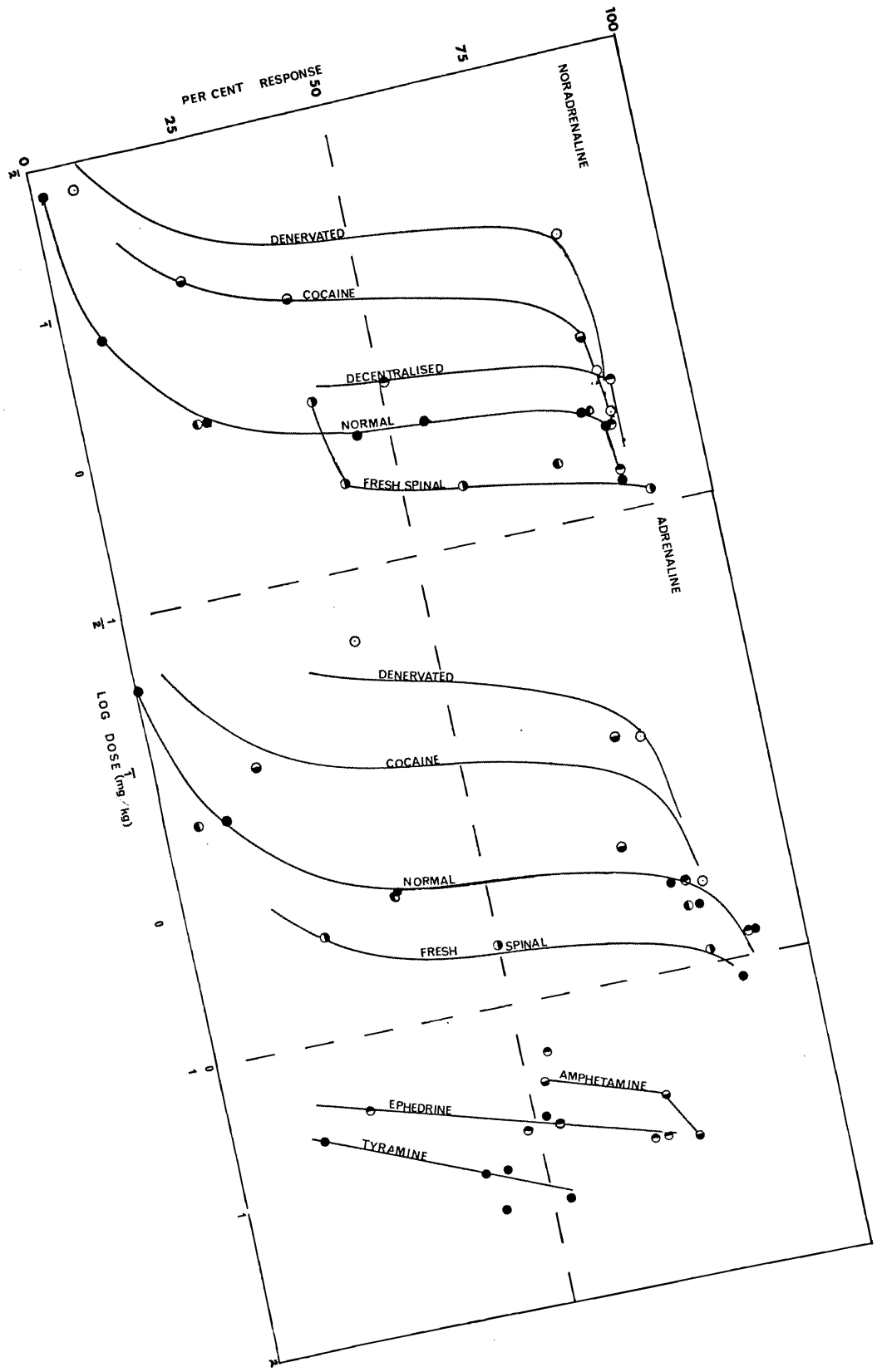

PER CENT RESPONSE
0
25
50
75
100
NORADRENALINE
DENERVATED
COCAINE
DECENTRALISED
NORMAL
FRESH SPINAL
ADRENALINE
LOG DOSE (mg. /kg.)
DENERVATED
COCAINE
NORMAL
FRESH SPINAL
AMPHETAMINE
EPHEDRINE
TYRAMINE

Fig. 101 The changes in potency of catechol or sympathomimetic amines on the minnow melanophores after various drugs and operations. Each column represents the change in sensitivity of the melanophores to a particular dose of an amine after modification of the chromatic response. The numbers represent the $\log_{10}$ dose of amine investigated and the letters refer to the hypotensive drug used. Thus the 31st column indicates that after five weeks chronic treatment of a minnow with reserpine, the response to amphetamine (10 mg/kg) is approximately 70[illegible] less than occurs in normal fish.

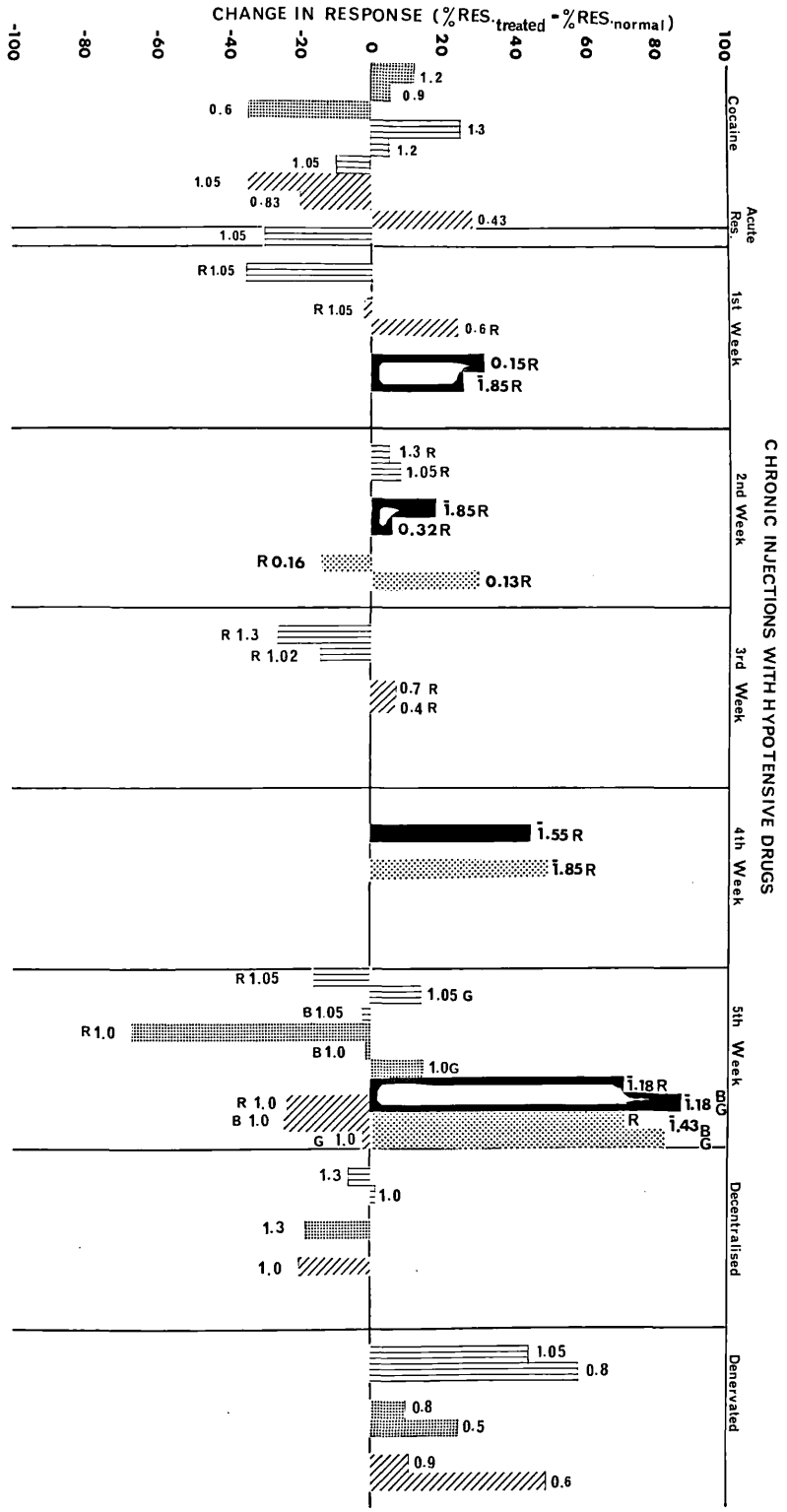

adrenaline


noradrenaline


tyramine


ephedrine


amphetamine


CHANGE IN RESPONSE (%RES.treated - %RES.normal)
-100
-80
-60
-40
-20
0
20
40
60
80
100
Cocaine
Acute Res.
1st Week
2nd Week
3rd Week
4th Week
5th Week
Decentralised
Denervated
CHRONIC INJECTIONS WITH HYPOTENSIVE DRUGS

(Section 1.324) which can be liberated by the indirect actions of sympathomimetic amines. Denervated melanophores were completely aggregated by injections of sympathomimetic amines but it is believed that these agents displaced small amounts of catecholamine from other unaffected regions of the body into the circulation to which the highly sensitive melanophores in the denervated region were able to react completely. After spinal section, which may be considered as decentralisation (Trendelenburg, 1963), the duration, but not the intensity, of the pallor after injection of sympathomimetic amines was increased. It is known that spinal section does lead to physiological changes in melanophores which make them unable to aggregate completely (Healey, 1951, 1954). Cocaine antagonized the effects of low doses of amphetamine and tyramine but potentiated higher doses. This action closely resembles the effects of competitive inhibition of indirect actions of sympathomimetic amines described for cocaine in mammals (Section 1.3261). Both reserpine and bretylium, but not guanethidine, antagonized the indirect actions of the amines (Fig. 102; p.294). The former drug is known to deplete adrenergic stores of neurotransmitter and bretylium is known to exert a cocaine-like action at the transfer site of the mammalian adrenergic store (Section 1.324).

From the studies on amphetamine and tyramine, using cocaine, reserpine and bretylium, it is possible to envisage an adrenergic store at or near the endings of the sympathetic paling fibres which closely resembles the store described in mammalian tissues (Section 1.324). However von Euler and Fänge (1961) found that vagotomy in the cod (Gadus callarias) did not deplete the adrenaline and noradrenaline content of the swim bladder but increased it. In mammals, denervation caused the disappearance from spleen and other tissues of catecholamines stored within the nerve fibres. These workers concluded that adrenergic stores of the kind described for mammals do not occur in the vagus/gas bladder nerve terminals of the cod. Studies of the nerve/melanophore junction in fish using the fluorescence techniques of Falck (1962) and the radioactive catecholamine studies of Axelrod and co-workers (1960 et seq.) are needed to clarify the nature of the adrenergic store. This knowledge is essential before sympathomimetic amines can be adequately classified in fish as exerting "direct" or "indirect" effects.

The observations of Scott (1965) on the potentiation of the aggregating effects of injected catecholamines in the skin of Scophthalamus by pyrogallol may be taken as strong evidence for metabolism of an adrenergic transmitter by catechol O-methyl transferase.

Section 4.14

Adrenergic receptors of the melanophores of the minnow

Gray (1955) suggested that the darkening of intact minnows after the injection of adrenergic blocking agents might involve adrenaline "reversal". Such reversal in mammals requires the presence in the end organ of antagonistic alpha and beta adrenergic receptors. Pye (1964a, b) found that, after ergotamine, electrical stimulation of the chromatic tract caused dispersion instead of the usual aggregation. Treatment of ergotized melanophores *in vivo* and *in vitro* with adrenaline or noradrenaline failed to produce such dispersion (Giersberg 1930, Pye 1964b). He concluded that darkening did not involve reversal of catecholamine effects. In the present study on the same species, the actions of isoprenaline and alderline do not support the concept of antagonistic adrenergic receptors. If two types of receptor are present they are most probably synergistic. Boyd *et al.* (1963) suggested that precise differentiation of autonomic receptors arose relatively late in vertebrate evolution.

On the other hand, Fujii (1961) found that in ergotized *Chasmichthys* melanophores, adrenaline was

reversed and caused darkening. Watanabe *et al.* (1962a) found that continued immersion of *Oryzias* scales in adrenaline solutions led to a secondary dispersion of the melanophores after an initial aggregation. The latter workers claimed that two mechanisms, pigment aggregating (p.c.ms) and pigment dispersing (p.c.m.) were involved. Both are believed to be adrenergic but the former has a lower threshold and fatigues more readily. Thus prolonged exposure to certain concentrations of adrenaline causes aggregation of melanophores through the greater response of the p.c.m. but subsequently the effects of the p.d.m. supercedes and causes dispersion. Their observations may support an alternative hypothesis. Scott (1965) found that metabolites of adrenaline from catechol-O-methyl transferase were chromatically inactive in *Scophthalamus*. It is possible that the accumulation of such metabolites in the *in vitro* preparations of Watanabe *et al.* might compete antagonistically at an adrenergic, aggregating receptor. Sufficient competition could block the active sites and allow an inherent dispersing mechanism of the unstimulated melanophore to supervene. Dispersion of melanophores in *in vitro* preparations of Watanabe *et al.* only occurred in higher concentrations of adrenaline.

Section 4.15

The site of action of adrenergic blocking agents in the minnow

The authors quoted in Section 3.24 have demonstrated that mammalian adrenergic blocking agents exert sufficient side-effects and lack of specificity in lower vertebrates to prevent conclusions being drawn from their use about the neurotransmitters involved at peripheral synapses without support from other experiments. The present work has not involved *in vitro* studies on isolated skin preparations and consequently nothing is known about the direct effect of adrenergic blocking agents on minnow melanophores. Both normal and spinal-sectioned fish are darkened by these blocking agents and this darkening is overcome by catecholamines and pituitary extracts. The nature of the darkening process is obscure. Blockade of aggregating fibres may unmask an inherent tendency for melanophores to disperse and in unoperated fish this may be supported by activity in an antagonistic, dispersing nerve tract. It seems unlikely, in the minnow at least, that the darkening involves a reversal of endogenous adrenaline. The effect of the blocking agents on pituitary mechanisms has been discussed in Sections 3.34, 3.44 and 3.5. The antagonism

between injections of blocking drugs and pituitary extracts may be caused by separate actions on the melanophore receptors which lead to dispersion and aggregation respectively. The adrenergic blocking agents might conceivably interfere with central mechanisms which coordinate pituitary paling hormone release.

The consistency with which the variety of adrenergic blocking drugs prevent paling by the aggregating fibres tends to support the suggestion that the endogenous transmitter is a catecholamine. The potent paling effects of catecholamines and sympathomimetic amines and the nature of the sensitivity changes after various agents and procedures should be taken into account when discussing the action of adrenergic blocking agents in the minnow. Further study of the possible side-effects of the agents is however necessary.

Section 4.2
Postulated mechanisms of colour change in the minnow

Section 4.21
Nervous paling mechanisms

The pathways of melanophore aggregating fibres from the medulla to the skin have been described for the minnow

(Section 1, Fig. 2; p.19). If this sympathetic system is similar to that found in mammals the preganglionic fibres which leave the spinal cord at vertebra 15 would be cholinergic. The potent ability of hexamethonium to darken white-adapted fish might represent a nicotinic blockade of cholinergic activity at sympathetic ganglia. The considerable pallor following nicotine may represent an overriding stimulation of chromaffin tissues (cf. mammals; Burn, 1960). This paling action would mask ganglionic blockade. The postganglionic paling fibres seem to be typically adrenergic. Catecholamines and sympathomimetic amines mimic their effects.
A wide variety of mammalian adrenergic blocking agents prevent paling in the minnow and the use of cocaine, hypotensive agents and lesions in the chromatic pathway affect the actions of catecholamines and sympathomimetic amines in a manner similar to that found for mammalian sympathetic tracts. The terminal branches of the paling fibre neurones, or a nearby structure, appear to store a catecholamine-like neurotransmitter which can be depleted by agents such as reserpine. The released transmitter, which is almost certainly a catecholamine (cf. Fujii, 1961; Watanabe *et al.* 1962b; Scheline, 1963; Scott, 1965) may pass to the postsynaptic membrane (the melanophore) or

into tissue fluids to affect neighbouring melanophores. Catecholamines have been found in teleost tissues (von Euler, 1952; von Euler and Fänge, 1961). The adrenergic receptor appears to bear a close resemblance to the alpha-receptor of mammals. If beta receptors exist they appear to be synergistic with the alpha-receptors and to play only a minor role in colour changes. It is possible that the adrenergic system contains only an undifferentiated type of receptor.

Activity in the paling fibres is brought about by illumination of the dorsal region of the retina (Section 1.21). If, as is suggested by von Frisch's observations on pineal and ventral retinal illumination, a set of antagonistic darkening fibres are continuously active in light, the activity of the paling system is determined by the albedo of the background. Adaptation to intermediate shades of grey would therefore be achieved by tonic discharge in the medullary paling centre related to the lightness of the background. The central pathways which link the retina to the medullary paling centre are not known.

It has been suggested that white-adapted spinal fish may be partly dependant on circulating catecholamines to maintain their pallor (Section 3.34). This catecholamine

level must be less effective during adaptation to a black background as they are potent aggregating substances to which melanophores are moderately supersensitive. Umrath and Walcher (1951) proposed that decentralised sympathetic ganglia develop a tonic discharge which causes melanophore aggregation. Such a mechanism would require an antagonistic darkening agency to allow darkening during adaptation to a black background.

Unoperated fish do not apparently depend on the pituitary gland for prolonged white background adaptation. Such fish are always darkened by adrenergic blocking agents. In fish which exhibit no fast (nervous) colour changes, that is fish treated with hypotensive agents or subjected to spinal section, it appears that the pituitary gland cannot maintain complete pallor. The action of the paling hormone must be reinforced by activity in the paling fibres or by catecholamines from other sources.

Section 4.22

Postulated nervous darkening mechanisms

Von Frisch concluded from his observations (Section 1.23) that a diencephalic centre in the minnow brought about darkening of the fish when illuminated from above by an

inhibition of the medullary paling centre. Other workers proposed that melanophores receive a dual innervation of autonomic fibres which respectively cause aggregation and dispersion.

In the present study (Section 3.1) it was found that minnows were able to darken rather rapidly even after a stay of many months on a white background. When transferred to a black background it is presumed that impulses in the sympathetic aggregating tract cease relatively quickly. However, although there may be an inherent tendency for melanophores to disperse in the absence of such stimulation, there must still be a high titre of paling hormone in the blood of these fish. The rapid darkening of such fish must involve an active darkening process. The unsupported activity of paling hormone is at least able to maintain the shade of the fish at an intermediate grey. Fish with no rapid colour changes (above) change shade extremely slowly.

Unoperated minnows on a white-background were found to darken when injected with adrenergic blocking agents. While it is not established that this darkening is due to the unmasking of an antagonistic set of darkening fibres, it was found that the degree of darkening in spinal sectioned fish was considerably less. Healey (1948 *et seq.*),

Gray (1956) and Pye (1964a) proposed that dispersing fibres in the minnow, if they exist, follow the path of paling fibres. Accordingly spinal section in the present study removed the active darkening stimulus found in fish in overhead illumination. Pye (1964a) found that minnows injected with ergotamine became pale on all backgrounds but darkened when chromatic fibres were stimulated electrically. Innes (1960) showed that natural ergot alkaloids exerted sympathomimetic effects by combining with adrenergic receptors. In the present study dihydroergokryptine was used which in mammals is a less potent stimulant of the receptors. The darkening of minnows after dihydroergokryptine is comparable with Pye's electrical stimulation experiment as the direct effect of ergotamine had to be overcome. It does not seem likely (Section 4.14) that the darkening is brought about by reversal of endogenous catecholamines.

Hypotensive agents were found to abolish fast background adaptations in both directions. Acute injections of reserpine and bretylium darkened white-adapted fish considerably. Injections of the same drugs into fish with no fast colour changes (spinal sectioned fish or chronically treated fish) did not cause this brief darkening.

Parker (1948) claimed that mechanical stimulation of darkening fibres occurred when chromatic tracts were cut. Other workers (Umrath and Walcher, 1951; Gray, 1955, 1956) attribute brief dispersion of denervated melanophores to this form of stimulation. In the present study it was found that sections in the chromatic tracts led to dispersion of the affected melanophores and that these melanophores were less sensitive to catecholamine injections (Sections 3.32 and 3.42). The ganglionic blocking agent, hexamethonium, antagonised the darkening which followed spinal section as did chronic pretreatment with bretylium and guanethidine which, in mammals, block conduction in terminal branches of adrenergic neurones.

In the introduction to this work (Section 1.23) the observations of Gray (1955, 1956a) and Pye (1964c) on caudal bands and local temperature responses in the skin of the minnow were described. Both workers concluded that darkening fibres were present in the chromatic system of the fish, basing their arguments on the speed of change of caudal band melanophores, on the assymetrical responses of caudal band melanophores and on the responses of innervated and denervated melanophores to local temperature changes.

All the above observations tend to support the presence of a peripheral tract of melanophore-dispersing fibres.

However the nature of the transmitter released by such fibres is not known. Apart from the dispersion which consistently followed injection of adrenergic blocking agents, hexamethonium and atropine, little darkening of white-adapted fish could be elicited with the drugs used in this study. Acetylcholine was never seen to cause darkening whilst more specific muscarinic agents such as methachol and bethanechol, which were only tested in unoperated fish with active paling fibres, produced only slight darkening with the doses used. There is little support in the present work for a cholinergic darkening system in the minnow although more rigorous investigation is obviously needed.

The failure of the fast darkening system after the hypotensive agents might be due to a central depressant effect, although bretylium and guanethidine exert mainly peripheral actions in mammals. Bretylium is known to accumulate specifically in mammalian adrenergic fibres. Should a similar property be found in the minnow it would imply that the postulated dispersing fibres are themselves adrenergic. Watanabe *et al.* (1962a, b) invoked the concept of two adrenergic receptors in *Oryzias* melanophores which brought about pigment aggregation or dispersion when stimulated by adrenaline.

It is clear that complete interpretation of the effects of the drugs used in this study must await the elucidation of the anatomy of the autonomic nervous system in fish and the identification of the transmitters involved in its nervous coordination.

Section 4.23

Chromatic hormones

The presence of paling hormone in the minnow pituitary is an established fact (Sections 1.24, 1.25) but the arguments for an antagonistic "intermedine"-like darkening hormone are inconclusive. Kent (1961) concluded that the teleost paling hormone contained the heptapeptide moiety essential for dispersing activity but that other amino acids in the hormone conferred the paling activity specific for teleost melanophores. In the present experiments it was suggested (Section 3.34) that catecholamines might reinforce the action of paling hormone to bring about complete pallor of white-adapted spinal minnows. However, it is necessary that the effect of such amines should be less during adaptation to a black background to allow the fish to darken adequately. Such lessened activity could be obtained if, as the titre of paling hormone falls as the fish adapts to a black background, a

dispersing hormone were released from the pituitary.

Fig. 102, p.294 summarises the proposed components of the colour change system in the minnow and Table 6, p.295 indicates the probable sites of action of the drugs used in this work.

Fig. 102 Diagram of the proposed chromatic effector system of the minnow.

A. adrenergic receptor(s); B. receptor which mediates nerve stimulated darkening; C. receptor for paling hormone; D. possible receptor for intermedine-like darkening hormone; E. receptor for direct stimulation by light or heat; ad.n., adrenergic neurones which aggregate melanophore pigment; b., retinal area which stimulates darkening; (B), systemic release of postulated darkening hormone; b.v., cardiovascular system; Chrom., chromaffin cells; c.m.t., chromatic nerve terminals; coel., coelomic cavity; c.t., spinal chromatic tract; g.r.c., grey ramus communicans; mel., melanophore; m.p.c. medullary paling centre; n.h., diffusing neurohumours from chronatic nerve terminals (B and W); pin., pineal organ; pit., pituitary gland; pr.f., preganglionic chromatic fibres leave the spinal cord in white rami communicantes in the region of vertebrae 12-15; sp.c., spinal cord; sp.n., spinal nerve; sp.p.c., von Frisch's spinal paling centre; symp.ch., sympathetic chain; s.g., sympathetic ganglion; w., retinal area which stimulates paling; W., systemic release of pituitary paling hormone; 1. transfer site to adrenergic store; 2. adrenergic store; 3. terminal branches of adrenergic neurone; 4. terminals of darkening fibres; 5. postganglionic membrane of ganglion synapse; 6. synapse of sympathetic ganglion; 7. hypothalamic centres controlling pigmentary activity of the pituitary gland.

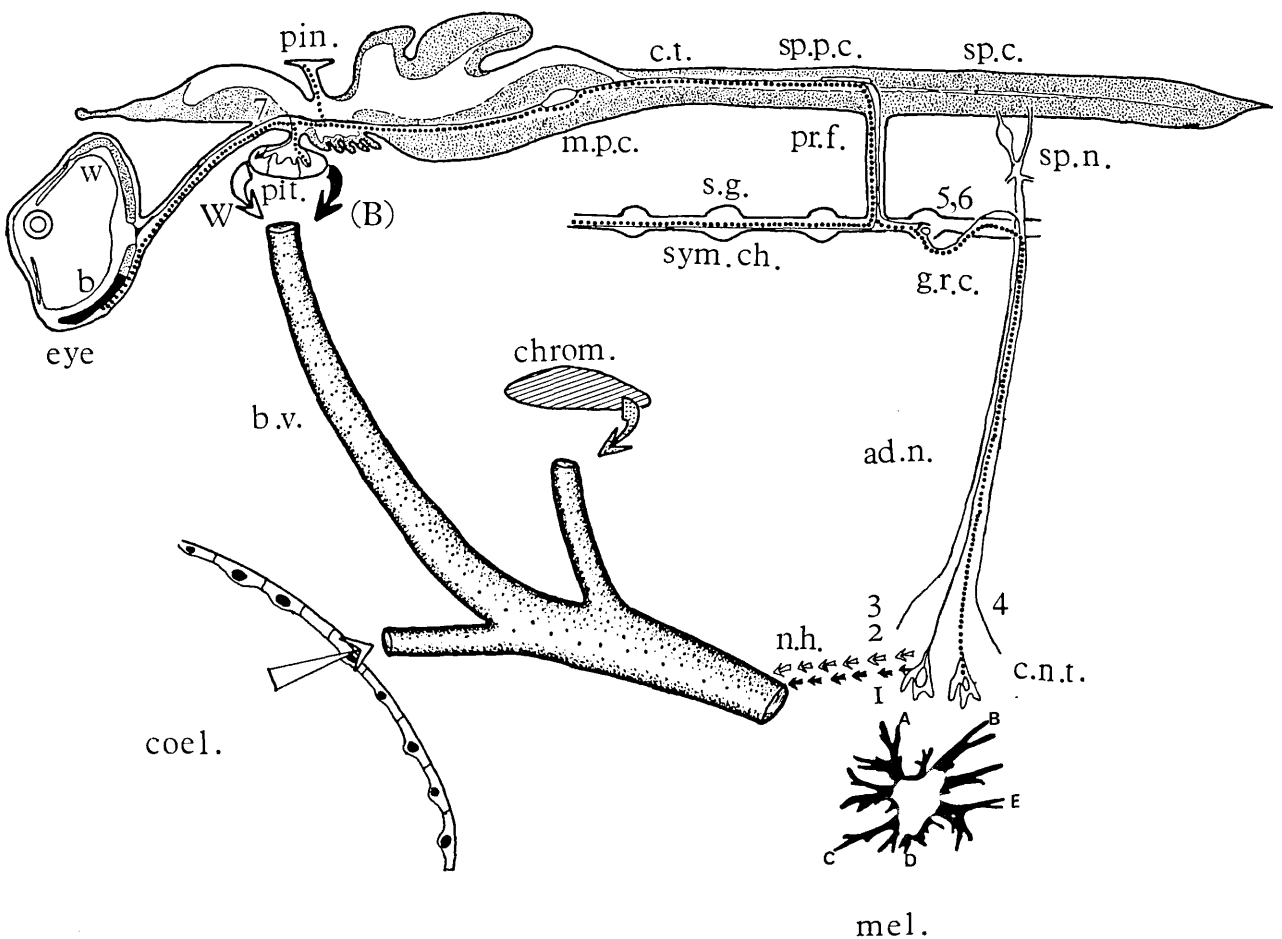

pin.
c.t.
sp.p.c.
sp.c.
m.p.c.
pr.f.
sp.n.
w
pit.
W
(B)
s.g.
5,6
b
sym.ch.
g.r.c.
eye
chrom.
b.v.
ad.n.
3
4
n.h.
2
c.n.t.
1
A
B
E
C
D
coel.
mel.

Table 6

The probable sites of action in the minnow of drugs used in the experimental section

(To be compared with Fig. 102 (p.294))

KEY:
- 0 No effect
- \+ Stimulant or potentiator
- (+) Weak or suspected stimulant
- \- Inhibitor
- (-) Weak or suspected inhibitor
- (?) Possible action at this site as inhibitor or stimulant
- B Causing melanophore dispersion
- W Causing melanophore aggregation

DRUG	A Adrenergic Receptor	B Nervous dispersing Receptor	C Paling hormone Receptor	D M.S.H. Receptor	E Receptor for heat or light	1 Transfer site to adrenergic store	2 Adrenergic store	3 Terminals of Paling fibres	4 Terminals of Darkening fibres	5 Post-synaptic membrane of symp. ganglion	6 Synapse of symp. ganglion	7 Hypothalamic centres	8 Chromaffin tissue
Adrenaline	+	0	0	0	0	+	+	0	0	0	0	(?)	0
Noradrenaline	+	0	0	0	0	+	+	0	0	0	0	(?)	0
Isoprenaline	+	0	0	0	0	(?)	(?)	0	0	0	0	0	0
Ephedrine	+	0	0	0	0	+	+	0	0	0	0	0	+
Tyramine	0	0	0	0	0	+	+	0	0	0	0	0	+
Amphetamine	0	0	0	0	0	+	+	0	0	0	0	0	+

Continued...

Table 6 continued

DRUG	Adrenergic Receptor A	Nervous dispersing Receptor B	Paling hormone Receptor C	M.S.H. Receptor for heat or light D	E	Transfer site to adrenergic store 1	Adrenergic store 2	Terminals of paling fibres 3	Terminals of darkening fibres 4	Post-synaptic membrane of symp. ganglion 5	Synapse of symp. ganglion 6	Hypothalamic centres 7	Chromaffin tissue 8
Phentolamine	-	(?)	0	0	0	(-)	0	0	(-)	0	0	0	0
Piperoxane	-	(?)	(-)	0	0	(-)	0	0	0	0	0	0	0
DHEK	-	(?)	0	0	0	(-)	0	0	0	0	0	0	0
Yohimbine	-	(?)	0	0	0	(-)	0	0	0	0	0	(?)	0
Dibenamine	-	0	-	0	0	(-)	0	0	0	0	0	(?)	0
Cocaine	+	0	0	0	0	-	0	0	0	0	0	0	0
Reserpine	0	0	0	0	0	0	-	0	(?)	(-)	0	0	-
Bretylium	(+)	0	0	0	0	-	(-)	-	(?)	(-)	0	0	(-)
Guanethidine	(+)	0	0	0	0	(?)	(-)	-	(?)	(-)	0	0	(?)
Acetylcholine	(+)	(?)	0	0	0	0	(+)	(+)	0	+	0	0	(?)
Eserine	0	0	0	0	0	0	0	0	0	0	+	0	0
Carbachol	0	0	0	0	0	0	0	0	0	+	0	0	(+)
Methachol	0	(+)	0	0	0	0	0	0	0	0	0	0	0
Bethanechol	0	(+)	0	0	0	0	0	0	0	0	0	0	0
Hexamethonium	0	(+)	0	0	0	0	0	0	0	-	0	0	0
Atropine	(-)	(+)	0	0	0	0	0	0	0	(-)	0	0	0
Homatropine	0	0	0	0	0	0	0	0	0	(?)	0	0	0
Pilocarpine	0	(+)	0	0	0	0	0	0	0	0	0	0	0
Nicotine	(?)	0	0	0	0	0	(+)	(+)	0	(-)	0	0	(+)
Paling hormone	0	0	±	0	0	0	0	0	0	0	0	0	0
Darkening hormone	0	0	0	(+)	0	0	0	0	0	0	0	0	0
Heat	0	0	0	0	B	0	0	-	0	0	0	0	0
Cold	0	0	0	0	W	0	0	0	-	0	0	0	0

SUMMARY

1. The observations of previous workers on the nervous and hormonal control of teleost colour changes are described and the effects on fish colour changes of autonomic drugs studied to date are tabulated.

2. The pharmacological actions of autonomic drugs in mammals are described and used as a model with which to compare the effects of the same drugs in fish. In addition, the nature of adrenergic stores in sympathetic neurones, of adrenergic receptors in effector tissues and sensitivity changes of such tissues after various agents and procedures are discussed.

3. Techniques used to inject minnows and to make lesions in the chromatic tracts and the macroscopic method for estimating melanophore activity with minimum distrubance of the fish are described.

4. Observations on the background adaptation of minnows shows that the rate is dependent on the temperature and previous history of the fish.

5. Injections of catecholamines (adrenaline, noradrenaline) were more potent agents than sympathomimetic amines

(tyramine, amphetamine, ephedrine). Groups of melanophores in the skin exhibited different thresholds to paling stimuli.

6. Competitive adrenergic blocking agents acting on mammalian alpha adrenergic receptors consistently darkened pale minnows. The beta blocking agent, pronethalol, did not darken pale fish but antagonized the paling action of isoprenaline. Alpha blocking agents were overcome by injections of noradrenaline.

7. The non-competitive alpha blocking agent, dibenamine, was only effective after several injections, whereupon pale fish were found to darken almost maximally.

8. Acute injections of hypotensive agents exerted brief sympathomimetic effects, then prevented complete paling and abolished fast colour changes. Hormonal colour changes were apparently unaffected. Chronic treatment with these agents potentiated the effects of catecholamines. The supersensitivity caused by reserpine slowly increased with time of treatment. The paling effects of sympathomimetic amines, on the other hand, were antagonized by reserpine and bretylium but not by guanethidine. In addition, chronic treatment decreased the amount of darkening which followed injection of dihydroergokryptine or

spinal section but failed to unmask any darkening activity of acetylcholine in the absence of sympathetic activity.

9. Cocaine enhanced the actions of adrenaline and noradrenaline and competitively antagonized the effects of tyramine and amphetamine.

10. The operation of spinal section darkened white-adapted fish which assumed an intermediate shade after a few days and did not achieve white background adaptation for several weeks. A small supersensitivity to noradrenaline developed slowly in such fish, but this was not so large as occurred in normal fish treated with cocaine. Immediately after the operation however, the operated fish were found to be subsensitive to catecholamines.

11. Sympathomimetic amines were able to pale black-adapted spinal fish whilst adrenergic blocking agents darkened white-adapted fish less effectively than normal fish.

12. Injections of hypotensive drugs into white-adapted spinal fish caused them to assume an intermediate shade of grey.

13. Sections of spinal nerves caused dark stripes to appear in the denervated area but these stripes disappeared within 24 hours. During this time the denervated melanophores were less sensitive to catecholamine injections but after several weeks developed a sensitivity which surpassed that caused by cocaine. Sympathomimetic amines were also potentiated in the stripes but this may have been the result of displacement of catecholamines from adjacent, normally-innervated areas.

14. Competitive adrenergic blocking agents darkened pale stripes by a direct action by blocking the effect of diffusing transmitters from adjacent areas.

15. The paling effects of plaice pituitary extract injections were unaffected by long term treatment with hypotensive agents but were antagonized to some extent by adrenergic blocking agents, especially dibenamine. No drug prevented the erythrophore dispersion which followed pituitary extract injections.

16. Acetylcholine, carbachol and pilocarpine did not darken pale minnows but more specific muscarinic agents (methachol, bethanechol) showed some indication of darkening activity.

17. Atropine darkened white-adapted fish and homatropine slowed the speed of background adaptations.

18. Ganglionic blocking agents were chromatically active. Hexamethonium darkened white-adapted fish, slightly paled black-adapted fish and antagonized the darkening which followed spinal section. Spinal white-adapted fish were darkened to some extent. Nicotine invariably paled black-adapted fish.

19. The results are discussed on the basis of previous workers studies on fish colour change and vertebrate pharmacology. It is concluded that aggregating chromatic fibres leaving the spinal cord of the minnow probably synapse with postganglionic, adrenergic fibres in the sympathetic ganglia. Such a synapse is likely to be cholinergic. The postganglionic fibres run to the skin melanophores in the spinal nerves and release an adrenergic mediator (dopamine, noradrenaline, adrenaline) at the neuro-effector junction. The store of adrenergic mediator is envisaged as not dissimilar from that envisaged for mammals, as the effects of tyramine and amphetamine are antagonized by cocaine, bretylium, dibenamine and reserpine. The adrenergic receptors are considered either as synergistic alpha and beta receptors or as primitive, undifferentiated receptors.

The presence of an antagonistic set of dispersing fibres is indicated from a variety of observations. Such fibres would appear to follow the same pathway as aggregating (paling) fibres but cannot with certainty be said to be cholinergic in the minnow.

The role of pituitary hormones in colour changes has not been examined systematically here. It appears that the peripheral actions of pituitary extracts are antagonized by adrenergic blocking agents in an unexplained way. The release of paling hormone might be affected by an action of adrenergic blocking agents and hypotensive agents on the hypothalamic/pituitary axis. There is no concrete basis in the present work to support the secretion by the pituitary of an intermedine-like darkening hormone in the minnow.

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