ON THE EXISTENCE OF TWO TYPES OF CHROMATIC BEHAVIOUR IN TELEOSTEAN FISHES

By R. M. NEILL

Department of Natural History, University of Aberdeen

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(With Seventeen Text-figures)

I. INTRODUCTION

It is well known that colour changes of animals may be brought about by (*a*) direct action of light, heat and other stimuli on the skin chromatophores, as, for example, in the "primary" photic response of Amphibia, and (*b*) action of stimuli on localized receptors.

Co-ordinated responses, i.e. responses of the second type, fall into two categories which offer a striking initial contrast in their time relations.

(i) *Rapid* type, e.g. the responses of the chameleon to local electrical stimulation and of the minnow to light reflected from its surroundings.

(ii) *Slow* type, e.g. the photic responses of Crustacea, Amphibia, Cyclostomes and Elasmobranch fishes.

II. SIGNIFICANCE OF TIME

In any co-ordinated response, a cycle of behaviour involves (*a*) the time taken for the stimulus to act on the receptor, (*b*) the time taken for the distribution of a disturbance through the co-ordinating mechanism, (*c*) the time taken for the effector to execute its response.

Where the eye is the receptor (*a*) is at most a matter of seconds.

If the mechanism is a nervous one (*b*) involves (i) transmission of the nervous impulse along the nerve trunks concerned, (ii) delay at synapses, (iii) delay at the nerve effector junction. A maximum distribution time value for these taken together is also at most a matter of seconds.

The maximum time for (*a*) and (*b*) together may therefore be taken as under 1 min., and any excess within the period of the complete cycle must be due to (*c*).

If, therefore, it is found that the total time for complete colour change, i.e. for the complete cycle, is of the same order as the effector-reaction time (*c*), then there is nothing to suggest that the co-ordinating mechanism is other than the nervous reflex.

If, however, the total time for the colour change is markedly greater than the effector time, then some other co-ordinating mechanism must be involved, e.g. the reflex liberation of hormone and its gradual accumulation in the blood stream to a certain limiting value.

Effector-reaction time can be assigned a maximum value on the basis of experiments with substances which bring about "contraction" or "expansion" of melanophores. For example, Osterhage (1932), employing a wide range of tissue extracts and other substances, found that the time taken to bring about contraction or expansion of melanophores from an intermediate state in *Gasterosteus* and *Rhodeus* ranged from 6 to 50 min. More recently Waring (unpublished), from perfusion experiments with fresh pituitary posterior lobe extract, has found the reaction time of fully contracted dermal melanophores in *Xenopus laevis* to be 75 min. for complete expansion. Vertebrate melanophores in general appear to react rather slowly as compared with muscle.

It may be concluded, therefore, that where the total time taken for colour change exceeds two hours hormonal co-ordination is indicated. On the other hand, a total change time of the order of 10 min. or less may be safely held consistent with predominantly nervous co-ordination, through the direct innervation of the melanophores themselves.

Within these limits, at least, the time relations of the chromatic behaviour of any vertebrate animal furnish a guide to the type of co-ordination involved.

The behaviour of dermal melanophores only is referred to in the present paper. Melanophores were assessed on the scale first introduced by Hogben for Amphibia. On this scale a melanophore index (μ) of 1·0 denotes complete contraction (aggregation of pigment) and a melanophore index $\mu = 5{\cdot}0$ denotes complete expansion (dispersion of pigment).

III. MELANOPHORE READINGS

As has been stated, the pigmentary effectors dealt with in this paper are the large dermal melanophores, analysis of whose mechanism is sought. Each recorded point of the time graphs presented is the mean of a number of animals whose melanophore indices were assessed on the above-mentioned scale after careful microscopic examinations of the state of a number of individual *dermal* melanophores, at a constant site, in the *living animal*.

This allows the state of the dermal melanophores at any given moment to be recorded quantitatively and accurately, irrespective of the general colour or shade of the whole animal.

In the eel, for example, two other classes of pigmentary organs at least are present in the skin—epidermal melanophores and xanthophores. All three classes of pigmentary effectors contribute to the naked eye or visible appearance of the animal.

The following protocol is relevant to this point. No two of the animals presented quite the same appearance to the eye.

Eel, white tank, 9° C. 168 *hr.*

Dermal melanophores	Epidermal melanophores	Xanthophores	Naked-eye appearance
2·5	1·5	1·5	Very pale
3·0	2·0	1·5	Medium
3·0	2·0	1·0	Pale
3·5	3·5	1·0	Dark medium
3·5	3·5	2·0	Darkish
3·5	3·0	2·5	Quite dark

The three classes of effectors must therefore be examined separately when nalysis of their mechanism is undertaken, otherwise serious error is bound to occur. Hogben's criticism of the photo-electric method of A. V. Hill and his co-workers (Hogben, 1936) is clearly justified. Hill's method has been tried and subsequently discarded by Wykes (1937) for small teleosts. Wykes aptly observes, "photo-electric measurements of this type record the *sum effect* of colour response only and give us no information as to the activity of the different pigmentary effectors concerned".

Wykes's own quantitative method—the measurement of *fixed* melanophores—is also, however, open to criticism. It involves killing the small fish used by immersion in boiling water or in formalin. In the present state of knowledge of the cytological behaviour and effector-reaction time of vertebrate melanophores this seems risky, especially where the existence of nervous control may be known or suspected. Further, in fixing generally a certain degree of change of size is involved, which may vary with the state of the tissue concerned. Melanophore readings will therefore be slightly different from those made on the living animal; and if a curve has a very protracted asymptote—for example, those of the eel in the regions between 4·0–5·0 and 2·0–1·0, it is far from certain that this will appear accurately in a graph based on fixed material.

In Amphibia at least the fixed condition of fully expanded melanophores is invariably *less than fully expanded*; and the fixed condition of fully contracted melanophores is always *less than fully contracted.* A melanophore index of 5·0 becomes 4·0 and a melanophore index of 1·0 becomes 2·0 (Hogben, unpublished). The risk of error in this case may be judged from Fig. 1, which represents a hypothetical case where readings from living material give the curve *A*. The dotted line (*A'*) shows the curve resulting from readings of the same material *after* fixation.

Where chromatic behaviour is of the slow type and the husbandry of the animals concerned is well understood, examination of the melanophores of the living animal at intervals may be safely undertaken.

Where, however, chromatic behaviour is of the rapid type accurate observation of the melanophores of the living animal frequently presents technical difficulties. The sensitiveness of some animals, e.g. many teleostean fishes, to chemical and mechanical stimuli seriously increases the difficulty of recording precisely the state of the melanophores at a given moment, and will readily bring about abnormal chromatic behaviour, unless precautions are taken.

A satisfactory method in such cases is one where the melanophore changes of single animals *in situ* are followed through to equilibrium under a bar binocular microscope without disturbance of the animal in any way. The mean melanophore indices at given time intervals of a number of animals thus read under uniform conditions furnish points of a representative curve.

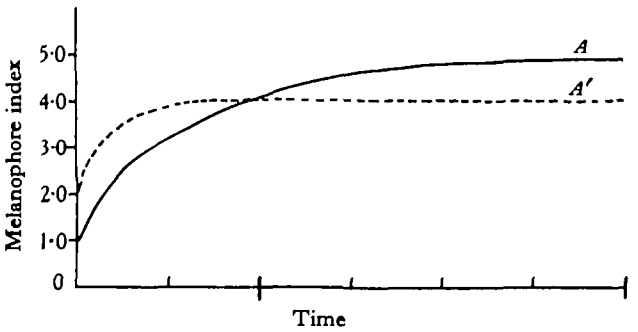

Fig. 1. Diagrammatic figure showing the curve resulting from melanophore readings on living (*A*) and fixed (*A'*) material.

Alternatively, or where the foregoing method is not feasible, or is impossible, e.g. in determining chromatic behaviour in total darkness, the method of Hogben and Landgrebe (in press) for observations on the chromatic behaviour of *Gasterosteus* may be adopted. By this method each point on the curve represents the mean melanophore index—read in the most suitably speedy fashion—of a number of living animals, e.g. six, used *once*, i.e. used for that point only and then discarded.

All three methods of determining the melanophore changes of the living animal have been used in the present research and are referred to in appropriate context.

IV. THE CHROMATIC BEHAVIOUR OF THE EEL (*ANGUILLA VULGARIS* L.)

Of the two contrasting types of behaviour known amongst Vertebrates—already referred to as *rapid* and *slow* respectively—rapid change is exhibited by some teleostean fishes and some reptiles and there is in these groups direct evidence of direct nervous control of melanophores: slow change, for which there is direct evidence of pituitary control, occurs in cyclostomes, elasmobranch fishes and Amphibia.

On the basis of existing work—especially that of Young—the latter is the archaic form of the chromatic function.

Petersen, whose work on the zoology of the eel is well known, wrote in 1894 of "eels, like most other fishes, being able to change colour quickly". Examination of the time relations of colour change in the eel shows that this is not so, and indicates that the eel, though a teleost, is, as regards its chromatic behaviour, of the physiologically archaic type.

(1) *Procedure*

The time graphs shown in Figs. 3–7 result from experiments with eels 45–70 cm. in length done under standardized conditions, using a constant artificial illumination, i.e. a 25 W. bulb 30 in. above the bottom of each tank, the top of the tank being covered with a sheet of ground glass. All other light was excluded. Animals were kept in porcelain tanks in running water throughout. The slowness of the colour response of the eel allows successive readings at intervals of the melanophore

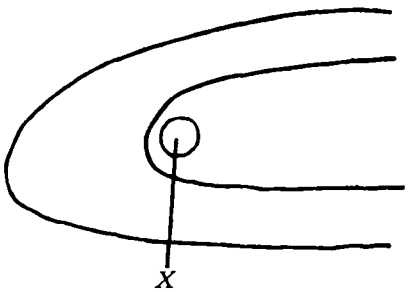

Fig. 2. Eel tail. X = site of readings.

indices of a group of animals to be undertaken under the conditions referred to without undue risk of error, and no difficulty was experienced in maintaining the animals over prolonged periods so long as they were properly handled. The following technique was adopted for reasons stated.

An eel was lifted from the tank by a capacious close-meshed net made of *soft* string, thick enough to remain sodden and flaccid. This was laid down on a solid surface, e.g. the glass top of an adjacent tank, and a *damp* cloth immediately laid over the animal, especial care being taken that the head was well covered. Thus treated animals readily become quiescent, and cloth and eel may be picked up and bent into a U shape convenient for holding, the tail-tip only protruding from the mass. This can only be done, however, without considerable resistance from the animal, provided that the head is completely but loosely covered, the action rapidly performed, and only *very light pressure* is employed. A firm grip invariably provokes resistance, and if persisted in will very frequently result in the death of specimens within a few days. The use of *dry* cloths invariably results in considerable mortality, owing to damage to the external mucus layer.

In the case of the larger specimens used in the present investigation, the mass of eel and cloth, too large for holding conveniently in one hand, was held lightly under the left arm, resting on the forearm and hand, while the tail-tip was read.

Reading was done by placing the tail-tip on wet glass plate covering the stage of a binocular microscope with concealed substage lamp; and thereafter the animal was released at once into its tank.

All readings were made at the site X (Fig. 2).

(2) *Chromatic behaviour*

The relevant time intervals are those involved in changing:

(*a*) From equilibrium on white background to equilibrium on black background in light.

(*b*) From equilibrium on black background to equilibrium on white background in light.

(*c*) From equilibrium in total darkness to equilibrium on white background in light.

(*d*) From equilibrium in total darkness to equilibrium on black background in light.

(*e*) From equilibrium on white background to equilibrium in total darkness.

(*f*) From equilibrium on black background to equilibrium in total darkness.

The mean equilibrium values of the eel were found to be:

	Melanophore index
On white background in light	1·2
On black background in light	4·8
In total darkness	3·5

Blinded eels kept for prolonged periods under standard illumination equilibrated at 3·7, thus showing a primary response of 0·2.

The response to change of background in light is very slow, taking 20 days on either white or black background at 8° C. (Fig. 3). A quite large rise of temperature

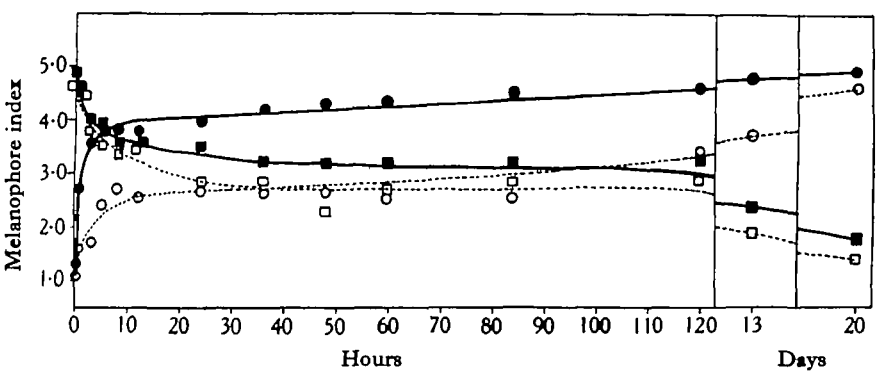

Fig. 3. Eel. Black and white background response in light. Temp. 8·3 ± 0·8° C. Standard lighting (see text). Each curve represents a group of six animals. Black circles and squares = dermal melanophores. Clear circles and squares = epidermal melanophores of the same animals.

has a relatively slight effect. Such effect is mainly seen in the early phases of equilibration. This is shown in Fig. 4 where are reproduced the initial part of the time graphs of two groups of eels, one carried through at 22° C., the other at 7° C.

As is well known in eels, the black and yellow pigmentation may give place to a black and silver coloration customarily believed to mark the onset of the reproductive phase of the life cycle. It would be more correct to state that this marks

the onset of migration to the sea. In point of fact there is no difference between the state of the gonads of silver and yellow eels of the same size, and the statement that the silver and black coloration is the breeding dress is only true in so far as eels do not become sexually mature unless they do migrate.

The initial reactions of the dermal melanophores of the "silver" phase are in general very slightly faster than those of the "yellow" phase of the life cycle. These are shown in Fig. 5. The difference is almost wholly in the early stages of response. Apart from this the chromatic behaviour of the two phases is similar, so far as dermal melanophores are concerned.

A striking difference, however, in the silver phase as compared with the yellow occurs in a different class of pigmentary effectors—the epidermal melanophores. With the onset of the silver phase a considerable proportion of these exhibit irregular or patchy distribution of pigment within the melanophores. In the expanded melanophores the normal stellate appearance is destroyed by uneven

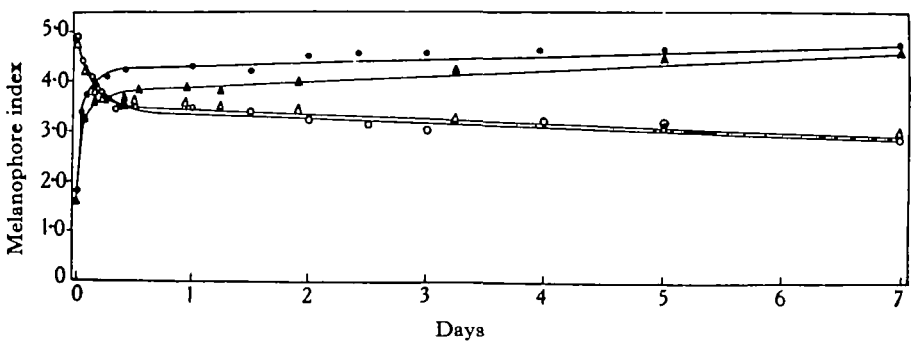

Fig. 4. Eel. Black and white background response in light at low and high temperatures. Each curve represents a group of six animals. Circles = at 6·7 ± 1·0° C., triangles = at 21·75 ± 0·5° C. Standard lighting.

dispersion of pigment. Under conditions inducing contraction aggregation of pigment may form a single mass or several. Further, in a proportion of the epidermal melanophores an appearance consistent with disruption of the melanophore supervenes and dispersal of its contents forms a grey blotch or blur of apparently inert pigment on and around the site of the melanophore.

The background responses in light of eels equilibrated for a prolonged period in total darkness and transferred to black and white background under standard illumination are shown in Fig. 6. Full pallor in white background is only attained after 22 days at 9° C. Complete darkening on black background takes 9 days at 6° C.

Eels transferred from a white or a black background in light to total darkness equilibrate at the intermediate figure of 3·5. Equilibration from white background is very slow, requiring 27 days at 8° C. Equilibrium is reached after 2 days from black background (Fig. 7).

The general similarity of these time graphs to those of Amphibia, e.g. *Xenopus laevis*, is very striking. The chromatic behaviour of both eel and *Xenopus* is of the slow type, though the latter is less slow in the final phases of equilibration.

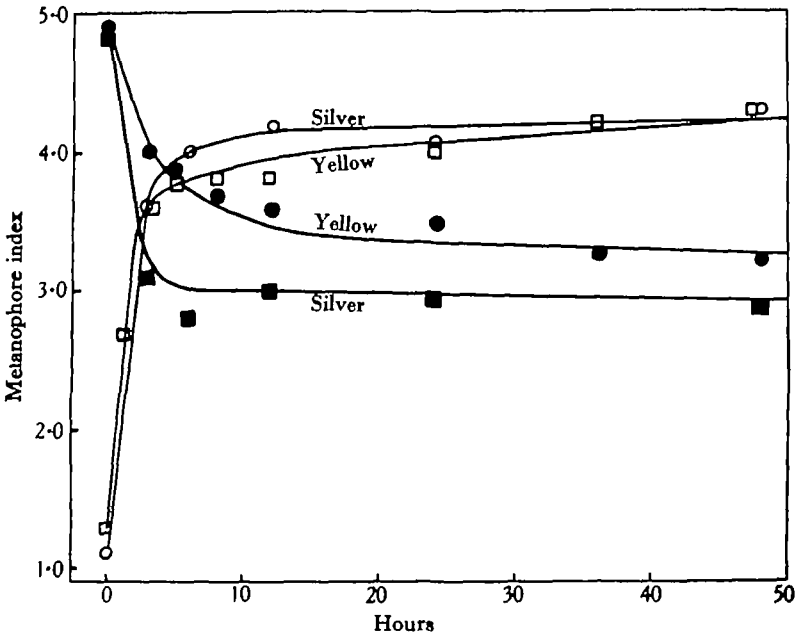

Fig. 5. Eel. Black and white background response in light of eels in the silver (squares) as compared with the yellow (circles) phase. Each curve represents a group of six animals. The early stages of the response are shown. The total time for complete response is similar in both phases. Temp.: silver 12·0 ± 0·4° C.; yellow 8·3 ± 0·8° C. Standard lighting.

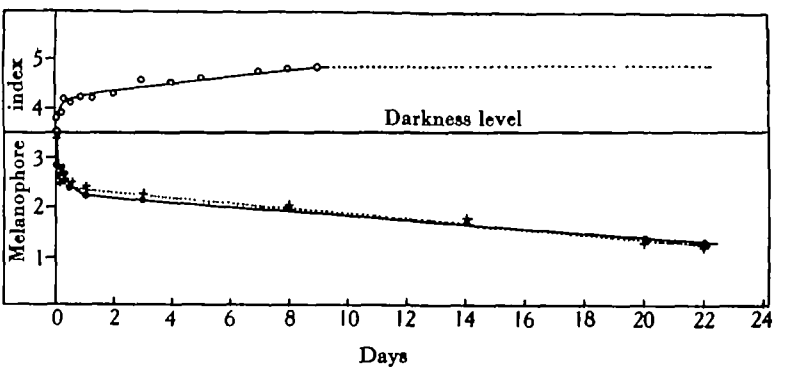

Fig. 6. Eel. Response to black (clear circles) and to white background (black circles) in light after 4 weeks in total darkness. Each curve represents a group of six animals. Temp. 6·0 and 9·0° C. respectively. Standard lighting. The dotted curve (crosses) represents a group of eels in the silver phase (temp. 9° C.).

The background responses of a group of *Xenopus* carried through under identical conditions of lighting and temperature (6·7° C.) to one of the two groups of eels recorded in Fig. 4 is shown in Fig. 8.

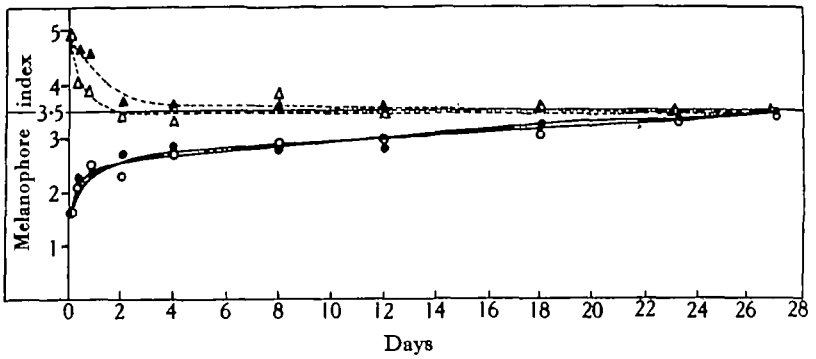

Fig. 7. Eel. Response on transference from black and from white background in light to tota darkness (clear triangles and circles). Each curve represents a group of six animals. Temp. 8·5 ± 0·7° C. Black circles and triangles show similar animals blinded immediately prior to transference to darkness.

Both species exhibit similar reactions on change from black to white background in light or vice versa. Comparison of Figs. 4 and 8 shows that in both there is a steep initial rise in reaction to black background and an absence of this steepness in reaction to white background.

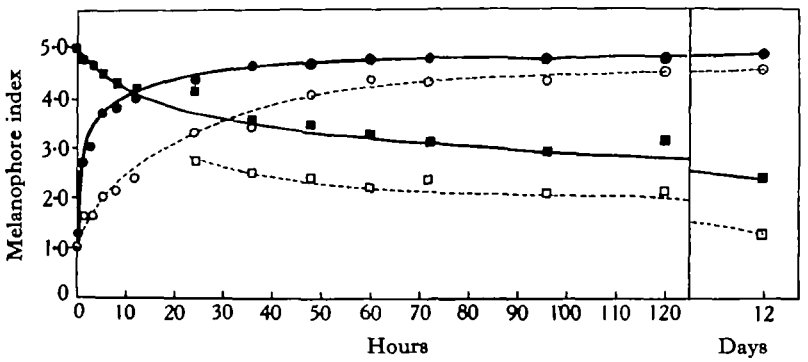

Fig. 8. *Xenopus*. Response to black and to white background in light of *Xenopus laevis* at 6·7 ± 1·0° C. Standard lighting. Black squares and circles = dermal melanophores. Clear squares and circles = epidermal melanophores. Each curve represents a group of six animals.

The similarity of response is equally marked in animals equilibrated in total darkness and transferred to black and white background in light (eel, Fig. 6; *Xenopus*, in Fig. 4), Hogben & Slome (1931). In both species response to white background is slower than response to black background.

The similarity of behaviour is again borne out by the behaviour following transference to total darkness of eels and *Xenopus* equilibrated on white and black backgrounds in light (eel, Fig. 7; *Xenopus*, Fig. 4), in Hogben & Slome (1936). In

both species the darkness equilibrium figure reached is intermediate, and in both the time taken to reach it from the white background condition is markedly in excess of that needed from the black background condition. In both also the primary response is small.

This correspondence is entirely consistent with what the time relations of the chromatic behaviour of the eel both in light and in darkness clearly indicate: that its chromatic behaviour is of the slow type where hormonal co-ordination is involved.

(3) *Nature of the mechanism*

It is evident that the chromatic behaviour of the eel is controlled by a hormonal mechanism, activated by localized photo-receptors in the retina.

In addition to bringing out which of the two alternative mechanisms previously discussed is concerned, the time relations of chromatic behaviour also throw light on the *complexity* of the mechanism involved, as shown by Hogben & Slome (1936) in Amphibia, Waring (1938) in elasmobranchs, and Smith (1938) in Crustacea.

(1) A convenient notation for the time intervals involved is as follows:

$_bT_w$ = The time taken to change from equilibrium on black background in light to equilibrium on white background in light.

$_bT_d$ = The time taken to change from equilibrium on black background in light to equilibrium in total darkness.

The relevant time intervals and the melanophore changes which accompany them are (using round figures for convenience):

Interval	Melanophore movement	Duration days	Interval	Melanophore movement	Duration days
$_bT_w$	5·0 → 1·0	20	$_wT_b$	1·0 → 5·0	20
$_bT_d$	5·0 → 3·5	2	$_wT_d$	1·0 → 3·5	27
$_dT_w$	3·5 → 1·0	22	$_dT_b$	3·5 → 5·0	9

In chromatic behaviour of the slow type either a single hormone is involved or more than one.

(*a*) If a single hormone is involved two alternative hypotheses present themselves:

(i) That the behaviour is controlled by a *melanophore expanding* hormone *B*, whose production is inhibited on a white background and augmented on a black background. This implies that the hormone concentration is highest on a black background ($\mu = 5{\cdot}0$), least on a white background ($\mu = 1{\cdot}0$) and intermediate in darkness ($\mu = 3{\cdot}5$).

Since $_wT_b$ is the time taken to raise μ from 1·0 to 5·0, i.e. for maximum increase in concentration of hormone, neither $_wT_d$ nor $_dT_b$ can be greater than $_wT_b$, as they both involve smaller increases in concentration.

Since $_bT_w$ is the time taken to lower μ from 5·0 to 1·0, i.e. for maximum decrease in concentration of hormone, neither $_bT_d$ nor $_dT_w$ can be greater than $_bT_w$.

In the eel ${}_{w}T_{d}$ (27 days) *is* greater than ${}_{w}T_{b}$ (20 days), and ${}_{d}T_{w}$ (22 days) *is* greater than ${}_{b}T_{w}$ (20 days).

Hypothesis (i) therefore fails.

(ii) That the behaviour is controlled by a *melanophore contracting* hormone *W*, whose production is augmented on a white background and diminished on a black background. This implies that the hormone concentration is highest on a white background ($\mu = 1{\cdot}0$), lowest on a black background ($\mu = 5{\cdot}0$) and intermediate in darkness ($\mu = 3{\cdot}5$).

Then since ${}_{b}T_{w}$ is the time taken to lower μ from 5·0 to 1·0, i.e. for maximum increase in concentration of hormone, neither ${}_{b}T_{d}$ nor ${}_{d}T_{w}$ can be greater than ${}_{b}T_{w}$; both involving smaller increases in concentration.

And since ${}_{w}T_{b}$ is the time taken to raise μ from 1·0 to 5·0, i.e. for maximum decrease in concentration of hormone, neither ${}_{w}T_{d}$ nor ${}_{d}T_{b}$ can be greater than ${}_{w}T_{b}$.

The time curves of the eel show, however, that ${}_{d}T_{w}$ (22 days) *is* greater than ${}_{b}T_{w}$, and ${}_{w}T_{d}$ (27 days) is greater than ${}_{w}T_{b}$.

Hypothesis (ii) therefore fails also.

The time relations therefore indicate that the hypothesis of a one-hormone mechanism will not cover the facts of colour change in the eel.

(*b*) If two hormones are involved the bi-humoral hypothesis put forward by Hogben & Slome (1931, 1936) to explain the chromatic responses of Amphibia may be next considered.

This hypothesis postulates that the melanophore index at any one time is due to the effective relative excess of one or other of two antagonistic hormones, each with its own rate of production and elimination, one of which, *B*, is a melanophore expanding hormone and the other, *W*, a melanophore contracting hormone, each activated by separate localized receptors in the retina.

If this hypothesis be well founded—as it has been shown to be in *Xenopus laevis*—the time relations of chromatic response are of primary significance.

The hypothesis implies that in an animal approaching equilibrium on a white background in light production of both *B* and *W* will be going on, *W* over-riding *B*.

The reactions taking place during the relevant time intervals may then be explained as follows:

		Initial state of melanophores	Final state of melanophores	Duration days
${}_{w}T_{b}$	Decrease of *W* (*B* increasing *in effect*)	1·0	5·0	20
${}_{b}T_{w}$	*B* constant. Increase of *W* (over-riding *B*)	5·0	1·0	20
${}_{b}T_{d}$	Decrease of *B*	5·0	3·5	2
${}_{d}T_{b}$	Increase of *B*	3·5	5·0	10
${}_{d}T_{w}$	Increase of *B*. Increase of *W* (over-riding *B*)	3·5	1·0	22
${}_{w}T_{d}$	Decrease of *B*. Decrease of *W*	1·0	3·5	27

Various degrees of interaction are therefore involved, implying that different orders of time will be required for their completion.

(1) Since ${}_bT_d$ and ${}_dT_b$ each involve only a single process of production or elimination of hormone, expectation would be that these time intervals would be relatively short.

(2) Since ${}_dT_w$ and ${}_wT_d$ each involve two *competing* processes, which in the one case are, and in the other case may be, in active and direct competition with each other, expectation would therefore be that equilibrium would be reached much more slowly than in (1).

If the time relations of the chromatic behaviour of the eel are consistent with these implications of the bi-humoral hypothesis stated, then the order of duration of ${}_dT_w$ and ${}_wT_d$ should significantly exceed that of ${}_bT_d$ and ${}_dT_b$. Reference to the right-hand column of the foregoing table will show that this is so.

Examination of the time relations of the chromatic behaviour of the eel discloses therefore that co-ordination is effected by a humoral mechanism, and that while the hypothesis of a one-hormone control is inadequate to account for the time intervals observed, the latter are consistent with an hypothesis postulating two antagonistic hormones whose interaction in varying concentrations is responsible for the state of the melanophores existing at any given time.

(2) *Behaviour in darkness.* Complete expansion (dispersion) of the melanophores of the eel does not take place on transference to darkness from equilibrium on white or black background in light.

Where activation of humoral control of chromatic behaviour is by photic stimulation of retinal receptors, it might be expected that the degree of response would be dependent on the amount of light reaching the retina: and that therefore an animal transferred from a white background in light ($\mu = 1{\cdot}0$) to a black background in light (involving a reduction in the amount of light falling on the retina) would exhibit a less degree of response than one transferred from a white background in light to total darkness (*absence* of light). This is not the case. Instead, equilibrium in darkness occurs at $\mu = 3{\cdot}5$ and the time taken to reach it is *relatively protracted* in comparison with the time required in light for the same range of melanophore movement.

This suggests that melanophore expansion and contraction in darkness may be a physiologically distinct process from expansion and contraction in light. Presumably, therefore, the difference between the white background and black background responses in light is not due to light intensity, but to the action of light on different parts of the retina.

In any case we have to distinguish between two categories of visually determined photic response, i.e.

(*a*) the transition from equilibrium in light to equilibrium in darkness, and vice versa;

(*b*) the transition from equilibrium on a white background with overhead illumination to equilibrium on a black background with overhead illumination, and vice versa.

V. THE CHROMATIC BEHAVIOUR OF *LEBISTES RETICULATUS* (PETERS) REGAN

The existence of the slowly reacting archaic humoral type of chromatic mechanism in a teleostean fish, the eel, is of great interest, since the group is one whose reactions have been generally believed, since the work of Pouchet, to be under nervous rather than endocrine control.

Nervous control of melanophores has been demonstrated in a number of teleosts (Sand, 1935). Chromatic behaviour of the rapid type occurs amongst others in *Lebistes reticulatus* (Peters) Regan. Study of the time relations of *Lebistes* provides a contrast to those of the eel, as background response in light is complete in less than an hour.

(1) *Procedure*

The time graphs shown in Fig. 10 show the response of two series of six *Lebistes* (♀) equilibrated in small glass containers to black and white background in porcelain tanks in running water, at 24 in. from a 40 W. opal bulb. On change of background the subsequent equilibration of each individual was followed through under a bar binocular without interference with the specimen. The time graphs shown in Fig. 11—equilibration to black and white background in light after 7 days' total darkness—were also constructed from readings made in this way. All readings of *Lebistes* were made at the site *X*, Fig. 9.

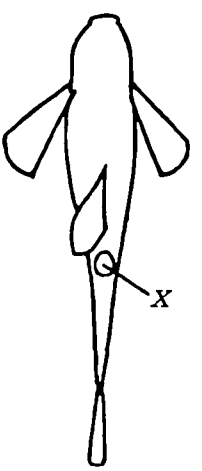

Fig. 9. *Lebistes*. *X* = site of readings.

In view of the rapidity of reaction of *Lebistes* to light, the curves shown in Fig. 12 were constructed differently. The fish were kept in total darkness in running water in small oval glass containers (Woolworth salt cellars) capped with a silk gauze cover secured by a rubber band. For reading, a container was gently lifted from the tank in darkness, a glass slip substituted for the gauze cap, and the container placed on the stage of a binocular microscope fitted with an enclosed substage lamp, the beam of which was reduced by a diaphragm to a fine pencil. On this lamp being switched on, rapid reading (5 sec.) of the melanophore index of the posterior region of the fish could be undertaken with a minimum risk of exposure of the specimen to direct light. At the same time a risk remained and accordingly each specimen was read *once* only and then discarded. Each point of the time graphs of Fig. 12 therefore represents the mean melanophore index of six individuals used for that occasion only. The starting figure of each batch of six was checked at the outset.

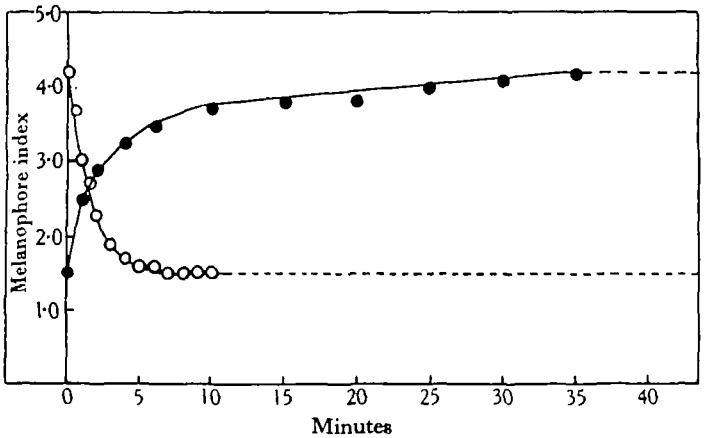

Fig. 10. *Lebistes*. Response to black and to white background in light. Each curve represents a group of six animals. Temp. 16° C. Lighting, 40 W. opal bulb at 24 in.

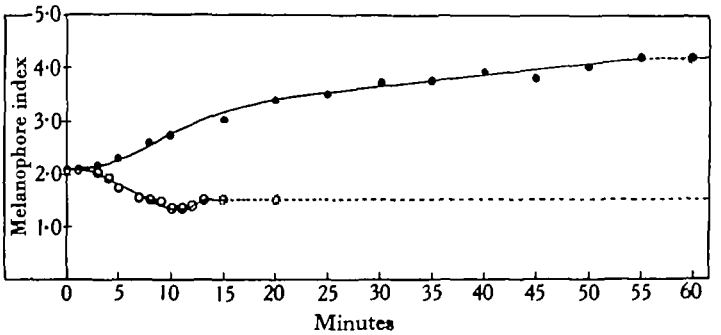

Fig. 11. *Lebistes*. Response to black and white background in light after 7 days in total darkness. Each curve represents a group of six animals. Temp. 16° C. Lighting, 40 W. opal bulb at 24 in.

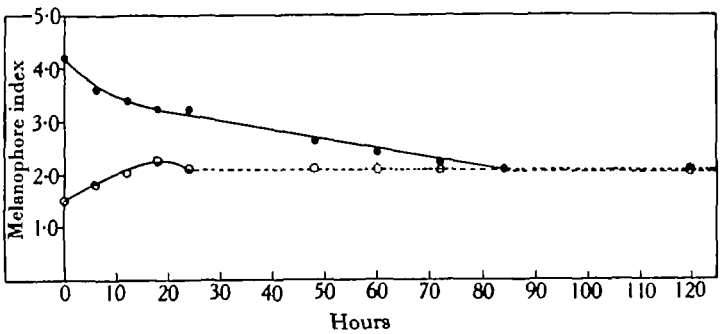

Fig. 12. *Lebistes*. Response on transference from black and white background in light to total darkness. Each point on the curves represents a separate group of six animals. Temp. 16° C.

(2) *Chromatic behaviour*

Mean equilibrium values	Melanophore index
On white background in light	1·5
On black background in light	4·2
In total darkness	2·1

Attempts to ascertain the primary response in *Lebistes* have been less conclusive, owing to the large mortality subsequent to removal of the eyes and before equilibrium may have been reached.

Eyeless animals kept under standard lighting behaved as follows:

		Surviving				
° C.	No.	48 hr.	72 hr.	96 hr.	7 days	10 days
16	20	5	4	4	2	1
Mean melanophore index		2·7	2·7	2·8	2·7	3·0

Since equilibration in total darkness takes place at 2·1 the range of the primary response would appear to be 0·6–0·9.

The response in light of *Lebistes* transferred from black to white background and vice versa is rapid (Fig. 10), especially the white background response which is complete in 7 min. Both black and white background responses slow down in the later stages of equilibration. In the former 35 min. in all are required for equilibrium to be reached.

In *Lebistes*, transferred from total darkness ($\mu = 2{\cdot}1$) to black and to white backgrounds in light, response is complete within 1 hr. (Fig. 11).

The behaviour in darkness of *Lebistes*, previously equilibrated on black and on white backgrounds in light (Fig. 12), shows a sharp contrast in *time* with its behaviour in light. Over 80 hr. are necessary for equilibrium to be reached following equilibration on black background, and 24 hr. from white background.

VI. THE CHROMATIC BEHAVIOUR OF *SALMO SALAR* L.

In the course of observations on the post hatching and subsequent parr stages of *Salmo salar* L and *S. trutta* L. it was noticed that the chromatic behaviour of these species appeared to be also of the rapid type. Observations on the latter also showed that background response does not occur in *Salmo* till approximately 3 weeks after hatching, at 7–8° C. This is consistent with the fact that the development of the eye in *Salmo* remains incompleted for some time after emergence from the egg.

For the time graphs presented in this paper *S. salar*, 3–4 months old (salmon parr), have been used.

(1) *Procedure*

The making of precise melanophore readings on these fish is rendered difficult by their extreme activity coupled with the rapidity under certain conditions of their colour response, and the readiness with which this may be disturbed by extraneous stimuli, e.g. by incautious manipulation. The following method was eventually used and found to work satisfactorily in practice.

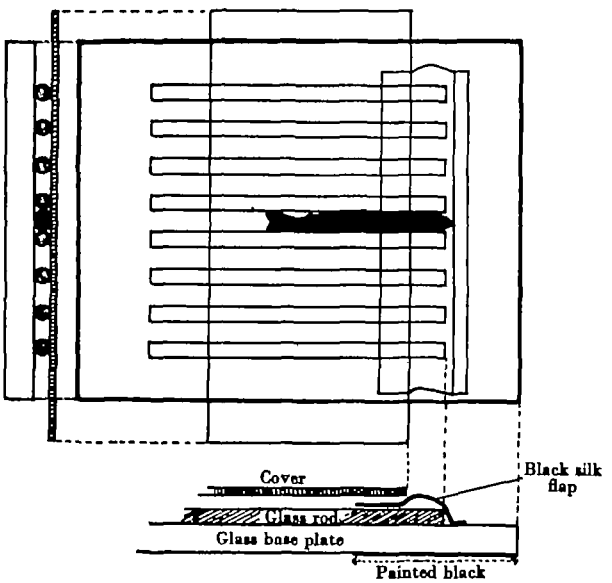

Fig. 13. Glass grid used for reading melanophores in living *Salmo*. Glass rods or lengths of tube are cemented to a glass base, the width of rods and interspaces being adjusted precisely to the dimensions of the animal. A flap of black silk gauze is cemented along one end, as shown. This acts as a stop when the fish is released on to the slightly tilted grid, surplus water draining away. A glass cover is placed over all, free of the anterior end of the fish. The underside of the base plate beneath the gauze flap is painted black.

Fish were equilibrated in running water in black and in white porcelain tanks in glass tubes capped with fine black or white silk gauze secured by a narrow rubber band, at 24 in. from a 40 W. electric bulb. For reading of the melanophore index a tube was taken from the tank, uncapped, and its contents poured rapidly, in subdued light, on to the glass grid shown in Fig. 13. A glass cover was added, the grid slid quickly under a binocular with enclosed substage lamp, and the reading made. The fish was then discarded. With a little practice reading could be done within 5 sec. of withdrawal from the background.

In determining the chromatic behaviour in total darkness it was found that for the operation of pouring on to the grid and covering the fish accurately, 2–3 sec. of very dim reflected light was desirable—and sufficient—in practice. A small area of bench between tank and microscope, fully screened from both, was used for this step.

Tests showed that this momentary departure from absolute darkness conditions had no appreciable effect on the melanophore readings.

Readings were made with a binocular microscope with enclosed substage lamp, as described for *Lebistes*.

All readings of *Salmo* were made at the site *X*, Fig. 14.

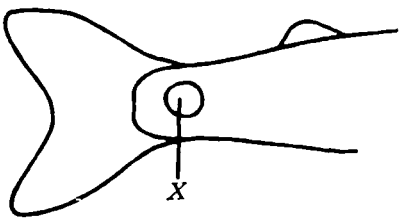

Fig. 14. *Salmo*. *X*=site of readings.

The time graphs shown in Figs. 15–17 are therefore constructed on the same principle as those of Fig. 12 above, i.e. each point of the curve represents the mean melanophore index of six fish used for that point only.

(2) *Chromatic behaviour*

Mean equilibrium values	Melanophore index
On white background in light	1·5
On black background in light	4·4
In total darkness	1·2

The primary response is moderate, the range being approximately 0·5. Eyeless fish kept under standard lighting showed the following figures:

		Surviving					
° C.	No.	24 hr.	48 hr.	96 hr.	1 week	3 weeks	6 weeks
10–14	20	11	4	4	4	4	2
Mean melanophore index		3·1	3·0	2·8	2·1	2·0	1·75

The background responses in light of *Salmo* are shown in Figs. 15 and 16.

The response of *Salmo* transferred from black background to white background in light is rapid, like that of *Lebistes*. Slowing down, however, occurs earlier, so that attainment of equilibrium at $\mu = 1{\cdot}5$ takes 30 min. The response of *Salmo* transferred from white to black background in light, however, is relatively slow, and requires 10 hr. for completion.

On transference to black and white background in light after 7 days in total darkness response to white background is complete within 1 hr. Equilibrium on black background is reached in 30 hr.

The chromatic behaviour of *Salmo* in darkness (Fig. 17), like that of *Lebistes*,

shows a time contrast to its behaviour in light. Fish transferred from white background in light to total darkness require 24 hr., and fish transferred from black background require 72 hr. to reach equilibrium at 1·2. The very low melanophore index of darkness equilibrium is significant, since it denotes a condition of pallor indistinguishable from that which occurs in fish placed on a white background in light.

VII. NATURE OF THE MECHANISM IN *LEBISTES* AND *SALMO*

The chromatic behaviour of *Lebistes* and *Salmo* presents striking contrasts with that of the eel. If the argument concerning the significance of time put forward at the beginning of this paper be accepted, the chromatic behaviour of the eel is clearly controlled by a humoral mechanism, while the existence of nervous co-ordination in *Salmo* and *Lebistes* is equally clear.

Salmo and *Lebistes* therefore appear to conform to the view that has been generally held hitherto concerning the nature of the co-ordinating mechanism in teleostean fishes.

Nervous control of melanophores in *Lebistes* and *Salmo* may readily be confirmed by weak electrical stimulation of the dorsal skin behind the head in animals equilibrated on a black background in light. Observed times of melanophore movement in animals so equilibrated, and hooded (with wet black paper) during the observation, were as follows:

	Melanophore movement	Mean time sec.	Recovery to initial state sec.
In the intact animal:			
Lebistes	4·0→1·5	32±5	73±7
Salmo	4·0→1·5	45±3	107±22
In the eviscerated animal:			
Lebistes	4·0→1·5	32±9	98±13
Salmo	4·0→2·0	49±5	135±11

With these reaction times in mind, further analysis of the mechanism of chromatic behaviour of *Lebistes* and *Salmo* may be essayed.

(*a*) *Behaviour in light*

Scrutiny of the time graphs of Fig. 10 shows that the response of *Lebistes*, transferred from a black background to a white background in light and vice versa, while rapid in its early stages subsequently slows down, so that the later phases of equilibration are distinctly more protracted than the earlier. This slowing down, more conspicuous in response to black background, suggests the possibility of a humoral element being involved also in co-ordination, and that the mechanism of the chromatic behaviour of *Lebistes* in light though predominantly nervous is reinforced by reflex hormonal control.

The same slowing down towards completion occurs in the response of *Salmo*

to white background in light (Fig. 15). In *Salmo*, however, the response of animals transferred from a white background to a black background is definitely prolonged

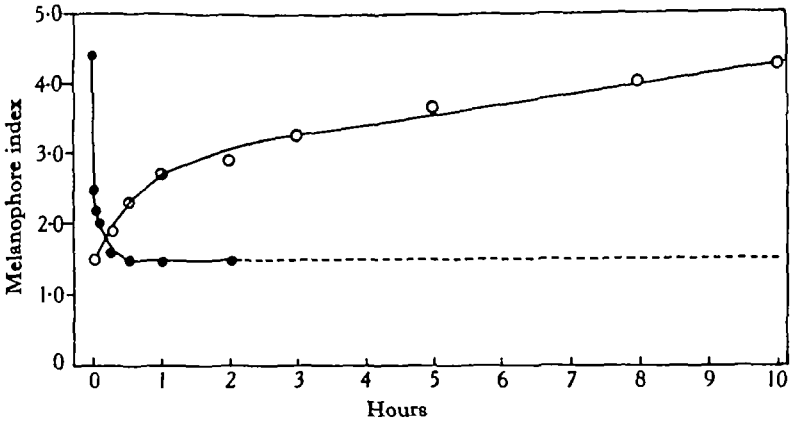

Fig. 15. *Salmo*. Response to black and to white background in light. Each point on the curves represents a separate group of six animals. Temp. 13·5° C. Lighting, 40 W. opal bulb at 24 in.

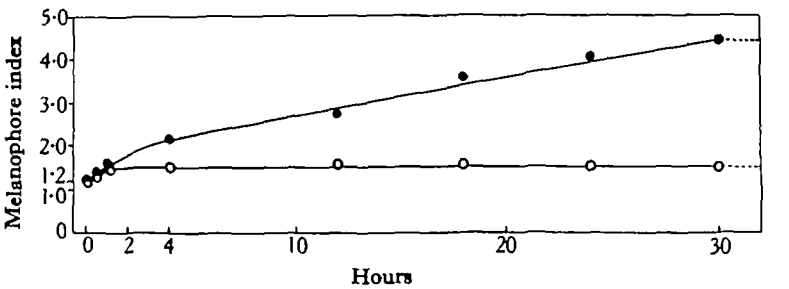

Fig. 16. *Salmo*. Response to black and white background in light of animals kept for 7 days in total darkness. Each point on the curves represents a separate group of six animals. Temp. 13·5° C. Lighting, 40 W. opal bulb at 24 in.

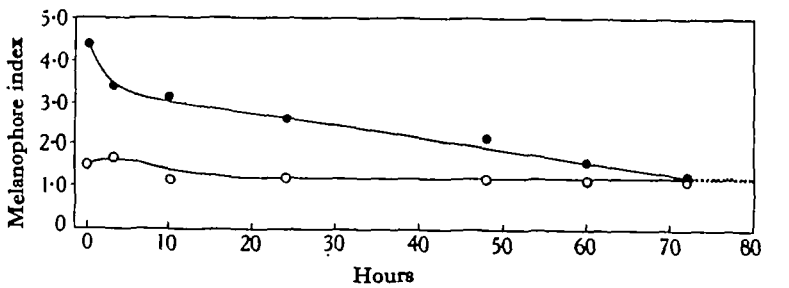

Fig. 17. *Salmo*. Response on transference from black and white background in light to total darkness. Each point on the curves represents a separate group of six animals. Temp. 13·5° C.

(10 hr.) in comparison with the white background response ($\frac{1}{2}$ hr.). *Ex hypothesi* it would appear to be co-ordinated less by nervous than by hormonal control. Control of chromatic behaviour of *Salmo* in light cannot then be described as wholly nervous.

This interpretation of the time relations of the chromatic behaviour of *Lebistes* and *Salmo* is consistent with the view that the control of the chromatic function in Vertebrates has evolved from a wholly humoral into a wholly nervous mechanism (as in some reptiles), and that both mechanisms should coexist in some forms, whether both be functionally significant or otherwise. The existence among teleostean fishes of two sharply contrasting modes of chromatic behaviour in light, as seen in the eel and in *Lebistes*, suggests that in this group intermediate conditions may be expected.

(*b*) *Behaviour in darkness*

The time relations of the chromatic behaviour, and the reactions of the melanophores themselves, of *Lebistes* and *Salmo*, *in darkness*, are not consistent with wholly nervous co-ordination in either species.

A wholly nervous mechanism activated by light is inadequate to account for the following facts:

(1) Pale *Lebistes* (1·5) transferred to darkness from white background in light darken only slightly to 2·1, require 24 hr. for the change, and remain at that figure (Fig. 12).

(2) Pale *Salmo* (1·5) similarly transferred to darkness from white background in light continue to pale to 1·2, requiring 24 hr. for the change. Absence, in darkness, of the primary response, *might* account for the lowered figure, but the time needed is significant (Fig. 17).

(3) Dark *Lebistes* (4·2) transferred from black background in light to total darkness eventually pale to 2·1, and remain at that figure. They take 84 hr. to reach it (Fig. 12).

(4) Dark *Salmo* (4·4) transferred from black background in light into darkness fall to 1·2—a lower figure than in light on a white background—taking 72 hr. (Fig. 17).

There are thus two sets of facts which indicate that co-ordination in both *Lebistes* and *Salmo* is not wholly nervous.

(1) The prolonged time required in each case to reach equilibrium in darkness as compared with that required in light. These time intervals are plainly out with the limits of a nervously controlled mechanism. On the other hand, following what has been said in relation to the chromatic behaviour of the eel, the duration of the interval ${}_bT_d$ in both species is consistent with humoral control being involved.

(2) The state of the melanophores (melanophore index) arrived at *in the absence of light*.

To retrace the argument put forward in regard to the chromatic behaviour of the eel (p. 85, supra), if chromatic response to photic stimulation were determined entirely by the amount of light which falls on the retina, i.e. were solely nervous reflex, it might be expected that an animal kept in total darkness would reach a higher melanophore index than an animal kept on a black background in light; certainly not a *lower* melanophore index, when appropriately corrected for the primary

reaction. This is not so, notably in *Salmo*, which is as pale in darkness as in light on a white background.

Thus the change from melanophore expansion to melanophore contraction, or vice versa, is not a single physiological process.

It is necessary therefore to distinguish between two distinct physiological processes in examining chromatic response to photic stimulation: to distinguish between

(i) what happens when an animal is brought from light into darkness, and vice versa; and

(ii) what happens when an animal kept in light on a white background is transferred to a black background in light, or vice versa.

(*c*) *Co-ordination*

To say that co-ordination of chromatic behaviour in *Lebistes* or in *Salmo* is not wholly nervous might therefore mean one of two things:

(*a*) that one of the two processes referred to above is mainly controlled by peripheral innervation and the other is mainly controlled by internal secretion;

(*b*) that humoral control reinforces nervous control of one or both of them.

The data summarized in this section indicate that we may now draw a distinction between *Lebistes* and *Salmo* from this standpoint. In *Lebistes* the first process is mainly, if not wholly, humoral, and the second is predominantly nervous. In *Salmo* the first process is mainly, if not wholly, humoral, and the second provides clear evidence for the coexistence of both humoral and nervous control, the latter reinforcing the former.

VIII. CONCLUSION

Examination of the time relations of the chromatic behaviour of *Anguilla vulgaris* furnishes evidence of the existence—hitherto unrecorded—among teleostean fishes of an exceedingly slow type of chromatic behaviour, co-ordinated by a humoral mechanism. This contrasts with the rapid type of chromatic behaviour exhibited by other teleosts where nervous control is involved, as in *Lebistes* and *Salmo*.

Study of the time relations of the two latter forms, however, indicates that co-ordination is not wholly nervous, especially in *Salmo* which provides clear evidence of the coexistence of both mechanisms, nervous and humoral.

These conclusions support the view which deems the evolutionary trend of the control of chromatic behaviour in vertebrates to have been from an archaic humoral mechanism, known to be present in cyclostomes, elasmobranchs and Amphibia, from which, through the superimposition of a nervous mechanism, the exclusively nervous control exhibited by some reptiles has been reached.

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