CHROMATIC BEHAVIOUR OF *SCYLLIUM CANICULA*

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(With Five Text-figures)

I. INTRODUCTION

CHROMATICALLY active forms are found in all classes of cold-blooded vertebrates. Changes in skin pigmentation result from 'expansion' and 'contraction' of dermal and epidermal chromatophores. Dermal melanophores are usually the most important in transition from the pale to the dark condition and vice versa. Most species exhibit two kinds of melanophore response to light: (*a*) a primary response of melanophore expansion in light; (*b*) a secondary response of melanophore expansion to overhead illumination in non-reflecting surroundings (black background) and of melanophore contraction to overhead illumination in surroundings which reflect light (white background). The eye is the receptor for the secondary response. The primary response is non-visual. The range of the primary is usually, but not always, slight in comparison with that of the secondary response. With regard to the control of the latter, it is customary to distinguish between humoral and direct nervous co-ordination of the melanophores. Recent work has shown that it is more precise to distinguish between species of two types:

(*a*) those which exhibit humoral control with no direct peripheral nervous intervention;

(*b*) others in which direct nervous control is superimposed to a greater or lesser extent on the more archaic humoral mechanism.

Hogben and Winton (Hogben, 1924) investigated the effect of hypophysectomy, injection of extracts, drugs, nerve section and nerve stimulation on the chromatic behaviour of Amphibia. They concluded that the hypothesis of 'pituitary secretion fluctuating in correspondence with the action of natural stimuli... is in the existing state of knowledge adequate, at least in adult amphibia, to interpret all the salient facts'. Lundstrom & Bard (1932), Hogben (1936), Parker (1937), Wykes (1936), and Waring (1936*a*, 1938) have since shown that elasmobranch melanophores also are under pituitary control. When Hogben's monograph was published (1924) the available data on melanophore expansion and contraction could be explained in terms of increased or reduced secretion of the melanophore expanding hormone.

In 1931, Hogben & Slome published results of an extensive investigation of the chromatic behaviour of *Xenopus laevis*. They postulated that the chromatic behaviour of this animal depends upon fluctuation of two antagonistic hormones, *B* ('expanding') and *W* ('contracting'). *B* is the very stable melanophore-expanding hormone from the intermediate lobe of the pituitary. *W* has not been identified with properties of gland extracts. Evidence for its existence comes mainly from three classes of experiments (Hogben & Slome, 1931, 1936):

(*a*) time relations of the responses of intact animals;

(*b*) responses evoked by the separate removal of the various lobes of the pituitary;

(*c*) differential tolerance of different classes of normal and operated pale animals to equal injections of *B*-containing extracts.

Similar but less complete evidence has been derived from studies on elasmobranchs (Hogben, 1936; Waring, 1936*a*, 1938) and *Anguilla* (Neill, 1940; Waring, 1940; Waring & Landgrebe, 1941). To explore more fully the issues raised in these investigations, the writers planned an investigation of the chromatic effector response of *Scyllium* with the object of (*a*) comparing its behaviour with that of *Xenopus* and *Anguilla*; (*b*) carrying out experiments of a new type to throw light on the ultimate fate of the *B* hormone in the animal body. Wartime restrictions led to withdrawal of boat permits when many of the experiments were incomplete; and there seems little likelihood of obtaining further supplies of fish in the near future. What follows is therefore an interim report on a theme which merits fuller treatment.

Waring (1938, 1942) and Abramowitz (1939) have already reviewed previous work on elasmobranchs. It includes hypophysectomy, injection of pituitary extracts, action of drugs, nerve section and nerve stimulation. About the normal responses of elasmobranchs we still know too little. There are no available data concerning time relations of intact fish during transition from complete darkness to white and black backgrounds with overhead illumination and vice versa.

II. RESPONSES OF INTACT *SCYLLIUM* TO CHANGE OF BACKGROUND AND COMPLETE DARKNESS

Unless kept in large tanks with abundant running water, elasmobranchs will not survive for long periods in captivity. The need to feed and clean in complete darkness is therefore a serious practical problem. The following procedure was adopted. Fish were caught on lines and brought into the laboratory in churns. They were stored in a large indoor concrete tank with completely light-tight cover. The fish were fed once a week with fresh herring and the tank was flushed out the following day. Both these operations were performed at dusk. To avoid even short exposure to diffuse light the fish were starved for 14 days before the beginning of the experiment. No animal was used until it had been under these conditions for at least a month. This precaution was taken to ensure complete equilibration. The tanks for providing white and black 'backgrounds' were supplied with abundant running water. An incandescent gas lamp was placed at a fixed distance above each

tank. Responses of the dermal melanophores are recorded in accordance with the Hogben scale.

Table 1 shows the mean equilibration values of *Scyllium*.

Table 1

	No. of fish	Melanophore index (μ)
On white background in light	15	1·4
On black background in light	15	4·9
In total darkness	15	2·9

The responses to change of background, etc. are summarized in Figs. 1, 2, 3 and Table 2.

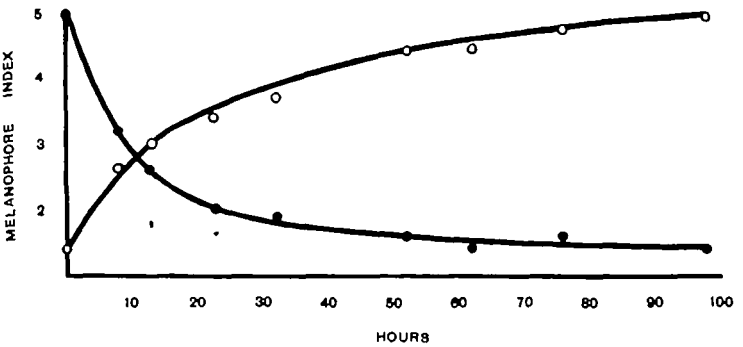

Fig. 1. *Scyllium canicula*. Background reversal in light. 16° C. Each point is average m.i. from six fish.

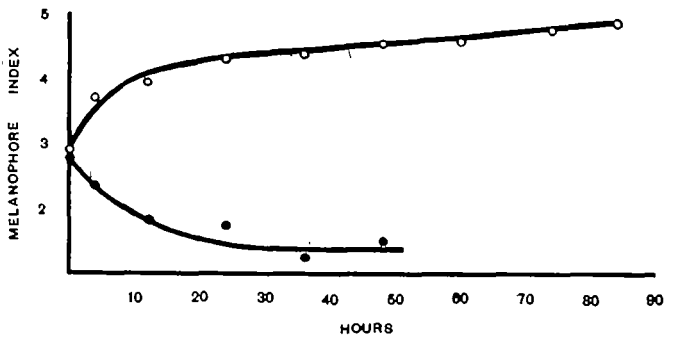

Fig. 2. *Scyllium canicula*. Transition from complete darkness to white and black tanks with overhead illumination. 15–16° C. Each point is average m.i. from six fish.

The time graphs are strikingly similar to those of *Xenopus* and *Anguilla*. Colour change of all three is slow. They all equilibrate at an intermediate condition in complete darkness. In dogfish the prolonged ${}_{w}T_{d}{}^{*}$ is similar to that of the other

* Notation adopted by Waring (1938) and Neill (1940) at Hogben's suggestion: ${}_{b}T_{w}$ = time taken to change from equilibrium on *black* background in light to equilibrium on *white* background in light, ${}_{d}T_{b}$ = time taken to change from equilibrium in complete darkness to equilibrium on black background in light, etc.

two species. We can summarize the chief points of difference between *Xenopus*, *Anguilla* and *Scyllium* as follows:

Xenopus	*Anguilla*	*Scyllium*
${}_wT_b < {}_bT_w$	${}_wT_b = {}_bT_w$	${}_wT_b > {}_bT_w$
${}_dT_b < {}_dT_w$	${}_dT_b < {}_dT_w$	${}_dT_b > {}_dT_w$
${}_dT_b < {}_bT_d$	${}_dT_b > {}_bT_d$	${}_dT_b > {}_bT_d$

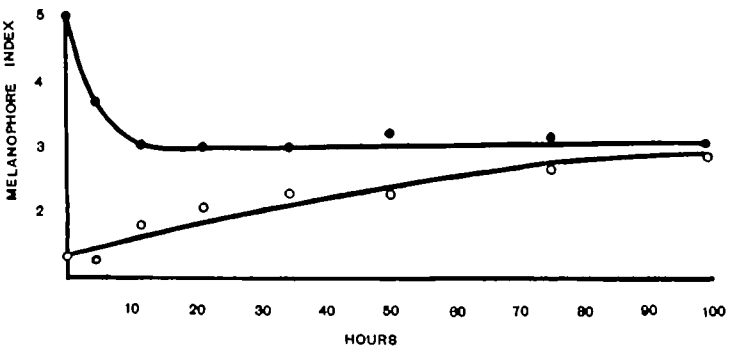

Fig. 3. *Scyllium canicula*. Transition from black and white tanks with overhead illumination to complete darkness. 15–16½° C. Each point is the average m.i. from six fish.

Table 2

	μ range	Time (hr.)
${}_bT_w$	5·0 1·5	60
${}_wT_b$	1·5 5·0	100
${}_dT_b$	3·0 5·0	85
${}_bT_d$	5·0 3·0	12
${}_dT_w$	3·0 1·5	35
${}_wT_d$	1·5 3·0	100

So far *Scyllium* is the only species for which ${}_wT_b$ is greater than ${}_bT_w$ and for which ${}_dT_b$ is greater than ${}_dT_w$. There is independent evidence that *B* is eliminated or destroyed very rapidly in this fish. An injection of *B* sufficient to evoke complete darkening (Waring, 1938) is removed from circulation much more rapidly than in *Xenopus* and *Anguilla*.

III. SIGNIFICANCE OF TIME

Hogben (1924), Hogben & Slome (1931, 1936) and Neill (1940) emphasize the importance of studying time relations before drawing far-reaching conclusions as to the nature of the co-ordinating mechanism concerned. In a co-ordinated response of the type under consideration, a complete cycle of behaviour involves (*a*) time taken for the stimulus to act on the eye, (*b*) time taken for the impulse to reach the pituitary, (*c*) time taken for the pituitary secretion to reach (or fall below) an excitant level in the circulation and (*d*) time taken for the effector to execute its response. The time for (*a*) and (*b*) may be taken as under a minute and any excess within the period of the complete cycle must be due to (*c*) or (*d*). (*d*) can be measured independently and, if significant, we can make due allowance for it.

The melanophore speed of Scyllium canicula

Waring & Landgrebe (1941) investigated the rates of melanophore contraction and expansion of *Anguilla* and *Xenopus*. They perfused whole preparations with (*a*) physiological saline and (*b*) saline containing either posterior lobe pituitary extract or adrenalin. The state of the melanophores was frequently under observation.

In preliminary experiments with *Scyllium* we used a similar technique. Owing to shortage of animals we were forced to adopt an alternative method. The fish remained for long periods in black tanks with overhead illumination. In these circumstances all the melanophores were fully expanded ($\mu = 5$). Rectangular pieces were cut from the edge of the pectoral fin. The cut edge of the skin was separated from the underlying muscle and skeletal elements and the whole dorsal skin with melanophores stripped free. Small pieces were observed microscopically in excavated glass blocks with abundant saline. The saline was changed frequently. When the melanophores were fully contracted, pituitary extract was added to the saline. The graph (Fig. 4) represents the results of one experiment. Each point is the average reading of the melanophores on four pieces of skin taken from the same animal. The lowest reading on the graph is $\mu = 2$. In about half of the experiments a lower figure (1·5) was obtained, but in all cases the lowest reading was reached within approximately 30 min.

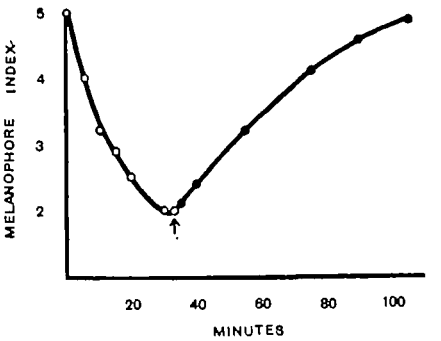

Fig. 4. *Scyllium canicula*. Pigmentary effector speed. 16° C. —○— skin immersed in elasmobranch Ringer. —●— skin immersed in elasmobranch Ringer plus pituitary extract. ↑ = addition of pituitary extract to saline solution. Each point is the average m.i. from four pieces of skin under identical conditions.

The melanophore speed of *Scyllium* as of *Xenopus* (Waring & Landgrebe, 1941) and *Rana* (Waring & McLeod, unpublished) is slow. A comparison of Fig. 4 with Figs. 1, 2 and 3 shows that *the slow effector speed is not the limiting factor in any of the normal responses of the intact animal.* Hence we are justified in interpreting the various time relations ($_bT_d$, $_bT_w$, etc.) as indices of *delay in the process of co-ordination.*

IV. NATURE OF THE CO-ORDINATING MECHANISM

Prior to the work of Hogben & Slome on *Xenopus* it was generally believed that only one pituitary hormone is involved in chromatic behaviour. This view is still that of some authors, and is usually referred to as the *one-hormone hypothesis*. According to the one-hormone hypothesis, production (or release) of the melanophore expanding hormone (*B*) by the pituitary is inhibited on a white background and augmented on a black background in light. This implies that the hormone concentration is highest on a black background ($\mu = 5$), least on a white background

($\mu = 1$) and intermediate in darkness ($\mu = 3$). Hogben believes that this hypothesis is incorrect for the following reasons (inter alia). If it is correct, ${}_{w}T_{b}$ is the time taken for *maximum* increase in concentration of hormone; and both ${}_{w}T_{d}$ and ${}_{d}T_{b}$ involve *smaller* increases in concentration. So neither ${}_{w}T_{d}$ nor ${}_{d}T_{b}$ can be greater than ${}_{w}T_{b}$. Similarly, ${}_{b}T_{w}$ is the time taken for *maximum* decrease in concentration of hormone. So neither ${}_{b}T_{d}$ nor ${}_{d}T_{w}$ can be greater than ${}_{b}T_{w}$. These deductions do not agree with observation. In *Xenopus* and *Anguilla* ${}_{w}T_{d}$ is greater than ${}_{w}T_{b}$. In *Anguilla* ${}_{d}T_{w}$ is greater than ${}_{b}T_{w}$. In *Scyllium* ${}_{w}T_{d}$ is the only time interval which conflicts with the requirements of the one-hormone hypothesis.

The alternative hypothesis suggested by Hogben postulates a second hormone (*W*) which is antagonistic to *B*. The existence of a contracting hormone (*W*) permits of a rational interpretation of the observed time relations (Hogben & Slome, 1931, 1936; Neill, 1940 and Waring, 1942).

For many animals it is established that the background response is due to the stimulation of localized photoreceptors (v. Frisch, 1911; Sumner, 1933, 1940; Hogben & Slome, 1936; Butcher, 1938; Hogben & Landgrebe, 1940). Practical difficulties have prevented similar work on elasmobranchs. In the absence of evidence to the contrary we may assume that

(*a*) photic stimulation of ventral retinal elements evokes release of *B* hormone into the circulation;

(*b*) stimulation of dorsal retinal elements either inhibits the release of *B* hormone (one hormone hypothesis) or evokes secretion of a contracting hormone *W* (bihumoral hypothesis).

Table 3 shows time relations set forth in Figs. 1, 2 and 3 tabulated in relation to the two hypotheses.

We can examine the claims of the two hypotheses from a different angle. If stimulation of the dorsal retinal elements inhibits the secretion of *B* into the circulation, such inhibition may take place in one of two ways: (*a*) inactivation of anabolic activity in the secretory elements; (*b*) prevention of release of the finished

Table 3

	One-hormone hypothesis	Two-hormone hypothesis
1. ${}_{w}T_{b}$	*Maximum* increase of *B* due to *release* from reflex inhibition by *dorsal* retinal elements	*Simple* decrease of *W* (while *B* remains constant. *B*: *W* ratio increasing)
2. ${}_{b}T_{w}$	*Maximum* decrease of *B* due to reflex inhibition by *dorsal* retinal elements	*Simple* increase of *W* while *B* remains constant. *B*: *W* ratio diminishing
3. ${}_{b}T_{d}$	Decrease of *B* due to non-stimulation of *ventral* field	Decrease of *B*
4. ${}_{d}T_{b}$	Increase of *B* due to stimulation of *ventral* retinal field *alone*	Increase of *B*
5. ${}_{d}T_{w}$	*Sub-maximum* decrease of *B* due to *prepotent* reflex inhibition of *B* through stimulation by dorsal field	*Concomitant* increase of *B*, and of *W*
6. ${}_{w}T_{d}$	*Sub-maximum* increase of *B* due to release from prepotent reflex inhibition by dorsal field	*Concomitant* decrease of *B*, and of *W*

product. If (*a*) is true, the one-hormone hypothesis implies that the gland of an animal equilibrated on a white background contains little *B*. So transition to darkness involves the *build up of B both in the gland and in the circulation* without retinal stimulation. We should expect this to be relatively slow. If (*b*) is correct, the one-hormone hypothesis implies that the gland of a fish equilibrated at $\mu = 1$ contains excess of *B*. Transition to darkness therefore involves *release into the circulation of B already stored in the gland.* We should naturally expect this to be rapid. So we can furnish a plausible interpretation of ${}_{w}T_{d}$ in terms of one hormone if the glands of fish equilibrated on a white background contain little *B*; and we can put this conclusion to a direct test.

Accordingly, fish were kept in complete darkness, and in white or black tanks with overhead illumination (13° C.). After 10 days the melanophores were read, the fish decapitated and the pituitaries immersed in acetone. Glands from animals kept in darkness were dissected in very dim light. The *B* content of each gland was determined and is expressed (Table 4) in the melanophore unit proposed by Landgrebe & Waring (1941).

Table 4

No. of fish	Tank	Dry weight of neuro-intermediate lobe in mg.*	Total L.W. melanophore units in neuro-intermediate lobe	L.W. units per mg.
1	Complete darkness	2·5	200	80
2	do.	2·2	180	82
3	do.	—	200	—
4	White tank with overhead illumination	2·9	50	16
5	do.	3·2	300	94
6	do.	2·0	240	120
7	Black tank with overhead illumination	2·6	150	57
8	do.	2·0	120	60
9	do.	—	200	—

* On a balance accurate to 0·05 mg.

The results are sufficient to indicate that there is a considerable store of *B* in the glands of fish kept on a white background. Previous work (Waring, 1936*b*) has shown that there is sufficient *B* in the neuro-intermediate of *Scyllium* from illuminated tanks fully to darken four pale fish. It is therefore extremely difficult to believe that the transition from an illuminated white background to darkness involves only the release of inhibition so that the stored *B* can enter the blood stream. If, then, we are prepared to interpret the chromatic behaviour of *Scyllium* in terms of one hormone, we must also conceive of more intricate optic-hypophysial connexions than what we can infer from available experimental evidence.

Many workers subject pituitary extracts and blood samples to caustic soda treatment before assaying their *B* content (Kleinholtz & Rahn, 1940; Levinson, 1940). Levinson estimated *B* activity from the *length of time* that pale animals remained

dark after injection. The degree of potentiation after caustic soda treatment is not necessarily correlated with the *B* content of the untreated material. So assays made after such treatment may be misleading. Our own assays were made on simple aqueous extracts. This is justified because elasmobranch glands contain little or no pressor substance. Caustic soda destroys pressor and oxytocic activity and modifies the melanophore excitant properties. Landgrebe & Waring (1941) have reviewed the literature and made new observations on this modification. Their chief conclusions were

(1) Caustic treatment of whole posterior lobe extracts increases (*a*) expanding power of the extract and (*b*) duration of the response evoked by sub-maximal doses.

(2) Destruction of the pressor autacoid is *not* the prime cause of these effects.

(3) Caustic soda does *not* modify the *B* molecule itself.

(4) 1 (*b*) certainly and probably 1 (*a*) also are due to the modification of some constituent of posterior lobe extract other than *B* or pressor.

In 1933 Koller and Rodewald observed that pituitaries from frogs kept in total darkness have a lower *B* content than those from frogs in an illuminated environment. Jores (1934) confirmed this result but claimed that after caustic soda treatment of the two extracts the difference disappeared. He suggested that the gland of a frog kept in darkness contains a store of *precursor B* and that the latter is activated by caustic soda. Assays of dogfish glands (Table 4) do not suggest a substantial reduction of *B* content in darkness. We ourselves have also tested the effect of caustic soda on glands from fish kept under different conditions. After the assays already recorded the remainder of the pulverized neuro-intermediate lobes from numbers 1, 2 and 3 were thoroughly mixed and a weighed quantity extracted with boiling water. After filtration the extract was divided into two equal parts. Both were made up to *N*/10 with normal caustic soda. One was immediately neutralized with *N* HCl and both were boiled in a water bath for 10 min. After cooling, the extract in the second tube was neutralized. Material from glands 4, 5, 6 and 7, 8 and 9 were similarly treated. The six extracts were assayed by injection into *Xenopus*. Landgrebe & Waring (1941) found that caustic soda treatment of extracts of dogfish glands results in a fourfold increase of potency. In sub-maximal responses such treatment prolongs the duration of response. In the present series we found no difference between the three groups either with respect to degree of potentiation or to duration of response.

V. EFFECT OF THYROIDECTOMY

The effect of thyroidectomy on chromatic function was investigated for two reasons:

(*a*) *B* is probably a complex polypeptide usually bound to a protein. Thyroxine acts on the cells themselves, primarily in facilitating changes in protein metabolism (Mansfield, 1935), and therefore possibly destruction of *B* in the tissues (cf. Landgrebe & Waring, 1941).

(*b*) Hogben & Slome (1936) found that after anterior lobe removal *Xenopus* is less tolerant to *B* substance. They postulated that the pars glandularis secretes a

substance *W* antagonistic to *B*. This differential tolerance has been confirmed (*Scyllium*, Waring, 1938; *Anguilla*, Waring, 1940). Abramowitz (1939) suggested that these results might be due to a 'differential metabolism of the injected *B* hormone'. The metabolic rate of mammals and frogs (Winton & Hogben, 1923) is in fact lower after hypophysectomy, and the fall may be largely attributed to diminished thyroid activity.

Experiments designed to explore the relation of the thyroid to chromatic co-ordination were of two kinds:

(i) *Effect of lighting conditions and hypophysectomy on the thyroid*

If photic stimuli affect thyroid activity they presumably do so by pituitary mediation as with the gonads (Marshall, 1936; Rowan, 1938). There is abundant evidence that the A.L.P. controls the thyroid of mammals, reptiles and amphibia. We have not encountered reports of similar work on elasmobranchs. So we examined the thyroids of three fish which had been hypophysectomized 1 month previously.

To test whether lighting conditions affect the thyroid the following experiment was set up. Three groups each of three intact fish were used. One group was kept in absolute darkness, a second group in illuminated white tanks, the third group in illuminated black tanks. After 8 days the fish were killed. The thyroid gland was dissected out, weighed and fixed in Bouin. Relevant data with reference to the size of the gland are in Table 5.

Table 5

	Melano-phore index	Sex	Weight of fish gm.	Length cm.	Thyroid Wet wt. (mg.) per kilogram body wt.
A	Intact in complete darkness	3·5	Male	590	61	41
B		3·0	Male	540	58	42
C		3·0	Female	515	57	97
D	Intact in illuminated white tank	1·5	Male	370	51	40
E		1·5	Male	450	52	44
F		1·5	Female	1150	68	91
G	Intact in illuminated black tank	5·0	Male	670	66	23
H		5·0	Male	480	55	65
I		5·0	Female	640	59	246
J	Completely hypophys-ectomized	1·0	Female	590	58	161
K		1·0	Female	928	66	98
L		1·0	Female	590	62	52

(A to I inclusive are the same fish as 1–9 inclusive of Table 4)

The thyroids were cut into complete serial sections. There was no consistent histological difference between glands of the different groups. Morphological signs of inactivity can be observed in mammals 1–2 weeks after hypophysectomy (Rowlands, 1935). Times for the involution of mature cold-blooded animals are not numerous. Adams & Martindale (1936) found that activity was completely suspended 5 weeks after hypophysectomy of *Triturus*. The epithelium of the glands from hypophysectomized *Scyllium* showed definite signs of activity. So either the

A.L.P. has no control over its activity or elasmobranch glands take longer to undergo involution than other species after removal of the pituitary stimulant.

(ii) *Effect of thyroidectomy*

The elasmobranch thyroid is accessibly situated immediately ventral to the union of the first afferent branchial arteries with the ventral aorta. The main body of the thyroid is a discrete mass attached to the surrounding tissues by a dozen or more distinct strands of connective tissue containing blood vessels. At the anterior end the thyroid is drawn out into a tongue which extends forward towards the union of the two Meckels cartilages. In all our experiments the whole gland was removed.

Thyroidectomized fish are capable of chromatic response. Table 6 shows the equilibration values under different conditions. Fig. 5 is the time graph of transition from white to black background and vice versa.

Table 6

	No. of fish	Average melanophore index
Complete darkness	6	2·1
White tank, Overhead illumination	12	1·1
Black tank, Overhead illumination	12	4·3

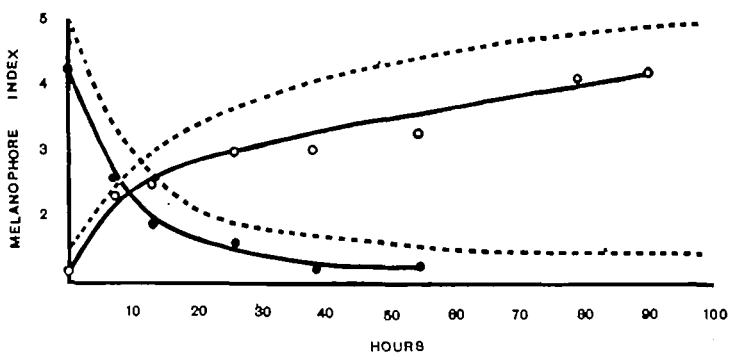

Fig. 5. *Scyllium canicula.* Background reversal of intact and thyroidectomized fish in light. Each point is the average m.i. from six fish. ——— thyroidectomized fish (14–15° C.). ---- intact fish (16° C.).

The data show:

(*a*) equilibration values of operated fish are lower than normal;

(*b*) the speed of response in either direction is approximately the same in operated and intact animals.

Observations on transition from complete darkness to black background with overhead illumination and vice versa were made on only three fish. They indicate no striking difference between normal and operated animals.

These findings are open to several interpretations. They lend no support to the view that thyreo-globulin facilitates the destruction of *B.* If it does, its action is

overruled by other agencies in this class of experiment. In any case, the data recorded do not support the objection of Abramowitz to the two-hormone interpretation of differential tolerance, after total or partial hypophysectomy.

SUMMARY

1. Dermal melanophores of intact *Scyllium* equilibrate at $\mu = 4{\cdot}9$ and $\mu = 1{\cdot}4$ respectively on black and white background with overhead illumination. They equilibrate at $\mu = 2{\cdot}9$ in complete darkness.

2. Normal responses are of the 'slow' type.

3. Expanded melanophores in skin strips immersed in saline contract in 30 min. In pituitary extract they expand in 60 min. So we may interpret the slow responses of intact fish as indices of the slow rise and fall of co-ordinating hormones in circulation.

4. The time relations of normal responses are not consistent with a one-hormone hypothesis.

5. Thyroids removed from animals hypophysectomized a month previously afford no evidence for pituitary control of thyroid activity.

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